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The Genomes of Three Uneven Siblings: Footprints of the Lifestyles of Three *Trichoderma* Species

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SUMMARY

The genus *Trichoderma* contains fungi with high relevance for humans, with applications in enzyme production for plant cell wall degradation and use in biocontrol. Here, we provide a broad, comprehensive overview of the genomic content of these species for "hot topic" research aspects, including CAZymes, transport, transcription factors, and development, along with a detailed analysis and annotation of less-studied topics, such as signal transduction, genome integrity, chromatin, photobiology, or lipid, sulfur, and nitrogen metabolism in *T. reesei*, *T. atroviride*, and *T. virens*, and we open up new perspectives to those topics discussed previously. In total, we covered more than 2,000 of the predicted 9,000 to 11,000 genes of each *Trichoderma* species discussed, which is >20% of the respective gene content. Additionally, we considered available transcriptome data for the annotated genes. Highlights of our analyses include overall carbohydrate cleavage preferences due to the different genomic contents and regulation of the respective genes. We found light regulation of many sulfur metabolic genes. Additionally, a new Golgi 1,2-mannosidase likely involved in *N*-linked glycosylation was de-

tected, as were indications for the ability of *Trichoderma* spp. to generate hybrid galactose-containing *N*-linked glycans. The genomic inventory of effector proteins revealed numerous compounds unique to *Trichoderma*, and these warrant further investigation. We found interesting expansions in the *Trichoderma* genus in several signaling pathways, such as G-protein-coupled receptors, RAS GTPases, and casein kinases. A particularly interesting feature absolutely unique to *T. atroviride* is the duplication of the alternative sulfur amino acid synthesis pathway.

INTRODUCTION

The genus *Trichoderma* has a long tradition of use in agriculture and industry, and fungi of this genus are among the most useful microbes for human welfare. Also, because of the relatively high growth rates and the ability to adjust to different environments, these fungi could succeed as workhorses and are among the most commonly isolated saprotrophic fungi (1–3). Their metabolic and physiological capabilities provide important perspectives to tackle the challenges of our century. The awareness that fossil energy dangerously raises the CO_2 footprint and pollutes the environment is increasing, and sustainable alternatives are being actively sought. One of them is the production of second-generation biofuels from cellulosic plant waste, for which efficient biopolymer-degrading enzymes are needed. *T. reesei* is applied for this task in the world's leading companies. However, although agricultural waste and other plant material regrows every year, it is far from unlimited. The competition between the use of land for food and feed production and production of energy and/or fuels is a continuous issue that has to be dealt with carefully. In this respect, losses in crop production due to plant pathogens aggravate this problem.

While *T. reesei* is a specialized species that is adapted to a saprotrophic lifestyle and is an efficient degrader of cellulosic plant matter, *T. virens* and *T. atroviride* are mycoparasites and biological control agents (4, 5). *T. atroviride* and *T. virens*, along with other fungal species capable of fighting plant-pathogenic fungi, a process called mycoparasitism, will become more important in the future as alternatives to synthetic pesticides, which can be harmful to human health and the environment (6, 7). The interaction of *Trichoderma* with plant roots can stimulate plant growth and prime the plant's immune system for better resistance against pathogens. Due to this induced systemic resistance, *Trichoderma* spp. are able to protect plants against foliar pathogens, in addition to soilborne pathogens (8–10).

In the genus *Trichoderma*, cellulase research has been mostly restricted to *T. reesei*, whereas several other *Trichoderma* spp. are mycoparasites and/or are able to directly interact with plants (7). Various mycoparasitic *Trichoderma* species, e.g., *T. atroviride*, *T. virens*, *T. harzianum*, and *T. asperellum*, have been investigated for enzymes involved in fungal cell wall degradation in recent decades. More recently, the direct interaction between these species and plants has also received much attention in research. CAZymes have already been shown to be involved in the cross talk between plants and *Trichoderma* spp. and in the induction of plant defense responses (11). This example shows how research with different *Trichoderma* species is now becoming increasingly interactive and comprehensive across species.

Besides being extensively used as biological control agents and biofactories (6), several members of the genus *Trichoderma* have emerged as fungal models for understanding (i) asexual reproduction (7, 12–14), (ii) light and oxidative stress signaling (12, 15), (iii) mechanisms of vesicle transport and secretory pathways (16, 17), and (iv) synthesis of lytic enzymes and antifungal secondary metabolites (16, 18–20). Furthermore, *Trichoderma* spp. serve as models to elucidate plant-microbe interactions of beneficial microorganisms (7).

All three species discussed in this review have been subjected to intensive research, with topics spanning virtually their whole physiology (5). While for species of the genus *Trichoderma* the names of the teleomorph species were used previously, i.e., *Hypocrea jecorina* (*Trichoderma reesei*), *Hypocrea atroviridis* (*Trichoderma atroviride*), and *Hypocrea virens* (*Trichoderma virens*), a decision made at the International Botanical Congress in July 2011, which became effective 1 January 2013, now requires the use of a single name independent of the availability of a sexual stage that is instead based on a priority of publication and a community vote. Hence, *Trichoderma* will be used in the future for members of the genus (for a review on this issue, see references 21 and 22).

The genome of *T. reesei* was the first of the genus to be sequenced, and these sequences revealed several surprising characteristics. Most importantly, despite its high efficiency in plant cell wall degradation, the genome of *T. reesei* contains the fewest cellulases among the most commonly studied ascomycetes. In many cases, genes encoding CAZymes are found in genomic clusters, which are located between regions of synteny with other sordariomycetes. Several of them, moreover, contain genes involved in secondary metabolism (23). Another intriguing finding was an apparent lack of active transposons, which indicates the operation of genome defense mechanisms, such as repeat induced point (RIP) mutations (24). Accordingly, the genome sequences of *T. atroviride* and *T. virens* showed evidence for the operation of RIP mutations as well, and the genes required for RIP mutations in *Neurospora crassa* are present in their genomes (4).

The different lifestyles as mycoparasites or efficient plant cell wall degraders of *T. atroviride*, *T. virens*, and *T. reesei* are reflected in their genomes, indicating that the high capability for mycoparasitism and defense was largely lost in *T. reesei* during evolution (4, 25). The genomes of the two mycoparasitic species, *T. atroviride* and *T. virens*, are somewhat larger than that of *T. reesei*, with sizes of 36.1 and 38.8 Mbp versus 34.1 Mbp, and also contain more than 2,000 additional predicted genes (11,865 genes for *T. atroviride* and 12,518 genes for *T. virens*), while *T. reesei* (9,143 genes) only contains around 500 unique ones compared to *T. atroviride* and *T. virens*. A screening for paralogous expansions in gene families revealed 46 expanded families, and 26 of them were only expanded in *T. atroviride* and *T. virens*. The largest of these expansions occurred for Zn_2Cys_6 transcription factors, major facilitator superfamily (MFS) transporters, short-chain dehydrogenases, S8 peptidases, and ankyrin domain proteins (4).

In silico predictions of the secretome of these three *Trichoderma* spp. indicated that roughly 10% of the encoded proteins are secreted (26). Besides the already-well-known inventory of hydrolytic enzymes, a rich proteolytic arsenal was also found. Additionally, the predicted secretome indicated the availability of oxidative enzymes for alternative degradation of lignocellulose during growth on cellulose. Interestingly, also a high number of small secreted cysteine-rich proteins was detected, among them a di-

verse group of hydrophobins (27) and important elicitor-like proteins (28, 29).

Here, our aim was an in-depth analysis and annotation of the genomic content of *T. reesei*, *T. atroviride*, and *T. virens* (http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html, http://genome.jgi-psf.org/Trire2/Trire2.home.html, and http://genome.jgi.doe.gov/Triat2/Triat2.home.html, respectively). We have consequently focused on topics which have not been reported in detail so far, such as genome defense mechanisms and chromatin, sexual development, glycosylation, nitrogen, sulfate, and mevalonate metabolism, environmental signaling, etc. Additionally, new perspectives on well-known gene groups are provided, for example, on CAZyme content, with an evaluation of carbohydrate degradation preferences. Besides annotation and investigation of homologs in the three species, we also screened available transcriptome data for evidence of transcription of genes and striking regulation patterns. In summary, this report shall serve as a basis for research with *Trichoderma* spp. not only dealing with well-known gene groups but also for more-detailed investigation of promising, but so far less-studied, subjects and their exploitation for improvement of *Trichoderma* spp. as hosts for protein production as well as biocontrol agents.

GENOME INTEGRITY

Genome Defense Mechanisms

For survival and competitiveness, it is essential that organisms can react when their genome integrity is being altered. There is strong experimental and genomic evidence showing that living beings have various intricate defense mechanisms that ensure genome integrity and reliable transmission of genetic material to each daughter cell. These mechanisms comprise protection against viruses, transposons, transgenes, and other factors potentially causing alterations in the structure of DNA. Such alterations can, however, also be provoked by exogenous and endogenous factors, as described below, some of which have great impact for our understanding of aging, as well as for research on various diseases, including carcinogenesis and neurodegeneration (30–36). Moreover, knowledge in this area impacts development and improvement of new tools to study gene function by use of functional genomics (36, 37).

RNA-dependent silencing. RNA-dependent silencing has been described as an effective genome defense mechanism against parasitic nucleic acids, for posttranscriptional RNA degradation, transcriptional gene silencing via heterochromatin formation and/or DNA methylation, or meiotic silencing by unpaired DNA (38, 39), in which the involvement of double-stranded RNA (dsRNA) and the RNA interference (RNAi) machinery is well documented.

The RNAi machinery consists at minimum of one so-called Argonaute protein (small interfering RNA [siRNA]-guided endonuclease), one Dicer protein (RNase III-like endonuclease), and one RNA-dependent RNA polymerase (RdRP) (40). In posttranscriptional silencing, long dsRNA is processed into siRNAs by Dicer, an RNase III-like endonuclease. siRNAs are then incorporated into a multiprotein complex, the RNA-induced silencing complex (RISC), in which Argonaute is the core component. RISC recognizes and cleaves mRNA complementary to the incorporated siRNA. RdRP generates dsRNA from single-stranded transcripts by amplifying the amount of dsRNA (41).

Filamentous fungi apply several mechanisms for defense against viral and transposon invasion. *Neurospora crassa* has three mechanisms, two of which are RNAi related, to suppress transposon invasion during the vegetative and sexual stages of its life cycle. Quelling (Q) is an RNAi silencing phenomenon that occurs in the vegetative stage when the introduction of repetitive DNA sequences triggers posttranscriptional gene silencing of all homologous genes (42). By means of forward and reverse genetics approaches, the core components of the quelling machinery were identified; these are *qde-1* (quelling deficient element-1), *qde-2*, *dcl-1* (dicer like-1), and *dcl-2*, which encode RdRP, Argonaute, and two Dicer-like proteins, respectively (36).

During the sexual cycle, two distinct silencing mechanisms are at work: RIP mutation, which takes place during the early stage of the sexual cycle and causes mutations of C·G to T·A (24, 43), and meiotic silencing by unpaired DNA (MSUD). MSUD is an RNAi-related mechanism that occurs later than RIP and can silence unpaired DNA/chromosomes (39). Genetics studies have identified a set of critical genes that are highly homologous to components of the quelling pathway but are specifically expressed in MSUD (M); these are *sad-1* (suppressor of ascus dominance-1) and *sms-2* (suppressor of meiotic silencing-2), which encode RdRP and Argonaute, paralogs of QDE-1 and QDE-2, respectively. Only DCL-1 (also called SMS-3) is required for meiotic silencing (36).

RNA-dependent silencing can also act as an antiviral defense, and this is well established in *Cryphonectria parasitica* (44) and *Aspergillus nidulans* (45).

A wide range of fungi have multiple RNA silencing components in the genome. The number of paralogous silencing proteins in the genome differs among the fungal species, suggesting that RNA silencing pathways have diversified significantly during evolution, in parallel with developing the complexity of the life cycle or in response to environmental conditions (46, 47).

Trichoderma genomes also comprise genes that encode these RNAi machinery components. *T. reesei*, *T. atroviride*, and *T. virens* each possess two Dicer-like proteins (protein identifiers: TR_69494, TR_79823, TA_292263, TA_291296, TV_171147, and TV_47151), three Argonaute proteins (TR_49832, TR_60270, TR_107068, TA_245602, TA_20708, TA_36522, TV_112874, TV_181363, and TV_37110), and three RdRPs (TR_67742, TR_103470, TR_49048, TA_321718, TA_225118, TA_317554, TV_122493, TV_28428, and TV_10390). At least in *T. atroviride* and *T. reesei*, there is evidence for the transcription of almost all of these genes. The role of this machinery in the defense of genome integrity has not yet been explored.

Phylogenetic analysis of the RdRP, Argonaute, and Dicer-like proteins was performed using fungal species in which at least one of these proteins has been evaluated experimentally in order to infer the possible function of the *Trichoderma* RNAi machinery (see Fig. S1 to S3 in the supplemental material). A paralogous grouping system has been proposed, which utilizes Q and M designations for the components of fungal RNA silencing pathways based on the two silencing pathways identified for *N. crassa*, quelling and MSUD (48).

Paralogs of the RNAi proteins in each *Trichoderma* species did not form a single cluster, but rather orthologous proteins from the three species of *Trichoderma* grouped together with their orthologs of other genera (see Fig. S1 to S3 in the supplemental material), implying that the corresponding genes separated and probably specialized early during evolution. *Trichoderma* Dcr1

proteins clustering with *N. crassa* DCL-1/SMS-3 belong to group M, and Dcr2 proteins are clustered in group Q (see Fig. S1).

The analysis of Ago proteins also reveals the two groups, where *Trichoderma* AGO1 and AGO2 clustered with *N. crassa* QDE-2, *Cryphonectria parasitica* AGL2, and *A. nidulans* Ago1, the role of which in RNA silencing has been proven (48, 49), so they belong to group Q. *Trichoderma* Ago3 clustered with *N. crassa* SMS-2, forming the M group (see Fig. S2 in the supplemental material).

The phylogenetic analysis of RdRp displayed three groups, of which two correlated with the grouping system Q and M. RDR1 of *Trichoderma* clustered with *N. crassa* QDE-1 as part of group Q. *Trichoderma* RDR2 clustered with SAD1 of *N. crassa* and RDR1 of *Schizosaccharomyces pombe* as part of group M, and the third group corresponded to the ortholog of RRP3 of *N. crassa* and the two RdRps of *A. nidulans*. The functions of these proteins are unknown, so we cannot infer the role of *Trichoderma* RDR3 in any mechanism of gene silencing for protecting genome integrity. However, in *T. atroviride*, RDR3 is involved in asexual development (50), suggesting its role in regulation of gene expression (see Fig. S3 in the supplemental material). These analyses suggest that the two silencing pathways are present in *Trichoderma*: quelling for genome defense against exogenous nucleic acids like viruses, transgenes, or transposons, and MSUD for the maintenance of genome integrity upon sexual reproduction.

The high efficiency of genome defense mechanisms in *Trichoderma* spp. is reflected in results of the genome analysis of the three species of the genus, because an extremely small proportion of transposable elements and no evidence of their activity were found (4). Thus, it is unlikely that the function of these RNAi components is the control of transposable elements. Alternatively, *Trichoderma* silencing pathways may have imposed a very strong shield avoiding invasion of parasitic nucleic acids. There has been no report of viruses that infect any *Trichoderma* species. Hence, dsRNA, an intermediate of viral replication, could initiate RNAi-mediated gene silencing in these fungi. Furthermore, RNAi-mediated gene silencing has been used as a tool to study gene function (51–54), which suggests that the machinery for processing dsRNA is functional.

The RNAi machinery plays an important role in gene regulation via small RNAs (sRNAs). Sequencing of sRNAs from many species of fungi has led to the discovery of many classes of them, e.g., siRNAs in *Schizosaccharomyces pombe*, *Saccharomyces castellii*, *Candida albicans*, *Cryptococcus neoformans*, and *Magnaporthe oryzae*; qiRNAs (QDE-2-interacting RNAs) in *N. crassa*; priRNAs (primal small RNAs) in *S. pombe*; ex-siRNAs (exonic-siRNAs) in *Mucor circinelloides* and *T. atroviride*; disiRNAs (Dicer-independent small interfering RNAs) in *N. crassa*, and milRNAs (miRNAs-like small RNAs) in *N. crassa*, *Sclerotinia sclerotiorum*, *Metharizium anisopliae*, *C. neoformans*, and *T. reesei*. The functional role of some of these classes of sRNAs has been proven, but for many others it still remains to be shown (50, 55–67). For *T. atroviride*, however, a functional characterization of the components of the RNAi machinery is already available. This mechanism was shown to control growth and asexual development (50), opening the possibility of the participation of the RNAi machinery of *Trichoderma* in both posttranscriptional and transcriptional gene regulation.

Conclusions. The analyzed species of *Trichoderma* have the same number of paralogs for each component of the genome defense mechanisms, and the phylogeny of each protein showed that the two main RNA-dependent silencing pathways, quelling and MSUD, could be functional in this genus. Interestingly, in *T. atroviride* this pathway is involved in gene regulation for vegetative and reproductive growth, suggesting that it may also participate in these processes in *T. reesei* and *T. virens*. Nevertheless, given the differences in the lifestyles of each one of these species and the finding of milRNAs in *T. reesei*, there is a strong possibility that the RNA-dependent silencing pathway controls gene expression for the survival of the different *Trichoderma* species in their particular habitats. The study of this pathway in this genus has just begun, but the results obtained so far indicate that *Trichoderma* could be an excellent model of study for understanding the mechanism used by this pathway for endogenous processes in the fungal kingdom beyond genome protection.

DNA Repair

Living organisms are continuously exposed to damaging agents, both from the environment and from endogenous metabolic processes, whose action results in modification of proteins, lipids, carbohydrates, and nucleic acids. Events that lead to DNA modifications include radiation (including light), hydrolysis, exposure to reactive oxygen or nitrogen species, and exposure to other reactive agents, like alkylating agents and lipid peroxidation products. Repair of DNA damage is essential for the maintenance of genome integrity and to allow accurate transmission of genetic information.

DNA repair mechanisms rely on a complex network of proteins responsible for maintaining DNA integrity. The tools employed for this purpose perform the following tasks: sanitization of the nucleotide pool, direct reversion of base modification, excision repair, and recombinational repair (68, 69). Although the biochemical mechanisms remained closely related through evolution, their complexity increased, including the number of players in higher organisms. Extensive information is available on DNA repair responses, mechanisms, and enzymes implicated in bacterial, yeast, and animal models. For filamentous fungi, our knowledge on DNA repair is still limited and has been best studied in *A. nidulans* and *N. crassa* (70–72).

Direct reversion of base modifications. DNA modifications provoked by UV irradiation and alkylating agents can be directly reverted. The cell contains enzymes, which detect such modifications and revert them to the original form. These DNA repair systems comprise reversion of thymine dimerization by DNA photolyases (73) and bases methylated using a suicide enzyme with methylase activity like Ada/Ogt and methylguanine methyltransferase (MGMT) from *Escherichia coli* and humans (32), respectively. The latter mechanism is less conserved in filamentous fungi, as described below, although there are several genes encoding proteins with homology to both systems that are implicated in protecting the cell from DNA modifications by alkylating agents.

Photoreactivation. Photoreactivation is an ancestral repair system with a simple mechanism, using the energy of light to revert the DNA damage caused by exposure of the cell to UV radiation. The main photoproducts generated when the cell is exposed to UV radiation, are cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4-PP), of which CPD is produced in higher yields in the cell. The enzymes catalyzing the reversion of these photoproducts are known as photolyases and are classified according to their activity (photoproducts repaired) as CPD and 6-4 photolyases, although recently a third type of enzyme (cryptochrome/photolyase DASH [*Drosophila*, *Arabidopsis*, *Synechocystis*, and hu-

man]) was identified that specifically repairs CPDs in UV-damaged single-stranded DNA (74, 75). With a few exceptions, including humans, CPD photolyases have been identified in all phyla, whereas 6-4 photolyases are less represented (73).

T. reesei, *T. atroviride*, and *T. virens* have genes encoding CPD photolyases (TR_107680, TA_302457, and TV_50747), 6-4 photolyases (TR_77473, TA_86846, and TV_37166), and DASH-cryptochrome/photolyases (TR_59726, TA_285589, and TV_50684). The *phr1* gene encodes a CPD photolyase, and its activity in photorepair has previously been described in *T. atroviride* (76, 77). In both *T. reesei* and *T. atroviride*, *phr1* transcript levels were shown to respond to light (76, 78). While other repair mechanisms can eliminate DNA damage provoked by UV irradiation, PHR1 photoreactivation is the most efficient mechanism (77). CPD photolyases homologous to PHR1 are present in all fungal genomes available in public databases, suggesting that this mechanism, comprising direct reversion of damaged DNA, is highly conserved in the kingdom of fungi. *Trichoderma* has a 6-4 photolyase, but orthologs are not present in all fungi, and *N. crassa* is one of them. In *Cercospora zeae-maydis*, one 6-4 photolyase has been described and was proven to have activity in DNA repair when the fungus was exposed to UV irradiation (79). Similarly, *Trichoderma* mutants lacking a 6-4 photolyase are more sensitive to damage provoked by UV irradiation (80). In mutants deleted in both photolyases, it was evident that the two enzymes are required for full repair of DNA damaged when the fungi were irradiated with UV (77, 80). In agreement with the observation that UV irradiation produces accumulation of more CPD than 6-4 photoproducts in DNA, the CPD photolyase has a more prominent role in reversion of the photodamaged DNA. Mutants lacking PHR1 are severely affected, whereas mutants from which the 6-4 photolyase-encoding gene (*cry-1*) has been deleted lose only around 30% of their photorepair capacity (80). There is evidence pointing to a possible regulatory role of photolyases in other repair systems which do not require light for their activity in repairing photoproducts generated by UV radiation (79, 81). Interestingly, analysis of recombinant strains overexpressing or lacking PHR1 in *T. atroviride* led to the proposal that this protein also has a regulatory role (77), similar to what has been observed for other fungi (82). It appears that fungal photolyases have conserved functions both in DNA repair and regulation which are evolutionarily distinct from those in higher organisms, such as plants and animals.

Repair of alkylated DNA. Alkylating agents comprise a group of mutagens and carcinogens that modify DNA by introducing methyl or ethyl groups at all potentially available nitrogen and oxygen atoms. It is now evident that some alkylating agents are not only widespread in the environment but are also produced intracellularly as by-products of normal metabolism. Alkyl-based lesions can arrest replication, interrupt transcription, or signal the activation of cell cycle checkpoints or apoptosis (32). One of the central molecules in metabolism is *S*-adenosylmethionine (SAM), a principal biological methyl donor that can spontaneously methylate DNA, generating mainly 7-methylguanine (7meG) and 3-methyladenine (3meA) and, to a lesser extent O^6-methylguanine (O^6meG). Furthermore, lipid peroxidation and amine nitrosation represent other internal sources of alkyl groups (32). To overcome the mutagenic and cytotoxic effects caused by alkylating agents, *E. coli* has developed an adaptive response (Ada response) (83). The associated component, Ada, a multifunctional protein showing O^4meT and O^6meG methyltransferase activities, functions as an activator of transcription of the genes encoding AlkA, AlkB, and AidB and feeds back to its own promoter (83). The Ada protein is composed of two major domains, a 19-kDa C-terminal domain (C-Ada19) and a 20-kDa N-terminal domain (N-Ada20), which can function independently in demethylating adducts by transfer onto Cys-321 and Cys-38, respectively (83, 84). Surprisingly, *T. atroviride* has four gene loci (TA_323331, TA_8455, TA_319764, and TA_8464) that encode proteins with N-Ada20 domains (Fig. 1), here named ADA1, ADA2, ADA3, and ADA4, which contain the conserved Cys-38 reactive residue for reacting with alkylating agents. *T. virens* has two genes, *ada1* and *ada2* (TV_138325 and TV_8980), and in *T. reesei* only one gene coding for ADA1 was detected (TR_64672). ADA1 and ADA4 have bipartite nuclear and mitochondrial location signals, whereas ADA2 has only the nuclear location signal and ADA3 seems to be a cytoplasmic protein. Considering that N-Ada20 and C-Ada19 domains can act separately in *E. coli* (83), the findings of this *in silico* analysis suggest that the protein set ADA1 and ADA4 can protect both nuclear as well as mitochondrial DNA, whereas ADA2 would act only on nuclear DNA. The findings also suggest that ADA1 is the main player present in all *Trichoderma* species analyzed. It will be interesting to investigate if the species of *Trichoderma* have different sensitivities to alkylating agents due to the different contents of ADA factors in their genomes.

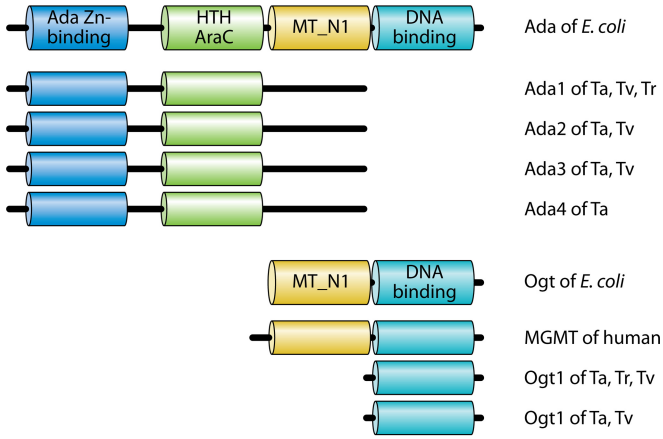

FIG 1 Comparison of Ada and Ogt/MGMT proteins with their homologs in *Trichoderma* spp. The protein sequences were analyzed using the SMART server to identify domains conserved in proteins of *Trichoderma* spp. and to compare functional domains among them. Colors show conserved domains, such as the Ada Zn binding and HTH AraC domains, which comprise the N terminal (N-ada20), and the MT-N1 and DNA binding domains, which comprise the C terminal (C-ada19) of the Ada protein from *E. coli*. Ta, *T. atroviride*; Tr, *T. reesei*; Tv, *T. virens*.

A role against alkylating agents has been shown for another protein, the O^6MeG DNA repair methyltransferase (MTase). *E. coli*, yeast, and humans have orthologous genes (Ogt/MGT) coding for this enzyme (30, 85) (Fig. 1). Two genes are present in each of the two species *T. atroviride* and *T. virens* (TA_221983 and TA_318229; TV_50154 and TV_49803) which encode proteins with MGT1/Ogt DNA binding domains, while only one is present in *T. reesei* (TR_5247). None of them comprises the MTase domain described for MGT1 and Ogt (86). It appears that the domains present in Ada and MGT/Ogt have been evolutionarily separated, and in some cases lost, in filamentous fungi.

DNA repair by excision. The excision repair pathway is in-

volved in the removal of damaged or mispaired DNA bases by excision. There are three subpathways to avoid alterations in the cell genetic code: base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). The mechanisms for recognition and excision of a damaged site are different among these subpathways, but in the final stage the three subpathways use similar components for gap filling (DNA polymerases) and sealing (ligase 1 [LIG1]) the repaired DNA strand (87).

(i) BER. BER is the major repair pathway involved in the removal of damaged DNA bases and repair of DNA single-strand breaks (SSBs) generated by environmental agents or spontaneously occurring during cell metabolism. Both modified DNA bases and SSBs with ends other than 3′-OH and 5′-P are repaired either by replacement of a single or several nucleotides in the processes called short-patch BER (SP-BER) and long-patch BER (LP-BER), respectively. BER proteins act upon structurally nondistorting and nonbulky lesions, e.g., oxidized or ring-saturated bases, alkylated and deaminated bases, apurinic/apyrimidinic sites, and also some mismatches (30, 34).

The BER system is an evolutionarily conserved mechanism used to remove mainly the damaged DNA bases. The first event in this mechanism is the formation of N-glycosidic bond breaks by DNA glycosylases, which recognize specific lesions on the DNA (30, 88). There are at least eight genes encoding DNA glycosylases in *Trichoderma* spp. (see Table S1 in the supplemental material), which are homologous and are generally divided into two types. The first type comprises homologs to AlkA (*Escherichia coli*), Ung1 (*Saccharomyces cerevisiae*), MutY (*E. coli*), TDGmug (*Homo sapiens*), and MBD4 (*H. sapiens*) monofunctional DNA N-glycosylases that remove an alkylated, deaminated, or mismatched base, leaving an apurinic/apyrimidinic (AP) site. Fpg (*E. coli*), Ogg1 (*S. cerevisiae*), and Nth1 (mice) bifunctional DNA N-glycosylases/AP-lyases belong to the second type, and their function is to remove oxidized or ring-saturated bases. In addition to the glycosylase activity, they have a 3′-AP-lyase activity which incises the phosphodiester bond at the 3′ side of the deoxyribose via β-elimination, leaving an SSB (30). Once the damaged base is excised, an AP site is generated, which is targeted by AP endonucleases (APEs). APEs generate 3′-OH and 5′-P ends suitable for filling by DNA polymerase I (Pol I) activity, renewing the damaged DNA bases (30, 89). Finally, DNA ligase I (LIG1; TR_22881, TA_133844, and TV_183439) seals the nick, forming a continuous double helix. *T. reesei*, *T. atroviride*, and *T. virens* have two genes encoding AP-endonucleases (APN1 and APN2) which can remove the deoxyribose and the phosphate moieties, forming a substrate targeted by Pol I and LIG1.

BER proceeds further via two alternative subpathways: SP-BER and LP-BER, which involves replacement of several nucleotides (30). The lesions removed by bifunctional DNA glucosylases are processed mainly by SP-BER, since the 3′-OH and 5′-deoxyribose phosphate (5′-dRp) ends may be readily filled. When modifications occur in the 5′-dRP moiety by oxidation or reduction, the lesion is further processed by LP-BER. PCNA is recruited together with Polδ or Polε, adding a few nucleotides to the 3′-OH end (often 6 to 13 nucleotides) (30, 90) and generating a flap containing the 5′-dRP end, which is then removed by Flap endonuclease activity (FEN1). Finally, the ends are sealed by DNA LIG1 (34).

Poly(ADP-ribose) polymerase enzyme (PARP) binds to an SSB immediately after its formation and dissociates after self-poly(ADP) ribosylation. PARP has been proposed to prevent cleavage of the strand break ends by nucleases, while the BER system repairs DNA damage, and PARP was also shown to stimulate LP-BER strand displacement synthesis by DNA polymerase (30, 91). Poly(ADP-ribose) glycohydrolase (PARG) is the main enzyme implicated in regenerating PARP proteins. In contrast to *T. reesei*, which has only a PARP protein (TR_22115) and similar to *A. nidulans* and *N. crassa*, *T. atroviride* and *T. virens* have two genes encoding PARP (TA_295780 and TV_89857) and PARG (TA_219648 and TV_4413) proteins, suggesting that PARG could be dispensable or that a different poly(ADP-ribose) hydrolysis pathway operates in *T. reesei*.

(ii) NER. NER is a versatile multistep repair pathway that serves to remove a broad range of bulky and helix-distorting DNA lesions caused by lipid peroxidation-induced DNA adducts, chemical mutagens, and other agents that lead to DNA adducts, such as mycotoxins produced by fungi. Further alterations targeted by this pathway are those resulting from the removal of oxidative DNA lesions and from CPD and 6-4 photoproducts introduced by UV irradiation. The main stages of NER are (i) DNA damage recognition, (ii) assembly of the protein complex that carries out excision of damaged DNA, and (iii) synthesis and ligation of a stretch of DNA for gap filling. The key event in eukaryotic NER is excision of an approximately 28-nucleotide DNA fragment containing the damaged site (92, 93). NER consists of two distinct subpathways, namely, global genome repair (GGR) and transcription-coupled repair (TCR), which are largely identical except for the mode of the DNA damage recognition. In TCR, RNA polymerase II (RNAP) stalled at the bulky damaged site constitutes the signal for the recruitment of DNA repair proteins, while in GGR the DNA damage-induced helical distortion is recognized by a specific protein complex. While TCR specifically repairs transcription-blocking lesions in actively transcribed DNA regions, GGR eliminates DNA lesions from the entire genome (94–96). *Trichoderma* genomes comprise XPC (xeroderma pigmentosum complementation group C in *Homo sapiens*; XPCa TR_61075, TA_293370, and TV_1243; XPCb TR_75205, TA_162061, and TV_194426) and HR23A (also known as UV excision repair protein RAD23 homolog A in *H. sapiens*; TR_102581, TA_298203, and TV_84601) homologs for recognition in GGR, and DNA-dependent ATPase, belonging to the SNF2 protein family, CSB (Cockayne syndrome type B in *H. sapiens*; TR_62057, TA_53490, and TV_160093) homologs to detect RNA polymerase stalled at damaged sites. Additionally, DDB1 (DNA damage binding protein 1, *H. sapiens*; TR_56582, TA_146031, and TV_80808), XPE/DDB2 (DNA damage binding protein 2; TR_57632, TA_127206, and TV_125686), and XPA (TR_69998, TA_237085, and TV_39247) can also act as sensors to detect damaged sites in GGR. Once GGR- and TCR-NER recognition proteins detect DNA damage, the transcription factor IIH (TFIIH) complex, XPA, and replication protein A1 (RPA1; TR_71468, TA_139326, and TV_85768) and RPA2 (TR_55902, TA_320493, and TV_84151) sequentially bind to the site of the damage to form a preincision complex. The two helicases XPB (TR_ 120753, TA_249572, and TV_41182) and XPD (TR_112413, TA_214371, and TV_214193), which are part of TFIIH, unwind the DNA double helix at the damaged site. The endonuclease XPG (TR_79304, TA_41742, and TV_208179) and the XPF-ERCC1 complex (TR_72108, TA_149406, and TV_29672), which hydrolyze phosphodiester bonds 2 to 8 nucleotides downstream and 15 to 24 nucleotides upstream of the damaged site, carry out the dual incision process.

All proteins implicated in both NER pathways are conserved in *Trichoderma* spp. (see Table S1 in the supplemental material). After incision of the damaged DNA, the resulting gap is filled by DNA polymerases δ/ε, which require a proliferating cellular nuclear antigen (PCNA), RPA, and replication factor C (RFC1-5) (34). Finally, the DNA fragments are sealed by DNA ligase 1 (34) by a mechanism apparently highly conserved in *T. reesei*, *T. atroviride*, and *T. virens*, since all orthologs were identified (see Table S1).

(iii) MMR. MMR is the major postreplicative DNA repair system, and it increases replication fidelity up to 1,000-fold (97). MMR removes primary replication errors that escaped DNA polymerase proofreading, such as base-base mismatches and small insertion-deletion loops (IDLs) that are most easily formed in long repetitive sequences, i.e., in microsatellites. Additionally, MMR also replaces modified bases, such as 8-oxoG and 2-oxoA, carcinogenic adducts, and UV photoproducts (31). Similar to *S. cerevisiae*, *Trichoderma* spp. contain six genes encoding homologs of MutS (*E. coli* MSH1, MSH2, MSH3, MSH4, MSH5, and MSH6), four homologs of MutL (*E. coli* MLH1, MLH2, MLH3, and PMS1), and one homolog of UvrD helicase (*E. coli*), which is absent in the animal and plant kingdoms (see Table S1 in the supplemental material). In humans, in contrast, only five MutSs (MSH2 to -6) and four MutL homologs (MLH1, MLH3, PMS1, and PMS2) have been described. Eukaryotic MMR proteins function as heterodimers, and it has been proposed that MSH2 to MSH6 form MutSα, which recognizes all eight single-base mismatches and small IDLs (up to about 10 unpaired nucleotides), whereas MSH2 and MSH3 (MutS) recognize larger IDLs (98). MutSα recruits MutLα ATPase (MLH1 and PMS1), which has been proposed to be important for coupling recognition with further MMR steps, and it consistently interacts with Exo1 and PCNA (99, 100). The final steps of MMR include mismatch excision and DNA resynthesis with the participation of a helicase (UvrD), proteins that stabilize the single-strand gap (RPA1 and RPA2) and hydrolyze DNA in the 5′ → 3′ direction via exonuclease (Exo1). Finally, the gap is filled by DNA polymerase δ (POLD1, POLD2, and POLD4) in the presence of a β-clamp loader (RFC1, RFC2, RFC3, RFC4, and RFC5), and the β-clamp (PCNA), and ends are joined by DNA ligase (LIG1) (31). *T. reesei*, *T. atroviride*, and *T. virens* have genes that encode all proteins described above (see Table S1).

Double-stranded break repair. Double-strand breaks (DSBs) are the most deleterious form of DNA damage. They can result from exposure to DNA-damaging agents, such as ionizing radiation and oxidative damage, or even occur spontaneously. Cells have developed two mechanisms for the repair of DSBs: homologous recombination (HR) and nonhomologous end joining (NHEJ) (101).

(i) HR. Recombinational repair is a highly conserved DNA repair mechanism thought to be present in all living organisms, and it is generally accurate (33). This system is specialized in recognition and repair of DNA breaks and consists of three stages which are common for prokaryotes and eukaryotes: presynapsis, where DSBs or gaps are formed and the resulting DNA end is prepared for recombination; synapsis, where physical connection between the recombinogenic substrate and an intact homologous duplex DNA template is generated, leading to formation of a heteroduplex molecule; and postsynapsis, where DNA synthesis from the invading 3′ end takes place followed by the resolution of junction intermediates (33). HR involves the exchange of DNA regions between homologous chromosomes. This process requires an extensive region of homology between the damaged DNA strand and an undamaged template. During HR, double-strand breaks are converted into 3′ single-stranded DNA (ssDNA) tails, which are bound by RPA proteins.

Processing of DSBs is thought to require homologs of mammal and *S. cerevisiae* MRE11p-RAD50p-NBS1p/XRS2p, respectively. RAD52 interacts with RPA and promotes binding of RAD51 to the ssDNA, which may be stabilized by RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 in humans; RAD55 and RAD57 in yeast) (101). Subsequently, the RAD51-bound ssDNA invades a homologous molecule in a reaction stimulated by RAD54, a member of the Swi2/Snf2 family of SF2 helicases (102). Rad54 remodels DNA structure, chromatin structure, and the Rad51-dsDNA complex. Rad51 paralogs participate in Holliday junction resolution (33), although studies in yeast and human cells have shown participation of a protein complex containing Mus81 with its partner MMS4 or Eme1 in resolving Holliday structures during meiosis (103). Central to DSB repair by HR is Rad51. Rad51 homologs were detected in *T. atroviride* and *T. virens* (TA_161197 and TV_112066); however, for *T. reesei* no gene model is available, although the genomic region on scaffold 12, spanning nucleotides 27383 to 28555, contains an open reading frame (ORF) that encodes a Rad51 homolog. Consequently, RAD51 is also present in *T. reesei*. Rad51 is the eukaryotic RecA homolog that catalyzes homology search and DNA strand exchange. In contrast to the mutation in *rad51* in yeast, mutants in the corresponding orthologs of *A. nidulans* and *N. crassa*, *uvsC* and *mei-3*, respectively, show a dramatic increment in spontaneous mutations. These observations suggest that the orthologs of *S. cerevisiae* Rad51p in filamentous fungi have additional functions that preserve genome integrity under "normal" conditions (72). For all genes that play key roles in HR, homologs were found in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 in the supplemental material).

(ii) NHEJ. In contrast to HR, the NHEJ pathway does not require homology and can rejoin broken DNA ends directly, end to end. NHEJ is an intrinsically error-prone pathway, while HR results in accurate repair (101). The first step in NHEJ is detection of the DSB by the heterodimer Ku70/Ku80 (TR_63200, TA_33616, TV_83371/TR_58213, TA_295291, and TV_232258), which recognizes and binds ends of dsDNA and then recruits the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs or PRKDC) to the ends (104). The association of two DNA-PKc molecules, one at each end, brings the broken ends together. The kinase activity of DNA-PKcs becomes activated upon DNA binding and association with another DNA-bound DNA-PK complex. Autophosphorylation of DNA-PKcs is required for DSB rejoining by NHEJ and is thought to result in a conformational change in DNA-PKcs. The altered conformation subsequently enables end-modifying enzymes to gain access to the ends, which eventually leads to complete dissociation of DNA-PK from the DNA. DNA ligase IV/XRCC4 and the recently discovered XLF component form the other complex constituted by the core proteins for NHEJ (105). NHEJ repair has evolved as the predominant pathway in filamentous fungi, as in other superior eukaryotes, such as plants and mammals (106). In *T. reesei* as well as in other filamentous fungi, some components of the NHEJ pathway (*mus53/lig4*; TR_58509, TA_83609, and TV_180472 and *ku70* homologs) have been the target of deletion in order to increase the proportion of

homologous recombination events upon transformation for targeted gene replacement experiments (107–109). Homologs of all genes involved in NHEJ are present in the genomes of the *Trichoderma* species analyzed here (see Table S1 in the supplemental material).

Sanitization of nucleotide pool. Various DNA-damaging agents react with nucleic acid bases present not only in DNA but also in precursors for DNA synthesis, i.e., 2′-deoxyribonucleotide-5′ triphosphates (dNTPs). Generally, bases in dNTPs are more easily accessible to damage than are bases in DNA (31). Modified dNTPs may induce mutations, since they are incorporated into DNA by DNA polymerases with an efficiency ranging from 10^{-5} to 10^{-2} of unmodified dNTPs incorporated (110–113). In fact, one of the most common oxidative modifications in the dNTP pool (8-oxodGTP) has been shown to be incorporated almost 24 times more efficiently in complementing A than the opposite template C by human polymerase β (112). Thus, misincorporated 8-oxodGTP opposite of A may lead to AT → CG transversions (114, 115). This modified ribonucleotide can also be incorporated during transcription and modify RNA sequences, and consequently their biological function (31).

To prevent mutations by incorporation of modified bases into DNA or RNA, cells are equipped with triphosphate nucleotide hydrolases that avoid incorporation of modified bases during replication or transcription (31). *Trichoderma* spp. have five proteins containing a NUDIX box signature sequence (PF00293), like MutT (see Table S1 in the supplemental material). Three of these genes encode homologs of MutT of *E. coli* and are reported to hydrolyze 8-oxodGTP, 8-oxodGDP, 8-oxoGTP, and 8-oxoGDP in order to avoid errors in replication and also in transcription. Additionally, *Trichoderma* spp. genomes contain homologs to human NUDT5 and NUDT15. NUDT5 has been demonstrated to hydrolyze 8-oxodGDP to 8-oxodGMP, which represents a critical step in avoiding availability of 8-oxodGTP for DNA polymerases (116).

Other mechanisms related to nucleotide pool sanitization are enzymes for elimination of dUTP and methylated nucleotides, such as 1-medATP, which can be repaired by dUTPase (deoxyuridine 5′-triphosphate nucleotidehydrolase) and oxidative demethylase AlkB, respectively (117, 118). Both mechanisms are present in *T. reesei*, *T. atroviride*, and *T. virens* to ensure integrity of the respective genome despite the potential presence of these modified nucleotides (see Table S1 in the supplemental material). Although not directly related to elimination or removal of modified bases, antioxidant enzymes such as superoxide dismutases (SODs) and catalases (CATs) also play an important role in protecting genome stability by elimination of reactive oxygen species (ROS), which can generate the oxidized nucleotides mentioned above. *T. reesei*, *T. atroviride*, and *T. virens* have three SODs (one manganese SOD and two Cu/Zn SODS), seven catalases, and three catalase peroxidases, which could maintain the cellular redox balance (see Table S1). Compared with *N. crassa* and *A. nidulans* as free-living organisms with four and three catalases, respectively, *Trichoderma* has a robust antioxidant system that is likely associated with the establishment of specialized interactions.

Postreplication repair: the Rad6 pathway. The Rad6 pathway is of major importance to postreplicative DNA repair in eukaryotic cells. Key components of this system comprise a ubiquitin conjugation enzyme, Rad6 (TR_120075, TA_301071, and TV_81725) and the heterodimer MMS-UBC13, which are recruited to chromatin by the RING-finger proteins RAD18 (TR_104000, TA_183923, and TV_135917) and RAD5 (119). The mutations in orthologs of *S. cerevisiae* Rad18p, i.e., *uvs-H* of *A. nidulans* and *uvs-2* of *N. crassa*, showed high sensitivity to gamma and UV irradiation as well as methylmethane sulfonate (MMS) alkylating agents (72). Such a phenotype is similar to that of mutants lacking *Rad18* in yeast, suggesting an essential function of the RAD6/RAD18 complex in fungi. The genomes of *T. reesei*, *T. atroviride*, and *T. virens* all have components of this pathway (see Table S1 in the supplemental material), showing that they constitute a highly conserved system. Interestingly, in yeast, components of this pathway perform functions similar to the widely studied SOS system of bacteria (35).

Checkpoint controls of damaged DNA organize responses of DNA repair. DNA repair is closely connected with cell cycle progression. DNA lesions induce signaling pathways that delay or halt the cell cycle before replication (G_1/S) or cell division (G_2/M) take place (120). Two signaling pathways have been conserved across evolution: the ATM (ataxia-telangiectasia mutated) and ATR (ATM and Rad3-related) pathways, which involve phosphatidylinositol 3-kinase-like proteins. ATM is activated by detection of double-strand breaks, while ATR recognizes single-stranded DNA regions originating from hairpins, which are caused by halted replication or by damage, for example, that results in pyrimidine dimers (121). A link between the stress kinase and the checkpoint pathways has been suggested. Stress kinases respond to various stimuli, such as UV radiation, oxidative stress, heat shock, and osmotic stress. It is, thus, plausible that the activation of stress kinases by the presence of DNA damage through the ATM or ATR pathways drives the cell to the inactivation of genes necessary for cell cycle arrest and for DNA repair (122). In fungi, there is evidence indicating that the stress-activated protein kinase (SAPK) has a role in protection from UV irradiation and many other stressors that could impact DNA integrity (123). In *T. atroviride* the stress mitogen-activated protein kinase (MAPK) gene *tmk3* (TR_45018, TA_301235, and TV_83666), a homolog of *S. cerevisiae Hog1/StyI/p38*, is regulated by light through the BLR complex (E. U. Esquivel-Naranjo, E. Medina-Castellanos, V. A. Correa-Pérez, J. L. Parra-Arriaga, F. Landeros-Jaime, J. A. Cervantes-Chávez, and A. Herrera-Estrella, unpublished data). The *T. reesei* homolog of *tmk3* is also regulated in response to light and by the light-regulated protein ENV1 (78). *T. atroviride* strains with mutations in the corresponding gene are sensitive to osmotic and oxidative stresses, as well as to UV irradiation. The latter effect specifically occurs in conidia, suggesting that TMK3 could regulate light-independent DNA repair systems, such as NER and/or MMR, whose abilities to repair thymidine dimers have been demonstrated (31, 34). Analysis of the expression of genes encoding proteins involved in different systems of DNA repair in the TMK3 mutant showed only a slight variation in their mRNA levels, compared to the wild-type strain, suggesting that such regulation of the repair systems is not at the transcriptional level but rather takes place through posttranslational modifications (Esquivel-Naranjo et al., unpublished).

Transcriptional regulation of DNA repair systems. As described above, several endogenous and exogenous environmental factors can interfere with genome integrity. To explore these factors, the transcriptomes regulated in response to different stimuli, such as light, injury, and mycoparasitism (*Trichoderma*-host fungus interaction) were analyzed. Several components of DNA re-

pair systems were found to be responsive to light, injury, and mycoparasitism, as revealed by transcriptome data (15, 22, 1313). Mycelial injury exerts a greater impact on genes involved in DNA repair (12 genes induced and 3 repressed) than light (7 genes induced) or mycoparasitism (7 genes repressed). Noticeably, light and injury are stimuli which induce genes coding for several DNA repair systems, whereas mycoparasitism has a negative effect on the expression of some genes related to DNA repair. Injury induces production of ROS through NADPH oxidase NOX1, which can directly oxidize nucleotides or indirectly promote DNA alkylation by lipid peroxidation (15, 32). Accordingly, genes encoding excision repair proteins (BER, NER, and MMR), as well as those involved in homologous recombination and pool sanitization, are induced by injury (15), and these are related to DNA repair caused by oxidation and alkylation. Overall, transcriptome analysis hints to coregulation of components of the repair systems, indicating that the cell specifically responds to different insults.

During *Trichoderma*-host fungus interactions, ROS (124) and mycotoxins (125) can be produced as defense mechanisms in fungi and can cause alterations in the DNA. Unexpectedly, genes encoding DNA repair systems are repressed during mycoparasitism in *T. virens*, making its genome vulnerable to toxins or other molecules that target DNA. In summary, genes encoding proteins involved in different mechanisms of DNA repair are tightly regulated in response to diverse stimuli in order to ensure genome integrity, although postranslational regulation also could be operative.

Conclusions. Genome sequence analyses showed that during evolution, *T. reesei* lost several genes involved in DNA repair, since they are present in both *T. atroviride* and *T. virens*. One of the more clearly affected DNA repair pathways is that used to deal with alkylated DNA, where *T. reesei* has only one *ada* gene (*ada1*). These shortages in the *T. reesei* genome are surprising, as efficient genome defense mechanisms were expected due to the low number of duplications in the genome (23). Interestingly, *T. virens* possesses only two *ada* genes, whereas the *T. atroviride* genome encodes four. Similarly, *T. reesei* has only one Mgt1/Ogt ortholog, while *T. atroviride* and *T. virens* have two.

CHROMATIN STRUCTURE, REARRANGEMENT, AND HISTONE MODIFICATION

Genomic DNA in eukaryotic organisms is compacted by an octamer of conserved basic proteins called histones, which together form the highly organized structure known as chromatin. Changes in chromatin structure may lead to short- or long-term alterations of the transcriptional activities of genes, and thus they are crucial for cell differentiation, metabolite production (126), and environmental adaptation (127). Epigenetic and chromatin rearrangement data obtained for higher eukaryotes or microorganisms such as yeast are often not applicable to filamentous fungi. In *Trichoderma* spp., the functions and the physiological relevance of chromatin rearrangements are not yet well understood. In *T. reesei*, nucleosome rearrangements at the *cbh2* (cellobiohydrolase 2) promoter in correlation with cellulase expression indicated a regulatory function in this process (128). More recently, a role for the putative protein methyltransferase LAE1 in regulation of cellulase gene expression was shown. However, *lae1* expression was not found to correlate with histone methylation patterns in CAZyme coding regions (129). Consequently, a better understanding of chromatin modification mechanisms that control transcriptional regulation in this important group of eukaryotes will allow us to fully exploit the possibilities for use of fungi in the production of agricultural and biotechnological products.

Chromatin Structure and Gene Regulation

Core histones. In the nucleus, the DNA is hierarchically packed, and the nucleosomes represent the first level of chromatin organization. The nucleosome is composed of two copies of each of the core histones, H2A, H2B, H3, and H4, which form an octameric core wrapped by 146 bp of DNA. Nucleosomes are separated by linker DNAs of variable lengths and are linked by the histone H1. The core histones of different organisms exhibit a high degree of conservation at the sequence level (see Fig. S4 in the supplemental material) (130, 131). Crystallographic studies focused on the core histone structures have identified a common folding motif. This histone-folding motif, which is an extended helix-turn-helix (HTH), directs the head-tail association of individual core histones in heterodimers to form the octamer (132). It has been demonstrated that the genes encoding those histones, which form dimers (H2A and H2B, H3, and H4) coevolved (133). Histones H3 and H4 are more closely related than H2A and H2B, probably because they form a tetramer that plays a critical role in the nucleosome formation. This is evident for *Trichoderma*, as shown in the phylogenetic tree, where H3 and H4 share an ancestor (see Fig. S4). Protists and plants form the first branch, followed by fungi and animals, which seem to have a common ancestor. As sequences of H3 and H4 evolved 10 times more slowly than H2A and H2B, the branching for the principal phylogenetic groups is less exact (133). The four core histones, H2A (TA_300145, TV_215760, and TR_121522), H2B (TA_300144, TV_92198, and TR_121516), H3 (TA_297965, TV_88789, and TR_124210), and H4 (TA_297966, TV_88786, and TR_82510), and the linker histone H1 (TA_293189, TR_190422, and TR_34402) were found in *Trichoderma* spp. This fact confirms that *Trichoderma* spp. have the same basic components forming the nucleosome as other organisms.

Histone variants. The histone variants H2A, H2B, H3, and H4 differ from the canonical ones by a few amino acids and have been implicated in the regulation of several cell functions, including transcription, DNA replication, and repair, as well as gene silencing (134).

In addition to the core histones, *Trichoderma* spp. have three histone variants in their genomes (see Table S1 in the supplemental material), including one H3 variant, homologous to CENP-A, Cse4p, and Cid from *Homo sapiens*, *S. cerevisiae*, and *Drosophila melanogaster*, respectively (135–137). There is also good evidence that they are involved in centromere formation. A mutation in *CSE4* provokes an arrest of the cell cycle in the mitosis phase (138). The recruitment of CENP-A to the centromere is RNAi dependent (139). The *Trichoderma* CENP-A proteins show a high degree of homology between them and with their homologs in *N. crassa* and *Gibberella zeae* (see Fig. S4 in the supplemental material). This high degree of homology between these proteins is also observed at a functional level, since Cse4 from *S. cerevisiae* is able to functionally complement CENP-A in human cells (140). Coevolution of histones, as postulated previously (133), is supported also by the phylogenetic analysis for *Trichoderma* showing that H3 and H4 share an ancestor (see Fig. S4). *Trichoderma* spp. also contain

an H2A variant (TR_124052, TA_296920, and TV_39222) that is homologous to H2A.Z and Htz1p from mammals and *S. cerevisiae*, respectively. In mammals, H2A.Z controls the localization of the heterochromatin protein HP1a and plays an important role in genome stability and chromosome segregation (141). In yeast, Htz1p is distributed over almost the whole genome (but not randomly), and its location depends on the SWR1 complex (the catalytic core of a multisubunit chromatin remodeling enzyme complex), which is preferentially localized in the intergenic regions (142). Htz1p also prevents the ectopic spreading of heterochromatin in the telomeric regions (143). Roles for H2A.Z have been reported in gene transcription, chromosome segregation, DNA repair, heterochromatin silencing, and progression through the different phases of the cell cycle (144).

An H4V histone variant was detected in *Trichoderma* genomes (TR_55201, TA_34079, and TV27515), but these histone variants are not closely related to those from *Neurospora* (see Fig. S4 in the supplemental material). Due to their low similarity with H4, this variant may as well be a pseudogene, as proposed for *N. crassa* (145).

Histone chaperones. The highly organized structures of nucleosomes are subject to changes by replacing or exchanging histones, which is crucial for access of the cellular machinery to DNA during gene transcription (146). Histones are highly basic proteins that cannot remain free inside the cell, as this could lead to deleterious effects by promiscuous interactions. When histones are not interacting with DNA, they are bound to specialized proteins called histone chaperones (HCs), which prevent unwanted interactions between histones and other factors (147, 148).

HCs are classified into three main classes: (i) chaperones capable of binding, transport, or transfer of histones without requiring additional molecules; (ii) chaperone complexes formed by subunits of histone chaperones; and (iii) chaperones that bind histones into enzymatic complexes (146, 149). Another property that distinguishes the HCs is their selectivity to bind to the different histones, i.e., those that preferentially bind to H3-H4 or to H2A-H2B, as well as to H3 or H2A isoforms (150, 151). HCs contain domains that are important for their interactions with histones, as well as low-complexity sequences that are rich in acidic amino acid residues (152). It is well known that conserved beta structures of HCs function as connecting junctions between H3-H4 (153–155). The *Trichoderma* HCs found in this work showed the presence of all these domains (Fig. 2).

Most information about HCs has been obtained in *S. cerevisiae*, *Drosophila*, mammalian cells, *Aspergillus*, and *Neurospora*. In *Trichoderma* spp., the class I histone chaperones are ASF-1 (TR_47838, TA_300330, and TV_64020), HIRA (TR_69960, TA_38546, and TV_141127), RTT106 (TR_64989, TA_239305, and TV_59389), NAP (TR_80498, TA_297644, and TV_211223), and SPT6 (TR_57295, TA_29617, and TV_88065). HC class II comprises CAF-B (TR_64684, TA_253063, and TV_114128), CAF-C (TR_103311, TA_127007, TV_34185), POB3 (TR_4004, TA_305357, and TV_82979), and SPT16 (TR_57600, TA_147719, and TV_80360). A sequence analysis of *Trichoderma* HC proteins showed a high degree of homology among them and with their orthologs in *N. crassa* and *G. zeae* (Fig. 2), which suggests a highly conserved role for HCs between *Trichoderma* spp. and other filamentous fungi.

Centromere Organization and Kinetochore Complexes

Organization of centromeres. The centromere is a specialized chromosomal DNA region that serves as the site for protein-DNA and protein-protein interactions to form the kinetochore. This structure joins two sister chromatids and is essential for the chromosome segregation during cell division. The DNA sequences of a centromere are composed of AT-rich α-satellite DNA repeats, which show low conservation among various model organisms. However, there is an overall structural similarity among proteins that form centromeres in evolutionarily diverse eukaryotes (156, 157).

The simplest centromeric DNA known, that of the budding yeast *S. cerevisiae*, consists of 125 bp and three protein binding motifs (CDEI, CDEII and CDEIII), which are necessary and sufficient to mediate accurate chromosome segregation during mitosis and meiosis (158). In *Schizosaccharomyces pombe*, *Drosophila melanogaster*, and *Homo sapiens*, the centromeres are larger and more complex, extending from 40 kb to megabases (159, 160). However, in contrast to *S. cerevisiae*, where centromere function depends on sequence-specific DNA-protein interactions, in the other organisms the centromere is determined by specialized chromatin domains whose formation at one site on each chromosome is controlled by epigenetic mechanisms (160). In filamentous fungi, the discovery of centromeric DNA sequences still remains a substantial challenge. According to analyses performed with *N. crassa*, putative centromeric DNA regions are composed of a complex, heterogeneous set of repetitive AT-rich sequences that can span between 30 and 450 kb.

There are several centromere/kinetochore proteins that have been defined in many model organisms (see Fig. S5 in the supplemental material). These proteins can be classified into several groups, belonging to different substructures, which execute their functions at different levels.

The histone H3 variant CENP-A is centromere specific and binds to the central core region of the centromere (see Fig. S5 in the supplemental material). This protein was first described in humans, and its homologous proteins have been found in other organisms, for instance, Cse4p in *S. cerevisiae*, *cid* in *Drosophila*, and *cnp1* in *S. pombe* (161). The three *Trichoderma* spp. under study each possess one ortholog to CENP-A (TR_57870, TA_212690, and TV_88204), which probably participates in the formation of the centromere.

CENP-B has a DNA binding domain of 125 amino acid residues close to its N terminus. The CENP-B domain binds with high affinity to a 17-bp DNA sequence, the CENP-B box (see Fig. S5 in the supplemental material). The CENP-B box is frequently found in human DNA α-satellites. CENP-B plays an important role in the higher fold order of the centromere (162). CENP-B is also necessary for *de novo* centromere formation, as well as in mediating centromeric chromatin modification (163). We found two putative CENP-B proteins for *T. reesei* (TR_75196 and TR_57756), *T. atroviride* (TA_280163 and TA_164783), and *T. virens* (TV_182621 and TV_12894), which are likely to be homologs (see Table S1 in the supplemental material) as reported for *S. pombe*. This fungus has three CENP-B homologs (Apb1, Cbh1, and Cbh2) that are structurally similar and display functionally redundant roles in chromosome segregation but different localizations *in vivo* (164). CENP-B homologs are also present in the filamentous fungus *A. nidulans* but are absent in *N. crassa*, *F.*

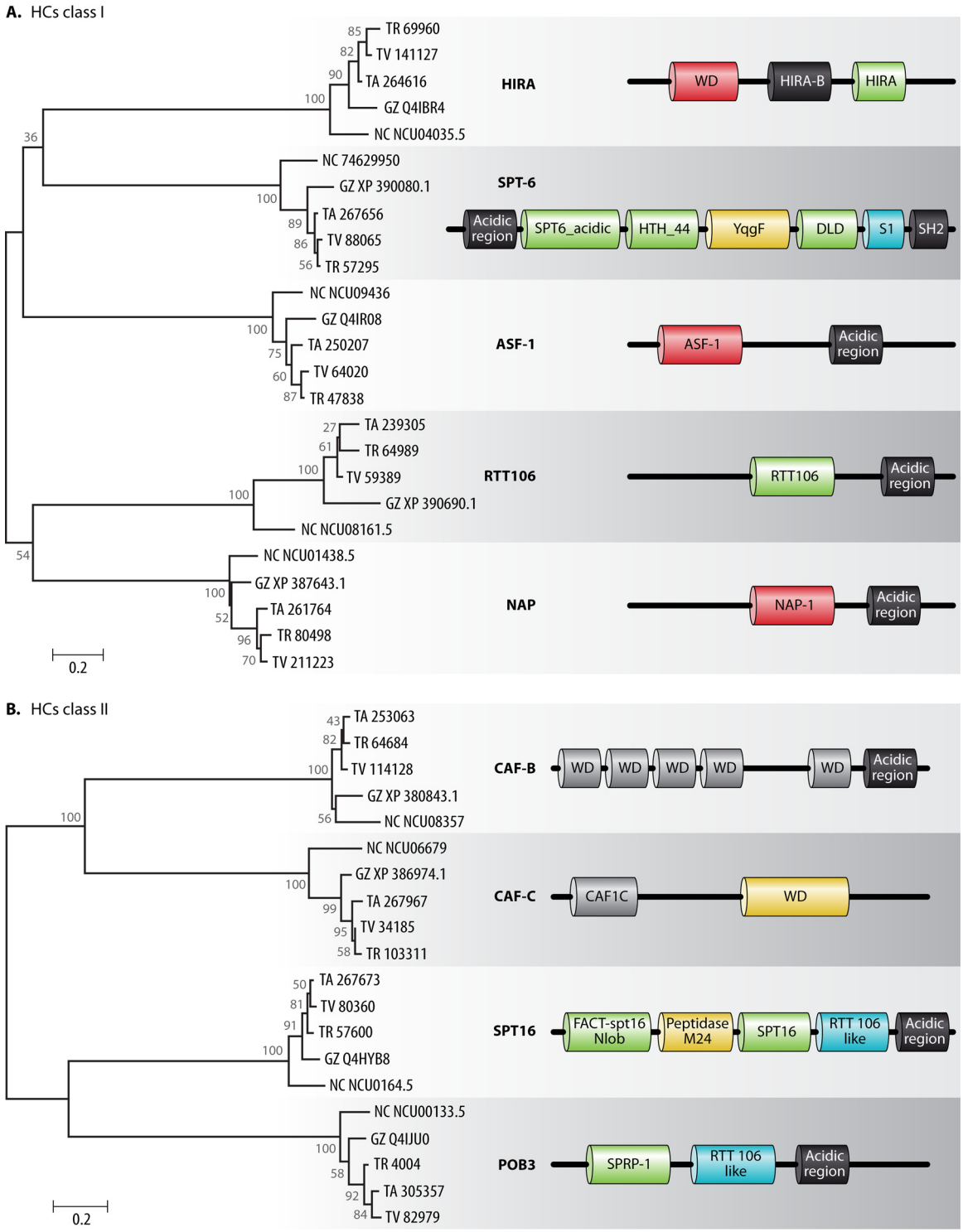

FIG 2 Schematic representation of different histone chaperones (HCs) found in *Trichoderma* spp. The HCs found in *Trichoderma* spp. are divided into two classes. (A) HC class I consists of HCs involved in binding, transport, or transfer. (B) HC class II consists of HCs capable of interacting with subunits of histone chaperones. The phylogenetic trees were constructed using the unrooted neighbor-joining method with the application of bootstrapping based on a multiple alignment of HC predicted protein sequences from *T. virens*, *T. atroviride*, and *T. reesei* genomes. *N. crassa* and *G. zeae* were used as outgroups. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4. Domain abbreviations: WD, structural motif with tryptophan (W) and aspartic acid (D); S1, RNA binding domain, peptidase; M24, metallopeptidase; SSRP1, specific structure recognition protein 1; HIRA B, histone regulatory homolog A binding; FACT-spt16 Nlob, Fact complex subunit spt16 N-terminal lobe domain; SPT6_acidic, acidic N-terminal SPT6; HTH_44, helix-turn-helix DNA binding domain of SPT6; YqgF, Holliday junction resolvase-like of SPT6; DLD, Death-like domain of SPT6; S1, S1 RNA binding domain; SH2, Src homology 2. All conserved domains in HCs were determined using Pfam (http://pfam.xfam.org/search) and SMART (http://smart.embl-heidelberg.de/).

graminearum, and *M. oryzae* (165), hence indicating differences in the mechanisms for centromere formation in filamentous fungi.

Kinetochore complexes. Several proteins that participate in kinetochore complex formation have been identified. *S. cerevisiae* has the Ndc80 complex (see Fig. S5 in the supplemental material), formed by Ncd80p, Nuf2p, Spc25p, and Spc24p. This complex is assembled in the yeast centromere and plays important roles in chromosomal interactions with microtubule segregation (166). Three of these proteins, Ndc80p, Nuf2p, and Spc24p, are present in *S. pombe*, and Ndc80p and Nuf2p have human orthologs. The Ndc80 complex is required for chromosome movement and kinetochore microtubule attachments in all described species (166). According to structural studies, the Ndc80 complex is a 56-nm-long rod-like molecule, with globular domains at either end of the rod (167, 168). We found that *T. reesei*, *T. atroviride*, and *T. virens* contain all of these components in their genomes (see Table S1 in the supplemental material). Hence, we can conclude that formation of kinetochore and chromosome segregation between yeast, vertebrates, and *Trichoderma* spp. is likely to follow a similar mechanism (166).

The Dam1/DASH complex has been discovered only in fungi and has the ability to form rings around the microtubule (MT) lattice (see Fig. S5 in the supplemental material) *in vitro*, connecting the centromere to the plus end of spindle microtubules throughout the cell cycle (169). The Dam1/DASH complex includes Duo1p, Dad1p, Dad2p, Spc19p, Spc34p, Ask1p, Dad3p, Dad4p, and Hsk3p proteins. We found orthologs for 8 of these 10 proteins in *T. atroviride* and *T. virens*, with Hsk3p and Dad1p orthologs missing (see Table S1 in the supplemental material). *T. reesei* has only seven orthologs of these proteins, additionally lacking the Duo1p ortholog. Interestingly, it has been demonstrated that all proteins are essential for proper kinetochore formation in *S. cerevisiae* (169). Consequently, the functions of Hsk3p, Dad1p, and Duo1p are either assumed by other factors in *Trichoderma* or their functions became dispensable during evolution in these fungi.

S. pombe Mis6p and Mis12p are required for correct spindle morphogenesis, determining metaphase spindle length. Mis6p is necessary to construct specialized chromatin present in the inner centromeres, whereas Mis12p plays a unique role in regulating the functional centromeres during the cell cycle (170). Genes encoding these two proteins were found in *T. reesei* (Mis12 [TR_107552] and Mis6 [TR_102403]), *T. atroviride* (Mis12 [TA_54174], and Mis6 [TA_288882]), and *T. virens* (Mis12 [TV_42852] and Mi6 [TV_34350]). The fact that *Trichoderma* spp. have proteins similar to those found in *S. pombe* may indicate that their centromeres have the same basic structure (170).

Cell cycle control. Proteins containing HORMA (Hop1p, Rev7p, and MAD2) domains participate in mitosis regulation and chromosome synapsis. It is believed that HORMA domains recognize chromatin states, which results from DNA adducts, double-stranded breaks, or nonattachment to the spindle and act as an adaptor that recruits other proteins (171). We found two proteins containing a HORMA domain in *T. reesei* (TR_104159 and TR_23446), *T. atroviride* (TA_188007 and TA_36067), and *T. virens* (TV_194423 and TV_190957), which are proposed to recognize the chromatin states that result from nonattachment to the spindle and act as adaptors to recruit other proteins. These proteins are related to MAD2 (mitotic arrest-deficient 2), a spindle checkpoint protein that prevents progression of the cell cycle upon detection of a defect in mitotic spindle integrity in several organisms from yeast to humans (172, 173). Consequently, the spindle checkpoint system appears to be evolutionarily conserved also in *Trichoderma* spp.

Chromatin Remodeling Factors

Multisubunit chromatin remodeling complexes constitute a mechanism of epigenetically mediated gene regulation. These complexes dynamically regulate transcription by utilizing the energy of ATP hydrolysis to reposition nucleosomes and thereby modulate accessibility of specific genes to the transcriptional machinery (see Fig. S6 in the supplemental material). Among the ATPase chromatin remodeling complexes, SWI/SNF is the most extensively studied example and is evolutionarily conserved from yeast to humans. SWI/SNF is comprised of 8 to 12 protein subunits (174).

SNF2. The Snf2p protein (TR_57935, TA_207565, and TV_214554) is the catalytic subunit of the multisubunit SWI/SNF (175, 176). The common feature of all Snf2 family proteins is a region of sequence similarity that includes seven helicase-related sequence motifs that are characteristic of members of the DEAD/H superfamily (superfamily 2) of nucleic acid-stimulated ATPases and DNA helicases. The helicase-like region in the Snf2 family is considerably longer than other helicases. The presence of other conserved blocks allows grouping of the Snf2 family members into 24 subfamilies on the basis of the primary sequence of the common helicase-like region (175, 177). These subfamilies form the groups Snf2-like, Swr1-like, Rad54-like, Rad5/16-like, and SSO1653-like proteins and a distant group.

T. reesei, *T. atroviride*, and *T. virens* have 22 to 28 predicted proteins related to the SWI/SNF ATPase/helicase domain (see Table S1 and Fig. S7a and b in the supplemental material), and although the number of gene models for *T. virens* is greater than the estimate for *T. atroviride* (4), the latter has more Snf2 family members.

In yeast, the SWI/SNF complex is nonessential, and it is present in relatively small amounts in the cell. Moreover, it is required for the expression of only a small portion of all the *S. cerevisiae* genes (178, 179).

In *Trichoderma* spp., the number of predicted proteins that belong to the Snf2-like, Swr1-like, and Rad54-like groups is comparable to that reported in *N. crassa* (see Table S1 in the supplemental material). Chromatin remodeling complexes are also involved in other processes that require alteration of chromatin structure, including DNA repair, DNA synthesis, mitosis, and genomic stability (174). Interestingly, most of the predicted proteins belong to the "Rad 5/16-like" group, members of which are involved mainly in DNA repair pathways (177).

Snf5. Analysis of *S. cerevisiae* revealed that Snf5p/INI1 (integrates important protein-protein interactions of the SWI-SNF complex and coordinates promoter recruitment and chromatin remodeling (180). In yeast, Snf5p has a single paralog called Sfh1p (for Snf5p homolog). Sfh1p cooperates with an essential Snf2p paralog as a member of an ATP-dependent nucleosome-restructuring complex, RSC (which is distinct from SWI/SNF). Mutation in Sfh1p1 arrests cells in the cell cycle, indicating that Sfh1p is required for cell cycle progression. Sfh1p is a phosphoprotein and its phosphorylation is regulated during the cell cycle (181).

Snf5p and Sfh1p are essential and show significant sequence identity to proteins found in humans, *D. melanogaster*, *Danio rerio*

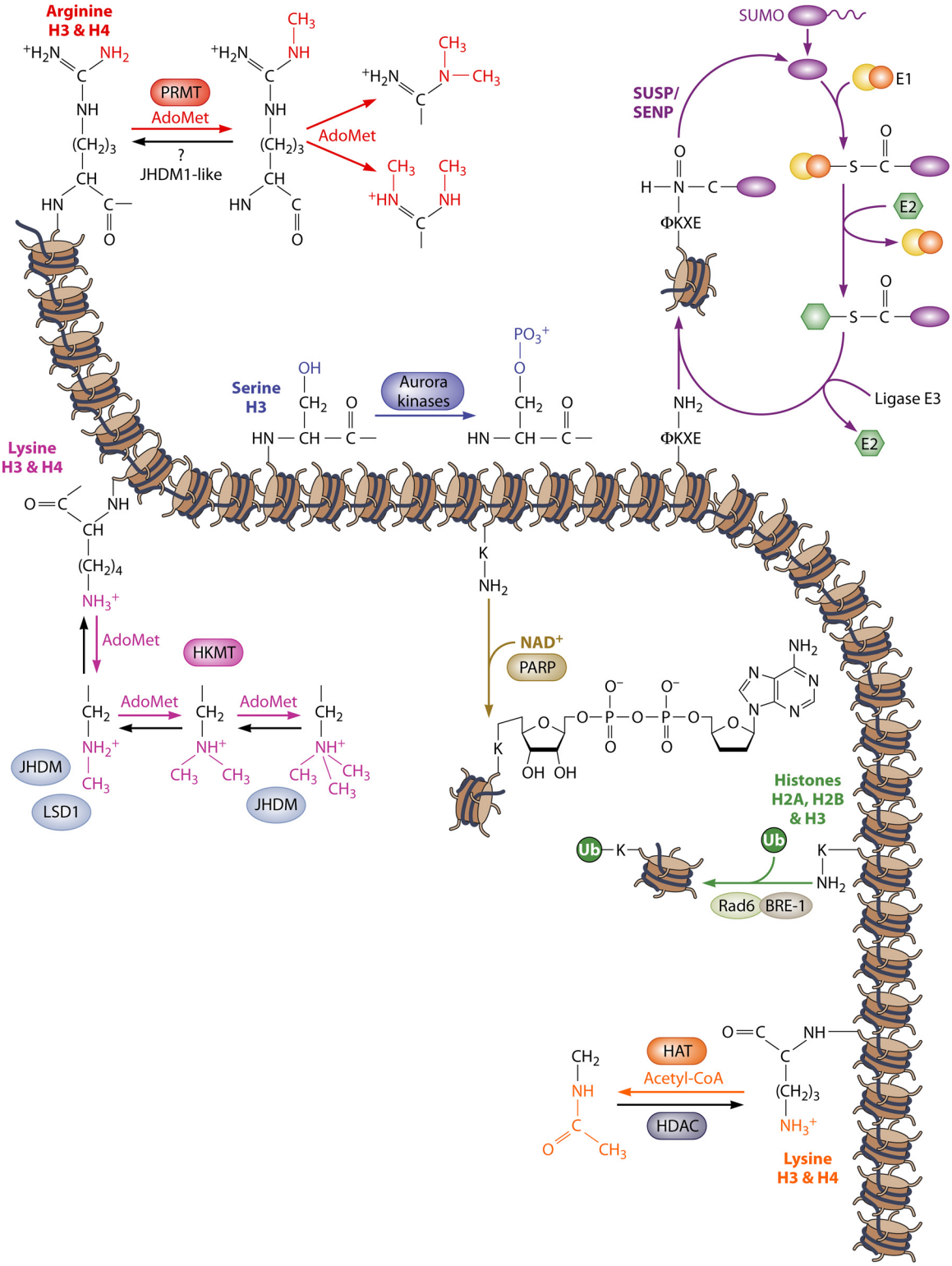

FIG 3 Histone-modifying enzymes. Histones can be modified in different ways to regulate gene expression and other DNA-templated processes, or to maintain genome integrity. Several distinct classes of enzymes can modify histones at multiple sites, catalyzing the addition or removal of an array of covalent modifications. The covalent modifications include acetylation/deacetylation, methylation/demethylation, phosphorylation, ADP-ribosylation, ubiquitylation, and SUMOylation. HATs catalyze the transfer of acetyl groups from acetyl-CoA to defined lysine residues of histones H3 and H4. Acetylation is a reversible process that depends on HDACs, some of which are NAD^+ dependent (see the text). Methylation involves the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to lysine and arginine of histones H3 and H4 mainly. Each lysine residue can be mono-, di-, or trimethylated by members of the SET domain-containing histone lysine methyltransferase (HKMT) family. Dot1 is another HMKT lacking the SET domain, which has a putative role in telomeric silencing. Arginine residues of histones H3 and H4 are methylated by members of the protein arginine methyltransferase (PRMT) family. Arginine can be mono- or

(zebrafish), *Caenorhabditis elegans*, *S. pombe*, *Arabidopsis thaliana*, and *Tetraodon fluviatilis* (pufferfish) (180, 181), suggesting that such essential activity for basic cellular processes has been conserved during evolution.

In *Drosophila*, SNR1 (Snf5) genetically cooperates with cyclin E/CDK2 to regulate the G_1/S transition and cell cycle progression. SNR1 also cooperates with histone deacetylase 1 (HDAC1) to regulate wing vein patterning. Snf5 loss may thus inactivate HDACs, and therefore impaired Snf5 could result in extensive epigenetic change (174).

The genomes of *T. reesei*, *T. atroviride*, and *T. virens* have 2 predicted proteins each, which are related to the Snf5 family members Snf5p (TR_54670, TA_173237, and TV_31111) and Sfh1p (TR_54598, TA_215727, and TV_61629) (see Fig. S8 in the supplemental material). These proteins are highly conserved among ascomycetes, with the *Trichoderma* proteins being more closely related to proteins of species with parasitic lifestyles, such as *G. zeae*, *Metarhizium acridum*, *Fusarium oxysporum*, *Nectria haematococca*, and *Cordyceps militaris*.

HMG domain proteins. The term high-mobility group (HMG) was originally coined for the HMG proteins because of their unusual solubility properties, their small sizes, and their high mobility relative to other chromatin proteins as demonstrated via polyacrylamide gel electrophoresis (182). HMG proteins are subdivided into 3 superfamilies: HMGB, HMGN, and HMGA. Each HMG superfamily has a characteristic functional sequence motif (see Fig. S9 in the supplemental material). The functional motif of the HMGB family is called the HMG box, that of the HMGN family is called the nucleosomal binding domain, and that of the HMGA family is called the AT hook, a DNA binding domain (183).

Members of the three families of HMG proteins are accessory "architectural factors" involved in modulating nucleosome and chromatin structure (see Fig. S6 in the supplemental material) and orchestrating the efficient participation of other proteins in such vital nuclear activities as transcription, replication, and DNA repair (184).

The HMGA proteins are characterized by three AT hooks and an acidic carboxyl-terminal tail (see Fig. S9 in the supplemental material). Through interactions with DNA and other proteins, they regulate transcription, influencing cell growth, proliferation, differentiation, and death (185). HMGB proteins comprise a single DNA binding domain, the HMG box, which can bind non-B-type DNA structures with high affinity. HMGBs are subject to posttranslational modifications which can fine-tune interactions of the proteins with DNA/chromatin. Association of HMGBs with chromatin is highly dynamic, and the proteins affect the chromatin fiber as architectural factors by transient interactions with nucleosomes, displacement of histone H1, and facilitation of nucleosome remodeling and accessibility of the nucleosomal DNA to transcription factors or other sequence-specific proteins (186). The six principal members of the HMGN protein family are found only in vertebrates (182).

The HMGB superfamily includes 10 predicted *Trichoderma* proteins (see Table S1 in the supplemental material), 3 of which have homology to mating peptides (see Fig. S9 in the supplemental material). In the *N. crassa* genome, eight HMGB proteins have been predicted and have been characterized as sex-determining transcription factors, MATA-3 and MAT**a**-1 (71). Besides, there are two members of the HMGA superfamily in *T. virens* and *T. atroviride* and only one predicted protein in *T. reesei* (see Table S1).

Histone acetyl transferases. Histone acetylation is performed by histone acetyl transferases (HATs) by transferring an acetyl group from acetyl coenzyme A (CoA) to lysine residues into the histone amino-terminal tails (Fig. 3). Histone acetylation correlates with gene transcription (187). *T. reesei*, *T. atroviride*, and *T. virens* have several orthologs of HATs that belong to the GNAT family, such as HAT1 (TR_120737, TA_295148, and TV_41535), GCN5 (TR_64680, TA_47901, and TV_211918), and ELP3 (TR_4989, TA_156760, and TV_46168). In *Drosophila* and HeLa14 cells, Hat1p is a type B HAT located in the cytoplasm, where it acetylates Lys5p and Lys12p residues at the *de novo*-synthesized histone H4 (188). Likewise, we found three members of the HAT MYST family, including SAS2, SAS3, and ESA1 (see Table S1 in the supplemental material), where orthologs of the first two are responsible for the silencing in telomeres and cell cycle progression in *S. cerevisiae* (189, 190). Esa1p is the catalytic component of the *S. cerevisiae* NuA4 histone acetyltransferase complex, which is involved in transcriptional activation by acetylation of nucleosomal histones H4, H3, H2B, H2A, and the H2A variant H2A.Z. Esa1p is also involved in DNA double-strand break repair by homologous recombination and in cell cycle progression (see Table S1). We also found homologs of the basal transcription factor TAFII250 that exerts HAT activity (see Table S1). Recently, it was demonstrated that histone acetylation is not only carried out at the amino terminus but also within the histone core. This function is performed by specific HATs. *Trichoderma* spp. have in their genome orthologs of Spt10p (TR_61081, TA_222395, and TV_124876) and Rtt109p (TR_60283, TA_251300, and TV_35965). In yeast, Spt10 is responsible for the transcriptional activation of genes encoding histones H2A and H2B (191, 192). Moreover, Rtt109p acetylates the *de novo*-synthesized histone H3, which subsequently is recruited to chromatin during DNA replication (193). In addition, Rtt109p is also involved in the response to DNA damage (194). Both HATs acetylate the lysine 56 on histone H3. In *Trichoderma* spp., phylogenetic analysis shows a high

dimethylated, the latter in a symmetric or an asymmetric way. Histone demethylation could be catalyzed by LSD1 or JmjC domain-containing histone demethylases (JHDMs). LSD1 can only remove mono- and dimethyl lysine modifications, whereas JHDMs can remove all three histone lysine methylation states. Arginine demethylation occurs as a deimination process, which may not play a role in fungi due the lack of homologous enzymes. However, the JHDM-1-like protein of *Trichoderma* spp. shares similarities to JMJD6 of mammals, the only arginine demethylase described so far. Serines of histone H3 are phosphorylated by members of the Aurora protein kinase family. In the ADP-ribosylation enzymatic reaction, NAD^+ is cleaved into nicotinamide and ADP-ribose, with the latter attached to lysine of histones by action of PARP. The SUMOylation occurs at lysines that are frequently close to a hydrophobic residue (Φ) and to a negatively charged environment (E). SUMO is synthesized as a precursor protein that is C-terminally processed. Subsequently, the conjugation to proteins involves the E1 enzyme (AOS1/UBA2) and the E2-conjugating enzyme (Ubc9), that form thioesters (—S—) with the modifier. E3 ligase stimulates the attachment to specific lysine residues of histones. The removal of SUMO is catalyzed by the SUSP/SENP isopeptidase family. Addition of ubiquitin (Ub) in lysines of H2A, H2B, and H3 occurs by a mechanism similar to SUMOylation. In this process, Rad6 is the E2 ubiquitin conjugase and BRE1 is the E3 ligase.

degree of conservation between HATs and with those of *N. crassa* and *G. zeae* (see Fig. S10 in the supplemental material).

We were unable to detect orthologs of the HATs Hpa2p from *S. cerevisiae* or PCAF, SRC-1, Tip60, MOZ, and ACTR from mice and humans. However, *T. reesei*, *T. atroviride*, and *T. virens* also possess acetyltransferases whose substrates are nonhistone proteins, such as ARD1, which participates in the ARD1-NAT1 complex that acetylates nascent polypeptides from the ribosome (195). We also found NAT5, a component of the *N*-acetyl transferase complex B (NatB), human orthologs of which acetylate methionine residues followed by asparagine (196). The *N*-acetyl acetyltransferase MAK3 from *S. cerevisiae*, which acetylates the N terminus of Gag protein (virus major coat protein) necessary for the assembly of L-A virus, is also present in the *Trichoderma* genomes (197).

Components associated with multiprotein chromatin remodeling complexes. In eukaryotes, proteins involved in remodeling and modification of chromatin do not act alone. They work together with other proteins, forming multisubunit complexes (i.e., HAT complexes), where each of these subunits contains proteins with characteristic domains that interact with each other or with histones to carry out either modification or remodeling. We detected *Trichoderma* proteins with different domains potentially involved in such interactions, including proteins containing bromodomains, PHD domains, or SANT domains (see Table S1 in the supplemental material). Some of these *Trichoderma* proteins are homologous to those present in other organisms, such as Gcn5p, which contains a bromodomain; however, most of them have not been described or characterized yet.

(i) Proteins containing a bromodomain. The bromodomain is a highly conserved 110-amino-acid domain found in proteins that interact with chromatin. They are commonly found in the subunits of the SAGA and SWI/SNF complexes (see Fig. S6 in the supplemental material) (198, 199). Proteins containing this domain recognize acetylated lysines in histone tails (200). *T. reesei*, *T. atroviride*, and *T. virens* share proteins that contain 1 or 2 bromodomains with *N. crassa* and *G. zeae*, including proteins belonging to the subfamily BET, characterized by the presence of two bromodomains, and a C-terminal region called extra terminal (ET) (see Table S1 in the supplemental material) (201). This protein is the homolog to Bdf1p from *S. cerevisiae*, where it is associated with the basal transcription factor TFIID (202) and located in zones with acetylated histone H4 (203). Genes encoding Bdr8- and BDRT-like proteins are present in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1) and exhibit high identity with their respective homologs in humans and mice. These proteins are also classified within the family BET (201). However, our analysis showed no presence of the characteristic 2 bromodomains of this subfamily (see Fig. S11 in the supplemental material). A phylogenetic analysis showed their proximity to BDF1 (see Fig. S11), and hence we conclude that these proteins belong to this subfamily. Moreover, we found homologs to Rsc4p (TR_122628, TA_322713, and TV_61838) and Spt7p (TR_77685, TA_37373, and TV_64004) that contained two bromodomains, which have been well characterized in *S. cerevisiae*. Rsc4p and Spt7p are subunits of the remodeling complex RSC and SAGA, respectively (204, 205). Spt7 is necessary to maintain the appropriate amounts of Spt20 and ADA1, components that maintain the integrity of the SAGA complex (199, 204). *T. reesei*, *T. atroviride*, and *T. virens* RSC4 proteins comprise two bromodomains in tandem (see Fig. S11) and exhibit high homology with those of *N. crassa* and *G. zeae*. These data support their putative relevance in the biology of these fungi, since mutations in *RSC4* are lethal in *S. cerevisiae* (206).

(ii) Proteins containing plant homeodomains (PHDs). The PHDs are C4HC3 zinc finger motifs found in many nuclear proteins and are thought to be involved in epigenetic and transcriptional regulation through chromatin modifications (207). Proteins containing this domain recognize and bind to trimethylated histone H3 at lysine 4 (H3K4me3) (208–210). The PHD fingers have also been found in chromatin remodeling proteins such as Yng1p (211). The genomes of *T. atroviride*, *T. virens*, and *T. reesei* contain 4 proteins bearing PHDs, as do the genomes of *G. zeae* and *N. crassa* (see Table S1 in the supplemental material). Additionally, we found orthologs of the well-characterized protein Epl1 (Enhancer of Polycomb-like 1, not to be confused with *Trichoderma* EPL1, a ceratoplatanin-like protein) from *S. cerevisiae*, a component of the NuA4 complex, which is essential for viability in *S. cerevisiae* (212). Epl1 is the homolog to *Drosophila* Enhancer of Polycomb [E(Pc)], which plays positive and negative roles in the epigenetic regulation of the homeotic genes. Furthermore, the E(Pc) mutation suppresses the position-effect variegation that results from chromosome rearrangements which translocate euchromatic genes close to the heterochromatin, and thereby causes silencing (213).

(iii) Proteins containing SANT domains. The SANT domain (switching-defective protein 3 [Swi3], adapter 2 [ADA2], nuclear receptor corepressor [N-CoR], and TFIIIB) is present in many chromatin remodeling proteins that regulate chromatin accessibility (214, 215). Accordingly, four proteins containing SANT domains (see Table S1 in the supplemental material), including RSC8 (chromatin remodeling complex present in the RSC) and ADA2 (subunit complexes having Gcn5 as a catalytic subunit) were found in *T. reesei*, *T. atroviride*, and *T. virens*. It has been proposed that the SANT domain interacts with unmodified histone tails (216), maintaining a no-acetylation state (217). Two of these proteins containing SANT domains have not been characterized; therefore, we have named them SANT1 and SANT2. Sequence and phylogenetic analyses confirmed the relationship between SANT1 and SANT2 with ADA2 and RSC8, including Ada2p and Rsc8p from *S. cerevisiae*. The SANT domains were also found to be highly conserved and exhibit the three aromatic residues, which correspond to each of the three helices predicted for the secondary structure of several proteins containing SANT domains (215) (see Fig. S12 in the supplemental material). However, SANT1 contains a conserved motif, which is not present in the other proteins. This could indicate that SANT1 has evolved to perform altered or additional functions compared to the group of RSC remodeling enzymes.

HDACs. HDACs remove acetyl groups of lysine residues from histone amino tails (Fig. 3), leading to higher DNA compaction and consequently to the repression of gene expression (218–220). The HDAC targets include lysines 9, 14, and 18 from histone H3 and lysines 8, 12, 16, and 20 from histone H4 (221). These proteins are assigned to 3 groups according to their function and the DNA sequence they recognize. Group I includes the classical HDACs, whereas group II includes enzymes dependent on NAD (NAD^+), usually named Sirtuins (see Fig. S13 in the supplemental material). The third group comprises the HD-tuins present exclusively in plants, which were originally identified in maize (*Zea mays*) (222–225).

The classical HDACs include RPD3-like proteins and a subset of HDA1-type enzymes. In *A. thaliana*, there are 12 members of this group, divided into different classes according to their domains (226). All these HDACs comprise a characteristic histone deacetylase domain (227). Previous works reported the presence of this group in fungi, including *A. nidulans*, *Aspergillus fumigatus*, and *C. neoformans* (228). *S. cerevisiae* contains in its genome five classical HDACs, i.e., Rpd3p, Hos1p, Hos2p, Hda1p, and Hos3p (229). Interestingly, Hos3p orthologs are exclusively found in filamentous fungi (230–232). The genomes of *T. reesei*, *T. atroviride*, and *T. virens* contain the four classical HDACs RPD3 (TR_48386, TA_163610, and TV_114500), HDA1 (TR_80797, TA_39952, and TV_88835), PHD1 (TR_57220, TA_212638, and TV_214375), and HOSB (TR_65533, TA_161985, and TV_182402), which are also present in *G. zeae* and *N. crassa* (see Table S1 in the supplemental material).

The Sirtuin class of HDACs is highly conserved from prokaryotes to mammals. In fungi, a common core domain of 200 to 275 amino acids in length has been identified. Most of these proteins are homologous to the Silent information regulatory protein 2 (Sir2p) from *S. cerevisiae*. This group of HDACs is NAD^+ dependent; NAD^+ is used as a cofactor in its catalytic domain (233, 234).

Although these enzymes are very similar to each other, they differ significantly in their biological functions, subcellular localizations (nucleus, mitochondria, or cytoplasm), and the kind of reaction they catalyze (235, 236). Several studies have indicated that these enzymes respond to different cellular stresses, suggesting that they are controlled by different signal transduction pathways in response to intra- and extracellular cues (237). Yeast Sir2p (TR_74366, TA_17234, and TV_33448) deacetylates lysine 16 in histone H4 and lysines 56 and 9 from histone H3 (238, 239). Furthermore, Sir2p exerts an NAD-dependent ADP-ribosyl transferase activity (233, 238), which is mainly involved in telomeric silencing, and the elimination of RNA-ribosomal DNA (rDNA) recombination (238–242). In *S. cerevisiae* the Hst1p to Hst4p sirtuins were identified as paralogs of Sir2p (243). There are few reports on the presence of these proteins in filamentous fungi such as *A. nidulans* (231) and *N. crassa* (244). *T. reesei*, *T. atroviride*, and *T. virens* contain HST1 (TR_120404, TA_51699, and TV_67405), HST2 (TR_50268, TA_166209, and TV_194055), HST3 (TR_54330, TA_215446, and TV_168419), HST5 (TR_67057, TA_299367, and TV_57675), and HST6 (TR_79919, TA_81752, and TV_168778), which are highly conserved (see Table S1 and Fig. S14 in the supplemental material), and hence their inventory of these genes is similar to that of *G. zeae* and *N. crassa*.

Histone methyltransferases and arginine-specific methyltransferases. Histones are methylated by the transfer of methyl groups from the methyl donor SAM to either the ε-NH_2 group of lysine or the ω- or δ-NH_2 of arginine residues (Fig. 3). Methylation of histone residues can either induce or inhibit transcription, depending on the modified residue and the number of methylations, since it could serve as a platform for the recruitment of effector proteins (245–247). In addition to its role in transcriptional regulation, methylation has also been linked to heterochromatin formation, X-chromosome inactivation, DNA repair, RNA processing, and nucleo-cytoplasmic localization (245, 248). Histone methylation is catalyzed by a group of histone methyltransferases (HMTases), and they are primarily classified into either lysine-specific (HMKT) or arginine-specific (PRMT) methyltransferases (245, 249).

A lysine can be mono-, di-, or trimethylated, or unmethylated, with different functional consequences for each of the four methylated forms (Fig. 3) (249). Many SET domain-containing methyltransferases that target site-specific lysines in histones have been identified and characterized (248). In humans, more than 60 proteins containing SET domains have been predicted, whereas nearly 40 were found in *A. thaliana* and about 10 HKMTs were found in *Drosophila*, *S. cerevisiae*, *S. pombe*, and *N. crassa* (71, 250). We found that *T. virens* contains 11 predicted HMKT protein-encoding genes, whereas *T. atroviride* and *T. reesei* contain 15 and 10, respectively. Interestingly, *T. atroviride* comprises three Set5p-like proteins, two of which are unique (TA_266615 and TA_267139) and the other one is similar to that also present in *T. virens*. In *T. reesei*, no Set5p homolog was detected (see Table S1 in the supplemental material).

The non-SET HKMT Dot1p (disruptor of telomeric silencing) has been identified as a telomeric silencing switch in *S. cerevisiae* and humans, since it catalyzes the methylation reaction of H3K79 (251, 252). Dot1 is conserved in *S. cerevisiae*, *N. crassa*, and higher eukaryotes, such as *D. melanogaster*, *A. gambiae*, *C. elegans*, and *H. sapiens*. For *T. reesei*, *T. atroviride*, and *T. virens*, one putative protein homologous to Dot1p (TR_48225, TA_53508, and TV_216816) was predicted, suggesting that the telomeric silencing switch DOT1 is also present in these fungi.

Concerning histone PRMTs, PRMT1, PRMT3, and PRMT5 have been highly conserved throughout eukaryotic evolution. Homologous genes have been identified in amoebae, protozoa, yeasts, molds, zebrafish, mice, and humans (253). Expression of PRMT1 is not required for cell viability in yeasts and mammals (254, 255). Nevertheless, this protein plays two important cellular roles in mRNA biogenesis and heterochromatin formation (253, 256). PRMT3 has a role in ribosome function, since the 40S ribosomal protein S2 (rpS2) is its physiological substrate (253). Methylation by PRMT5, which can target both H3 and H4, has been implicated in transcriptional repression (257). Our genome analysis revealed one member of each PRMT in *T. atroviride*, *T. virens*, and *T. reesei*.

Jumonji domain proteins. The level of complexity within the histone methylation system might in part define the corresponding number and complexity of demethylase enzymes that antagonize these modifications. Histone methylation was thought to be irreversible for many years, because the proposed mechanisms would require either passive or active histone exchange to revert to the original unmethylated state. This idea prevailed until the discovery of lysine-specific demethylase 1 (LSD1), which removes mono- and dimethyl groups from H3K4 via an oxidation reaction (258). Subsequent to the discovery of LSD1, Jumonji proteins were proposed as histone demethylases. These enzymes contain a Jumonji C (JmjC) domain and catalyze lysine demethylation of histones through an oxidative reaction that requires iron Fe(II) and α-ketoglutarate as cofactors (Fig. 3) (259, 260). The JmjC domain-containing histone demethylases can remove all three histone lysine methylation states (260, 261).

T. reesei, *T. atroviride*, and *T. virens* have two proteins that are members of the JARID (Jumonji/ARID domain-containing) family (see Fig. S15 in the supplemental material) (TR_62964, TA_291656, and TV_163714; TR_22117, TA_229470, and TV_47924) that remove methyl groups from H3K4me3 in yeast,

plants, and animals (260–262). Like their homologs in yeast, *N. crassa*, animals, and plants, the architecture of *Trichoderma* JARID-like proteins present a JmjN domain, a JmjC domain, and a C5HC2 domain (249, 260, 262), but only one presents a PHD and a PLU-1 domain (see Fig. S15). It has been shown that the histone demethylases containing the PLU domain can demethylate mono-, di-, or trimethylated H3K4 (263).

Another histone-demethylase target is H3K36, and its methylated state is antagonized by JHDM1 or JMJD5 (262). In *T. reesei*, *T. atroviride*, and *T. virens*, the JHDM1 protein (TR_58851, TA_135826, and TV_38658) also contains the F-box domain (see Fig. S15 in the supplemental material), which has been reported to cooperate with S-phase kinase-associated protein 1A (SKP1) to form the SKP1–cullin–F-box protein E3 ubiquitin ligase complex, which suggests that JHDM1 might link histone demethylation to protein ubiquitylation (260). On the other hand, JMJD5 proteins (TR_70310, TA_293380, and TV_36532) belong to the "JmjC domain-only" group, and their functions remain largely unknown (260). However, recent findings in *Arabidopsis* have revealed that JMJD5 is involved in gene expression regulated by the circadian clock (262).

Histone phosphorylation. Phosphorylation is another important postranslational modification (PTM) that occurs on serines (S), threonines (T), and tyrosines (Y) in all four core histones that constitute the histone octamer, as well as histone H1 and some of the histone variants (Fig. 3). This histone modification regulates two apparently opposite processes: condensation of mitotic chromosomes associated with a transcriptionally repressive state as well as signal-induced gene activation during interphase (264, 265). For instance, phosphorylation of serines 10 and 28 and threonines 3 and 6 of histone H3 has been associated with condensation of chromosomes during mitosis and meiosis (266, 267). The H3S10 phosphorylation also has been reported to activate transcription in response to extracellular signaling of the MAPK pathway (268, 269). Kinases (see also the section on protein kinases) that have a role in histone phosphorylation include Aurora- and Ipl1-like protein kinases, haspin, glycogen synthase kinase-3 (GSK3), NimA, and the SNF1 kinase complex, composed of Snf1p, Snf4p, and Gal83p. All these kinases are conserved in eukaryotic linages, including animals, fungi, amoebozoa, and plants (265, 270, 271). The reverse reaction is catalyzed by protein phosphatase 1 (PP1) (Fig. 3) (272).

During mitosis, the dynamics of chromatin architecture ensures the accurate segregation of the sister chromatids. In this process, the phosphorylation of serine 10 in H3 by Aurora- and Ipl1-like protein kinases is considered an essential step of modification (267, 270), since it permits the dissociation of HP1 from chromatin in M phase (273). In filamentous fungi, the kinase NimA/Nim-1 (TR_68364, TA_292097, and TV_158156) can phosphorylate histone H3S10 (274). The kinase haspin (TR_54171, TA_5069, and TV_114758) phosphorylates histone H3Thr3 during prometaphase and metaphase, and this modification appears to be important for metaphase chromosome alignment (266). Histone H1 is also phosphorylated in mitosis, specifically at threonine 10 by GSK3 (TR_74400, TA_297064, and TV_73199) during prometaphase, and disappears during telophase (275).

In *S. cerevisiae*, Snf1p (TR_45998, TA_227921, and TV_40990) regulates transcription of genes required for growth in different carbon sources, i.e., low-glucose medium or sucrose, through H3S10pho, which leads the acetylation of promoters mediated by Gcn5p and recruiting of TATA binding protein (268, 276, 277).

T. reesei, *T. atroviride*, and *T. virens* contain a single gene for all these kinases and the phosphatase (see Table S1 in the supplemental material), suggesting that the mechanisms that regulate chromatin condensation could be similar to those previously reported in other eukaryotic organisms. However, some differences could be present, since in *T. reesei* the homolog of Snf1 does not phosphorylate histone H3 (278).

Histone ubiquitination. Another posttranslational modification of histones is the conjugation of ubiquitin to the COOH termini of the core histones H2A, H2B, and H3 (Fig. 3). Ubiquitinated forms alter chromatin structure locally, and this modification has been correlated with increased transcriptional activity, replication, and meiosis (279, 280). Monoubiquitylation of H2B is linked to transcriptional activation and gene silencing, a mechanism conserved from yeast to humans. Moreover, ubiquitylation of H2A is important for transcriptional repression in higher eukaryotes, and both histones play key roles in DNA repair (281). Histone ubiquitination further acts as a prerequisite modification for other histone modifications that alter the structure and function of chromatin (282).

In yeast, the histone H2B ubiquitination is catalyzed by the ubiquitin-conjugating E2 enzyme Rad6p and the ubiquitin E3 ligase Bre1p (279, 283). Both proteins are present in a complex which also contains Lge1p, which is required for H2B ubiquitination (Fig. 3). In *T. reesei*, *T. atroviride*, and *T. virens*, three putative E2 ubiquitin-conjugating enzymes were identified, and only one of them is a Rad6-like protein (see Table S1 in the supplemental material). It has been reported that the Rad6p protein of *S. cerevisiae*, the Rhp6 protein of *S. pombe*, and MUS-8 of *N. crassa* have a very similar function (284). Therefore, it can be assumed that the *Trichoderma* Rad6-like proteins (TR_120075, TA_301071, and TV_81725) are involved in DNA repair functions, especially UV-induced mutagenesis, and also in sporulation.

Bre1 not only serves as an E3 ligase for H2B ubiquitination but is also required for the recruitment of Rad6 to chromatin (283). We found only one BRE1-like protein in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 and Fig. S16 in the supplemental material).

Histone sumoylation. SUMO (small ubiquitin-related modifier) is a family of around 100-amino-acid-long proteins which are highly conserved in eukaryotes and very similar to ubiquitin at the tridimensional level (285). Like ubiquitin, SUMO also binds covalently to its target proteins to modify their function. This posttranslational modification plays important roles in several processes in the cell through the covalent binding of SUMO (Fig. 3), including histones. In this sense, sumolyation of histones is generally associated with transcriptional repression (286). Binding of SUMO to its target is similar to that of ubiquitin (see Fig. S17 in the supplemental material). *T. reesei*, *T. atroviride*, and *T. virens* possess all the components involved in the sumoylation pathway in their genomes (see Table S1 in the supplemental material), including the SUMO protein and the homologs to Smt3p from *S. cerevisiae*. From yeast to mammals, eukaryotes comprise the heterodimer Aos1/Uba2, called E1, which activates SUMO by binding it through thioester bridges. Upon SUMO activation, it is conjugated with the Ubc9 protein (also present in the genome of *Trichoderma* spp.), which ligates SUMO to its target protein. In *S. cerevisiae* Siz1p (SIZA) Siz2p, Cst9p, and Mms21p protein ligases,

called E3 by analogy with the ubiquitin system, apparently confer specificity to the ligation of SUMO to its targets (287–289). The Siz1p and Siz2p ligases are paralogs, probably raised during the genome duplication of the *S. cerevisiae* ancestor. In *T. reesei*, *T. atroviride*, and *T. virens*, we found just the Siz1 ortholog (TR_62004, TA_214582, and TV_183126) (see Fig. S18 in the supplemental material), likely because the *Trichoderma* ancestor did not suffer a genome duplication event. We hypothesize that the Siz1p ortholog in *Trichoderma* spp. confers specificity in histone sumoylation in these fungi, since deletion of Siz1p and Siz2p in *S. cerevisiae* provokes a considerable reduction in sumoylation of histones H2B and H4 (290). We also found orthologs to Mms21p ligase, which is not related to histone sumoylation, in *Trichoderma* spp. This ligase could be involved in sumoylation of nonhistone proteins that regulate growth and DNA damage repair, as reported for *S. cerevisiae* (287).

ADP ribosylation. ADP ribosylation is a postranslational covalent modification carried out by ADP-ribosyltransferases (Fig. 3), which regulate the cell cycle, response to DNA damage, replication, and transcription. Histones are subject to ADP ribosylation by ADP-ribosyltransferases in specific amino acids located within histone tails. The ADP-ribosyl hydrolases and poly(ADP)ribose glucohydrolases degrade the ADP-ribose polymers (Fig. 3) (291). The ADP ribosylation is read by zinc finger domains or macrodomains, which regulate the chromatin structure and transcription accordingly. Furthermore, the histone ADP ribosylation can be considered an additional component of the histone code (292–294).

PARP-1 and PARP-2 are members of the PARP family, the proteins of which synthesize ADP-ribose polymers by using β-NAD^+ as a substrate and transfer them onto E, D, or K residues of target proteins (295, 296). Mass spectrometry analysis of histone peptides showed that lysine K13 of H2A, K30 of H2B, K27 and K37 of H3, and K16 of H4 are specifically ADP ribosylated by PARP-1 (294). The poly(ADP)ribose polymerase superfamily is characterized by the presence of the PARP catalytic domain, which is comprised of 17 members (297). The PARP domain catalyzes the ADP ribose transfer from NAD to the target proteins (296).

Recently, a new nomenclature for these proteins was proposed, based on their catalytic domain structure: the diphtheric toxin ADP-ribosyltransferase family (ARTD) (298). These domains were formerly found in the nonclassic histone macroH2A (299, 300). The ADP ribosylation cycle involves the synthesis of specific acceptor residues, recognition, and degradation of the poly (ADP)ribose. To date, only the human ARTD1, ARTD3, and ARTD10 have been described as histone ADP-ribosyltransferases, whereas ARTD2 is unable to ADP ribosylate histones *in vitro* (294, 301).

ARTD1 is a multifunctional nuclear zinc finger protein that participates in the rapid response to DNA-damaging agents in mammalian cells (302). The ADP-ribose polymerization activity of this enzyme (elongation and branching) is substantially increased when double-stranded DNA is broken or cut (303, 304). Therefore, ARTD1 covalently modifies a number of nucleic acid binding proteins with a strong polyanion (305). The human hARTD1 promotes chromatin compaction in supranucleosomal structures in a way dependent on the amino termini of essential histones. The ARTD1 DNA binding domain (DBD) is necessary and sufficient for its binding to the nucleosomes. However, it is unable to promote the chromatin compaction and partially represses RNA polymerase II-dependent transcription *in vitro*. Furthermore, the ARTD1 catalytic domain, which is unable to bind to the nucleosomes by itself, cooperates with the DBD to promote chromatin compaction and transcriptional repression (306). These proteins are present in a wide range of eukaryotic organisms, and they have been widely studied in mammals, although they also have been found in prokaryotes as well as in viruses (291). In these organisms, ARTD proteins play an important role in DNA repair, genome integrity, and epigenetic regulation (307).

One putative protein containing the poly(ADP-ribose) polymerase catalytic domain, as well as a DBD and ADP-ribosyl transferase domain, was identified in *T. reesei* (TR_22115), *T. atroviride* (TA_295780), and *T. virens* (TV_89857). These proteins contain all the domains described for ARTD family proteins and share high degrees of homology between them and with their counterparts in *N. crassa and G. zeae*, as well as with several *Fusarium* species. Together, these data indicate that ARTD proteins are evolutionarily conserved, which led us to conclude that they could be responsible for ADP-ribosylation of lysines K13 of H2A, K30 of H2B, and K27 and K37 of H3, as well as K16 of H4, to maintain genome integrity and transcriptional regulation in *Trichoderma*.

Conclusions. We identified 153, 159, and 147 putative chromatin structural and modifying proteins for *T. virens*, *T. atroviride*, and *T. reesei*, among which most are shared between the three *Trichoderma* species as well as with the phytopathogenic fungus *Giberella zeae* and the necrotrophic fungus *N. crassa*. In this group were found the highly conserved core histones H3, H4, H2A, and H2B. We also identified proteins involved in such modifications, like HATs and HDACs, as well as components that influence chromatin dynamics, such as ATP-dependent remodeling proteins and those that modify the DNA by methylation (methylases) and demethylation (demethylases). Six proteins were found exclusively in *T. atroviride*: Snf2 (TA_86511), Rad5 (TA_270730), Set5-like (TA_86045, TA_286297, and TA_94162), Set9-like (TA_79382), and HMGA (TA_297915), including HMT and chromatin remodeling proteins. Furthermore, we found that *T. virens* and *T. atroviride* share eight orthologous proteins that were not found in *T. reesei*: Duo1 (TV_194602 and TA_261226), Rad5/16 (TV_149973 and TA_224421; TV_188178 and TA_11002), Rad5 (TV_172559 and TA_193915), HMGA3 (TV_229777 and TA_271281), HMGB5 (TV_228694 and TA_321166), Set5-like (TV_42740 and TA_183670). In summary, the two mycoparasitic species share more orthologous proteins with each other than they do with the saprophytic *T. reesei*. These subtle differences between them could dictate the differences in their lifestyle ranking from saprophytism to mycoparasitism and rhizosphere competence.

METABOLISM AND TRANSPORT

CAZymes

The CAZy classification (carbohydrate-active enzymes; www.cazy.org) (308) relies on amino acid similarities and therefore enables an assignment of protein sequences to various families without the need of a prior biochemical characterization of the respective protein. In view of the large numbers of microbial genomes that are being sequenced, the prediction of enzyme activities and protein functions, based on sequence similarities to already characterized proteins, is an important means for *in silico* genome

analysis. The CAZyme genome content of an organism (CAZome) represents an important source of information for selection of interesting targets for further biochemical screening approaches.

CAZymes are categorized into different families in the CAZy database (308) (Cazypedia [http://www.cazypedia.org/]). These families describe structurally related catalytic and carbohydrate binding modules (or functional domains) of enzymes that degrade, modify, or create glycosidic bonds. Enzyme categories in the CAZy database include glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs). GHs hydrolyze or rearrange glycosidic bonds, GTs form glycosidic bonds using activated sugars as donors (e.g., UDP-linked sugars), PLs cleave uronic acid -containing sugars via a β-elimination mechanism, and CEs catalyze the de-*O*- or de-*N*-acylation of substituted saccharides. In addition, the CAZy database contains a classification of carbohydrate binding modules (CBMs), which are involved in the adhesion of the enzymatic module to different carbohydrates. A CBM is defined as a contiguous amino acid sequence within a carbohydrate-active enzyme with a discrete fold having carbohydrate-binding activity (309, 310). Recently, some CAZy families have been redefined in the new class of auxiliary activities (AA). This new class comprises, among others, lytic polysaccharide monooxygenases (AA9 to AA11 and AA13) that were previously found in families GH61 (reclassified as AA9) and CBM33 (reclassified as AA10) (310, 311).

The hydrolysis (by GHs) and formation (by GTs) of glycosidic bonds can proceed via retention or inversion of the anomeric carbon (C-1) of the substrate after cleavage. In an inverting enzyme, the hydrolysis of a β-glycosidic bond creates a product with the α-configuration, and vice versa, whereas in retaining enzymes the initial configuration of the anomeric carbon atom is preserved. In GHs in general, two amino acids at the opposite sides of the sugar plane are the key factors for the nucleophilic attack, stabilization of the transition state, and finally hydrolysis of the glycosidic bond. Often, acidic side chains of the two catalytic amino acids are important for the reaction mechanism. A special case of the retaining mechanism is the neighboring group participation, where a side group of the substrate itself assists in the catalytic mechanism (312). Chitinolytic enzymes (e.g., proteins from GH families 18 and 20) use this catalytic mechanism. GHs with a retaining mechanism also commonly have transglycosylating abilities and are able to form oligosaccharides from their hydrolytic reaction products when they are present in high concentrations. In contrast to that, GTs, for which so far predominantly inverting mechanisms have been reported instead use activated carbohydrate monomers (e.g., UDP-activated sugars for cell wall biosynthesis) to create glycosidic bonds and thus long carbohydrate chains (313).

Studies on three-dimensional structures of GH enzymes have so far largely verified that members of a GH family share the same overall protein fold, including a common catalytic site, and the same reaction mechanism, i.e., retaining or inverting (314). Some GH families contain exo- and endoenzymes with the same carbohydrate cleavage specificity, e.g., GH 6 contains cellobiohydrolases and endoglucanases. Both cleave the β(1→4) bonds of cellulose, but with different preferences for cleavage at the cellulose chain ends or centers, respectively. For some families only one enzymatic activity has so far been reported, whereas in other families different enzymatic activities have be found.

Lytic polysaccharide monooxygenases (LPMOs), currently found in AA families 9 to 11 and 13, are enzymes secreted by a variety of fungal and bacterial species (311, 315–320). These enzymes cleave polysaccharide chains via an oxidative mechanism of action and thereby disrupt the structures of recalcitrant polysaccharides. They are able to attack flat molecular surfaces of the polysaccharide and tightly packed carbohydrate chains that would not be easily accessible for hydrolases. These enzymes significantly accelerate the degradation of polysaccharides into oligosaccharides, and their discovery provided an answer to the long-sought-for question about how the initial attack on cellulose or chitin was carried out by microorganisms (318). Major research efforts are currently focused on investigating further details of the enzymatic mechanisms of LPMOs and discovering new members of these families with different substrate specificities. Fungal LPMOs that oxidize cellulose belong to AA9 (formerly GH61) (315, 319, 321), and more recently a fungal LPMO that oxidizes chitin has been characterized for AA11 (322). In addition, an LPMO active on starch was reported and is now classified as AA13 (323). Chitin and cellulose are β(1→4)-linked polymers with flat molecular surfaces, whereas starch contains α(1→4) linkages (amylose) and α(1→6)-linked side chains (amylopectin). The finding that LPMOs can also attack this type of polysaccharides shows that the oxidative enzyme mechanism of glycosidic bond cleavage is more widespread than initially expected. Sequence homology between these families is very low. Nevertheless, the available structures of LPMOs show that two histidine residues, termed the histidine brace, coordinate a copper center. Besides this copper ion, which is the native metal cofactor for these enzymes, they also need a biological redox partner for their enzymatic action, which can be another enzyme, e.g., cellulose dehydrogenase (CDH), or a reducing agent such as ascorbate (320, 321).

LPMOs have been shown to oxidize either the C-1 or C-4 atom of the β(1→4) glycosidic bond on the surface of chitin or cellulose (315, 317, 318) and C-1 of starch (323), resulting in the cleavage of this bond and the creation of new chain ends that can be subsequently processed by hydrolytic chitinases and cellulases. Already before the mode of action of LPMOs was recognized, it was shown that the addition of these proteins (i.e., GH61 and CBM33 proteins) to enzyme mixtures significantly enhanced the degradation of cellulose and chitin in synergism with the respective hydrolases (324–326). The groundbreaking discovery of the mechanistic details of LPMOs has opened up new possibilities on the discovery of even more enzymes with these properties and has boosted the research of their application for the conversion of biopolymers into biofuels and other renewable chemicals.

***Trichoderma* CAZomes.** To provide a uniform annotation with respect to existing families and cutoff E values of the CAZomes of *T. atroviride*, *T. reesei*, and *T. virens*, the respective protein catalogs were downloaded from the JGI genome websites and submitted to dbCAN v2.0, which is a Web server for automated CAZy annotation that was published recently (327). The protein IDs, including ortholog assignments and gene names of the CAZymes of *T. atroviride*, *T. reesei*, and *T. virens* can be found in Table S1 in the supplemental material.

Industrial *T. reesei* strains secrete high levels of cellulases under inducing growth conditions and are therefore used for the biotechnological production of these enzymes. Most of the cellulase genes in *T. reesei* are regulated in a coordinated way, but the relative ratios of their expression may differ in higher-production

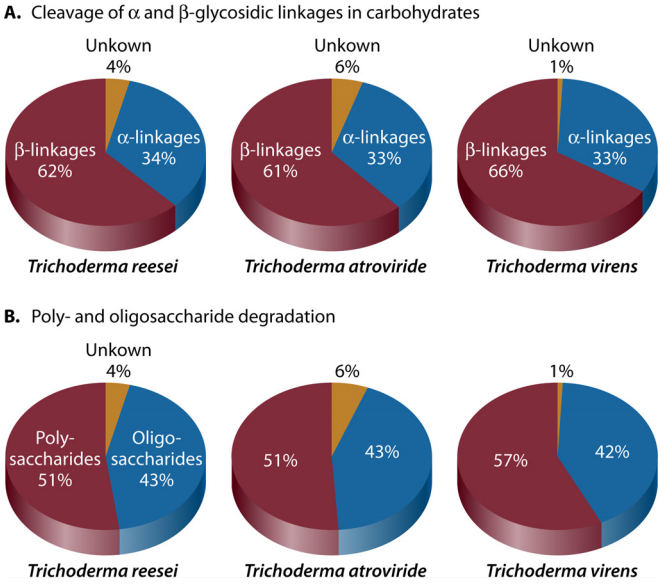

FIG 4 Distribution of CAZymes with cleavage preferences for α- and β-glycosidic linkages in carbohydrates (A) and poly- and oligosaccharide degradation (B) between *T. atroviride*, *T. virens*, and *T. reesei*.

mutants (328). *T. reesei* serves today as a model organism for the regulation and biochemistry of (hemi)cellulose degradation (329–331).

In comparison to *T. reesei*, which has despite its high cellulolytic potential a rather limited number of cellulose-degrading enzymes and GHs in general, the numbers of GH-encoding genes in *T. virens* and *T. atroviride*, respectively, are significantly higher, which ranks them on the forefront for ascomycetes (4). The numbers of GTs, PLs, and CEs in these three *Trichoderma* spp. are similar to those in other filamentous fungi. Detailed CAZome comparison showed that the total numbers of GHs are higher in the mycoparasites *T. atroviride* and *T. virens*, mainly because of expansions of a few GH families, e.g., GH 18 (chitinases) and GH 55 (glucanases) (see also below, and Fig. S19 in the supplemental material). Further, in contrast to *T. reesei*, whose genome has a low number of CBM-containing proteins among the compared Sordariomycetes, *T. atroviride* and *T. virens* have high numbers of CBMs, and most of them are found in GH proteins, indicating a good ability of these enzymes to adhere to insoluble substrates (4).

These CAZome data show that *T. atroviride* and *T. virens* have ca. 20 and 25%, respectively, more CAZymes than *T. reesei*. This suggests that the two mycoparasitic species have a different carbohydrate degradation potential than the predominantly saprotrophic species *T. reesei*. In particular, the number of GHs is strongly increased in the mycoparasites.

Global carbohydrate degradation potentials of *Trichoderma* spp. Carbohydrates can be generally grouped into α- or β-linked carbohydrates and poly- or oligosaccharides. We therefore tested whether the mycoparasites are adapted to degrading one of these general types of carbohydrates better than *T. reesei*. GH families were grouped according to the enzymatic properties for the respective GH family members to preferentially degrade α- or β-linkages and for their main preferences toward oligo- or polysaccharides (Fig. 4). The analysis revealed that the overall carbohydrate cleavage preferences for these different general types of carbohydrates were not significantly different between the saprotrophic species *T. reesei* and the two mycoparasites, *T. atroviride* and *T. virens*.

Mycoparasitism was shown to be the ancestral state of the genus *Trichoderma*, and the CAZomes of mycoparasites seem to be well adapted for fungal cell wall degradation. Several of the GH families that are expanded in the mycoparasites are connected to fungal cell wall degradation. The structural scaffold of the fungal cell wall consists of chitin and β-1,6-branched β-1,3-glucan and, accordingly, increased numbers of enzymes involved in the degradation of these polysaccharides are encoded in the genomes of *T. atroviride* and *T. virens*. Although chitinase and glucanase genes had already been cloned from *Trichoderma* spp. (332), CAZome analysis revealed genes for many more members of the respective GH families. Further, several expanded CAZyme families were identified whose activities may also be related to fungal cell wall degradation, but these had not been studied yet in *Trichoderma*.

In the following sections, we will compare the cellulase and chitinase/glucanase systems of *T. atroviride*, *T. virens*, and *T. reesei* to illustrate differences among these species.

Cellulases. *T. reesei* is an efficient degrader of cellulosic plant matter, but its pool of cellulases and xylanases and associated hemicellulase activities is relatively small in comparison to other ascomycetes (23). Instead, in *T. reesei* the secret of success for cellulose degradation appears to be its ability to express some of its cellulases at very high levels. The cellobiohydrolases CEL6A (CBH2; TR_72567, TA_44894, and TV_78675) and CEL7A (CBH1; TR_123989, TA_88458, and TV_90504), which cleave dimers from cellulase chains, are the two most abundantly secreted proteins in *T. reesei* under cellulase-inducing growth conditions. These proteins are known to account for 70 to 80% of the total *T. reesei* secreted proteins, with CEL7A typically accounting for around 60% of the total secreted proteins (333). Other abundant components of the *T. reesei* cellulase complex are CEL7B and CEL5A, with up to 10% each.

The number of identified cellulases and xylanases has not significantly expanded in *T. virens* and *T. atroviride*, although the mycoparasites have slightly more cellulases than *T. reesei*. This suggests that the observed low variety of cellulases and xylanases is a common feature of the genus *Trichoderma*. *T. atroviride* and *T. virens* have one and two, respectively, more members of family GH12 and *T. virens* has an additional GH45 cellulase containing a CBM1 at the C terminus. Further, in addition to the two GH61 proteins (reclassified as lytic polysaccharide monooxygenases belonging to family AA9 [311]) that have so far been found to be expressed during cellulose degradation, all three *Trichoderma* species possess two more GH61 (AA9) proteins. Due to their radical-based enzymatic mechanism, they are able to attack the crystalline parts of cellulose better than hydrolase, and addition of AA9/GH61 proteins to cellulase mixtures was shown to significantly increase glucose yields from cellulose degradation (324, 334).

To determine the differentiation of the cellulase machineries between *T. atroviride*, *T. reesei*, and *T. virens*, the protein sequences of *Trichoderma* β-glucosidases and cellulases from GH families 1, 3, 5, 6, 7, 12, 45, 61, and 74 (xyloglucanase) were compared and analyzed in detail. Protein similarities between *T. atroviride*, *T. reesei*, and *T. virens* were in a similar range for all tested enzymes associated with cellulose degradation (see Fig. S19 in the supplemental material). On average, 82% amino acid identity and 90% amino acid similarity/positives were found for the compared

proteins. In particular, the most strongly expressed cellulases in *T. reesei*, CEL5A, CEL6A, CEL7A, and CEL7B, and the investigated β-glucosidases fell within this range, whereas the cellulases CEL12A, CEL45A, and CEL61A were slightly less similar to each other, with 77% amino acid identities. CEL61C showed the lowest similarity among the compared cellulose-degrading proteins. It is annotated as a cellulase, but it was not among the cellulase genes that were found to be upregulated under cellulase-inducing conditions (328). For those cellulases where active site residues have already been experimentally determined in detail, e.g., CEL6A and CEL7A, the respective amino acids were found to be completely conserved between the three *Trichoderma* species. Within the genus *Trichoderma*, *T. reesei*, *T. atroviride*, and *T. virens* belong to different sections (*T. reesei*, section Longibrachiatum; *T. virens*, section Pachybasium; *T. atroviride*, section Trichoderma), but phylogenetically, *T. reesei* and *T. virens* are more closely related to each other than to *T. atroviride*. We were therefore interested whether this is reflected in the protein sequences of cellulases and compared them in a phylogenetic analysis. Of the 15 cellulose-degrading enzymes that were compared, the highest similarity pairs were between *T. reesei* and *T. virens* for 8 of them, between *T. reesei* and *T. atroviride* for 4 of them, and between *T. atroviride* and *T. virens* for 3 of them. Thus, the majority of the tested cellulose-degrading proteins reflect the phylogeny of the genus (see Fig. S20 in the supplemental material). Interestingly, among the *T. atroviride*/*T. virens* pairs, the three highly expressed cellulases CEL7A, CEL7B, and CEL5A can be found, whereas CEL6A belongs to the *T. reesei*/*T. virens* pairs.

Fungal cell wall-degrading enzymes: chitinases and glucanases. While the numbers of cellulases and xylanases are not considerably different between *T. atroviride*, *T. reesei*, and *T. virens*, a greatly increased number of enzymes involved in fungal cell wall degradation are encoded in the genomes of *T. atroviride* and *T. virens* compared to *T. reesei*. The chitinolytic system of *Trichoderma* has already received considerable attention, and the chitinase system of mycoparasites is clearly more diversified than that of *T. reesei* (4, 335). Although mycoparasites have a large variety of different chitinolytic enzymes, similar sets of chitinases seem to be expressed under different types of inducing conditions, e.g., mycoparasitism, growth on chitin, starvation, and autolysis. The chitinases ECH42, CHIT33, and CHIT36 (all three GH family 18) and the *N*-acetylglucosaminidase NAG1 (GH 20) seem to be the most abundant chitinolytic enzymes under these growth conditions. It is striking that the same proteins that are involved in mycoparasitism are also mainly responsible for exogenous chitin decomposition and recycling of chitin of the fungus' own cell walls during autolysis and starvation (335). Some members of the chitinases were only identified based on the recent genome analyses. As described more recently, some members of GH family 18 do not exhibit chitinase activity but instead have endo-*N*-acetylglucosaminase (ENGase) activity and cleave between two *N*-acetylglucosamine residues in the carbohydrate antennae of proteins (336). They are therefore involved in protein deglycosylation. *Trichoderma* genomes contain two genes encoding ENGases, of which one (Eng18A [Endo T]) is secreted and may thus be responsible for postsecretorial modifications of glycan structures on endogenous glycoproteins, such as cellulases (336). The second ENGase (Eng18B) does not contain a signal peptide and is therefore an intracellular protein, presumably involved in the endoplasmic reticulum-associated protein degradation pathway (ERAD) of misfolded glycoproteins. Disruption mutants of Eng18B in *T. atroviride* show defects in vegetative growth, tolerance to abiotic stress, conidiation, chitin utilization, and mycoparasitism of *Botrytis cinerea* (337).

Glucanases, e.g., the β-(1,6)-glucanase TvBGN3, have been reported to be induced during mycoparasitism and potentially involved in the mycoparasitic attack (338). Genome analysis revealed that the mycoparasites have higher numbers of glucanases than *T. reesei*, but most of them have not been characterized so far.

Protein comparisons also showed that chitinases and glucanases are strongly conserved between *T. atroviride*, *T. virens*, and *T. reesei* (see Fig. S21 in the supplemental material). Amino acid identities were >82% and similarities/positives were >91% for most tested proteins. Only ECH30, which is not as strongly expressed as the other chitinases under comparable conditions, and the GH 18 protein EndoT, which is involved in extracellular protein deglycosylation in *T. reesei* (336), were slightly less conserved. Analogous to cellulases, also the active sites of chitinases were completely conserved among the three *Trichoderma* species.

Phlyogenetic analysis showed that from 10 compared proteins, 6 showed the highest similarities between *T. atroviride* and *T. virens*, 4 between *T. reesei* and *T. virens*, and none between *T. atroviride* and *T. reesei*. These ratios are strongly different from what we found for the cellulases (see Fig. S22 in the supplemental material). This suggests that evolutionary constraints for substrate specificity may be an important aspect for evolution of chitinases in the mycoparasites. For more details on the phylogeny and evolution of the GH 18 gene family in *Trichoderma*, the reader is referred to reference 339.

Gene expression of CAZymes in *Trichoderma*. Gene expression of CAZymes in *Trichoderma* has already achieved considerable attention in several genome-wide studies using microarrays or expressed sequence tag (EST)-based approaches (328, 340–343).

In *T. reesei*, several transcription factors that are important for the induction or repression of cellulase and hemicellulase genes have been well described, including XYR1, CRE1, ACE1, and ACE2 (for a detailed review on this topic, see references 344 and 345). More recently, LAE1 (a protein methyltransferase) and VEL1 (a member of the VELVET protein family) were found to be important for the regulation of cellulase gene expression in *T. reesei* (129, 346); in *T. reesei*, CAZyme-encoding genes are often coregulated upon induction, especially in the presence of cellulosic plant material (328, 340, 347, 348). The largest number of genes and CAZy families induced was detected in the cultures with the hemicellulosic material, bagasse, xylans, and wheat straw (68 to 124 genes in 39 to 47 CAZy families), whereas cultivation in the presence of cellulosic or cellulose-derived materials, Avicel cellulose, pretreated spruce, or sophorose, resulted in a clearly smaller number of genes induced (43 to 58 genes in 28 to 36 families).

The common core of genes induced in the presence of the lignocellulose substrates (as judged by induction of gene expression by both cellulose and xylan, and by induction in the presence of at least 70% of the substrates used) included genes encoding characterized or predicted functions, such as GH6 cellobiohydrolase, GH5 endoglucanase, xylanases of families GH10, GH11, and GH30, GH5 β-mannase, GH3 family β-glucosidases and β-xylosidases, GH27 α-galactosidases, GH2 β-mannosidases, acetyl xylan esterases of families CE3 and CE5, glucuronoyl and acetyl esterases of families CE15 and CE16, GH31 α-glucosidases/α-xy-

losidases, GH54 and GH43 α-L-arabinofuranosidases (or β-xylosidase/α-L-arabinofuranosidases), GH61 polysaccharide monooxygenases, GH55 β-1,3-glucanases, GH67 α-glucuronidase, GH79 β-glucuronidase, GH105 rhamnogalacturonyl hydrolase, GH95 α-L-fucosidase, GH89 α-*N*-acetylglucosaminidase, and chitinases of families GH18 and GH20.

In *T. reesei*, 30% of the CAZome was found to be only induced on wheat straw and not on cellulase-inducing lactose (347). Two-thirds of the CAZome was expressed both on wheat straw as well as on lactose, but 60% of it was at least >2-fold higher on the former. Major wheat straw-specific genes comprised xylanases, chitinases, and mannosidases. The latter two CAZyme families were significantly more highly expressed in a strain in which *xyr-1* was nonfunctional. Interestingly, most of the genes encoding enzymes that cleave hemicelluloses side chains [α-L-arabinosidases, α-(methyl)-D-glucuronidases, α-D-fucosidases, and polysaccharide deacetylases] were equally well expressed on lactose and wheat straw (transcript group E). Finally, it was noted that the presence of wheat straw also specifically induced an array of GH 18 chitinases, particularly those that also contain a cellulose binding domain (CHI18-14, CH18-16, and CHI18-17 [349]), GH2, and GH47 β-D-mannosidases and GH55 endo-β-1,3-glucanases.

Light has also been shown to have a pronounced influence on GH gene expression in *Trichoderma* spp. This has also been mainly studied in *T. reesei* so far, but it is also known to occur in other *Trichoderma* spp., e.g., *T. atroviride*. In strains deficient in genes encoding components of the heterotrimeric G-protein pathway, the number of glycoside hydrolase genes differentially regulated in light and darkness was even higher than in the parental strain QM9414 (350). The obtained data indicated that the signaling proteins PhLP1 (a phosducin-like protein), GNB1 (G-protein beta-subunit), and GNG1 (G-protein gamma-subunit) are specifically involved in triggering the expression of GHs in light. More than 50% of the GH-encoding genes were found to be regulated by light in the parental strain QM9414 and/or one or more mutant strains. This suggests that these genes can be expected to be potentially regulated in response to light and to a nutrient signal as transmitted via the heterotrimeric G-protein pathway. Members of all GH families detected in *T. reesei* were found to be potentially subject to light regulation, and many of them are regulated by the photoreceptors BLR1, BLR2, or ENV1 (351).

CAZymes are also differentially regulated during various developmental stages of fungal growth. In *T. reesei*, several genes encoding CAZymes were observed to be upregulated during conidiation (352). Upregulated genes comprised 57 GHs involved in cellulose and hemicellulose degradation, 3 CEs, and 3 chitinases. The cellulose-degrading proteins included 6 of the 9 cellulases (CEL5A, CEL6A, CEL7A, CEL7B, CEL12, and CEL45), two lytic polysaccharide monooxygenases of GH family 61 (now AA9; CEL61A and CEL61B), SWO1 (an expansin with a CBM1), CIP1 (containing a CBM1), glucosidases, and xylanases (XYN2, XYN3, XYN4, and XYN5). The remaining upregulated GHs (21 of 28) comprised glycosidases and glycanases active against various side chains in hemicelluloses, including two pectinases. All of these genes exhibited a strong peak of expression early during sporulation (at 3 h), after which most of them declined. Cellulase transcription during conidiation, but not conidiation itself, was found to be controlled by the major regulator XYR1. *T. reesei* conidia were found to contain cellulase activities (353, 354) that enhanced germination in media with cellulose as the carbon source. Thus, the conidium-located cellulases enable the fungus to germinate in the presence of a cellulosic carbon source. Moreover, the sporulation-associated transcriptome is nonrandomly distributed in the genome (352).

Genome-wide gene expression during mycoparasitism has been studied, mainly with microarray and EST-based approaches (22, 25, 355, 356). Besides more indirect growth conditions related to mycoparasitism, e.g., growth on fungal cell walls or chitin as carbon source, the experimental design often used to study mycoparasitism entails plate confrontation assays.

Several interesting findings were reported from the available transcriptome studies of the mycoparasitic attack. In a study where EST libraries of *T. atroviride* from the different stages of mycoparasitism described above were evaluated (25), only a few genes encoding CAZymes (e.g., chitinase, β-glucanases) were found to be upregulated during the onset of mycoparasitism, although the mechanism of mycoparasitism has frequently been discussed in terms of involvement of extracellular enzymes capable of hydrolyzing the host's cell wall (332).

Recently, the transcriptome during the stages of the mycoparasitic attack was compared between *T. atroviride*, *T. virens*, and *T. reesei* using microarrays (22). In general, a comparison of the gene families that showed upregulation revealed very little common responses between the three *Trichoderma* spp., also, not between the two strong mycoparasitic species. *T. atroviride* increased the transcription of β-glucanases of the GH 16 (AA9) family, among which some also contain a carbohydrate binding domain of the lectin superfamily. Unfortunately, their properties have not been studied in sufficient detail to offer an interpretation for why *T. atroviride* overexpresses just this battery of β-glucanases and not one of the several others in its genome (4). In contrast, the transcriptome of *T. reesei* before contact revealed a unique response to the presence of the prey. A massive upregulation of cellulolytic and hemicellulolytic CAZys was shown. Interestingly, the expression of the same cellulase and hemicellulase genes was strongly downregulated in *T. atroviride* and highly induced in *T. reesei*, while *T. virens* remained unaffected. It is commonly believed that the expression of cell wall lytic enzymes (particularly chitinases but also β-glucanases and proteases) and secondary metabolites are the major determinants of mycoparasitism (8). However, also in this study, the role of hydrolytic enzymes in this process was not clearly confirmed, and these aspects probably need to be revisited. Nevertheless, the study already clearly demonstrated that the two mycoparasites *T. atroviride* and *T. virens* employ completely different strategies to antagonize their prey.

Conclusions. CAZyme-encoding genes are among the most crucial ones for fungi living in a forest habitat such as *Trichoderma* spp. Analysis of gene content showed considerable differences between *T. reesei*, *T. atroviride*, and *T. virens* in the numbers of CAZymes, including numerous unique genes of every species and a generally higher number in *T. atroviride* and *T. virens*. Nevertheless, there are also unique CAZyme-encoding genes in *T. reesei*, including *xyn5*. With respect to carbohydrate degradation preferences, we found that the strongly increased number of CAZymes, especially glycoside hydrolases, did not lead to a shift between degradation of alpha- or beta-linkages or altered preferences toward oligo- or polysaccharides in the mycoparasitic *T. atroviride* and *T. virens* versus the saprotrophic *T. reesei*.

Nitrogen Metabolism

Many fungi can use a wide variety of compounds as nitrogen sources and are able to express, upon demand, catabolic enzymes of many different pathways. Ammonia, glutamate, and glutamine are favored nitrogen sources in fungi such as *N. crassa*, *A. nidulans*, and *S. cerevisiae* (357). However, these fungi are capable of utilizing other secondary sources when the primary ones are not available or are present in low concentrations, e.g., nitrate, nitrite, purines, proteins, most amino acids, and amides (358–360). The use of secondary nitrogen sources requires *de novo* synthesis of permeases and catabolic enzymes, which involves indication of nitrogen limitation and induction of pathway-specific enzymes by a substrate. In the presence of primary nitrogen sources, there is no expression of genes required for the assimilation of secondary sources; this is known as nitrogen catabolite repression (NCR) (361). Under nitrogen deprivation, derepression occurs (357, 362). Without enough nitrogen, fungal cells are capable of assimilating nitrates by reducing nitrate to ammonia, which is then converted to glutamate or glutamine. Thus, primary nitrogen metabolism can take place when nitrates are the main nitrogen source (357, 363). Movement of nitrogen compounds, as well other nutrients, occurs around the fungal colony and is bidirectional in some cases (364, 365). Hyphal cells store and translocate amino acids, which permits the regulation of developmental genes by controlling the intracellular N status (365).

In filamentous fungi, primary nitrogen sources based on amino acids or ammonium are routinely used to induce nitrogen catabolite repression, whereas the presence of KNO_3 or other nitrates results in nitrogen derepression (357, 362).

Assimilation of different nitrogen sources and photoconidiation are related processes in some species of *Trichoderma*. Primary sources of nitrogen strongly promote photoconidiation in *T. asperellum*, *T. atroviride*, and *T. pleuroticola*, but not *T. hamatum* or *T. virens*. It was also demonstrated that depending on the light conditions both the N:C ratio and derepression can stimulate conidiation (366). Sudden nitrogen deprivation also stimulates conidiation in *T. atroviride* independently of the BLR-1/BLR-2 complex, indicating that nitrogen derepression promotes starvation-induced conidiation (52).

Also for mycoparasitism, the nitrogen source availability likely plays a role in *T. atroviride*. The (yet-unidentified) receptors, which sense the nitrogen status of the medium, are thought to be modulated by components derived from the host fungus and thereby simulate nitrogen limitation (25). The induction of the *prb1* gene, which encodes a protease of *T. atroviride* and whose overexpression enhances the mycoparasitic ability, depends on nitrogen limitation (367). On the other hand, the induction of some chitinases is not only influenced by the presence of fungal cell walls but also by the nitrogen source and starvation (349, 368–370). Together, these results suggest that the transcriptomic response to a prey fungus resembles the gene expression pattern upon induction by nitrogen starvation.

Nitrate assimilation. (i) Structural genes. Nitrate is one of the main forms of assimilable nitrogen in most ecosystems on Earth. Its uptake and reduction to ammonium involves the enzymes nitrate reductase (NR) and nitrite reductase (NiR). Nitrate assimilation requires significant energy for transport and conversion of the nitrogen from the +5 to the −3 oxidation state and incorporation of ammonium into carbon-containing compounds (371). Nitrate is transformed to nitrite in a two-electron transfer reaction, performed by NR. To prevent accumulation of the intermediate nitrite, which is highly toxic and mutagenic, and to harmonize its assimilation with other interdependent metabolic pathways, fungi have evolved complex regulatory networks to control gene expression and enzyme activity in this pathway. In *Aspergillus*, *Neurospora*, and other fungi, inorganic nitrate serves as an excellent nitrogen source but will not be utilized unless there is insufficient ammonia, glutamate, or glutamine (359, 371–374). Two cytoplasmic enzymes comprise the assimilatory nitrate pathway: nitrate reductase, which reduces nitrate to nitrite, and nitrite reductase, which catalyzes the reduction of nitrite to ammonia. Both enzymes are synthesized *de novo* requiring nitrogen derepression and specific induction by nitrate or nitrite (357, 375). Nitrate reductase is a large redox enzyme which contains a molybdopterin-containing domain, where the actual reduction of nitrate to nitrite takes place. This enzyme binds FAD (flavin adenine dinucleotide) as a cofactor (376). In *A. nidulans*, the structural genes are clustered as *cnrA-niiA-niaD*, which encode NR and NiR, respectively (373, 377–379).

The genome of *N. crassa* encodes one NR, *nit-3* (TA_291159, TV_177810, and TR_81955) and one NiR, *nit-6* (TV_168068, TA_256104, and TR_69291) and several genes which are required for synthesis and assemble of the molybdopterin cofactor (Mo-C): *nit-1* (TV_67305, TA_297199, and TR_57002), *nit-7* (TV_56439, TA_226825, and TR_58837), *nit-8* (TV_43650, TA_299371, and TR_54508), and *nit-9* (TV_58736, TA_238021, and TR_78561)). In *A. nidulans*, mutations in the structural genes (*nit-3* and *nit-6*) lead to the inability to utilize nitrate, and the absence of *areA*, which encodes a positive regulator, results in lack of both enzymes (359, 380). The structural genes are controlled at the transcriptional level and accumulate only under nitrogen deprivation and nitrate induction, which depend on the functional products of *nit-2* and *nit-4* (372, 373, 381).

The nitrate transporter genes in *N. crassa* (*nit-10*) and *A. nidulans* (*nrtA*) are also present in *T. virens*, *T. atroviride*, and *T. reesei* (TV_14210, TA_217165, and TR_111724). *nit-10* and *nrtA* encode proteins of 541 and 483 amino acids, respectively. In contrast with *nrtA*, *nit-10* is not clustered with the other nitrate metabolism-related genes (*nit-3*, *nit-6*, etc.). In the absence of *nit-10*, *N. crassa* is unable to grow on nitrate as the sole nitrogen source, although higher concentrations of nitrate enable wild-type growth (382).

nit-10 and *nrtA* are subject to repression by nitrogen metabolites and require nitrogen induction. Moreover, expression of *nit-10* requires functional products of *nit-2* and *nit-4* (positive regulators of *nit-3*), whereas *nrtA* requires the products of *nirA* (nitrate induction control gene), *areA* (nitrogen metabolite repression control gene), and *niaD* (involved in autoregulation of nitrate reductase). Furthermore, *A. nidulans* possesses an additional high-affinity nitrate transporter, encoded by the *nrtB* gene. Mutants in *nrtB* grow normally on nitrate as sole nitrogen source, whereas a double mutant failed to grow on nitrate (379, 382). As putative homologous genes in *Trichoderma* spp. are found (see Fig. S23 and Table S1 in the supplemental material), it can be assumed that these fungi regulate nitrogen metabolism in a similar way as *A. nidulans* and *N. crassa*.

(ii) Regulatory genes. In *N. crassa* and *A. nidulans*, the major positive-acting regulatory gene *nit-2/areA* (TV_113059, TA_224741, and TR_76817) mediates global nitrogen repression/de-

repression. *nit-2* and *areA* are highly similar and encode GATA-type transcription factors. They function as global-acting factors that activate various genes encoding enzymes and permeases needed to utilize secondary nitrogen sources when ammonium and glutamine are absent from the cultivation broth (357). Biosynthesis of NR and NiR requires the lifting of nitrogen catabolite repression as signaled by NIT-2, and the simultaneous induction by nitrate, which is mediated by *nit-4* (372). The transcription factor NIT-4/NirA (TV_154783, TA_226133, and TR_62787) acts in a positive manner, and mutants of *nit-4* lack both NR and NiR as well as other catabolic enzymes (380, 383–385). Neither NIT-2 nor NIT-4 is capable alone of activating gene expression, but together they cooperate to trigger strong activation (386). Interestingly, the DNA binding of NirA depends on nitrate concentration and on a functional AreA protein. Furthermore, AreA is necessary for intracellular nitrate accumulation and could be exerting its effect indirectly on NirA via inducer exclusion (387).

In *N. crassa* and *A. nidulans*, the negatively acting regulatory gene *nmr1* (for nitrogen metabolic regulation)/*nmrA* (TV_176019, TA_297054, and TR_74375) encodes a repressor protein, which modulates the activity of the positively acting NIT-2 (AreA) protein probably by interfering with DNA binding under nutrient-sufficient conditions. *nmr1/nmrA* encode a protein that has no characteristic DNA binding or protein kinase motifs (388). Mutants in *nmr1*/*nmrA* of *N. crassa* and *A. nidulans*, respectively, show a partially derepressed phenotype (389). Direct interaction between the NMR1 and NIT-2 proteins has been demonstrated in *N. crassa* (390, 391).

Furthermore, it has been described in *A. nidulans* that NirA and AreA act synergistically on the bidirectional promoter of *niaD* and *niiA*. Intriguingly, this promoter undergoes chromatin rearrangements upon induction (389). In medium lacking nitrate, NirA is located in the cytoplasm, and addition of an inducer causes relocalization to the nucleus. However, the replacement of nitrate by neutral (urea or arginine) or repressing (ammonium) nitrogen sources causes NirA to accumulate in the cytosol (392). Therefore, NirA interacts with the nuclear export factor KapK, and addition of the inducer (nitrate) disrupts such an interaction. However, when NirA is exported from the nucleus, KapK associates again with NirA (393).

The fact that all known regulatory and structural proteins were found in *T. reesei*, *T. atroviride*, and *T. virens* and show high phylogenetic similarity suggests that these proteins have important roles in these fungi to take up and use different nitrogen sources for growth and development.

The purine catabolic pathway. The use of purines requires the *de novo* synthesis of a set of highly regulated enzymes that are produced only upon nitrogen derepression. The metabolism of purines, which results in the production of ammonia, is accomplished via a multienzyme pathway (394, 395) (Fig. 5).

In *A. nidulans*, at least eight structural genes that encode permeases (*uapA*, *azgA*, and *uapC*) and enzymes for purine uptake and degradation are controlled by the *uaY* gene, which mediates uric acid induction (396, 397). The AzgA protein transports adenine, guanine, hypoxanthine, and the analogs 8-azaguanine and purine with high capacity. The UapA transporter is specific for uric acid and xanthine, their thioanalogs, 2-thiouric acid and 2-thioxanthine, allopurinol, and oxypurinol. The UapA and UapC proteins are 62% identical and belong to a distinct family of purine-pyrimidine carriers. On the other hand, UapC is a wide-specificity, low-capacity carrier which transports all natural purine analogs (398, 399). The UapC protein is mainly located in the plasma membrane and secondarily in internal structures (400). The expression of catabolic enzymes requires a functional AreA protein and nitrogen derepression. For instance, the *uaP* and *uaZ* genes that encode a purine-specific permease and a urate oxidase require functional *uaY* and *areA* genes and are controlled by uric acid induction and nitrogen derepression (396, 397). Interestingly, the expression of *uaY* does not require induction, nitrogen derepression, or functional AreA (397). In *A. nidulans*, *uaX* and *uaW* encode enzymes that catalyze the conversion of the product of urate oxidation by urate oxidase, 5-hydroxyisourate, to optically active allantoin (401).

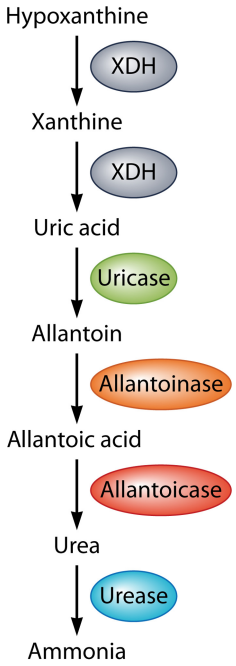

FIG 5 Purine catabolic pathway in fungi. The xanthine dehydrogenase XDH (TR_78797, TA_141294, and TV_40214) is the major enzyme oxidizing hypoxanthine to xanthine and xanthine to uric acid. Subsequently, uricase (TR_76381, TA_145570, and TV_78632) catalyzes the enzymatic oxidation of uric acid into allantoin, and allantoinase (TR_123795, TA_300514, and TV_89297) catalyzes the hydrolysis of allantoin into allantoic acid. The enzyme allantoicase (TR_78010, TA_148414, and TV_214821) breaks down allantoic acid into urea, and finally the urease (TR_76381, TA_145570, and TV_78632) converts urea into ammonia.

In *N. crassa*, the purine pathway comprises hypoxanthine, xanthine, uric acid, allantoic acid, ureidoglycolate, urea, and ammonia as intermediates (Fig. 5). The structural genes that encode the enzymes of this pathway are not clustered and include genes for xanthine dehydrogenase (XDH), uricase, allantoinase, allantoicase, and urease (Fig. 5) (395). During the catabolism of uric acid to ammonia, the three initial enzymes are repressed by ammonia, whereas the last two enzymes are constitutively synthesized. Interestingly, uric acid, which is an intermediate in the pathway, induces uricase and allantoicase, whereas allantoinase is induced by uric acid and allantoin (402, 403). *N. crassa* PCO-1 (TR_35454, TA_172139, and TV_54183) regulates the expression of several genes encoding enzymes required for the catabolism of purines (404). As a Zn(II)Cys_6 binuclear cluster transcription factor,

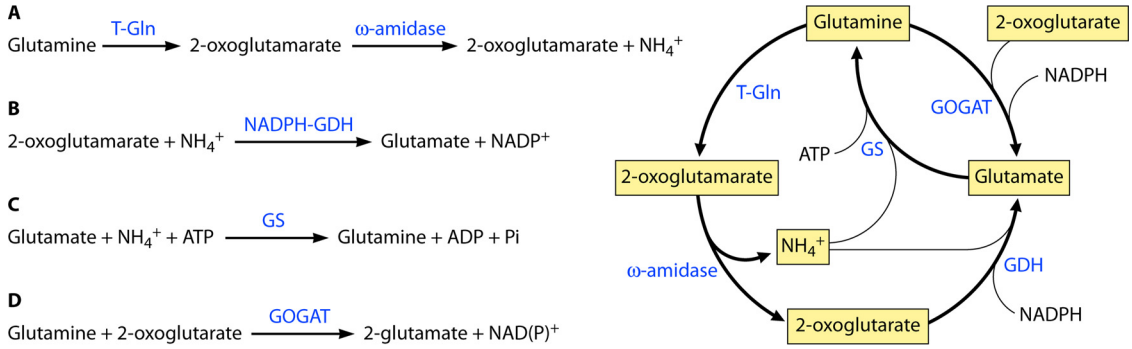

FIG 6 Glutamine assimilation and glutamine as nitrogen donor in *N. crassa*. (A) Glutamine is transaminated via glutamine transaminase (T-Gln; TR_106250, TA_84459, and TV_79678), and subsequently the 2-oxoglutarate produced is hydrolyzed to 2-oxoglutarate and ammonium through the participation of a ω-amidase (TR_104803, TA_29974, and TV_40527). (B) Thereafter, the NADP-glutamate dehydrogenase (NADP-GDH; TR_73536, TA_297025, and TV_73234) synthesizes glutamate via reductive amination. (C) Finally, the synthesis of glutamine is carried out by a glutamine synthetase (GS; TR_122287, TA_141213, and TV_87581), which incorporates ammonium into the γ-carboxyl of glutamate after being activated by ATP. (D) The final step completes the glutamine-glutamate cycling. As a result of this cycle, glutamine and glutamate are continually resynthesized, but glutamate can also be synthesized in a reaction catalyzed by glutamate synthase (GOGAT; TR_122287, TA_141213, and TV_87581). (E) Schematic representation of glutamine-glutamate cycling in *N. crassa*. 2-Oxoglutarate can be interconverted into ammonium (NH_4^+) by the ω-amidase, glutamate by the GOGAT, and GDH, whereas ammonia can be converted into glutamate through two pathways. It may be coupled directly to 2-ketoglutarate to form glutamate by GDH in the presence of NADPH. Furthermore, NH_4^+ is also incorporated into glutamine via GS in the presence of ATP. Glutamine and 2-ketoglutarate are converted to glutamate through GOGAT in the presence of NADH. In addition, glutamate is degraded to 2-ketoglutarate and ammonia by GDH in the presence of NAD. All the glutamine-glutamate cycling enzyme-encoding genes were found in the *Trichoderma* spp.

PCO-1 binds to elements located in the upstream promoter region of four genes encoding purine catabolic enzymes XDH, uricase, allantoinase, and allantoicase. A functional *nit-2* gene product was found to be essential in the expression of enzymes needed to utilize other secondary nitrogen sources (405), but not in the case of XDH induction, which is a key enzyme at an early step in purine degradation (404). XDH activity is subject to nitrogen repression, which is dependent on *nmr* but not *nit-2* (406).

Predicted homologous proteins involved in purine metabolism, XDH (TR_78797, TA_141294, and TV_40214), uricase (TR_76381, TA_145570, and TV_78632), allantoinase (TR_123795, TA_300514, and TV_89297), allantoicase (TR_78010, TA_148414, and TV_214821), and urease (TR_76381, TA_145570, and TV_78632)) were detected in *T. reesei*, *T. atroviride*, and *T. virens*. High homology among the proteins was demonstrated by the distances in the neighbor-joining phylogenetic tree (see Fig. S24 in the supplemental material), suggesting that during evolution of nitrogen metabolism pathways, the enzymes involved in purine metabolism remained conserved.

Glutamine assimilation. Ammonium is the preferred nitrogen source for most fungi and is converted into glutamine and glutamate, which act as nitrogen donors to produce other nitrogen-containing compounds by transamination and transamidation. Glutamine synthetase (GS) catalyzes the synthesis of glutamine from ammonium and glutamate in a reaction driven by hydrolysis of 1 ATP molecule per 1 NH_4^+ ion assimilated (Fig. 6). Glutamine is a key compound of nitrogen catabolite repression, a regulatory circuit responsible for predominant utilization of reduced nitrogen sources, including glutamine and ammonium, instead of more complex and energy-demanding compounds, such as nitrate, purines, and proteins. Ammonium assimilation is accomplished either via NADP-dependent glutamate synthesis catalyzed by glutamate dehydrogenase (NADP-GDH), or a second pathway via GS (GS-GOGAT). NADP-GDH incorporates ammonium into a molecule of 2-oxoglutarate to produce glutamate. In the GS-GOGAT pathway, GS incorporates first a molecule of ammonium into glutamate to produce glutamine and later the GOGAT enzyme converts glutamine and 2-oxoglutarate to two molecules of glutamate (Fig. 6) (407, 408).

Glutamine is a nitrogen donor and the final product of ammonium assimilation. It also plays a role as a repressor of nitrogen catabolism (Fig. 6) (409, 410, 411). Because two molecules of glutamate are formed, one can be used as a substrate by GS to synthesize glutamine again. The glutamine-glutamate cycle functions in resting cells as well as in growing cells. It has a determinant role in protein turnover, as inhibition of glutamine synthesis and its cycling impairs protein synthesis (Fig. 6) (410).

In *N. crassa*, lack of the GS gene *gln1* results in derepressed levels of nitrite and nitrate reductase activities upon growth in the presence of glutamate, ammonium, and glutamine, suggesting that GS could be involved in sensing nitrogen status and signaling (412, 413). Accordingly, a role in sensing was attributed to GS in the nitrogen regulation network of *Fusarium fujikuroi* (414).

In *N. crassa*, the ω-amidase pathway converts glutamine to 2-oxoglutarate and ammonium through the action of a glutamine transaminase. Furthermore, the GDH and GS participate in the assimilation of ammonium obtained by the ω-amidase pathway (Fig. 6). The activities of these enzymes result in a cycling of glutamine that is essential for cell growth (409, 411).

A phylogenetic analysis (see Fig. S25 in the supplemental material) shows clusters of possible *N. crassa* homologous proteins found in *T. reesei*, *T. atroviride*, *T. virens*, and *G. zeae*. These homologs include glutamine synthetase (GS; TR_122287, TA_141213, and TV_87581), glutamate dehydrogenase (GDH; TR_73536, TA_297025, and TV_73234), glutamate synthase (GOGAT; TR_122287, TA_141213, and TV_87581), ω-amidase (TR_104803, TA_29974, and TV_40527), and glutamine transaminase (TR_106250, TA_84459, and TV_79678).

Conclusions. The main model systems used to study nitrogen metabolism have been *N. crassa*, *A. nidulans*, and recently *F. fujikuroi*. Profound knowledge of this physiological process is needed at the different regulatory levels, since this metabolic pathway has

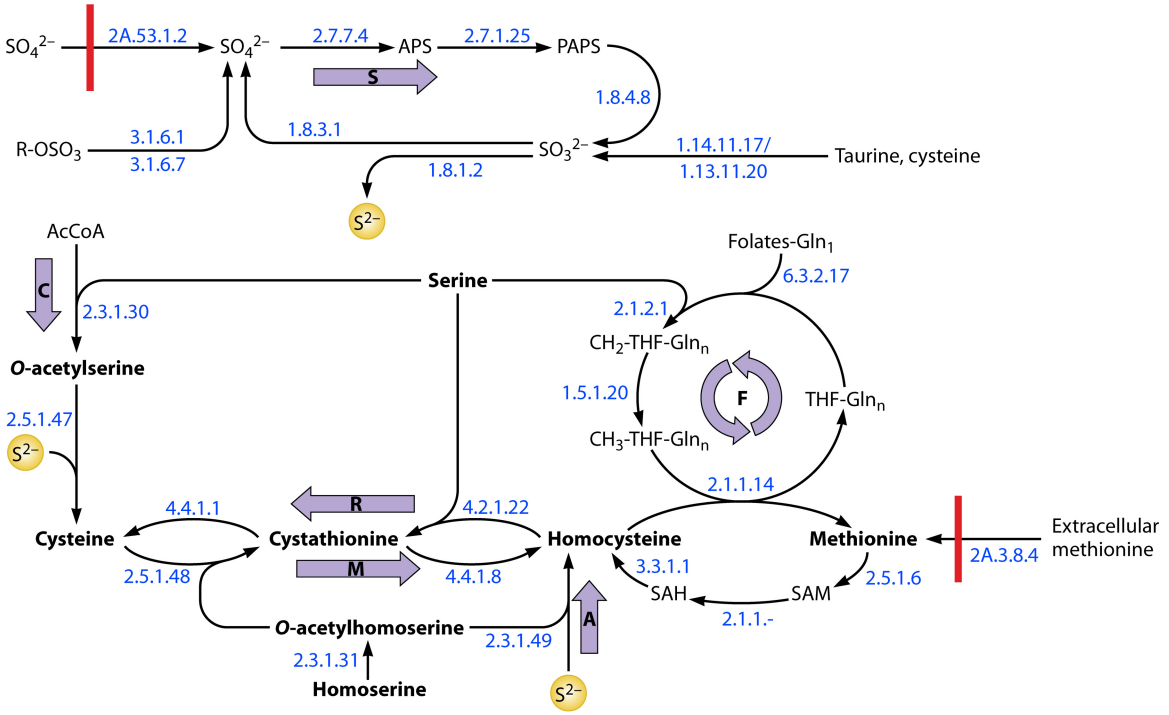

FIG 7 Sulfur metabolism in filamentous fungi. Putative *Trichoderma* spp. enzymes (also indicated by EC designation in the figure) and their gene ID numbers are as follows. For assimilation of sulfate and other sulfur sources pathway (S): sulfate permease (EC 2A.53.1.2; TR_79741, TR_57088, TR_62285, TA_45766, TA_148686, TA_41966, TV_158532, TV_59747, and TV_193580); arylsulfatase (EC 3.1.6.1; TR_61121, TA_37606, and TV_190530); choline sulfatase (EC 3.1.6.7; TR_78320, TA_140398, and TV_69180), sulfate adenylyltransferase (EC 2.7.7.4; TR_47066, TA_300938, and TV_83979); adenylylsulfate kinase (EC 2.7.1.25; TR_75704, TA_82974, and TV_210664); 3-phosphoadenosine-5-phosphosulfate (PAPS) reductase (EC 1.8.4.8; TR_65410, TA_299393, and TV_216652); sulfite reductase, alpha, beta chain (EC 1.8.1.2; TR_81576, TR_45138, TA_223153, TA_128510, TV_82368, and TV_87662); methionine permease (EC 2A.3.8.4; TR_60144, TR_54865, TR_110316, TR_68831, TA_31104, TA_297834, TA_217038, TA_293457, TV_47944, TV_177947, TV_183616, and TV_200088); taurine dioxygenase (EC 1.13.11.20; TR_112567, TR_69529, TR_103012, TR_30776, TR_123979, TA_186979, TA_132655, TA_152183, TA_49608, TA_184293, TA_139690, TV_39626, TV_232090, TV_153908, TV_189583, TV_17533, and TV_77829); cysteine dioxygenase (EC 1.13.11.20; TR_120176, TA_258544, and TV_28127); sulfite oxidase (EC 1.8.3.1; TR_106695, TR_76601, TR_62367, TA_146350, TA_313009, TV_231246, TV_215055, and TV_213747). Cysteine biosynthesis pathway (C): serine *O*-acetyltransferase (EC 2.3.1.30; TR_66517, TA_45036, and TV_214328); cysteine synthase (EC 2.5.1.47; TR_76018, TA_147947, and TV_40598). Methionine biosynthesis pathway (M): cystathionine gamma-synthase (EC 2.5.1.48; TR_3641, TA_137525, and TV_37054); cystathionine beta-lyase (EC 4.4.1.8; TR_82547, TA_298488, and TV_90179); methionine synthase (EC 2.1.1.14; TR_121820, TA_136004, and TV_87179). Alternative sulfur amino acids pathway (A): homoserine *O*-acetyltransferase (EC 2.3.1.31; TR_123633, TA_299508, TA_260926, and TV_50363); *O*-acetyl-L-homoserine sulfhydrylase (EC 2.5.1.49; TR_122301, TA_195487, TA_93352, and TV_74905). Reverse transsulfuration pathway (R): *S*-adenosylmethionine synthetase (EC 2.5.1.6; TR_46238, TA_301763, and TV_87758); various methyltransferases (EC 2.1.1.-); SAH lyase (EC 3.3.1.1; TR_110171, TA_129664, and TV_82877); cystathionine beta-synthase (EC 4.2.1.22; TR_81089, TA_134949, and TV_81462); cystathionine gamma-lyase (EC 4.4.1.1; TR_63919, TA_299967, and TV_176825). CH_3-folate metabolism (F): glycine/serine hydroxymethyltransferase (EC 2.1.2.1; TR_65295, TR_121686, TA_301356, TA_300337, TV_190230, and TV_111115); methylenetetrahydrofolate reductase (EC 1.5.1.20; TR_81824, TR_55055, TA_93509, TA_33767, TV_229117, and TV_90038); folyl polyglutamate synthase (EC 6.3.2.17; TR_ 64029, TR_ 28050, TA_ 167387, TA_ 252788, TV_ 15479, and TV_ 81938).

been associated with several other processes, such as secondary metabolism and development. To the best of our knowledge, genes encoding regulatory and structural proteins involved in nitrogen metabolism have not been studied in *Trichoderma* spp. Our findings suggest that these proteins have been conserved during evolution and that *T. reesei*, *T. atroviride*, and *T. virens* share most characteristics with other filamentous fungi. However, some differences have been determined in the organization and expression of nitrogen metabolism genes in *N. crassa*, *A. nidulans*, and recently in *F. fujikuroi*. Understanding of *Trichoderma* spp. nitrogen metabolism requires a long-term investment by the *Trichoderma* research community in order to dissect the metabolic complexity and gene function.

Sulfur Metabolism

Different sulfur-containing compounds are taken up from the environment and transported via many routes into the fungal cell. Sulfate assimilation has not yet been studied in detail in *Trichoderma*. However, initial studies indicate that sulfate uptake is regulated by light in *T. reesei* and different upon growth on cellulose or glucose (415).

Sulfur assimilation. The first step of sulfate assimilation in all prototrophic organisms is an ATP-dependent process executed by specific transporters located in the cell membrane, the sulfate permeases (416) (Fig. 7). In eukaryotes (like fungi), sulfate uptake is carried out by sulfate permeases from the SulP family (416–419). These proteins are assigned to the TC 2.A.53 class according to the Transporter Classification (TC) system (420).

T. reesei, *T. atroviride*, and *T. virens* have three putative sulfate permeases similar to the known fungal sulfate transporters from the SulP family (TC 2A.53 class) (see Table S1 in the supplemental material). All of them resemble the *N. crassa* conidial permease CYS-13 more than the mycelial CYS-14 described in reference

(421). Homology among all three *T. reesei* putative sulfate permeases and CYS-13 was reported previously (415).

Numerous genes encoding enzymes of the sulfate assimilation pathway are significantly upregulated by light, as shown by transcriptomic microarray studies (350) and analyses of EST libraries constructed under various light conditions (25). In *T. reesei* and *T. atroviride*, the transcript level of the gene coding for one sulfate permease (TR_57088 and the orthologs TA_148686, respectively) is augmented by light, in *T. reesei* increasing 2-fold in comparison with darkness. On the other hand, transcription of genes encoding the two other permeases in these *Trichoderma* spp. is not regulated by light.

Sulfate can also be assimilated after hydrolysis of its organic esters. This function is performed in fungal cells by aryl- and choline sulfatases (422, 423) (EC 3.1.6.1 and EC 3.1.6.7, respectively), which are also found in *T. reesei*, *T. atroviride*, and *T. virens*. Transcriptomic data showed that the gene encoding *T. reesei* choline sulfatase (TR_78320) is upregulated by light to twice the level found in darkness (350). Also, numerous ESTs encoding sulfate adenylyltransferase (EC 2.7.7.4; TA_300938) were found in an EST library from *T. atroviride* cultured after stimulation by light (25). No induction of sulfate adenylyltransferase has been observed, however, in *T. reesei* (350).

Another sulfate assimilatory enzyme whose homologs have been found in all *T. reesei*, *T. atroviride*, and *T. virens* is adenylylsulfate kinase (EC 2.7.1.25), which is responsible for sulfate activation to a form suitable for reduction by 3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductase (EC 1.8.4.8). Microarray data from *T. reesei* showed that the PAPS reductase transcript abundance increases by roughly 20% when *T. reesei* is grown in the presence of light (350). Similar regulation occurs in *T. atroviride*, since corresponding ESTs have been found to increase in abundance upon illumination when two libraries from fungi grown in darkness and in light were compared (25).

The pivotal enzyme in the assimilation pathway, which catalyzes the final reduction of sulfite to sulfide, is a two-subunit sulfite reductase (EC 1.8.1.2). Both subunits have been found in all *Trichoderma* spp. studied (see Table S1 in the supplemental material). Moreover, in *T. reesei* the transcript encoding the beta-subunit of sulfite reductase (TR_45138) is light regulated, since its level also increases by about 30% following exposure to light (350).

Analysis of three *Trichoderma* genomes reveals the presence of four putative methionine permeases (see Table S1 in the supplemental material), which is reminiscent of the genome content of *A. nidulans* rather than that of *N. crassa*. One of the identified putative methionine transporters (TR_68831, TA_293457, and TV_200088) shares homology with *S. cerevisiae* Mup3p, a low-affinity permease. The remaining two appear to be high-affinity transporters homologous to *S. cerevisiae* Mup1p. These transporters may also take up cysteine as Mup3p does in *S. cerevisiae* (424). In *T. reesei* the regulation of one of these Mup1p-like transporters, TR_60144, appears to be light dependent, since its transcript level increased over 3-fold under light conditions (350).

One of the principal natural growth environments of *Trichoderma* spp. is decaying plant material, a poor source of small amounts of oxidized derivatives of sulfur-containing amino acids. Moreover, the sulfate anion is rarely available in natural soil, constituting less than 5% of total sulfur. However, sulfonates comprise up to 20 to 40% of organic sulfur in forest soil (425). One of the most widely occurring natural sulfonic acids is taurine, a product of cysteine oxidation and degradation. *Trichoderma* spp. have only one putative cysteine dioxygenase (EC 1.13.11.20) responsible for mobilization of sulfite from cysteine, similarly to what was found in *N. crassa* (71). Putative cysteic acid decarboxylases (EC 1.13.11.20), formerly attributed to glutamate decarboxylases with a broad spectrum of substrates which can generate taurine from cysteic acid, are widely represented in the three genomes studied. *Trichoderma* spp. do not possess enzymes corresponding to the putative alkanesulfonate monooxygenases of *N. crassa*, which utilize more complex sulfur compounds. They are well equipped with a broad repertoire of putative taurine dioxygenases that can liberate sulfite from taurine. In contrast to four taurine dioxygenases in *N. crassa* (71), five proteins in *T. reesei* and six in *T. atroviride* as well as in *T. virens* (EC 1.14.11.17) (see Table S1 in the supplemental material) were detected. However, the role of these enzymes in *Trichoderma* spp. needs to be considered with caution, due to their high homology to xanthine dioxygenases.

Two sulfite oxidases are present in *T. atroviride* (EC 1.8.3.1; TA_146350 and TA_313009; similar to *N. crassa*), whereas *T. reesei* and *T. virens* possess three each. Interestingly, a gene encoding one of these enzymes in *T. atroviride* (TA_146350) was found in an EST library to be regulated both by light and mycoparasitic growth (25, 352), whereas none of the three sulfite oxidase mRNAs in *T. reesei* was regulated in this way (350).

T. reesei, *T. atroviride*, and *T. virens* also have a putative mitochondrial oxaloacetate/sulfate/thiosulfate transporter (TC 2.A.29.15.1; TR_119845, TA_128804, and TV_70356), which in *S. cerevisiae* takes up sulfate (426), although it appears not to exert an important function in sulfur metabolism.

Cysteine, homocysteine, and methionine biosynthetic pathways. Protein-coding genes were identified from *T. reesei*, *T. atroviride*, and *T. virens* for the following five pathways: cysteine and methionine biosynthesis, alternative sulfur amino acid biosynthesis, reverse transsulfuration, and CH_3-folate metabolism (Fig. 7; see also Table S1 in the supplemental material). The main cysteine biosynthesis pathway involves serine *O*-acetyltransferase (EC 2.3.1.30) and cysteine synthase (EC 2.5.1.47). These enzymes and their genes have been well characterized in *N. crassa* and *A. nidulans* (427–429). They synthesize cysteine by incorporating inorganic sulfide into the serine backbone.

Cystathionine gamma-synthase (EC 2.5.1.48), which has been well characterized in *N. crassa* (430), makes cystathionine from cysteine and *O*-acetylhomoserine, which is then converted by cystathionine beta-lyase (EC 4.4.1.8) into homocysteine. Both enzymes have their orthologs in all three *Trichoderma* spp. Due to its toxicity, homocysteine occurs in cells in small quantities and is converted to methionine by cobalamine-independent methionine synthase (EC 2.1.1.14), which is found as a single enzyme in *T. atroviride*, *T. virens*, and *T. reesei*. A transcript encoding this synthase was detected during mycelial growth of *T. reesei* and *T. atroviride* (25, 350).

A particularly interesting feature absolutely unique to *T. atroviride* is the duplication of the alternative sulfur amino acid synthesis pathway (Fig. 7, pathway A). In *T. atroviride*, this pathway comprises two homoserine transacetylases (EC 2.3.1.31; TA_299508 and TA_260926) activating homoserine into *O*-acetylhomoserine, which then serves in homocysteine biosynthesis catalyzed by a pair of *O*-acetyl-L-homoserine sulfhydrylases (EC 2.5.1.49; the main, TA_195487, and the minor, TA_93352).

Interestingly, differential EST analysis of *T. atroviride* during mycoparasitic growth revealed that the genes encoding cystathionine gamma-synthase, methionine synthase, and cystathionine beta-synthase (EC 4.2.1.22, an enzyme of the reverse transulfuration pathway), were noticeably induced under these conditions (25). This derepression may help counterbalance the rising homocysteine level generated by two homoserine sulfhydrylases during pathogenic attack.

The CH_3-folate circuit that delivers methyl moieties for methionine biosynthesis usually involves two sets of isoenzymes, one mitochondrial and one cytosolic. Such organization is also observed in other filamentous fungi, like *N. crassa* and *A. nidulans* (431–433). Similarly, *Trichoderma* spp. express two folyl polyglutamate synthases (EC 6.3.2.17), two glycine/serine hydroxymethyltransferases (EC 2.1.2.1), and two methylenetetrahydrofolate reductases (EC 1.5.1.20). Their subcellular localization (one mitochondrial and one cytosolic) (see Table S1 in the supplemental material) has only been predicted by *in silico* analysis of the respective signal peptide signatures (via the TargetP 1.1 server [434]).

The reverse *trans*-sulfuration pathway is responsible for the synthesis of cysteine from methionine. All enzymes participating in this pathway have been found in *T. reesei*, *T. atroviride*, and *T. virens*. The conversion starts with SAM synthetase (EC 2.5.1.6) and SAH lyase (EC 3.3.1.1), which generates homocysteine. This methionine-homocysteine circuit has a special cellular function, since SAM is the main metabolite of the bulk of cellular methionine (435, 436) and serves as a general agent in polyamine biosynthesis, nucleic acid methylation, and other processes. In yeast, this part of sulfur metabolism has been shown to be under the control of lipid metabolism (437).

T. reesei, *T. atroviride*, and *T. virens* express cystathionine beta-synthase (EC 4.2.1.22), which is an enzyme of the reverse transsulfuration pathway responsible for homocysteine conversion into cystathionine. In *T. atroviride*, transcription of the gene encoding this enzyme is slightly increased by light and, as previously noted, by mycoparasitic attack (25). Cystathionine is then converted into cysteine by cystathionine gamma-lyase (EC 4.4.1.1). In *A. nidulans*, genes coding for cystathionine beta-synthase and cystathionine gamma-lyase are derepressed by excess methionine or homocysteine. Hence, they are considered members of the "homocysteine regulon," which comprises genes regulated by elevated levels of the toxic homocysteine and responsible for its detoxification (438).

Additional enzymes connected with sulfur metabolism. Analysis of genomic data has enabled the identification of several genes coding for putative glutathione *S*-transferases (GSTs; EC 2.5.1.18), the enzymes responsible for maintaining redox homeostasis and inactivation of toxic compounds in the cell. In contrast to *N. crassa*, with three identified GST loci (71), *T. reesei*, *T. atroviride*, and *T. virens* possess five GST-like proteins (see Table S1 in the supplemental material). One of the three *N. crassa* GST-encoding genes (NCU02888) is overrepresented by three orthologs in *T. reesei*, *T. atroviride*, and *T. virens*: TR_22453, TR_81979, and TR_53153; TA_223166, TA_41876, and TA_142566; TV_89283, TV_51640, and TV_77999. Surprisingly, none of the three *Trichoderma* spp. analyzed possesses a mitochondrial isoenzyme. All their GSTs seem to have a microsomal localization, judging from the presence of an appropriate signal peptide motif (TargetP 1.1 server [434]). Similar to *N. crassa*, *T. reesei*, *T. atroviride*, and *T. virens* have one putative thioredoxin reductase (EC 1.6.4.5), although they are well equipped with numerous putative thioredoxins and proteins bearing thioredoxin domains. Thioredoxins are responsible for sustaining a proper redox potential. Of particular interest are two kinds of thioredoxin-harboring enzymes. The first one comprises a PITH domain (439) present in filamentous fungi (TR_75568, TA_301402, and TV_52324) and is a putative component of the proteasome. The second one (TR_122900, TA_317925, and TV_213844) is a mitochondrial ribosomal small-subunit protein homologous to *S. cerevisiae* Rsm22p and contains a SAM-dependent methyltransferase domain fused with thioredoxin. A similar domain organization is also found in *Fusarium* proteins, *G. zeae* (FG09447), *N. haematococca* (XP_003046162), and *F. oxysporum* (EGU83953), as well as in *Magnaporthe*-related species, *M. oryzae* (XP_001409898 and EHA46106) and *Grosmannia clavigera* (EFX01860). Further enzymes related to the management of sulfide and sulfhydryl moieties have also been found in *T. reesei*, *T. atroviride*, and *T. virens*, such as sulfide dehydrogenase (TR_76215, TA_299260, and TV_83404), cysteine desulfurase (TR_75845, TA_301514, and TV_75378), and methionine sulfoxide reductase (TR_78264, TA_208224, and TV_50657).

Not belonging to the main pathway, but very important for the cell redox balance and crucial for detoxification of sulfide, is the mitochondrial enzyme sulfide:quinone reductase (SQR) (440). It is a flavoprotein belonging to the disulfide oxidoreductase (DiSR) family (441) and represents an ancient prokaryotic type of enzymes. *T. reesei*, *T. atroviride*, and *T. virens* have a single ORF encoding SQR (TR_76215, TA_299260, and TV_83404). In *T. reesei* its transcript abundance is 2-fold increased under light conditions (350).

Regulation of sulfur metabolism. The main component of the sulfur regulatory circuit is a positively acting bZIP transcription factor, which in yeast is a heterodimer consisting of two proteins, Met28p and Met4p (442–444). In filamentous fungi, like *N. crassa* and *A. nidulans*, one homodimeric bZIP activator (*N. crassa* CYS-3, *A. nidulans* MetR) is responsible for sulfur network regulation (445, 446). However, recent studies revealed that *A. nidulans* and other members of the *Eurotiales* may possess exchangeable homo-/heterodimeric regulatory networks comprised of MetR and paralogous bZIP transcription factor MetZ (447). When a preferred organic sulfur source such as cysteine or methionine appears in the environment, the sulfur metabolite repression (SMR) system designates the bZIP protein for inactivation (Fig. 8). The SMR system consists of a negatively acting SCF (Skp1/cullin/F-box) ubiquitin ligase complex, which is composed of a crucial F-box protein responsible for recognizing and targeting bZIP (*N. crassa* SCON-2 [448], *A. nidulans* SconB [449], the Skp1 protein (S-phase kinase-associated protein 1; *N. crassa* SCON-3 and *A. nidulans* SconC [450, 451]), the Rbx1 protein, and an E2/E3 ubiquitin-conjugating protein. All these components are held on cullin, a scaffold protein (446, 451). Activation of the SCF ubiquitin ligase results from an increased level of cysteine, its positive effector ligand (452, 453). In *A. nidulans*, most of the genes regulated by the SMR system are involved in sulfate assimilation and sulfide incorporation. All genes encoding subunits of the SCF and the bZIP transcription factor have orthologs in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 in the supplemental material).

The *T. reesei* homolog of the sulfur controller SCON-2, encoded by *lim1*, is upregulated upon sulfur limitation and negatively regulated by the photoreceptor ENV1 (78). Its transcript

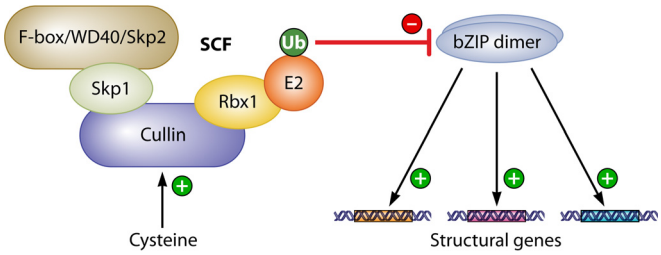

FIG 8 Sulfur metabolite repression system in fungi. The SCF ubiquitin ligase complex consists of several core components: the adaptor protein Skp1 (TR_73823, TA_146592, and TV_215651), scaffold protein cullin (TR_55706, TR_2707, TR_82651, TR_5148, TA_130811, TA_289394, TA_30100, TA_41324, TV_170804, TV_160722, TV_37896, and TV_43883), an F-box protein (TR_77795, TA_29353, and TV_56502), the RING finger protein Rbx1 (TR_121950, TA_297990, and TV_216834), and the ubiquitin-conjugating enzyme E2. The interchangeable F-box proteins determine the complex specificity. Identified F-box proteins involved in sulfur metabolism include *S. cerevisiae* Met30p, *N. crassa* SCON-2, and *A. nidulans* SconB, targeting ubiquitination of the bZIP protein at high concentration of cysteine. The red circle with a minus sign is a low-molecular-weight effector of the SCF complex. The ubiquitinated bZIP activator (in *Trichoderma* spp., TR_119759, TA_297702, TV_119501, and TV_180261) is directed to degradation by the 26S proteasome or inactivated.

levels decrease upon induction of cellulase gene expression and increase in light (351, 415). In *T. atroviride* the gene encoding the Skp1p ortholog (TA_146592) is probably derepressed by light and during mycoparasitic growth, since numerous clones have been found in an EST library constructed under those conditions (25). In all three *Trichoderma* spp., a CYS-3/MetR-orthologous transcription activator was found (TR_119759, TA_297702, TV_119501, TV_180261). Of particular interest is *T. virens*, which possesses two highly similar bZIP activators that evolved probably via gene duplication. These two encoded proteins (TV_119501 and TV_180261) share 58% similarity between each other. As bZIP proteins frequently act as heterodimers rather than homodimers (454), a heterodimeric bZIP factor may be involved in regulation of all or some of the SMR genes in *T. virens*, similar to the situation in *S. cerevisiae* (444) or in *A. nidulans* (447). The described bZIP components may have evolved independently as a convergent adaptation, or two different homodimeric factors could act redundantly or specialize in regulation of subsets of SMR genes. No experimental data to differentiate between these possibilities are available yet.

Four highly similar cullins are found in *T. atroviride*, *T. virens*, and *T. reesei* (TR_55706, TR_2707, TR_82651, and TR_5148 in *T. reesei*; TA_130811, TA_289394, TA_30100, and TA_41324X in *T. atroviride*; TV_170804, TV_160722, TV_37896, and TV_43883 in *T. virens*), but which of them organizes the SCF complex controlling sulfur metabolism remains to be determined (see Table S1 in the supplemental material). Transcriptomic analysis of *T. atroviride* has revealed that the gene encoding a putative cullin, TA_130811, is stimulated both by light and during mycoparasitic growth (25). Thus, it could be involved in light-induced conidiation and presumably also in sulfur regulation. Finally, one should mention that bZIP proteins are also crucial for regulation of secondary metabolism (455). In this respect, it is noteworthy that among *Trichoderma* spp. only *T. virens* produces the sulfur-containing secondary metabolite gliotoxin (456). Moreover, the TV_180261 gene encoding a second MetR-like bZIP regulator and the *gliJ* gene (TV_216163) involved in toxin biosynthesis are located tandemly in genome. This suggests that a secondary metabolite cluster is at least partially under the control of the SMR system.

Conclusions. All essential genes required for sulfur prototrophy are present in the three *Trichoderma* spp. A unique feature of *T. atroviride* is the duplication of the alternative sulfur amino acid synthesis pathway. Keeping in mind that during mycoparasitic growth the genes of homocysteine regulon are derepressed, the duplication of this biochemical pathway suggests sulfur deficiency under these conditions. A duplicated biosynthetic pathway for sulfur amino acids might meet the demand, for instance, in maintaining redox balance or mitochondrial iron-sulfur assembly. Interestingly, the methionine concentration in the medium has a different effect on cellulase gene expression in light and darkness in *T. reesei* (415). Only *T. virens* has a duplicated *metR* ortholog, located in the genome in the vicinity of the *gliJ* gene from the gliotoxin biosynthesis pathway. Hence, a connection of sulfur metabolism with secondary metabolism warrants further investigation.

Mevalonate Metabolism

The mevalonate pathway (Fig. 9) involves condensation of acetyl-CoA molecules, resulting in the synthesis of isopentenyl diphosphate (IPP), which then isomerizes to dimethylallyl diphosphate (DMAPP). These two five-carbon molecules serve as the substrates for the synthesis of thousands of products of the mevalonate pathway. Organisms such as higher plants and some algae synthesize isoprenoids using both the mevalonate and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways (457).

Intensive studies of the mevalonate pathway are mainly connected with cholesterol biosynthesis because of its relevance to human health. Cholesterol in humans and ergosterol, its analog in fungi, are essential for structure and function of cellular membranes. Polyprenols are important in protein glycosylation and in cell wall biosynthesis, and ubiquinone participates in electron transport. In this section, the role of metabolites synthesized in the mevalonate pathway in biocontrol activity characteristic of some *Trichoderma* strains (458) is discussed.

Synthesis of farnesyl diphosphate (FPP). (i) Acetoacetyl-CoA thiolase (AAT) Erg10p (EC 2.3.1.9). Acetoacetyl-CoA thiolases (Erg10p) are located in the cytoplasm, where they participate in the mevalonate pathway, and in mitochondria, where they regulate the pool sizes of acetoacetyl-CoA originating from fatty acid catabolism and of acetyl-CoA entering the tricarboxylic acid cycle (459). Search of genomic sequences of *T. atroviride*, *T. virens*, and *T. reesei* using the two *ERG-10* sequences from *S. cerevisiae* revealed homologous sequences coding for two acetoacetyl-CoA thiolases, one located in the cytoplasm (TR_79439, TA_303080, and TV_72521) and the other from mitochondria (TR_120079, TA_149280, and TV_169943) (see Table S1 in the supplemental material). Analysis of two thiolases from *T. reesei* (TR_79439 and TR_120079), which participate in different pathways, showed 48% identity and 66% similarity of their sequences. Further analysis of the cytoplasmic thiolase TR_79439, the one taking part in the mevalonate pathway, gave, respectively, 91and 94% identity with *T. atroviride* and *T. virens* (TA_303080 and TV_72521) enzymes. The predicted thiolase proteins from *T. reesei*, *T. atroviride*, and *T. virens* displayed high identity with thiolases from other filamentous fungi, such as *F. oxysporum* or *G. zeae* (both 82%

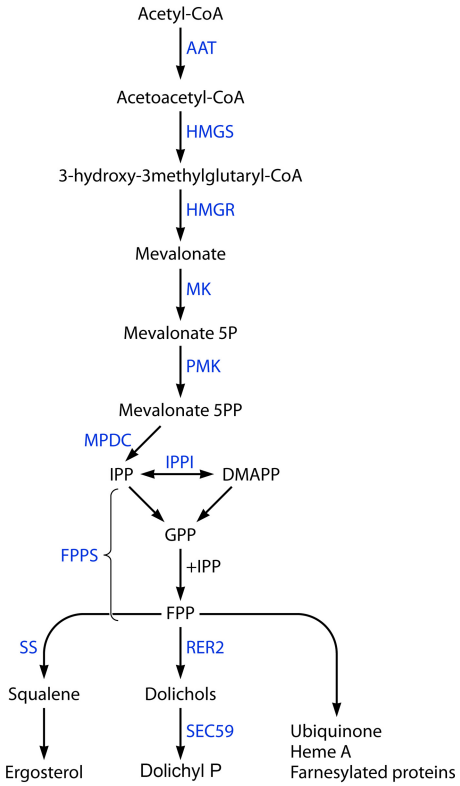

FIG 9 Schematic representation of the mevalonate pathway Abbreviations of enzymes shown in the scheme are as follows: AAT (acetoacetyl-CoA thiolase; EC 2.3.1.9; TR_79439, TA_303080, TV_72521, TR_120079, TA_149280, and TV_169943); HMGS (hydroxymethylglutaryl-CoA synthase; EC 2.3.3.10; TR_75589, TA_130709, and TV_70450); HMGR (hydroxymethylglutaryl-CoA reductase; EC 1.1.1.34; TR_71380, TA_143946, and TV_71643); MK (mevalonate kinase; EC 2.7.1.36; TR_121374, TA_295691, and TV_142066), PMK (phosphomevalonate kinase; EC 2.7.4.2; TR_74711, TA_34690, and TV_28802); MPDC (diphosphomevalonate decarboxylase; EC 4.1.1.33; TR_22798, TA_81597, and TV_47159); IPPI (isopentenyl diphosphate delta-isomerase; EC 5.3.3.2; TR_120106, TA_301103, and TV_84157); FPPS (farnesyl diphosphate synthase; EC 2.5.1.10; TR_49399, TA_134836, and TV_166777); RER2 (*cis*-prenyltransferase; EC 2.5.1.20; TR_21534, TA_299001, and TV_65319); SEC59 (dolichol kinase; EC 2.7.1.108; TR_121295, TA_287169, and TV_36104); SS (squalene synthase; EC 2.5.1.21; TR_122653, TA_303098, and TV_81989). IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate.

identical with the *T. reesei* protein). AAT activity together with HMGS and HMGR activities were shown to be regulated in response to the ergosterol level, although the activity of HMGR was affected only slightly (460). In an excess of sterol, obtained under anaerobic conditions, the activity of AAT is strongly decreased. The gene for acetoacetyl-CoA thiolase, *erg-10*, in *S. cerevisiae* (459) was shown to be overexpressed during cold shock due to a high demand for sterols in the cell membranes (461). Furthermore, Erg10p was required for optimal growth of yeast at low temperatures.

(ii) 3-Hydroxy-3-methylglutaryl-CoA synthase (HMGS) Erg13p (EC 2.3.3.10). HMGS (TR_75589, TA_130709, and TV_70450) catalyzes the transfer of acetyl-CoA onto the growing chain of acetoacetyl-CoA with formation of hydroxyl-methylglutaryl-CoA. Characterization of yeast mutants blocked for mevalonic acid formation enabled the isolation of two genes, *erg10* and *erg13*, encoding, respectively, acetoacetyl-CoA thiolase (AAT) and HMGS (462). Furthermore, it was shown that the activities of HMGS and AAT were up- or downregulated by lack or excess of ergosterol, respectively (460). This finding suggested an important role of HMGS activity in the synthesis of sterols in yeast. Interestingly, in *T. reesei* the HMGS-encoding gene is positively regulated in light on cellulose (350). The amino acid sequence of *T. reesei* HMGS (TR_75589) is 94% and 93% identical to homologous proteins from *T. virens* (TV_70450) and *T. atroviride* (TA_130709), respectively. Moreover, the *T. reesei* HMGS protein is 61% identical and 75% similar to the Erg13p protein from *S. cerevisiae*.

(iii) 3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) Hmg1p and Hmg2p (EC 1.1.1.34). The HMGR enzyme catalyzes the biosynthesis of mevalonate by reductive deacetylation of the thioester group of HMG-CoA to the primary alcohol mevalonate (MVA). In mammalian cells, the activity of HMGR is subjected to regulation at the levels of transcription, translation, posttranslational modification, and degradation (463–465). The regulation includes also cross-regulation by independent biochemical processes and contraregulation of separate isozymes (466). The feedback regulation by-products of the mevalonate pathway coordinate the synthesis and degradation of the HMGR protein. When pathway flux is lowered, both transcription and translation of HMGR-encoding genes are increased and the degradation rate is slowed. In yeast there are two isozymes of HMGR, both regulated by feedback as well as cross-regulated by oxygen. Hmg1p is the primary source of HMGR activity during aerobic growth of *S. cerevisiae* (465). It was demonstrated that farnesyl diphosphate is a source of positive signaling for Hmg2p degradation via modulation of the enzyme stability (464).

HMG-CoA reductase-encoding genes were cloned from some ascomycetous fungi, including *Trichoderma harzianum* (467). *T. atroviride*, *T. virens*, and *T. reesei* revealed sequences encoding proteins with high homology to the HMG protein from other organisms (see Table S1 in the supplemental material). The amino acid sequence of HMGR from *T. virens* is 47% identical to that of *S. cerevisiae* Hmg1p. Analysis of the protein sequences of Hmg1p and Hmg2p from *S. cerevisiae* showed 62% identity, but their C-terminal domains were 95% identical. Comparison of the C-terminal domains of *S. cerevisiae* Hmg1p or Hmg2p proteins with their orthologs from *T. reesei*, *T. atroviride*, and *T. virens* revealed much higher identity than that obtained for the whole protein. The C terminus of the hypothetical HMGR from *T. reesei* (NCBI accession number EGR52932.1) was 70% identical and 84% similar to its analog from yeast. All domains essential for catalysis, such as the substrate binding pocket, inhibitor binding sites, and catalytic residues, are predicted to be localized in this region of HMGR. In *T. harzianum*, the *hmgR* ORF contains 3,518 bp with one 65-bp intron and encodes a protein of 1,150 amino acids (Pfam accession number PF00368) (467). Partial silencing of the *hmgR* gene in *T. harzianum* resulted in reduction of HMGR activity by up to 23%. Reduced activity of HMGR caused lower production of metabolites such as terpenoids and decreased antifungal activity of *T. harzianum* silenced strains (467). These results clearly point to a direct correlation of the mevalonate pathway activity and antifungal activity of some *Trichoderma* strains. Furthermore, we have also identified putative sequences coding for HMGR from *T. reesei*, *T. atroviride*, and *T. virens* (TR_71380, TA_143946, and TV_71643), which appear to have one HMGR

gene, which is expected to be essential (see Table S1 in the supplemental material). Predicted HMGR proteins from *T. reesei* were 87% and 92% identical to orthologous proteins from *T. atroviride* and *T. virens*, respectively. A mevalonate reductase, EC 1.1.1.32, was not detected in *T. reesei*, *T. atroviride*, or *T. virens*.

(iv) Mevalonate kinase (MK) Erg12p (EC 2.7.1.36). Mevalonate kinase is located in the cytosolic fraction and has been characterized in *N. crassa* (468). Mevalonate kinase requires ATP as a cosubstrate and Mg^{2+} ions for full activity and is inhibited by geranyl diphosphate (GPP). Sequences homologous to Erg12p (469) from *S. cerevisiae* were also found in *T. reesei*, *T. atroviride*, and *T. virens* (TR_121374, TA_295691, and TV_142066). Comparison of these proteins revealed, respectively, 95% and 88% identity of *T. virens* and *T. atroviride* proteins to the *T. reesei* putative mevalonate kinase.

(v) Phosphomevalonate kinase (PMK) Erg8p (EC 2.7.4.2). Mevalonate 5-phosphate (mevalonate 5P), a product of mevalonate kinase (Erg12p), is phosphorylated by phosphomevalonate kinase (Erg8p). The identity of predicted PMK proteins of *Trichoderma* spp. varied from 90 to 78% for *T. virens* (TV_28802) and *T. atroviride* (TA_34690), respectively, in comparison to the *T. reesei* (TR_74711) amino acid sequence. Identity of the *T. reesei* (TR_74711) protein with *S. cerevisiae* Erg8p was only 34%. Erg8p-like mevalonate kinase (Erg12p) requires ATP and Mg^{2+} as cofactors (470). Furthermore, *S. cerevisiae* Erg8p was found to have orthologs in fungi (*S. pombe*, *C. albicans*), in Gram-positive bacteria, and in plants, but not in humans (471). The deduced molecular masses of the human PMK protein and Erg8p from yeast are approximately 22 and 47 kDa, respectively (472). The fact that human PMK differs from the one found in fungi, including *C. albicans* and bacteria, makes these enzymes attractive as targets for antifungal and/or antibacterial drugs.

(vi) Mevalonate-5-diphosphate decarboxylase (MPDC) Erg19p (EC 3.1.1.33). MPDC catalyzes decarboxylation of mevalonate diphosphate to form isopentenyl diphosphate. The reaction requires energy from ATP and Mg^{2+} as a cofactor (473). Homology modeling with *S. cerevisiae* MPDC provided models for other MPDCs plus various kinases, including MK and PMK. Modeling results suggest that the three enzymes catalyzing subsequent conversion of mevalonate to isopentenyl diphosphate (MK, PMK, and MPDC) could arise from a common precursor and may represent an example of retrograde evolution (473, 474). *S. cerevisiae* MPDC is encoded by the *ERG19* gene (475). A single point mutation drastically impairs the oligomerization of MPDC in yeast, which normally forms a fully active homodimer *in vivo* (476).

Sequences homologous to the yeast Erg19p were also found in *T. reesei*, *T. atroviride*, and *T. virens* (TR_22798, TA_81597, and TV_47159). Comparison of the *T. atroviride* and *T. virens* protein sequences with the *T. reesei* putative decarboxylase revealed 91% and 94% identity, respectively. The *T. reesei* TR_22798 protein exhibited over 80% identity with diphosphate mevalonate decarboxylases from other filamentous fungi, such as *F. oxysporum* (83%) and *G. zeae* (82%). Sequences of much lower identity with the *T. reesei* putative MPDC were found in *A. niger* and *A. nidulans* (66%), *S. cerevisiae* (57%), and *Candida glabrata* (60%).

(vii) Isopentenyl diphosphate:dimethylallyl isomerase (IPP isomerase) Idi1p (EC 5.3.3.2). IPP isomerase catalyzes transposition of hydrogen in isopentenyl diphosphate to form dimethylallyl diphosphate (DMAPP). IPP isomerases are grouped as IPP isomerase type I or type II (477).

Overexpression of the *IDI1* gene coding for IPP isomerase (478) in yeast resulted in a 6-fold increase in IPP isomerase activity. Disruption of the *IDI1* gene demonstrated that it is an essential single-copy gene in *S. cerevisiae* (479). The protein sequence of the Idi1p from *S. cerevisiae* is 54% identical to the *T. reesei* putative IPP isomerase (TR_120106). Corresponding sequences were also found in the other *Trichoderma* spp. (TV_84157 and TA_301103). Putative IPP isomerases from *T. virens* and *T. atroviride* revealed 98 and 93% identity with the *T. reesei* protein, respectively.

(viii) Farnesyl diphosphate synthase (FPPS) Erg20p (EC 2.5.1.10). FPP synthase catalyzes head-to-tail condensation of dimethylallyl diphosphate (DMAPP) and IPP to form geranyl diphosphate (GPP). The addition of IPP to GPP to produce the 15-carbon compound FPP is catalyzed by FPP synthase as well. FPP synthase is localized at the branch point of the mevalonate pathway and provides a substrate for synthesis of all the end products of the pathway. However, it was shown that fluctuation of the intracellular concentration of FPP influences mostly the synthesis of sterols, since squalene synthase has a low affinity for FPP (480). Furthermore, FPP is a feedback regulator of HMGR, as indicated above. FPP synthase is encoded by the *ERG20* gene, which was cloned from *S. cerevisiae* (481), and some filamentous fungi, such as *N. crassa* and *F. fujikuroi* (482). In yeast, *ERG20* is a single-copy essential gene, whereas at least five copies of the FPP synthase gene exist in rat liver (483). Only one FPP synthase-encoding gene was found in *T. reesei* (NCBI accession number JX845568; TR_49399) (484) and other *Trichoderma* spp. (TA_134836 and TV_166777). The *erg20* gene from *T. reesei* encodes a protein of 347 amino acids. An alignment of ERGXX protein sequences from *T. reesei*, *T. atroviride*, and *T. virens* revealed that the highest identity among them occurs at the middle region of the protein from amino acids 85 to 267 and reaches 87% identity and 96% similarity with an analogous region of ERG20 from *N. crassa*. When the whole proteins from *T. reesei* and *N. crassa* were compared, the identity and similarity decreased to 80 and 93%, respectively. ERG20 from *Trichoderma* also has quite high identity with the *S. cerevisiae* Erg20p primary structure, since the identity reaches 56% with 75% similarity. Comparison of the regions comprising functional domains from *Trichoderma* and *S. cerevisiae* resulted in 69% and 84% identity and similarity, respectively. The identity of the whole FPP synthase protein sequence between *T. reesei* and other *Trichoderma* strains is 94 and 88% for *T. virens* and *T. atroviride*, respectively.

The dolichol biosynthesis pathway. (i) *cis*-Prenyltransferase (cis-PT) Rer2p (EC 2.5.1.20). *cis*-Prenyltransferase (dehydrodolichyl diphosphate synthase; cis-PT) is a key enzyme in dolichol synthesis and represents the first enzyme of the polyprenyl branch of the mevalonate pathway. The enzyme catalyzes the elongation of the polyprenol chain by sequential addition of IPP to farnesyl diphosphate (485–487). Polyprenyl diphosphate formed in this reaction is then dephosphorylated by isoprenoid phosphatase, the α-prenyl residue is saturated, and the resultant dolichol can be installed in the structure of cell membranes. Dolichol can also be phosphorylated by dolichyl kinase (Sec59p), and in this form it takes part in N- and O-glycosylation and GPI anchor synthesis as a carbohydrate carrier (488).

cis-Prenyltransferases are classified into three subfamilies with respect to product chain length, i.e., short (C_{15}), medium (C_{50} to C_{55}), or long (C_{70} to C_{120}) (489). In the yeast *S. cerevisiae*, cis-PT is encoded by *RER2* and *SRT1* (490–492). Rer2p is responsible for

the main activity of cis-PT and is active mainly in the early logarithmic phase of growth. Moreover, the Δ*rer2* mutation in *S. cerevisiae* results in morphological changes in cellular organelles, such as accumulation of endoplasmic reticulum (ER) membranes and their extreme elongation and the ring-like structure of Golgi apparatus membranes (490). It is known that in Δ*rer2* cells, *SRT1* compensates for Rer2p function, although the polyprenols produced by Srt1p are longer (C_{85} to C_{110}) than those produced by Rer2p (C_{70} to C_{85}) and are not converted to dolichols efficiently (491). Srt1p, a long-chain cis-PT, has an additional 5 amino acids in a regulatory domain critical for determination of the ultimate product chain (helix −3), which is not present in the short-chain cis-PTs.

Overexpression of the yeast *RER-2* gene in *T. reesei* resulted in overproduction of dolichols, increased activities of enzymes using dolichyl phosphate as an acceptor of carbohydrate residues, and hyperglycosylation of secretory proteins (493). *T. reesei*, *T. atroviride*, and *T. virens* have only one gene coding for cis-PT (TR_21534, TA_299001, and TV_65319). Alignment of cis-PT protein sequences showed that RERII from *T. atroviride* and *T. virens* are 92 and 89% identical to the *T. reesei* predicted cis-PT. Similar sequences are also found in other filamentous fungi, such as *F. oxysporum* (76% identity with TR_21534) or *G. zeae* (72% identity with TR_21534). The RERII protein from *T. reesei* (TR_21534) is 47 and 44% identical to *S. cerevisiae* Rer2p and Srt1p, respectively; however, both proteins from yeast are only 39% identical.

(ii) Predicted polyprenyl phosphate phosphatases Dpp1 and Lpp1 (EC 3.1.3.4). There are two enzymes, Dpp1p and Lpp1p, that have been predicted to participate in polyprenol phosphate dephosphorylation in yeast (494). LPP1p and DPP1p account for most of the hydrolytic activities toward dolichyl-P-P, dolichyl-P, farnesyl-P-P, and geranylgeranyl-P-P, but yeast could contain other enzymes capable of dephosphorylating these essential isoprenoid intermediates. Sequences similar to the yeast Dpp1p and Lpp1p were predicted for *T. reesei*, *T. atroviride*, and *T. virens* (TR_69858, TA_88402, and TV_72904; TR_29346, TA_278433, and TV_128286).

(iii) Predicted polyprenol reductase Dfg10 (EC 1.3.1.94). In yeast cells, the alpha polyprenyl residue in the dephosphorylated polyprenol is saturated and dolichol is formed. In humans, steroid 5 alpha-reductase type 3 encoded by the *SRD5A3* gene was found to participate in the saturation of the double bond in the alpha-isoprene unit of polyprenols. However, the presence of residual dolichol in cells depleted of this enzyme suggests the existence of an alternative enzyme for dolichol synthesis (495). The protein Dfg10p, similar to the human SRD5A3p, was found in *S. cerevisiae*, and its predicted function is to convert polyprenols to dolichols. However, there are no proteins similar to Dfg10p in *T. reesei*, *T. atroviride*, or *T. virens*.

(iv) Dolichyl kinase SEC59 (EC 2.7.1.108). Dolichyl kinase catalyzes the terminal step in dolichyl monophosphate formation. The yeast *sec59* mutant accumulates inactive and incompletely glycosylated secretory proteins at the restrictive temperature (496). The amount of dolichyl phosphate in the membranes of the *sec-59* mutant at the nonpermissive temperature was less than 10% of the wild-type level. A *sec59-1* mutant bearing a single amino acid substitution (Trp^{332} to Gly) has a thermosensitive phenotype, which was revealed in the presence of 1 M sorbitol in the medium (497). The cell wall defect of the *sec59-1* mutant was confirmed by its increased sensitivity to Calcofluor white, a fluorescent agent that binds to chitin and interferes with cell wall assembly. It was shown that this mutant was impaired in dolichyl phosphate mannose synthase and *N*-acetylglucosamine transferase activities and that *RER2* overexpression could partially suppress the *sec59-1* mutation (497).

Activity of dolichyl kinase in *T. reesei* QM9414 was increased when *Trichoderma* was cultivated in a medium supplemented with choline or Tween 80 (498). It was suggested that these compounds enriched the membranes with phosphatidyl choline and oleic acids, influencing the environment of the membrane-bound enzymes.

Proteins homologous to the yeast Sec59p were found in *T. reesei*, *T. atroviride*, and *T. virens* (TR_121295, TA_287169, and TV_36104). The predicted amino acid sequences of dolichyl kinases from *T. virens* and *T atroviride* are, respectively, 84 and 80% identical to the *T. reesei* TR_121295 protein sequence, while the protein encoded by the gene from *N. crassa* (NCU03771) revealed only 55% identity. Very little identity (up to 31%) between *T. reesei* and *S. cerevisiae* dolichyl kinases was found, only at the C terminus of the proteins.

Conclusions. In *T. reesei*, *T. atroviride*, and *T. virens*, genes encoding all enzymes required for synthesis of dolichols, with exception of polyprenol reductase, were detected. However, saturated dolichols were isolated from *T. reesei* (470), suggesting the existence of an alternative enzyme catalyzing this reaction. Almost nothing is known about factors regulating expression of genes of the polyprenol synthesis pathway. The only available information refers to *hmgs* (*ERG13*) of *T. reesei*, the expression of which is positively regulated in light during cultivation on cellulose (343).

It is noteworthy that the two enzymes of polyprenol synthesis, HMGR and cis-PT, are represented in *Trichoderma* by single proteins, while the synthesis of mevalonate (HMGR) and elongation of the polyprenol chain (cis-PT) in yeast are catalyzed by two isoenzymes, Hmg1p/Hmg2p and Rer2p/Srt1p, respectively. Only two genes (*hmgR* and *erg20*) encoding key enzymes of the mevalonate pathway have been cloned and functionally analyzed in *Trichoderma* (444, 461).

Lipid Metabolism

Lipids constitute a large portion of cellular membranes and play important roles in cell signaling and energy metabolism. *Trichoderma* species have not been extensively used as model organisms for lipid metabolism. However, there is considerable interest in harnessing the power of *Trichoderma* for industrial uses involving both catabolic and anabolic lipid processes and for understanding the role of lipids during plant-fungus and fungus-fungus interactions. While lipid metabolism hasn't been extensively studied in *Trichoderma*, conclusions based on gene homology can be reached by comparison with the well-studied sordariomycete *N. crassa* and the more distantly related yeast *S. cerevisiae*. Roughly 95% of the extractable fatty acid methyl esters from *T. reesei* are made up of palmitic (length:saturation, 16:0), palmitoleic (16:1), steric (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids (499). Lipids comprised primarily of 16- and 18-carbon chains are common throughout the fungal kingdom, with some variance in their relative amounts (500). Factors affecting the lipid content and composition of lipids in *Trichoderma* have been investigated. *T. reesei* was found to accumulate a higher concentration of more-saturated lipids when grown at 26°C, with

decreasing saturation and concentration when the temperature was lowered toward 10°C or raised toward 35°C (499). Studies in *T. viride* as well as *T. harzianum* found that nitrogen-deficient medium stimulated lipid accumulation (501), as has been found for many oleaginous fungi (502). The composition and quantity of both polar and nonpolar lipids are also subject to change during conidiation and sporulation of *T. viride* (503), demonstrating that lipid metabolism is dynamic and responsive to the environment in these species.

Trichoderma spp. have been demonstrated to utilize extracellular lipids as an energy source. For instance, linoleic acid is rapidly incorporated into neutral lipids when *T. viride* or *T. harzianum* is cultured with this fatty acid (504). More recently, *Trichoderma* species with the capability to produce extracellular lipase (505) and cholesterol esterase (506) activities have been identified and are of interest for production of industrial enzymes and bioremediation. Examination of potentially secreted triglyceride lipases (class III lipases) identified a single lipase with a conserved signal sequence in *T. reesei*, *T. atroviride*, and *T. virens* (TR_106405, TA_322056, and TV_36225) and two lipases with predicted signal sequences in 2/3 of the species (TR_79010, TA_131743, and TV_188672; TR_37368, TA_298014, and TV_183117). Some species of *Trichoderma* are able to utilize alkanes as a carbon source in addition to fatty acids. This process is dependent on ER alkane-inducible cytochrome P450 in *T. harzianum* (507). The ability to metabolize alkanes in an industrial context is attractive and would allow the production of value-added products from contaminated substrates, as has been explored using an oleaginous strain of *T. reesei* (508).

Trichoderma species are popular biocontrol agents for crop plants against pathogenic fungi (6, 509, 510), and both *T. virens* and *T. atroviride* have been well studied in this respect (29, 338, 511, 512).

A number of studies have highlighted the role of lipid metabolism during the fungus-plant interaction aspect of biocontrol, including during root colonization of tomato plants by *T. harzianum* (513) and *T. asperelloides* (514). Additionally, lipid metabolism is important to mycoparatism in *T. atroviride*, during which fatty acyl-CoA dehydrogenase is upregulated (25).

Fatty acid synthesis and acyl group transfer. Single-copy genes for *de novo* lipid biosynthesis (Fig. 10) from acetyl-CoA, including acetyl-CoA carboxylase (TR_81110, TA_147404, and TV_78374), type I fatty acid synthase subunits alpha (TR_48788, TA_85662, and TV_48659), and beta (TR_78591, TA_226146, and TV_171412), and β-keto-acyl synthase (TR_120712, TA_32217, and TV_82746) are readily identifiable in the genomes of *T. reesei*, *T. atroviride*, and *T. virens*. Six fatty acid desaturases are predicted in all three *Trichoderma* species (see Table S1 in the supplemental material) and exhibit significant homology with those in *N. crassa*. An additional fatty acid, Δ6 desaturase, is predicted in *T. reesei*, *T. atroviride*, and *T. virens*, suggesting this enzyme may have been duplicated (71). The initial step of *de novo* lipid biosynthesis in the glycerolipid pathway is incorporation of acyl-CoA onto glycerol-3P to make 1-acyl-*sn*-glycerol-3P. Three enzymes in *T. reesei*, *T. atroviride*, and *T. virens* are predicted to have this activity (SCT1) (TR_109086, TA_127885, and TV_190817, and two others, TR_21279, TR_109980, TA_156351, TA_144174, TV_71725, and TV_80938). Addition of a second acyl-CoA chain to make 1,2-diacyl-*sn*-glycerol-3P is achieved either by the activity of lysophospholipid acyltransferase (ALE1), of which all three species have a

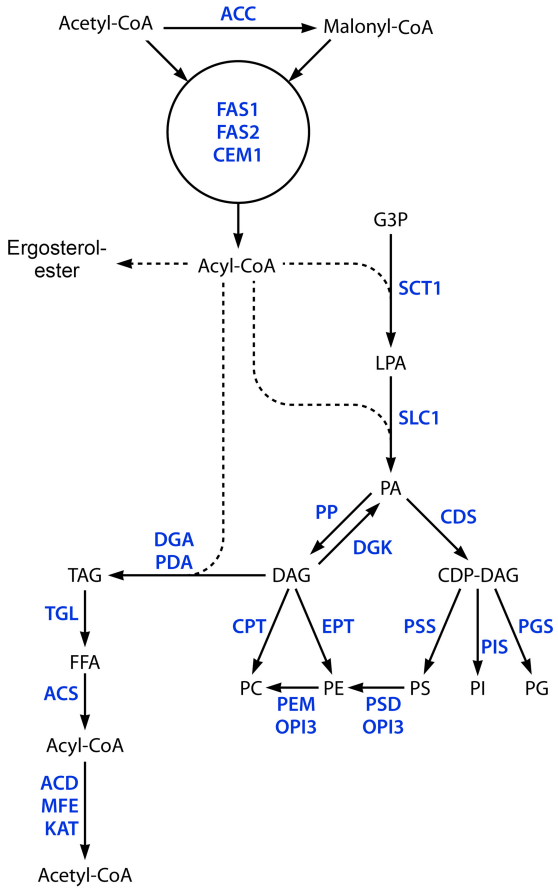

FIG 10 Schematic representation of lipid metabolism. Abbreviations of metabolites and enzymes (in blue) shown in the scheme are as follows: ACC, acetyl-CoA carboxylase; TR_81110, TA_147404, and TV_78374); FAS1 (fatty acid synthase alpha; TR_48788, TA_85662, and TV_48659); FAS2 (fatty acid synthase beta; TR_78591, TA_226146, and TV_171412); CEM1 (beta-keto-acyl synthase; TR_120712, TA_32217, and TV_82746); SCT1 (glycerol-3-phosphate 1-*O*-acyltransferase; TR_109086, TA_127885, and TV_190817); SLC1 (1-acyl-sn-glycerol-3-phosphate acyltransferase; TR_107258, TA_130298, TA_30732, TV_56309, and TV_41116); PP (phosphatidate phosphatase; TR_79560, TA_89543, and TV_143439); DGK (diacylglycerol kinase; TR_77915, TA_295259, and TV_217264); DGA (diacylglycerol acyltransferase; TR_120566, TA_157028, and TV_216961); ARE (acyl-CoA:sterol acyltransferase; TR_50607, TR_61705, TA_213158, TA_301974, TV_58320, and TV_87128); PDA (phospholipid:diacylglycerol acyltransferase; TR_121546, TA_244255, and TV_37216); CDS (CDP-diacylglycerol synthase; TR_51806, TA_39363, and TV_33697); PSS (phosphatidylserine synthase; TR_121550, TA_150107, and TV_36484); PIS (phosphatidylinositol synthase; TR_74023, TA_297712, and TV_71870); PGS (phosphatidyl glycerol phosphate synthase; TR_3600, TA_28801, and TV_213484); PSD (phosphatidylserine decarboxylase; TR_110040, TR_80958, TA_156864, TA_151310, TV_196625, and TV_77855); PEM (phosphatidylethanolamine methyltransferase; TR_44868, TA_285860, and TV_71510); OPI3 (methylene-fatty-acyl-phospholipid synthase; TR_49864, TA_300563, and TV_45525); CPT (cholinephosphotransferase; TR_55627, TA_300979, and TV_71348); EPT (sn-1,2-diacylglycerol ethanolamine- and cholinephosphotranferase; TR_122644, TA_303084, and TV_32034); TGL (triacylglycerol lipase; TR_80191, TR_67507, TR_71259, TR_122091, TR_107263, TA_193414, TA_221800, TA_281797, TA_172817, TA_30218, TV_133319, TV_59787, TV_30485, TV_49387, and TV_74421). ACS, acyl-CoA synthetase; ACD, acyl-CoA dehydrogenase; MFE, 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase (TR_2392, TA_33165, and TV_70294); KAT, 3-ketoacyl-CoA thiolase; G3P, glycerol-3-phosphate; LPA, 1-acyl-sn-glycerol-3-phosphate; PA, phosphatidic acid; DAG, diacylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; TAG, triacylglycerol; FFA, free fatty acid.

single copy, or a 1-acyl-sn-glycerol-3P acyltransferase (SLC1). Interestingly *T. atroviride* and *T. virens* have two genes predicted to have this activity (TA_130298, TA_30732, TV_56309, and TV_41116), while *T. reesei* has only one, though it is the most similar to *slc1* in *S. cerevisiae* (515), suggesting this may be the most important of the two for lipid synthesis. Dephosphorylation of 1,2-diacyl-sn-glycerol-3P to 1,2-diacyl-sn-glycerol is predicted to be achieved by a group of three phosphatases in *T. reesei* (TR_79560, TR_69858, and TR_29346) or four in *T. atroviride* (TA_89543, TA_88402, TA_278433, and TA_294663) and *T. virens* (TV_143439, TV_72904, TV_128286, and TV_232165). However, in yeast this activity is mainly achieved by the lipin homolog *pah1* (TR_79560, TA_89543, and TV_143439) and is functionally conserved in mammals, suggesting it is likely to be conserved across fungi (516, 517). Phosphorylation of 1,2-diacyl-sn-glycerol back to 1,2-diacyl-sn-glycerol-3P is achieved by diacylglycerol kinase (DGK1; TR_77915, TA_295259, and TV_217264) and represents an important regulatory step in lipid homeostasis in yeast (518). Triglycerides are the major form in which acyl chains are stored in lipid droplets and are made by transferring an acyl group to 1,2-diacyl-sn-glycerol. Homologs of enzymes utilizing either acyl-CoA (diacylglycerol acyltransferase DGA1 (TR_120566, TA_157028, and TV_216961), acyl-CoA:sterol acyltransferase 1 ARE1 (TR_50607, TA_213158, and TV_58320), acyl-CoA:sterol acyltransferase 2 ARE2 (TR_61705, TA_301974, and TV_87128) (519, 520), or phosphatidylcholine (lecithin cholesterol acyltransferase LRO1 [TR_121546, TA_244255, and TV_37216]) (521) as the acyl donor are present as a single copy in *T. reesei*, *T. atroviride*, and *T. virens*. Two serine palmitoyltransferases are present in all three *Trichoderma* species (TR_22148, TA_299162, TV_215751; TR_46463, TA_300124, and TV_47144) and are predicted to transfer acyl groups to serine as the first step in sphingolipid biosynthesis (522).

Phospholipid synthesis and turnover. Phosphatidatecytidylyl transferase (CDS1; TR_51806, TA_39363, and TV_33697) activates 1,2-diacyl-sn-glycerol-3P to CDP-diacylglycerol for phospholipid biosynthesis and is present as a single copy in all three *Trichoderma* species (523). CDP-diacylglycerol is a substrate for the production of phosphatidylserine via CHO1 (TR_121550, TA_150107, and TV_36484), phosphotidylinositol via PIS1 (TR_74023, TA_297712, and TV_71870), and phosphatidylglycerol via PGS1 (TR_3600, TA_28801, and TV_213484). Phosphatidylserine is converted to phosphatidylethanolamine by PSD1 (TR_110040, TA_156864, and TV_196625) and PSD2 (TR_80958, TA_151310, and TV_77855) and subsequently to phosphatidylcholine by CHO2 (TR_44868, TA_285860, and TV_71510) and OPI3 (TR_49864, TA_300563, and TV_45525). Phosphatidylcholine and phosphatidylethanolamine are also synthesized directly from diacylglycerol by CPT1 (TR_55627, TA_300979, and TV_71348) and EPT1 (TR_122644, TA_303084, and TV_32034) (524). All the enzymes for the synthesis of major phospholipids are present in a single copy in all three *Trichoderma* species, reinforcing the fact that they are under strong selection. Interestingly, an enzyme with homology to PIS1 is present in *T. atroviride* and *T. virens* (TA_133333 and TV_72847) but not *T. reesei*, suggesting an as-yet-uncharacterized phosphatidyltransferase activity present in these species. Phospholipid hydrolysis is an important aspect of membrane homeostasis, lipid turnover, and cell signaling (71). All three *Trichoderma* species have 13 identifiable phospholipases: one lysophospholipase, one phospholipase A, two phospholipase B, six phospholipase C, and three phospholipase D proteins.

Triglyceride utilization. Utilization of storage lipids is an essential aspect for cell division and recovery from stationary-phase growth (525). *T. reesei*, *T. atroviride*, and *T. virens* species have homologs of the four lipid particle (TGL1, TGL3, and TGL4) and mitochondrial (TGL2) triacylglycerol lipases present in *S. cerevisiae* that catalyze the initial step in storage lipid mobilization. Interestingly, a second triacylglycerol lipase with homology to TGL4 of *S. cerevisiae* is present (TR_107263, TA_30218, and TV_74421). Fatty acids freed by lipases are activated by the addition of a CoA group by one of many acyl-CoA synthases identified (16 in *T. reesei*, 18 in *T. atroviride*, and 19 in *T. virens*). Recent investigations in *N. crassa* have elucidated the location and specificity of homologs for a few of these enzymes (526), but much work remains to be done to determine their individual functions in *Trichoderma*. Activated fatty acids are free to enter beta-oxidation through the action of acyl-CoA dehydrogenase which, like acyl-CoA synthase, is represented by a large family of enzymes (6 in *T. reesei*, 8 in *T. atroviride*, and 8 in *T. virens*) with potentially overlapping specificity (see Table S1 in the supplemental material). A single copy of MFE1 which performs the 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase reactions of beta-oxidation is present in all three *Trichoderma* species. Four enzymes predicted to function as 3-ketoacyl-CoA thiolases in *T. reesei* (TR_75368, TR_123720, TR_3835, and TR_5270) or five in *T. atroviride* (TA_133790, TA_299697, TA_222949, TA_138745, and TA_44587) and *T. virens* (TV_80821, TV_73014, TV_10779, TV_50177, and TV_194445), which release acetyl-CoA from the shortening acyl-CoA chain, finish off beta-oxidation.

Conclusions. Many of the genes involved in the synthesis of neutral lipids and phospholipids are present in at least, and usually at most, a single copy in *T. reesei*, *T. atroviride*, and *T. virens*. Conservation suggests their essential functionality, as would be expected of the genes involved in making and maintaining cellular membranes. Areas of genetic diversity are particularly evident in enzyme classes involved in catabolism of lipids and include secreted and intracellular triglyceride lipase content, acyl-CoA synthases, acyl-CoA dehydrogenases, and 3-ketoacyl-CoA thiolases. The substrates and activity levels for these enzymes await characterization.

Secondary Metabolism

Filamentous ascomycetes are well known for the diversity of secondary metabolites that they produce. *Trichoderma* species are no exception, as their genomes show a huge potential for the production of a diverse group of secondary metabolites. It is thought that secondary metabolite pathways produce an important set of molecules involved in *Trichoderma* interactions with fungi, other microbes, and plants for several activities that include attack, defense, communication, mating, and nutrient assimilation (527–530). Recent reviews have provided an overview about the catalog of genes potentially associated with secondary metabolite production in the genomes of *T. reesei*, *T. atroviride*, and *T. virens* (7, 18).

Among the major classes of secondary metabolite-producing enzymes are terpenoid synthases, polyketide synthases (PKSs), nonribosomal peptide synthetases (NRPSs), and PKS-NRPS hybrids (4). The first *Trichoderma* genome to be sequenced was from *T. reesei* and it contained 2 NRPS-PKS hybrid-encoding genes and the fewest number of genes for terpenoid synthases (12 genes),

NRPS (8 genes), and PKS (11 genes) (23). Interestingly, 9 out of 11 of PKS-encoding genes and all of the NRPS and terpenoid synthase domain-encoding genes of *T. reesei* have predicted orthologs in *T. virens*. Moreover, *T. virens* has the most secondary metabolite genes in every class, with 22 NRPS, 18 PKS, 16 terpenoid synthase domain, and 4 NRPS-PKS hybrid-encoding genes. Finally, the genome of *T. atroviride* is predicted to contain genes for 14 NRPSs, 18 PKSs, a single NRPS-PKS hybrid, and 14 terpenoid synthase domains (4, 23) (see Table S1 in the supplemental material). Phylogenetic analysis has been conducted on the inventory of both PKSs and NRPSs predicted in these genomes, and the results recapitulate much of what has been seen previously from similar multigenome studies looking at a wider breadth of ascomycetes (4, 531–533). In particular, the variability of secondary metabolite backbone genes both in total and for each class is not only found across the ascomycetes with sequenced genomes but also within the genomes from the genus *Trichoderma*.

The diversity of secondary metabolites in *Trichoderma* is thought to play a role in the interactions of these organisms with bacteria, plants, and other fungi. This was illustrated in a comparative study of *T. reesei*, *T. atroviride*, and *T. virens* confrontation with *Rhizoctonia solani* (22). In *T. virens*, the gliotoxin-associated NRPS (TV_78708) is upregulated, while in *T. reesei* (TR_60118) and *T. atroviride* (TA_134224 and TA_134224) PKS genes in each fungus are upregulated (22). In *T. atroviride*, a lipoxygenase (TA_33350) that is thought to be responsible for γ-pentyl-pyrone (a coconut-smelling antifungal compound) is also upregulated (22, 504). One commonality shown by this study was the downregulation of NRPS-encoding genes (including the peptaibols) (22). Thus, each species has a specific response wherein only a subset of secondary metabolite genes are upregulated at a given time or condition.

Because transformation tools are available for *T. reesei*, *T. atroviride*, and *T. virens*, reverse genetics studies of secondary metabolite regulation have been performed. Based on results from studies initially performed in *A. nidulans* and which led to the discovery that *laeA* and VELVET play important roles in secondary metabolite regulation, genes encoding LAE1 (TR_41617, TA_302782, and TV_31676) and/or VEL1 (TR_122284, TA_42972, and TV_164251) have been characterized in *T. reesei*, *T. atroviride*, and *T virens* (129, 346, 534–538). Deletion and overexpression studies in *T. reesei* indicated that LAE1 was involved in secondary metabolite regulation but not to the extent found in other ascomycetes (129, 535, 537). Similar to LAE1 in *T. reesei*, the *T. atroviride* homolog has a limited role in regulating secondary metabolite production. When *lae1* is deleted, the lipoxygenase (TA_33350) predicted to be involved in γ-pentyl-pyrone production and production of two polyketides is downregulated (537). Deletion of VEL1 in either *T. reesei* or *T. virens* produces a pleiotrophic phenotype that includes changes to expression of known and predicted genes whose products are predicted to be involved in secondary metabolite production (346, 534, 538). In *T. reesei*, metabolite analysis revealed that VEL1 both positively and negatively influences secondary metabolite production, and this contributes to mate recognition upon initiation of sexual development (534).

While at the genomic level the inventory of secondary metabolite genes has been characterized, there are only a few studies that have associated secondary metabolites with their biosynthetic pathways. A terpene cyclase deleted from *T. virens* (*vir4*; TV_56195) leads to a lack of production of volatile sesquiterpenes from this fungus (539). The deletion of the polyketide predicted by phylogenetic analysis to be involved in *T. reesei* pigment production leads to a pleotropic phenotype, including nonpigmented or "albino" conidia and changes to fruiting bodies (540). A well-characterized group of secondary metabolites found in these fungi are large nonribosomal peptides called peptaibols (541). A database of peptaibols has been established (542). The peptaibol peptides contain between 10 and 25 amino acids. The first peptaibol synthetase-encoding gene was cloned from *T. virens* (TV_66940), and there are predicted peptaibol synthetases as well in *T. reesei* (TR_23171 and TR_123786), *T. atroviride* (TA_317938 and TA_323018), and *T. virens* (TV_66940, TV_10003, and TV_69362), (541, 543). The genes encoding the peptaibol NRPSs are over 50 kb in length and rank as the largest known genes in fungi. Another well-characterized secondary metabolite from the genus *Trichoderma* is gliotoxin, a nonribosomal peptide from the epidithiodioxopiperazine (ETP) class. ETPs have a dioxopiperazine ring with a disulfide bridge (126, 544). This ETP NRPS (TV_78708) compound was initially characterized over 70 years ago, and genes for its biosynthesis in *T. virens* have been identified (545–547). An ETP-type NRPS (TR_24586) has also been noted in the genome of *T. reesei*, but the compound it makes has not been characterized (23, 544).

Conclusions. The significance of shared and "unique" orthologous secondary metabolite genes and gene clusters between these three *Trichoderma* species is largely unclear due to the small number of studies linking secondary metabolite gene clusters to metabolite structures and metabolite functions. In addition, research addressing how secondary metabolite production is regulated under a variety of growth conditions and how this differs from secondary metabolite regulation in other fungi such as in the genus *Aspergillus* must continue. Future studies aimed at understanding regulation and function of secondary metabolites will be critical in determining how the chemical production potential of *Trichoderma* relates to its ecological niche and industrial microbiology roles, such as biocontrol and enzyme production. The paucity of chemical genetic studies linking genes to secondary metabolites represents a significant knowledge gap and therefore an excellent opportunity for future basic and applied research.

Glycosylation

After protein phosphorylation, glycosylation is the most frequent posttranslational modification in eukaryotic cells, including of course fungi. The main types of protein glycosylation found in the fungal cell are *N*-linked and *O*-linked glycosylation (Fig. 11), where an oligosaccharide is covalently attached to Asn and Thr/Ser residues, respectively, and the addition of GPI at the C terminus, which is known as a GPI anchor (548–550). These biosynthetic pathways are essential for cell viability, to maintain proper cell wall structure and organization, and for virulence (549–551).

***N*-linked glycosylation.** The biosynthesis of *N*-linked glycans begins at the cytosolic face of the ER, where the glycolipid $Man_5GlcNAc_2$-PP-dolichol is synthesized (548). These enzymes transfer the sugar units from GDP-Man and UDP-GlcNAc to dolichol in a stepwise process. Then, the $Man_5GlcNAc_2$-PP-dolichol is translocated to the ER lumen by a flippase activity, whose encoding gene has not been identified yet (552). This glycolipid ($Man_5GlcNAc_2$-PP-dolichol) is further modified by glycosyl transferases that transfer sugars from the donors

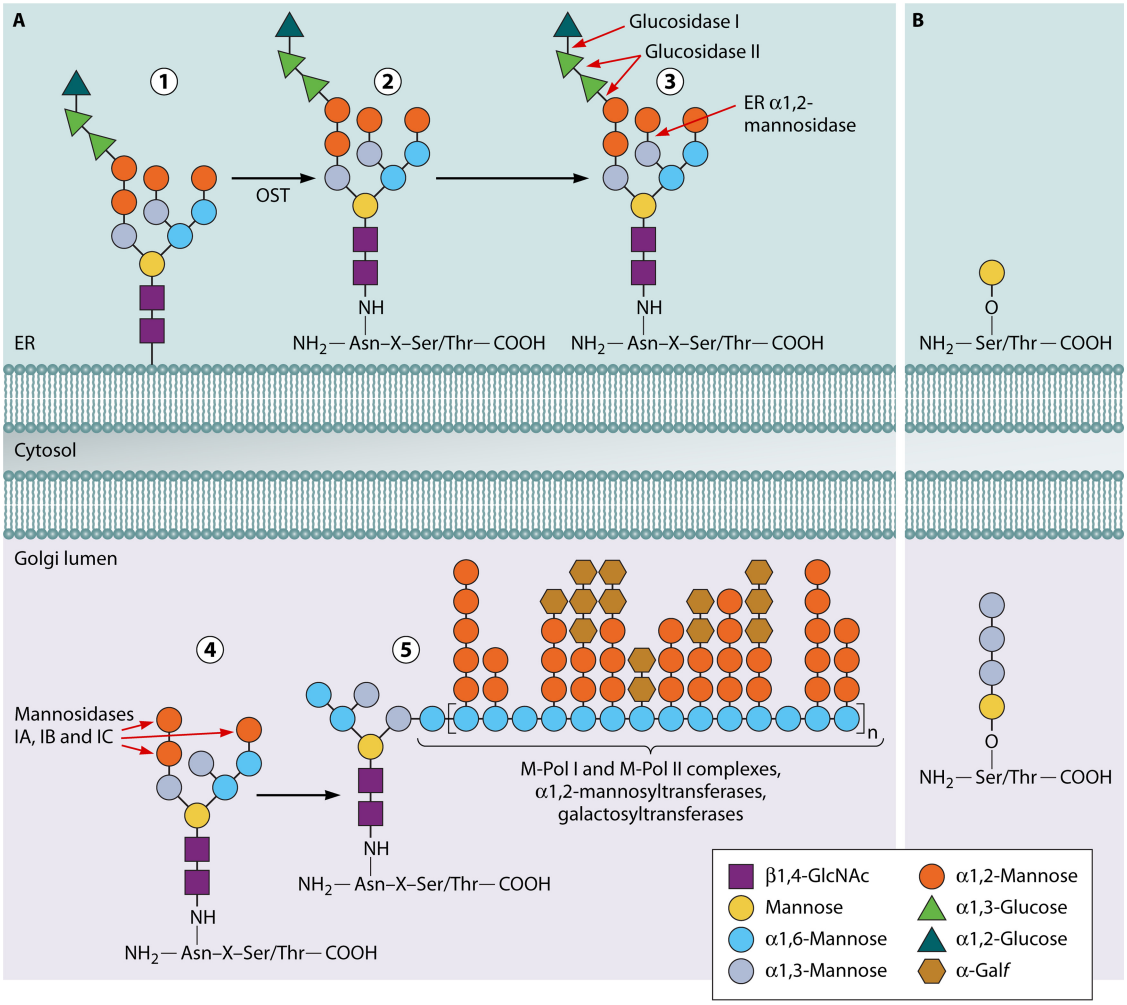

FIG 11 Representation of the putative pathways involved in the elaboration of *N*-linked glycans (A) and *O*-linked glycans (B) in *Trichoderma*. (Step 1) The elaboration of *N*-linked glycans begins in the ER with the synthesis of the oligosaccharide Glc3Man9GlcNAC2 attached to dolichol. (Step 2) Then, this oligosaccharide is transferred to the nascent proteins by the oligosaccharyl transferase complex (OST) and is processed by the ER glucosidades (TR_121351, TA_77708, and TV_86135) and α-1,2-mannosidase (TR_2662, TA_234256, and TV_82921), generating Man8GlcNAC2 (Step 3). Proteins that are expected to form part of OST are TR_119600, TR_33342, TR_45732, TR_120807, TR_64600, and TR_80584 in *T. reesei*; TA_298272, TA_148232, TA_301378, TA_148028, TA_155537, and TA_298708 in *T. atroviride*; TV_79118, TV_121404, TV_111960, TV_75107, TV_85177, and TV_88481 in *T. virens*. (Step 4) Next, the glycoproteins are transported to the Golgi complex, where the *N*-linked glycan undergoes further processing by Golgi complex mannosidases IA, IB, and IC (TR_22252, TR_79044, and TR_79960; TA_284729, TA_161121, and TA_40546; TV_112062, TV_86342, and TV_157800), generating Man5GlcNAC2. (Step 5) Proteins with significant similarity to the Golgi complex mannosidases. Finally, this structure is utilized as molecular scaffold by mannosyltransferases and galactosyltransferases to synthesize the *N*-linked glycan outer chain. Putative proteins participating in this step are TR_65646, TR_4561, TR_80340, TR_46443, TR_58609, TR_81211, TR_82551, TR_105557, TR_79832, TR_109361, TR_21576, TR_69868, TR_69211, TR_46421, TR_66687, and TR_48178 in *T. reesei*; TA_41037, TA_132346, TA_133591, TA_132601, TA_30858, TA_143140, TA_132930, TA_301583, TA_46185, TA_81367, TA_297159, TA_38402, TA_83451, TA_240794, TA_291006, and TA_301560 in *T. atroviride*; TV_66986, TV_32495, TV_89619, TV_231261, TV_87298, TV_82029, TV_83396, TV_184845, TV_77126, TV_31851, TV_76470, TV_33900, TV_73292, TV_83426, TV_183497, and TV_111228 in *T. virens*. The elaboration of *O*-linked glycans starts in the ER, where members of the PMT family transfer a mannose unit to Ser/Thr residues. Then, glycoproteins are exported to the Golgi complex, where mannosyltransferases elongate the *O*-linked glycan. It is likely that the *O*-linked glycans are also decorated with galactose units.

dolichol-PP-Man (DPM) and dolichol-PP-Glc, generating $Glc_3Man_9GlcNAc_2$-PP-dolichol (548, 553). Once synthesized, the oligosaccharide is transferred *en bloc* by the oligosaccharyl transferase complex, which recognizes the Asn residue within the *N*-linked glycosylation sequon Asn-X-Ser/Thr (where X can be any amino acid except Pro) (554, 555). The *N*-linked glycan attached to proteins then undergoes a series of modifications by ER α-glycosidases. Glucosidase I removes the outermost α1,2-glucose unit (556), glucosidase II trims the two remaining glucose residues (557), and finally one mannose residue from the middle branch of $Man_9GlcNAc_2$ is removed by the ER α1,2,-mannosidase (557, 558). The genomes of *T. reesei*, *T. atroviride*, and *T. virens* contain all the genes required for synthesis of $Glc_3Man_9GlcNAc_2$, transfer to nascent proteins, and processing by ER α-glycosidases (see Table S1 in the supplemental material). Among the genome sequences of the three *Trichoderma* species, we did not find any differences in the number of genes involved in these biosynthetic steps when compared to other eukaryotic organisms.

Then, the glycoproteins are transported to the Golgi complex where they undergo different modifications by species-specific

glycosyl hydrolases and transferases. In lower eukaryotic cells such as *S. cerevisiae* there are no Golgi glycosyl hydrolases and thus the *N*-linked glycans do not suffer any further hydrolytic processing (559). However, in filamentous fungi such as *Aspergillus* and *Penicillium*, there are mannosidases that process $Man_8GlcNAc_2$ to $Man_5GlcNAc_2$ (549, 560, 561). These are enzymes classified within family 47 of the glycosylhydrolases (562), and along with the ER α1,2-mannosidase form the class I mannosidases (563). When biochemically characterized, these enzymes have similar enzymatic properties but differ in their processing profiles for the $Man_9GlcNAc_2$ oligosaccharide. The ER enzymes selectively remove one mannose residue from the middle branch, while the Golgi complex enzymes remove the other α1,2-mannose residues present in $Man_8GlcNAc_2$, generating $Man_5GlcNAc_2$ (563). This selective processing by ER α1,2-mannosidase is due to the presence of an Arg at position 273 (within *S. cerevisiae* Mns1) (564). *T. atroviride*, *T. virens*, and *T. reesei* contain the orthologs of Golgi complex mannosidases IA, IB, and IC (see Table S1 in the supplemental material), which indicates that these organisms modify the *N*-linked glycan core in a similar way as other filamentous fungi. Accordingly, the *N*-linked glycans from some secreted proteins of *Trichoderma* can contain $Man_{5-9}GlcNAc_2$ (565). Interestingly, *T. reesei* and *T. atroviride*, but not *T. virens*, contain a fourth gene that likely encodes a Golgi complex α1,2-mannosidase (TR_111953 and TA_155013), as they contain a Met and Leu at the equivalent position of Arg^{273}. Other filamentous fungi analyzed so far do not have this extra mannosidase activity within the Golgi complex (549, 563); thus, it is currently unknown how this enzyme could participate within the *N*-linked glycan elaboration.

In *S. cerevisiae*, the *N*-linked glycan core is further modified by mannosyltransferases that generate high-mannose-content *N*-linked glycans (566). The first step during the *N*-linked glycan outer chain elaboration is carried out by the α1,6-mannosyltransferase Och1, which starts the elaboration of the outer chain backbone α1,6-polymannose, and this is further extended by the α1,6-mannosyltransferase complexes MolP-I and MolP-II (566). *T. reesei*, *T. atroviride*, and *T. virens* contain all the elements for these biochemical processes, indicating elaboration of this *N*-linked glycan outer chain (see Table S1 in the supplemental material). As with other filamentous fungi, such as *A. fumigatus*, the genomes of *T. atroviride*, *T. virens*, and *T. reesei* contain the *OCH-1*-like gene family, including *OCH-2*, *OCH-3*, and *OCH-4*, although it has been reported that they do not participate in the classic mannosylation pathway described for *S. cerevisiae* (567). Since the *Trichoderma* genomes contain orthologs of members of the *KRE2/MNT1* gene family, it is likely that the outer chain backbone is further modified by branches composed of α1,2-mannose residues (568). However, no ortholog for α1,3-mannosylation or phosphomannosylation were found for *T. atroviride*, *T. virens*, or *T. reesei*, suggesting the N-linked glycan outer chain lacks these sugar modifications.

The fact that *T. atroviride*, *T. virens*, and *T. reesei* have three class I Golgi complex mannosidases indicates that these organisms have the potential to generate hybrid and complex *N*-linked glycans (569). Accordingly, their genomes contain a gene encoding a protein with high similarity to *N*-acetylglucosaminyltransferase III (see Table S1 in the supplemental material) that adds the bisecting GlcNAc residue found in both hybrid and complex *N*-linked glycans from higher eukaryotes, such as mammals (570). However, their genomes do not contain any ortholog for Golgi complex mannosidases from family 38 of glycosylhydrolases, which trim the α1,3- and α1,6-mannose forms $Man_5GlcNAc_2$, a fundamental step during elongation of complex *N*-linked glycans (569). Therefore, it is likely that only elaboration of hybrid and high-mannose *N*-linked glycans takes place in *Trichoderma*. Finally, as the genomes of these organisms contain the ortholog of *A. nidulans ugmA*, the gene encoding the UDP-galactopyranose mutase involved in the generation of UDP-galactofuranose, the building unit of galactomannans in *Aspergillus* (571), it is likely that *Trichoderma* can also synthesize galactose-containing *N*-linked glycans.

Glycoprotein quality control. The ER α-glycosidases participating in the *N*-linked glycosylation pathway also have a role in glycoprotein endoplasmic reticulum-associated degradation, where misfolded proteins are labeled for degradation by the cytosolic proteasome (572). Once transferred to the protein backbone and processed by glucosidase I, the $Glc_2Man_9GlcNAc_2$ oligosaccharide is trimmed by glucosidase II, generating $GlcMan_9GlcNAc_2$ (550). This monoglucosylated oligosaccharide is recognized by calnexin and calreticulin, which in turn interact with ERp57/Pdi1, facilitating protein folding (572). Glucosidase II then removes the last glucose residue from the *N*-linked glycan core, releasing the glycoprotein from interaction with calnexin/calreticulin. If the glycoprotein is not properly folded, then it is recognized by the UDP-glucose:glycoprotein α-glucosyltransferase that reglucosylates the $Man_9GlcNAc_2$ oligosaccharide, allowing interaction again with calnexin/calreticulin (572). This cycle is disrupted by the action of ER α1,2-mannosidase and EDEM proteins (ER degradation-enhancing α-mannosidase-like protein) that demmannosylate the *N*-linked glycan core, generating a structure unable to be recognized by UDP-glucose:glycoprotein α-glucosyltransferase. If the glycoprotein is still misfolded, it is labeled for retrograde transport to the cytosolic compartment and degradation (573). *T. reesei*, *T. atroviride*, and *T. virens* contain genes for all the components for this ER quality control system, including one putative ortholog of calnexin, one of a protein disulfide isomerase, one of UDP-glucose:glycoprotein α-glucosyltransferase, and EDEM proteins (see Table S1 in the supplemental material). In the latter, we found differences among the genomes of *Trichoderma* species: while *T. reesei* has putative orthologs of EDEM1 (TR_64285), EDEM2 (TR_45717), and EDEM3 (TR_65380), *T. atroviride* contains only orthologs of EDEM1 (TA_89446) and EDEM2 (TA_127412), but no obvious ortholog for EDEM3, and *T. virens* has all three orthologs (TV_31872, TV_78530, and TV_54636) as well as a putative gene duplication for EDEM3 (TV_193426). Whether these changes have a significant impact in the ER quality control mechanisms for glycoproteins remains to be investigated.

***O*-linked glycosylation.** The pathway for *O*-linked glycosylation begins in the ER, but in contrast to *N*-linked glycosylation, there is not a sequon to add sugar residues to Ser or Thr residues. In fungi, this pathway involves the participation of protein mannosyltransferases, which are encoded by members of the *PMT* family that transfer one mannose group from DPM (dolichol-PP-Man) to the target protein (574). The proteins encoded by this gene family do not have redundant activity *in vivo*, as each member has specific substrate specificities, and thus they are classified into subfamilies: *PMT1*, *PMT2*, and *PMT4* (574). In *S. cerevisiae* there are seven members in this gene family, but in filamentous fungi there is only one member in each subfamily (574–576), in-

cluding in *T. reesei*, *T. atroviride*, and *T. virens* (551, 577) (see Table S1 in the supplemental material). Then, the *O*-linked glycans are further elongated by members of the *KRE2/MNT1* gene family which, as in *N*-linked glycosylation, adds α1,2-mannose residues, forming linear α1,2-mannose polymers that in *Trichoderma* can contain up to three monosaccharide units (565, 578). Analysis of *S. cerevisiae* and *C. albicans KRE2/MNT1* gene family members indicated that *Trichoderma KTR1* and *KRE2* orthologs are likely to be involved in the *O*-linked glycosylation pathway (568, 579, 580). Since some family members are quite promiscuous in terms of substrate specificity, and the functional roles of *S. cerevisiae* Ktr4 and Ktr5 have not been clearly established (568), it is possible that the orthologs found in *Trichoderma* could also participate in the biosynthesis of these glycans.

The *O*-linked glycosylation activity is regulated by DPM synthase (EC 2.4.1.83), a key enzyme in this process. In *Trichoderma*, DPM synthase forms an enzymatic complex of three proteins, DPMI, DPMII, and DPMIII (581, 582), which contrasts with the situation in yeast, where DPM synthase consists of a single Dpm1 protein (583, 584). The yeast Dpm1 protein is an equivalent of the DPMI catalytic subunit of *Trichoderma* DPM synthase (582). This difference in DPM synthase structures was a reason for grouping the enzymes into two groups: the *S. cerevisiae* group, having a single-protein enzyme, and the human group, with an enzymatic complex of DPM synthases (585). The predicted *T. reesei* DPMI protein shows 65% identity and 82% similarity to the homologous human protein but only 28% identity to the *S. cerevisiae* Dpm1. It was shown that DPM1 from *Trichoderma* could not rescue the *dpm1*Δ mutation in *S. cerevisiae* (581). Comparison of the Dpm1 proteins from yeast and *Trichoderma* showed that the DPMI protein from *Trichoderma* lacks the C-terminal transmembrane domain present in yeast Dpm1. Furthermore, a chimeric *T. reesei* DPMI protein with the yeast transmembrane domain at the C terminus was also not functional in a yeast *dpm1*Δ mutant (581). Expression of different combinations of the *dpm1*, *dpm2*, and *dpm3* genes from *Trichoderma* in yeast revealed that besides the DPMI catalytic subunit, DPMIII subunit is indispensable for rescuing the *dpm1*Δ mutation (582). In contrast, the Dpm1 protein from *S. cerevisiae* itself is functional in *Trichoderma*. Overexpression of the yeast *DPM1* gene in *T. reesei* resulted in 2-fold-higher activity of DPM synthase and increased *O*-linked glycosylation abilities (493, 586).

GPI synthesis. The elaboration of GPI anchors, as the early steps in *N*-linked glycosylation, is quite conserved from *S. cerevisiae* to mammalian cells (587). The pathway begins in the ER cytosolic face, where phosphatidylinositol (PI) is modified with a GlcNAc residue in a reaction catalyzed by the phosphatidyl inositol:*N*-acetylglucosaminyl transferase complex (PI-GnT) (588), and this sugar moiety is subsequently de-N-acetylated by Gpi12 (589). This glycolipid is then translocated to the ER luminal face by a flippase activity, followed by acetylation of the inositol moiety and addition of one α1,4-mannose residue to the GlcN moiety by Gwt1 and Gpi14, respectively (587, 590). This glycolipid is further mannosylated and then modified with ethanolamine phosphate groups, generating the characteristic GPI structure (see reference 587 for details).

Finally, the transfer of the glycolipid to the polypeptide is carried out by the GPI transamidase, a complex composed of five ER proteins that removes the C terminus of the target protein, which contains the GPI signal sequence, and covalently attach the GPI anchor (587). *T. reesei*, *T. atroviride*, and *T. virens* contain all the genes involved in this biosynthetic pathway, including the glycosyltransferases and transamidases found in other fungal systems (see Table S1 in the supplemental material). We could not find any difference in terms of the quantity of putative genes involved in GPI elaboration among the *Trichoderma* species, stressing the high degree of conservation of this pathway in eukaryotic cells.

Conclusions. The protein glycosylation pathways are well conserved in *T. reesei*, *T. atroviride*, and *T. virens*, as in other eukaryotic organisms, and no significant differences are predicted from the genomic analysis. There is a small difference in the number of EDEM family members among the *Trichoderma* species, i.e., a lack of EDEM3 in *T. atroviride* and an additional ortholog in *T. virens*, but transcriptomic analysis did not show any significant overexpression or negative gene regulation. It remains to be addressed whether these changes have a significant impact on the ER control quality mechanisms.

Transport

Filamentous fungi are well known for their ability to utilize a wide range of carbon and nitrogen sources. They are well adapted to harsh environments, surrounded by toxins, which are synthesized by other microorganisms (6, 591). Hence, it is not surprising that fungi developed transport systems for efficient uptake of nutrients and ions for efficient efflux of antifungal compounds and for communication (592). Successful competition of *Trichoderma* in its natural habitat is speculated to be supported by the strong expansion of genes coding for proteins involved in transport (4).

Among fungal transporters, ABC (ATP-binding cassette) transporters and MFS transporters have been most intensively studied (593–595). Transporters of the ABC transporter superfamily are primarily ATP-dependent efflux transporters, which often contribute to multidrug resistance (MDR) in (fungal) pathogens (596). In general, the structures of ABC transporters comprise a nucleotide binding domain and a transmembrane domain. In contrast, MFS transporters lack a nucleotide binding domain and are consequently smaller in size. They transport small molecules in response to chemiosmotic ion gradients (592, 596).

ORFs encoding transporters were identified in the genome of *T. reesei* according to the community annotation of the genome (23). Additionally, the predicted gene models of *T. reesei* along with sequences from known transporters of other fungi were used for BLAST searches in the genomes of *T. atroviride* and *T. virens*. Putative homologs (E value cutoff, 1e−10) were checked by bidirectional BLAST analysis and the presence of transmembrane domains before being considered for further analysis. However, soluble ABC proteins lacking transmembrane domains were also identified in the genomes of fungi, even if their functions are not related to transport (596). Hence, only predicted transporters comprising transmembrane domains are discussed below.

The total number of transporters identified in the individual *Trichoderma* genomes was in good agreement with the respective genome size (*T. virens* > *T. atroviride* > *T. reesei*). In summary, we found for *T. reesei* 138 transporters, whereas 158 transporters and 186 transporters were predicted in *T. atroviride* and *T. virens*, respectively (see Table S1 in the supplemental material).

The largest group of transporters belongs to the class of sugar transporters, with 50 identified in *T. reesei*, 61 in *T. virens*, and 64 in *T. atroviride*, followed by ABC transporters, with 46 in *T. reesei*, 61 in *T. virens*, and 46 in *T. atroviride* (Table 1).

TABLE 1 Overview of transporters identified in genomes of *Trichoderma* species

Transporter classification	No. of transporter type identified in species		
	T. reesei	*T. virens*	*T. atroviride*
Sugar transporter	50	74	64
ABC transporter	46	61	46
General substrate transporter	14	17	16
Transporter (sulfur metabolism)	5	5	6
Mg^{2+} transporter	3	3	3
Oligopeptide transporter	4	5	5
Tetrapeptide transporter	8	10	9
UDP-glucose/galactose transporter	3	3	3
Zinc transporter	5	8	6
Total	138	186	158

Transporters associated with sulfur assimilation and transport of sulfate, methionine, and thiosulfate are discussed in more detail in the section dealing with sulfur metabolism. Both TR_75475 and TR_81546 have similarity to sulfate transporters of the SulP family. However, the similarity to known and experimentally confirmed sulfate transporters is below 30%. Consequently, TR_75475 and TR_81546 were not further analyzed (see the "Sulfur metabolism" section).

ABC transporters. *T. reesei*, *T. atroviride*, and *T. virens* encode a higher number of ABC transporters than *N. crassa*, *S. cerevisiae*, or *S. pombe* (71, 597, 598). The largest set of ABC transporters was found in *T. virens*, with 61 in total and 12 unique ones with no homologs in *T. reesei* and *T. atroviride*. In contrast, genomes of *T. reesei* and *T. atroviride* contained a considerably smaller number of ABC transporters, and unique ABC transporters were predicted. Three unique ABC transporters were identified for *T. reesei* and two for *T. atroviride*.

The role of ABC transporters in fungi has so far been studied in the contexts of secretion of secondary metabolites, resistance to toxic compounds, and cell signaling. In addition, ABC transporters were recently suggested to be involved in biological control of pests for plant protection (22). A comparative transcriptomic study revealed that ABC efflux transporters are transcribed in *T. reesei*, *T. atroviride*, and *T. virens* at several stages of mycoparasitism (22). In another study, the *T. atroviride* ABC transporter TA_53468 (TAABC2) was demonstrated to be upregulated in the presence of pathogen-secreted metabolites, mycotoxins, and fungicides (599). Deletion mutants of TA_53468 were analyzed and showed an important role for this ABC transporter in antagonism and biocontrol (599). Homologs of TA_53468 were predicted both in *T. reesei* and *T. virens*. In a plate confrontation assay, another ABC transporter of *T. atroviride*, TA_289170, was slightly downregulated before physical contact with the host compared to the control samples. Interestingly, genes involved in transport and metabolism of secondary metabolites were shown to be downregulated during mycoparasitic interactions (355). No homologs of TA_289170 were predicted in the genome of *T. reesei* or *T. virens*. As *Trichoderma* strains are well-known producers of antagonistic metabolites against fungi and bacteria, the observed downregulation of these transporters is surprising.

ABC transporters are known to contribute to multidrug resistance in microbial pathogens and tumor cells, but so far fungal ABC transporters have been barely studied in this context (596). In *N. crassa*, four homologs (CDR4, ATRB, ATRF, and ATRF-2) of *S. cerevisiae* Pdr5p were identified. However, only CDR4 was found to be important for azole resistance (600). Homologs for *N. crassa* CDR4, ATRB, ATRF, and ATRF-2 were identified in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 in the supplemental material). Several *Trichoderma* species, e.g., *T. longibrachiatum*, are known to cause infections in immunosuppressed humans. In antifungal therapy, *Trichoderma* spp. have shown low susceptibilities to amphotericin B and available azole derivatives (601, 602). To our knowledge azole resistance in *Trichoderma* has not been studied yet at the molecular level. It might be interesting to investigate azole susceptibility in the nonpathogenic *T. reesei*, which is phylogenetically closely related to *T. longibrachiatum*.

Trichoderma ABC transporters have been shown to be important for cell-to-cell communication, e.g., the pheromone transporter STE6 in sexual development (350). The yeast ABC transporter Ste6p was shown to catalyze ATP hydrolysis coupled to **a**-factor transport, which promotes mating (603). The *T. reesei* Ste6p homolog (TR_62693) is positively regulated by the phosducin-like protein (PhLP1) and was consequently suggested to impact mating efficiency (350). In another study, TR_62693 was strongly upregulated during growth on lactose (604), supporting an effect of the carbon source on sexual development. Homologs of TR_62693 were identified in the genomes of *T. virens* and *T. atroviride*.

MFS transporters (sugar transporters/general substrate transporters). A paralogous gene expansion of solute transporters of the MFS family in *Trichoderma* was recently reported (4). The set of sugar transporters was considerably smaller in *T. reesei* than in *T. atroviride* and *T. virens* (4). In fact, 50 sugar transporters were predicted in *T. reesei*, while there were 61 in *T. virens* and 64 in *T. atroviride*.

Interestingly, no single unique sugar transporter was predicted in *T. reesei*. In contrast, 5 unique transporters were predicted in *T. atroviride* and 10 unique transporters in *T. virens*. For 11 transporters of *T. virens*, homologs were exclusively identified in *T. atroviride*, whereas only for 3 transporters of *T. virens* homologs were predicted in the genome of *T. reesei*. Only one transporter predicted for both *T. reesei* and *T. atroviride* showed no homolog in *T. virens*.

Mycoparasitism-related genes of *T. atroviride* were identified in a comparative transcriptome analysis during confrontation of *T. atroviride* with *R. solani* (355). Two genes encoding sugar transporters (TA_133590 and TA_150280) were shown to be downregulated compared to control conditions, independent of physical distance. However, the function of these sugar transporters is still unclear. Homologs of both TA_133590 and TA_150280 were predicted in the genome of *T. reesei* and *T. virens*, but evidence for their transcription is not available.

Cellulases and hemicellulases produced by *Trichoderma* are used in a variety of industrial applications (6). Lactose is a commonly used soluble carbon source to induce these enzymes at an industrial level. Although the molecular mechanism of induction is not fully understood, lactose was found to induce a high number of putative MFS transporters in *T. reesei* (604). Deletion of the 14 putative transporters that were upregulated upon growth on lactose led to identification of one gene (TR_3405) that is essential for lactose uptake and utilization. It was hypothesized that in the *T. reesei* hypercelluloytic strain PC-3-7, TR_3405 plays a pivotal

role in the induction of cellulase genes but is not directly involved in the uptake of cellulase inducers or lactose (605). MFS sugar transporters TR_77517, TR_3405, and TR_79202 were identified to be critical for cellulose production in lactose cultures (605). TR_77517 shows high similarity to lactose transporters, whereas TR_3405 and TR_79202 are both related to sucrose transporters (605). Homologs of TR_3405, TR_77517, and TR_79202 were found in the genomes of *T. atroviride* and *T. virens*. Interestingly, the respective homologs in *T. atroviride* were shown to be upregulated in the early response to mechanical damage (15). In the same study, transcription of genes encoding sugar transporters was detected in *T. atroviride*. Homologs of these genes were analyzed in *T. virens* but not in *T. reesei*.

Regulation of glycoside hydrolase genes in *Trichoderma* is affected by nutritional signals and environmental signals, including light (4). Hence, it is not surprising that sugar transporters (TR_76800 and TR_121441), a putative carboxylic acid transporter (TR_10556), and a predicted oligopeptide transporter (TR_44278) are targets of light signaling in *Trichoderma* (351). Homologs of these light-dependent regulators were predicted for both *T. virens* and *T. atroviride*. The *T. atroviride* homolog of TR_44278 was found to be upregulated in response to mechanical damage, confirming the important role of transporters in the injury-response mechanism of *Trichoderma*. Apart from sugar transporters, ABC transporters were also shown to be regulated by light (351).

Tetrapeptide transporters/zinc transporters. A gene expansion of tetrapeptide transporters in *Trichoderma* was observed in this study. The set of peptide transporters was considerably larger in the genome of *T. atroviride* than in that of *T. reesei* and *T. virens*. The putative *T. reesei* tetrapeptide transporter TR_59364, which was shown to be regulated in response to light, shares no homologs with *T. virens* and *T. atroviride*. However, the function of TR_59364 is not yet known, and its role in transport remains to be determined.

Five putative zinc transporters were predicted in the genome of *T. reesei*. Respective homologs were identified in both *T. atroviride* and *T. virens*. Evidence for transcriptional activity of the corresponding genes was reported in *T. reesei*. A higher number of putative zinc transporters was identified in *T. virens*. However, no evidence for transcription of these genes is available (22).

Conclusions. A large paralogous gene expansion was observed both for solute MFS transporters and ABC transporters of *T. atroviride* and *T. virens*, but not for *T. reesei* compared to other ascomycete genomes. ABC transporters are suggested to be involved in resistance to toxic compounds and cell signaling, whereas MFS transporters demonstrate an essential role in nutrient uptake. Hence, the strong expansion of transporters in *T. virens* and *T. atroviride* reflects the adaption to their natural habitat and lifestyle, i.e., mycoparasitism and the competition with other saprotrophs for limiting nutrients.

ENVIRONMENTAL SIGNALING

A proper reaction to a changing environment is crucial for survival and competition of fungi. From spore germination to nutrient sensing to detection of pheromones for initiation of a mating response or activation of chemical warfare to fight competitors, fungi send signals and react to signals (13, 606, 607). These extracellular signals are received by uptake of low-molecular-weight substances and intracellular activation of a signaling pathway or by binding of a ligand to a membrane receptor which is responsible for signal transmission. Reception of diverse environmental signals initiates a complex network, which is responsible for signal integration and aimed at a defined response in terms of gene regulation to optimally react to the conditions at hand. In order to achieve this task, the signaling pathways described below act sequentially or in parallel, with interconnections at several nodes; however, these pathways and nodes require considerable further research to be fully understood.

The Heterotrimeric G-Protein Pathway

Cell signaling mediated by heterotrimeric G-proteins is highly conserved in eukaryotes and involves three essential elements: a G-protein-coupled membrane receptor (GPCR), a heterotrimeric G-protein (composed of the three subunits, Gα, Gβ, and Gγ), and an effector (608). The GPCR, often also referred to as the 7-transmembrane (7-TM) receptor, is located at the cell surface, where it acts as a sensor. GPCRs are typically composed of seven membrane-spanning regions connected by intra- and extracellular loops, with typically the N terminus located outside and the C terminus inside the cell (609). In the classic paradigm, the recognition of an external stimulus by the GPCR causes the exchange of the GDP bound to the Gα subunit of the heterotrimeric G-protein with GTP, releasing the Gβγ dimer. The released dimer and/or the Gα subunit then interact with effectors, such as the adenylate cyclase and MAP kinase cascades, which affect various downstream targets (608, 610). Over the past decade, however, it became evident that G-protein-independent interaction partners are also capable of mediating GPCR-derived signals (610, 611).

Heterotrimeric G-proteins have been studied in detail in several filamentous fungi, revealing their involvement in the regulation of cellular responses, such as growth, mating, morphogenesis, cell division, and pathogenic development (610). In contrast, little information is available on the role of fungal GPCRs, although a considerable number of these 7-TM receptors are encoded in the genomes of filamentous fungi (610).

Heterotrimeric G-proteins. Similar to most filamentous fungi (610), three highly conserved Gα, one Gβ, and one Gγ subunit (see Table S1 in the supplemental material) are encoded in the genomes of *T. reesei*, *T. atroviride*, and *T. virens*, respectively (607, 612). It was previously shown that signaling via the adenylate cyclase inhibiting Tga1 (TA_299351, in *T. atroviride*) and TgaA (TV_80697, in *T. virens*) and the adenylate cyclase-activating Tga3 (TA_34532, in *T. atroviride*) and GNA3 (TR_21505, in *T. reesei*) Gα proteins play essential roles in regulating mycoparasitism-relevant processes, such as attachment to and coiling around host hyphae, production of cell wall-lytic enzymes, and secondary metabolites (53, 613–615). However, despite the considerable 99% conservation between *T. atroviride* Tga1 and *T. virens* TgaA proteins, deletion of the corresponding genes resulted in quite different phenotypic consequences. *T. atroviride* Δ*tga1* mutants showed constitutive sporulation, reduced growth, and an avirulent phenotype against *R. solani* (613), whereas *T. virens* Δ*tgaA* mutants grew and sporulated like the wild type and were fully pathogenic against *R. solani*, but had a reduced ability to colonize *S. rolfsii* sclerotia (616). Similar to Δ*tga1* mutants, *T. atroviride* mutants missing the *tga3* gene showed light-independent conidiation, reduced growth, and alterations in the production of chitinases and antifungal metabolites. Tga3 was further found to be essential for the transmission of signals that regulate recognition of the prey

fungus and attachment to its hyphae (614). In *T. reesei*, both GNA1 (TR_123302) and GNA3 are involved in the regulation of cellulase gene transcription by light (617, 618). However, the fact that constitutive activation of GNA3 resulted in a 10-fold upregulation of cellulase gene expression in light but did not lead to inducer-independent cellulase formation rules out that GNA3 transmits the crucial signal for the presence of cellulose (618). Thereby, light-dependent regulation of *gna1* and *gna3* is mediated by ENV1, either by an influence on transcript levels (*gna3*) or via a positive feedback loop upon activation (619).

Functional characterization of the Gβ (GNB1; TR_46469) and the Gγ (GNG1; TR_75949) subunits of *T. reesei* revealed that they function in the same pathway together with the class I phosducin PhLP1 (TR_58856), thereby regulating the transcription of glycoside hydrolase genes. The finding that all three proteins, PhLP1, GNB1, and GNG1, are required for the proper regulation of light responsiveness in *T. reesei* confirmed the function of the class I phosducin in efficient folding of the beta-gamma subunits in response to light, according to the function of these proteins in higher eukaryotes (350). Interestingly, *phlp1*, *gnb1*, and *gng1* are involved in regulation of the pheromone gene *hpp1* and the putative pheromone transporter homolog *ste6*, and they influence sexual and asexual development, albeit they are not essential for these processes (350). Moreover, PhLP1 was found to be one factor involved in connecting the nutrient response pathway with the light response pathway (620).

Similar to other filamentous fungi, a high number of proteins with Gβ-like WD40 repeats are encoded in *T. reesei*, *T. atroviride*, and *T. virens*. One of these proteins, CPC-2, is involved in the general amino acid (cross-pathway control) pathway in *N. crassa* (621), and in *T. reesei* the *cpc2* transcript has been found to be upregulated by light (78). In addition, *cpc2* transcription is induced in *T. reesei* upon confrontation with the plant-pathogenic fungus *R. solani* in the precontact stage and after contact but not in the direct contact stage (22).

G-protein-coupled receptors. Recent genome mining of the mycoparasites *T. atroviride* and *T. virens* resulted in the identification of 65 and 76 GPCR-like proteins, compared to 58 candidates in the saprophyte *T. reesei* (54, 622). Phylogenetic analyses grouped the identified putative GPCRs into 14 classes based on established classification systems (610, 622–624). Whereas in classes I to VII (pheromone receptors, putative carbon and nitrogen sensors, cyclic AMP [cAMP] receptor-like proteins, and GPCRs with an RGS domain) and classes X to XII (GPCRs similar to PTM1 and GPCR89 and family C-like GPCRs), orthologous triplets are present in the three species, the other classes show less conservation within the genus. The mycoparasitic species *T. virens* and *T. atroviride* show an expansion of receptors of the PAQR family (class VIII) compared to *T. reesei* and of DUF300 domain-containing (class XIII) and PTH11-like receptors, respectively (622).

So far, the functional characterization of only a single GPCR-encoding gene of *Trichoderma* has been published. *T. atroviride gpr1* (TA_160995) encodes a GPCR belonging to the class of cAMP receptor-like proteins (class V) which was not only shown to be essential for vegetative growth and conidiation (54) but also to govern mycoparasitism-related processes, such as coiling and expression of chitinase-encoding genes upon induction by the living host (625).

Receptors related to human steroid receptor mPR, which respond to progesterone and adiponectin as ligands, have previously been classified as PAQR (progestin–adipo-Q receptors). This family includes 11 mammalian paralogs but also prokaryotic hemolysin-type proteins and the yeast proteins Izh1, Izh2, Izh3, and Izh4 (626, 627). While two to three members of the PAQR family are present in filamentous fungi like *N. crassa*, *A. nidulans*, *F. graminearum*, and *M. grisea* (610), the *T. reesei* genome encodes five (TR_119819, TR_68212, TR_70139, TR_82246, and TR_56426), the *T. virens* genome encodes six (TV_30459, TV_47976, TV_160502, TV_194061, TV_92622, and TV_180426), and the *T. atroviride* genomes encodes seven (TA_290047, TA_210209, TA_142946, TA_46847, TA_152366, TA_142943, and TA_136196), with all members bearing the hemolysin III motif (622). Transcriptional analysis showed a response to the presence of *R. solani* for several of these genes: while *T. virens* TV_160502 and TV_180426, *T. atroviride* TA_152366 and TA_210209, and *T. reesei* TR_56426 were upregulated upon contact with the prey fungus, TA_142946 and TA_136196 in *T. atroviride*, TV_92622, TV_47976, and TV30459 in *T. virens*, and TR_119819 in *T. reesei* showed downregulation (622). The extraordinary expansion of the PAQR family, especially in the mycoparasitic species *T. atroviride* and *T. virens*, together with the prey-dependent transcriptional regulation of several members make these proteins interesting as mediators in the communication of *Trichoderma* with other fungi.

PTH11-like proteins represent the largest class of GPCRs in *Trichoderma*, with 52 members in *T. virens*, 38 members in *T. atroviride*, and 35 members in *T. reesei* (622). The PTH11 receptor was first identified in *M. grisea* (*M. oryzae*) and is characterized by an extracellular amino-terminal CFEM domain followed by seven transmembrane regions. In *M. oryzae*, PTH11 is required for development of the appressorium and it is proposed to act upstream of the cAMP pathway, which is required for pathogenicity (628). A putative role of PTH11-like receptors in governing virulence-related functions is also indicated by the higher number of these proteins encoded in the genomes of pathogenic fungi like *M. grisea* and *F. graminearum*, with 61 and 106 members, respectively, compared to the saprophyte *N. crassa*, with 25 members (610). Among the PTH11-like proteins identified in *T. atroviride*, *T. virens*, and *T. reesei*, only a subset (TV_28615, TV_218092, TV_51635, TV_44825, TA_300847, TA_252553, TA_30137, TR_27992, TR_27983, and TR_62462) contains the fungus-specific CFEM domain, which is in accordance with other fungi of the subphylum Pezizomycotina, to which PTH11-like proteins are restricted.

Interestingly, several PTH11-like GPCRs showed significant upregulation in *T. atroviride* during confrontation with *R. solani* before or at direct contact with the prey (TA_156014, TA_45731, TA_130989, TA_156579, TA_85568, and TA_153140), whereas this was not the case in *T. virens* (22). These differences in the transcriptional regulation of genes putatively involved in host sensing in the two mycoparasites are in accordance with their overall completely different transcriptomic responses. While *T. atroviride* uses a strategy of parasitic interaction primarily involving antibiosis and hydrolytic enzymes, gliotoxin-producing *T. virens* strains are suggested to directly aim to kill the host at a distance by poisoning *R. solani* with gliotoxin (22).

In *T. atroviride*, the expression of several GCPR-encoding genes is triggered by mechanical injury. These include certain PTH11-like receptors (TA_46092, TA_130873, and TA_91914)

and members of classes I to XIII, i.e., the RGS domain-containing GPCRs TA_40423 and TA_210761 (class VI), the PAQR family proteins TA_210209 and TA_142946 (class VIII), and the microbial opsin TA_210598 (class IX). Among the latter, TA_40423 showed the strongest response, as it was 13-fold induced after injury compared to no injury (15). Mechanical injury has been shown to result in enhanced oxidative stress and in an increase in calcium signaling and oxylipin synthesis, leading to the model that the mycelial damage response could use oxylipins as signaling molecules (15). Recently, oxylipin signaling has been reported to depend on the class III GPCR GprD in *A. nidulans* (629). However, the corresponding *T. atroviride* class III member (TA_246916) shows only a moderate transcriptional response to injury.

In *S. pombe*, the PQ-loop containing GPCR Stm1 couples to the Gpa2 Gα subunit and is required for the proper recognition of nitrogen starvation signals (630). Quantification of mRNA levels of the two Stm1-like receptor-encoding genes TA_300620 and TA_238619 by reverse transcription-quantitative PCR revealed >2.5-fold-enhanced transcription upon contact with *R. solani* compared to a self-confrontation control. In addition, the transcription of both genes was >5-fold enhanced upon cultivation on minimal medium with sodium nitrate as the nitrogen source, compared to growth of *T. atroviride* on complete medium (C. Escobar and S. Zeilinger, unpublished data).

Regulators of G-protein signaling. G-protein signaling is regulated not only by interactions of the G-protein subunits with GPCRs but also by phosducins, GTPase-activating proteins, and other proteins (631).

RGS (regulator of G-protein signaling) proteins are GTPase activators which increase the rate of GTP hydrolysis at the Gα subunits, thereby negatively regulating the activity of heterotrimeric G-proteins (631). In the filamentous fungus *A. nidulans*, four RGS proteins are encoded in addition to the RGS domain-containing GprK GPCR (624). Whereas RgsB (TR_78314, TA_152907, and 181927) and RgsC (TR_65607, TA_154987, and TV_212406) are still uncharacterized, FlbA (TR_54395, TA_192509, and TV_72112) was shown to promote asexual sporulation through negative regulation of the FadA Gα protein, and RgsA (TR_72259, TA_41236, and TV_83004) regulates colony growth, aerial hypha formation, and pigmentation by negatively impacting GanB (632, 633). Eight RGS and RGS-like proteins have been identified in the plant pathogen *M. oryzae* that regulate growth, differentiation, and pathogenicity (634). For two of those *M. oryzae* proteins (MoRgs7 and MoRgs8), the RGS domain is linked to a 7-TM motif, and they represent class VI GPCRs.

In each of the three *Trichoderma* species, four RGS proteins could be identified besides the three RGS domain-containing GPCRs present in their genomes. Phylogenetic analysis revealed them as orthologs of the four RGS proteins of *A. nidulans*. RGS1 (TR_54395) as well as the class VI RGS domain-containing GPCR TR_81383 have previously been found in *T. reesei* to be light-independent targets of the class I phosducin PhLP1 (350). Analysis of the available transcriptome data further revealed that the RgsA-like protein TR_72259 is downregulated in *T. reesei* upon confrontation with *R. solani* both before and after contact (22).

Activation of G-protein signaling by GPCRs is based on the guanine-nucleotide exchange activity of the receptors, which are GEFs (guanine-nucleotide exchange factors) for Gα subunits. In addition to the membrane-localized GPCRs, cytoplasmic nonreceptor GEFs, such as *N. crassa* RIC-8 (635), have recently been identified which bind to the G-protein and accelerate guanine-nucleotide exchange. In *M. oryzae*, MoRic8 was shown to interact with and positively regulate MagB (636), and in *N. crassa* RIC-8 exhibited GEF activity toward the GNA-1 and GNA-3 Gα proteins (635). Another protein with GEF activity, Arr4p/Get3p, has been described in *S. cerevisiae*. Arr4p/Get3p is homologous to the catalytic subunit of ArsA, a bacterial ATP-dependent arsenite extrusion pump; it was shown to bind selectively and directly to the Gα subunit Gpa1p, thereby amplifying the pheromone-response pathway in yeast (637).

In each of the three *Trichoderma* species, one RIC8 ortholog (TR_62401, TA_89180, and TV_90332) and one Arr4p/Get3p ortholog (TR_75859, TA_301914, and TV_232207) were identified in the respective genomes, confirming the existence of cytoplasmic nonreceptor activators of G-protein signaling in this fungal genus.

Conclusions. In summary, significant differences between the three *Trichoderma* species exist concerning the number of putative GPCRs, whereas the number of genes encoding heterotrimeric G-protein subunits and RGS proteins is highly conserved. To date, only a few *Trichoderma* genes assigned with the G-protein signaling pathway have been functionally characterized, and for only two of them (TA_tga1/TC_tgaA/TR_gna1 and TA_tga3/TR_gna3), results from more than one species are available. Despite the high sequence conservation, these show clear species-specific differences in the functions of the studied Gα subunits, even between the two mycoparasites.

The cAMP Pathway

The cAMP pathway is a highly conserved signaling route that entails the secondary messenger cAMP as a coincident signal which can serve for integration of different pathways. This pathway has broad functions in fungi, including growth, development, nutrient sensing, and virulence (638). Light also impacts the cAMP signaling pathway, where it is well established that cAMP as a second messenger is produced by adenylyl cyclase, an enzyme whose activity is modulated by heterotrimeric G-protein α-subunits, highlighting the possibility that a photoreceptor coupled to G-proteins (GPCR-like) could be involved in this signaling pathway. Artificially increased intracellular cAMP levels in the dark mimic a light effect in *T. atroviride* (639) and in *T. viride*, illumination causes a transient rise in cAMP levels and protein kinase A (PKA)-dependent phosphorylation (640). Additionally, cAMP levels modulate cellulase gene expression (641) and induce coiling (53) in *Trichoderma* spp.

cAMP levels are modulated by the G-protein α-subunits GNA1 and GNA3 in *T. reesei* (617, 618) and *T. atroviride* (613, 614). The genomes of *T. reesei*, *T. atroviride*, and *T. virens* contain an adenylate cyclase (ACY1; TR_124340, TA_318748, and TV_211694), one adenylyl cyclase-associated protein (CAP1; TR_124234, TA_142327, and TV_77527), and two phosphodiesterases, one high-affinity cyclic nucleotide phosphodiesterase (PDE2; TR_102655, TA_315287, and TV_132865) and one low-affinity cyclic nucleotide phosphodiesterase (class II; PDE1; TR_3873, TA_31692, and TV_38143). With respect to protein kinase A, *T. reesei*, *T. atroviride*, and *T. virens* have one regulatory subunit (PKAr1; TR_75878, TA_29552, and TV_214426) and two cata-

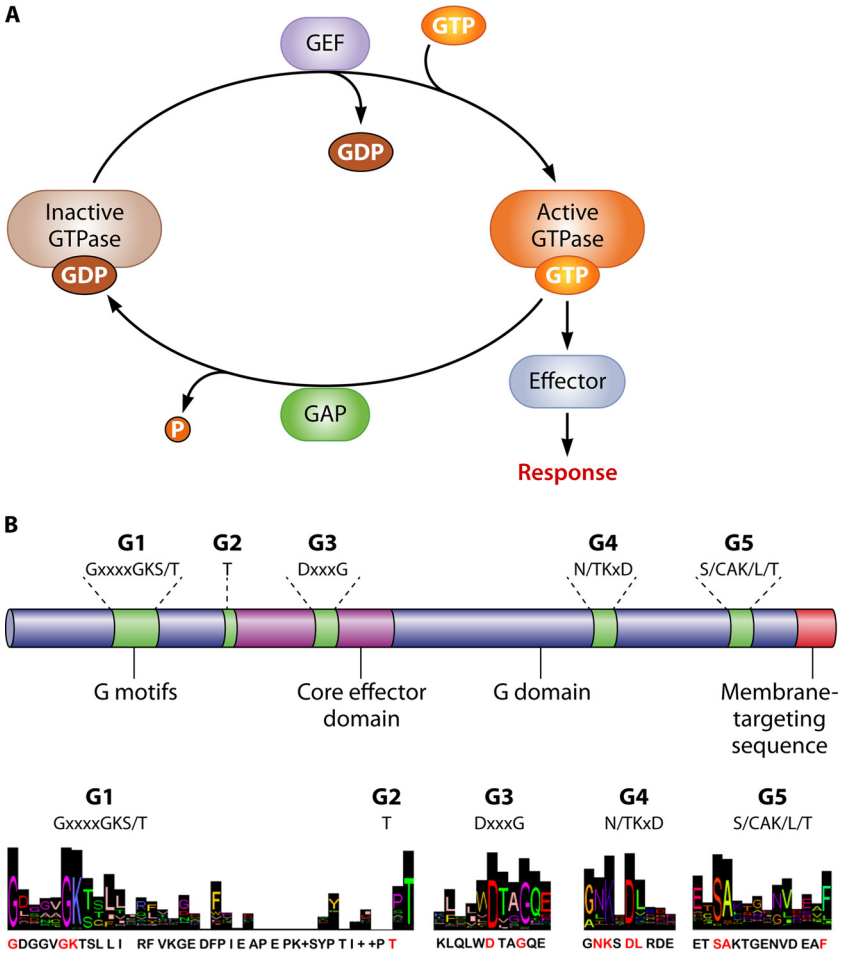

FIG 12 The small GTPase regulatory cycle and structure. (A) RAS- related GTPases cycle between an active (GTP-bound) and inactive (GDP-bound) state. The intrinsic GTPase activity of Ras-related GTPases is stimulated by specific GTPAse activation proteins (GAPs), which accelerate the inactivation of their regulatory activity. GEFs activate the small GTPases, which consecutively interact with specific effectors to mediate downstream pathways. (B) These small GTPases have conserved signature domains. All the *Trichoderma* spp. small GTPases were aligned using Clustal W with default parameters in Jalview, and the conserved domains were exported.

lytic subunits (PKAc1, via TR_57399, TA_164264, and TV_184045; PKAc2, via TR_65873, TA_52331, and TV_214212). ACY1 and PKAc1 are involved in regulation of vegetative growth, cellulase gene expression, and development but are not essential for sexual development in *T. reesei* (642). In *T. atroviride*, protein kinase A is involved in regulation of conidiation and induction of early light response genes (52).

Ras-GTPases

Small Ras-GTPases (rat sarcoma guanosine triphosphatases) are found in all eukaryotic organisms, and they are involved in almost every cellular process (643). They are important regulators of diverse cellular processes ranging from cell division, differentiation, trafficking, and adhesion to growth and cell death (644), but only a few studies in *Trichoderma* spp. are available (17, 20, 645).

Ras-GTPases modulate the activity of proteins called effectors, which normally are downstream components of diverse signaling cascades. The ability of the small GTPases to act as signaling switches resides in their ability to cycle between an active GTP-bound state and an inactive GDP-bound state. This cycling is regulated in a precise manner by GEFs, which stimulate the replacement of GDP by GTP and of GTPase activation proteins (GAPs) that stimulate the intrinsic GTP hydrolysis of the GTPase (646) (Fig. 12A). Although virtually all small GTPases share this common principle of regulation, each subfamily of GTPases has its own set of evolutionarily unrelated GAPs and GEFs (647) (see Table S1 in the supplemental material). Moreover, guanine nucleotide dissociation inhibitors (GDIs) block GDP dissociation from members of the RHO subfamily, representing additional regulatory molecules (see Table S1).

Members of the Ras-like superfamily of GTPase have been categorized as low-molecular-mass proteins (21 to 30 kDa) which contain a core conserved GTP/GDP binding and hydrolysis domain with five characteristic signature motifs (known as G1 to G5) (646) (Fig. 12B). G1 [Walker A/P-loop; GxxxxG K(S/T)] is responsible for binding of α- and β-phosphate groups of the nucleotide, G2 [Switch I; x(T/S)x] binds Mg^{2+}, G3 (Walker B/Switch II; DxxG) interacts with the nucleotide γ-phosphate and Mg^{2+}, G4 [(N/T)KxD] binds directly to the nucleotide at the K and D sites, and the weakly conserved G5 is

involved in guanine base recognition (648). The majority of Ras superfamily proteins undergo posttranslational modifications by lipids, and some members (Rho and Rab) contain a C-terminal CAAX domain (C is Cys, A is an aliphatic amino acid, and X is any amino acid) which is suitable for modification by farnesyl and geranyl transferases. These modifications allow associations with membranes, but it has been recognized that some other members do not require such lipid modification in order to associate with the membrane (646).

According to their primary amino acid sequence and biochemical properties, the Ras superfamily is divided into different families: RAS (rat sarcoma), RHO (RAS homologs), RAB/YPT (rat brain), ARF/SAR (ADP ribosylation factors), RAN (RAS-related nuclear) (649), ERA-like (*Escherichia coli* RAS-like protein), SPG1/TEM1 (septum-promoting GTPase), and MIRO-like (mitochondrial Rho-GTPases) (650, 651). In *T. reesei*, *T. atroviride*, and *T. virens* there are 105 genes encoding proteins of the RAS superfamily that are distributed in the different families mentioned above (36 genes in *T. atroviride*, 34 in *T. reesei*, and 35 in *T. virens*) (Fig. 13; see also Table S1 in the supplemental material). Among them, the RHO, RAS, RAB, and ARF/SAR families contain the majority of the members; this suggests complex functions or specific roles in different aspects in *Trichoderma* spp., such as secretion, endocytosis, and differentiation, which are processes that require more than one small GTPase. Even though these proteins might be crucial determinants in *Trichoderma* spp. fate, there have been remarkably few efforts to understand their roles in these fungi (17, 20, 645).

RHO family. Small GTPases of the RHO family, also called Cdc42/Rho-GTPases, are absent in eubacteria and archaea and are specific to eukaryotes (652). These proteins are implicated in the regulation of diverse cellular processes such as actin organization, formation and maintenance of cell polarity, cell cycle progression, membrane trafficking, and gene expression (653).

Unlike yeast, in which six distinct Cdc42/Rho GTPases have been identified (Cdc42 and Rho1 to Rho5) (654), filamentous fungi contain a second Rho subfamily, represented by RAC1 (TR_47055, TA_300919, and TV_81671), which is closely related to CDC42 (TR_50335, TA_298630, and TV_111456). Interestingly, we found that in *T. reesei*, *T. atroviride*, and *T. virens* there are two main phylogenetic branches, one of them comprising CDC42, RAC1, RHO4, RHO1, RHO2, and RHO3, while RHO5 and two novel subfamily members, RHO6 (TV_164189, TA_215113, and TR_41009) and RHO7 (TV_37243) reside in the second branch (Fig. 13). RHO6 members are present in other filamentous fungi, although they have not been described so far; on the other hand, RHO7 seems to be exclusive to *T. virens*. These two novel Rho members are phylogenetically related to RHO5 (TA_300047, TV_188860, and TR_5278) (Fig. 13). However, two major differences distinguish the Rho6 family from the rest of the Rho members. First, they contain an unusual N-terminal extension region (~130 amino acids in length) before the G1 domain, and second, the highly conserved lysine sequence GXXXXG**K**(S/T) from the G1 domain is replaced by a glutamine (Q). Mutations in G1 (or the P-loop) domain could compromise GTP binding and decrease affinity for GDP, suggesting that Rho6 members might have different affinities for GTP and GDP compared to the rest of the small GTPases. In *T. reesei*, *rho6* (TR_41009) is repressed by constant light in strains lacking either the G-protein beta-subunit GNB1, the G-protein gamma-subunit GNG1, or the phosducin-like protein PhLP1, and accumulates to higher levels in darkness compared to the wild-type strain (350). This could be related to the phenotypes observed in the deletion mutants of *gnb1*, *gng1*, and *phlp1*, which are involved in light modulation of gene expression and in the regulation of nutrient acquisition and sexual development.

It is known that polarized growth in fungi is dependent on a number of different small GTPases of the Rho family. In *A. niger*, six GTPases were identified that participate in diverse mechanisms, with sometimes overlapping functions (651). RhoA (homolog to RHO1; TR_119871, TA_298907, and TV_89761) is involved in the establishment of cell polarity and viability, while RacA (homolog to RAC1; TR_47055, TA_300919, and TV_81671) contributes to asexual development, septation, and branching mechanisms (651). Asexual reproduction in *A. niger* is also regulated by RhoB (homolog to RHO2; TR_21294, TA_156444, and TV_71661), RhoD (homolog to RHO4; TR_53562, TA_143775, and TV_31417), and CftA (homolog to CDC42; TR_50335, TA_298630, and TV_111456). On the other hand, CftA and RacA share overlapping functions, such as in cell polarization growth and cell wall biosynthesis (651), while RhoD has an essential role in septum formation. Deletion of *rho3* (*rhoC*) in *A. fumigatus* caused no discernible phenotypic changes, and deletion of *rho2* and *rho4* (*rhoB* and *rhoD*, respectively) are related to cell wall stress and integrity (651). In *A. fumigatus*, Rho1 seems to be essential, and deletion of *rho2* and *rho4* alters the cell wall integrity (655).

The small GTPase RHO3 (TR_123258, TA_146469, and TV_75844) is conserved in fungi and plays a key role in the control of cell polarity, exocytosis, and vesicle secretion. In filamentous fungi, Rho3 homologs are not essential for establishing and maintaining polarized hyphal tip growth. In *A. gossypii*, Rho3 is involved in regulation of growth at the hyphal tip, and mutation of this gene causes periodic swelling of hyphal tips that is overcome by repolarization (656). In *M. grisea*, Rho3 deletion causes no obvious defects in vegetative growth, but *rho3* mutants are nonpathogenic, their conidia are narrower, and conidium germination is delayed. They still form appressoria, but these are morphologically abnormal and defective in plant penetration. This indicates that in *M. grisea* Rho3 is a key regulator in appressorium penetration and infectious growth (657). In *T. reesei*, RHO3 is involved in secretion. Disruption of this gene causes no mutant phenotype during growth in glucose, but growth and protein secretion of *T. reesei* in cellulose cultures were remarkably decreased in *rho3* mutants compared with the parental strain (658).

Cdc42 from fungal plant pathogens participates in pathogenicity and polarized growth by coordinating processes such as cytoskeleton organization, polarized secretion, and endocytosis (659). Investigation of several Cdc42 orthologs (660–663) demonstrated that Cdc42 is not required for plant penetration but is involved in early stages of plant colonization and that the deletion mutants of *cdc42* are unable to survive in the host plant postpenetration (660). In *U. maydis*, the Cdc42 signaling network (including its GEF DON1; TR_120482, TA_234463, and TV_160649) and its downstream effector DON3 (TR_71315, TA_215231, and TV_208567) are required for appropriate septum formation and in appressorium development (664–666), but not necessarily for polarized growth (661).

A closely related homolog to yeast Cdc42p, called Rac1, is pres-

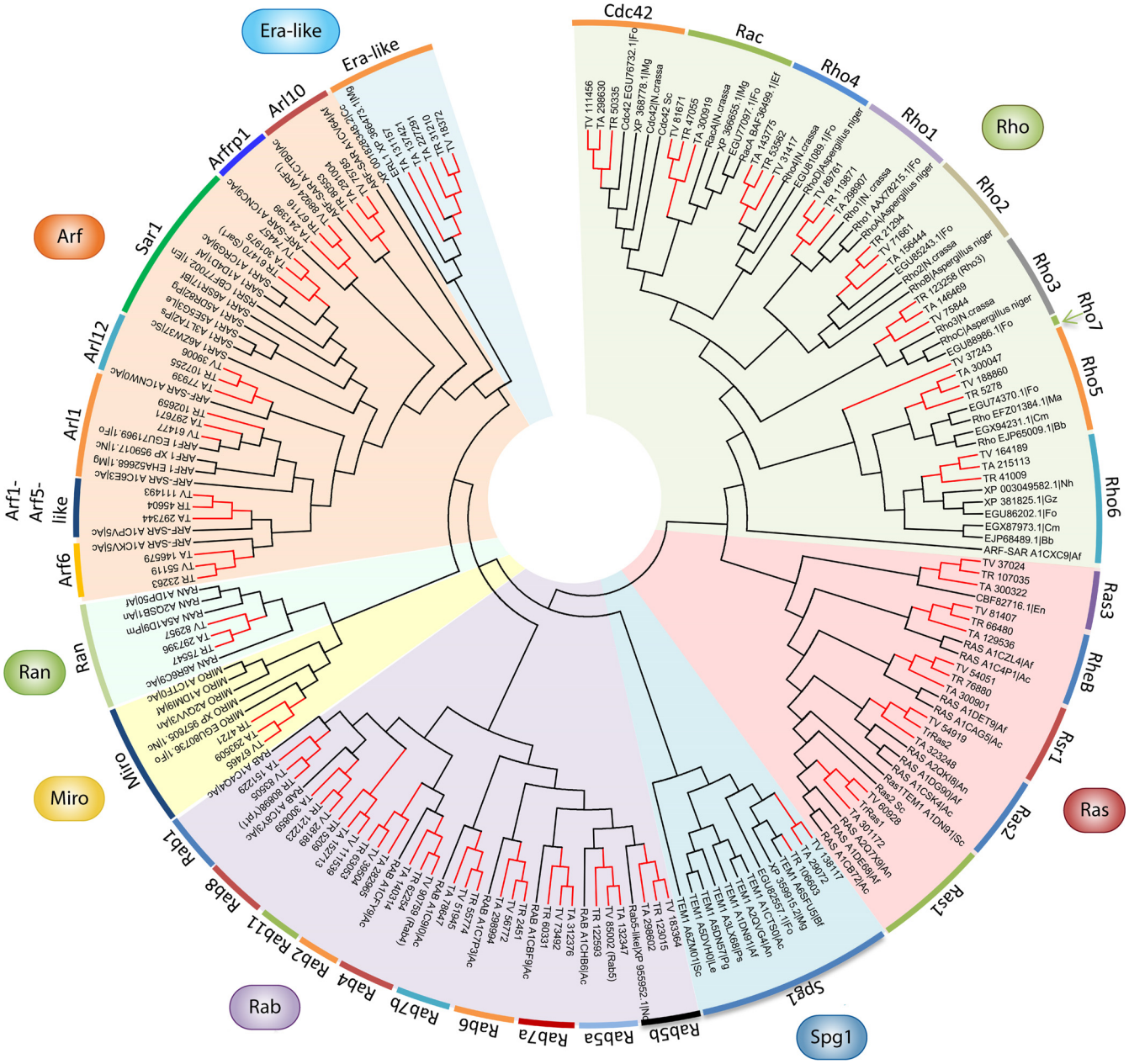

FIG 13 Evolutionary relationships of small GTPases from fungi. The evolutionary history was inferred using the neighbor-joining method. The optimal tree with a sum of branch length of 33.40865216 is shown. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 195 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1,079 positions in the final data set. Evolutionary analyses were conducted using MEGA5. GenBank/EMBL/DDBJ database accession numbers are indicated after the name of each protein in the tree. The protein ID numbers from *Trichoderma* spp. shown in the figure were from the DOE Joint Genome Institute database along with the prefix for the species: TR, *T. reesei*; TV, *T. virens*; TA, *T. atroviride*.

ent in filamentous fungi. In some instances they share common tasks; however, they have specific roles in differentiation and pathogenicity. Rac1p is essential for pathogenicity and proper filament formation. In yeast, Cdc42p is activated by the GEF CDC24 (TR_4751, TA_162118, and TV_158848) and returned to its inactive GDP-bound state by the different GAPs that include RGA1 (TR_74848, TA_247705, and TV_132439), RGA2 (TR_45689, TA_320897, and TV_ 112137) (667, 668), and BEM3 (TR_121071, TA_78316, and TV_11632) (667).

RacA, the homolog of RAC1, plays a crucial role in activation of Nox family NADPH oxidases by their interaction in both animals and fungi (651, 669–672). ROS play a significant role in morphogenesis and in fungal plant colonization (673, 674). An important source of ROS is NADPH oxidase (NOX), which is a multicomponent enzyme. In fungi, it has been reported that this complex consists of a transmembrane catalytic subunit (most species have 2 to 3 genes encoding catalytic Nox subunits), a cytosolic subunit NoxR, which is the regulator of the catalytic subunit, a

small G-protein RacA, and some scaffold proteins that include GEFs Cdc24p and Bem2p (669). In *A. nidulans*, it has been postulated that Cdc42 might influence the localization of NoxR, the regulator of the NADPH oxidase (Nox) complex, while RacA activates this complex (671). In *T. atroviride* production of ROS is essential for conidiation induced by mechanical injury, and it has been suggested that ROS (in particular H_2O_2) might work as signaling molecules in this fungus (15). The generation of ROS during mechanical injury is dependent on NOX1 (TR_79498, TV_32702, and TA_302802) and its regulator, called NOXR (TR_120969, TV_192142, and TA_315943) (15). Remarkably, NOX1 and NOXR are dispensable for conidiation induced by light, suggesting that the source of ROS during light induction is independent of the NOX complex (15). That study identified new morphological stages during conidiation. Perhaps the most remarkable observation was the formation of thinner hyphae that are generated from cells neighboring the point of injury (15). Additionally, NOXR was suggested to be an effector of RAC1/RacA, which participates with the Nox complex in signaling transmission induced during the injury response in *T. atroviride* (15).

Rho dissociation inhibitors (RDIs) are also widely studied, because they impose an additional level of regulation by preventing GTPase association with membranes, which is a requirement for their activity. Moreover, Cdc42/Rho GTPases are implicated in a signaling cascade downstream of a Ras GTPase (653, 654, 675). In *T. atroviride*, *T. virens*, and *T. reesei*, as in other fungal systems, there is only one Rho dissociation inhibitor (TR_2537, TA_297147, and TV_111588) (see Table S1 in the supplemental material).

The RAS family. Members of the Ras family of GTPase proteins are involved in morphogenesis and virulence in many organisms, including several species of pathogenic fungi (676). *S. cerevisiae* contains two highly similar Ras homologs, RAS1 (TR_120150, TA_301172, and TV_60928) and RAS2 (TR_110960, TA_323248, and TV_54919), and both have redundant cellular functions. In contrast to the multiple effectors in mammalian cells, *S. cerevisiae* Ras1p and Ras2p appear to have a single effector, namely, the adenylyl cyclase Cdc35p (ACY1; TR_124340, TA_318748, and TV_211694). Mutants of *S. cerevisiae* lacking either Ras1p or Cdc35p are unviable because both proteins are required for activating cAMP synthesis, which is essential for cells to progress through the G_1 phase of the cell cycle. ACY1 mutants of *T. reesei* are viable but show a severe growth defect phenotype (642). Ras1p plays an important role in regulating pseudohyphal growth by activating a MAPK cascade and the cAMP/PKA cascade (677). Similar roles for Ras homologs have also been reported for the filamentous morphogenesis of other fungi where the *rasA* (*ras1*) gene products regulate events in germination, including mitosis, as well as in completion of the asexual developmental cycle and polarized growth of hyphae (676, 678–680). Other roles for Ras1 homologs include activating cAMP synthesis in *C. albicans*, causing the yeast-to-hypha transition (677), high-temperature growth, and virulence in *C. neoformans* (681), mating and haploid filamentous growth (678, 679) and the pheromone response in *U. maydis*, where the expression of a dominant-active allele of *ras1* ($Ras1_{Q67L}$) induced the transcription of the pheromone precursor gene *mfa1* with no other apparent phenotypic alteration (682).

RAS2 proteins represent a second group of proteins within the Ras subfamily (Fig. 13; see also Table S1 in the supplemental material) that occupy a separate phylogenetic clade from Ras1. Together, RAS1 and RAS2 are the most extensively studied Ras proteins from this subfamily. These two groups of proteins share low identity but have conserved motifs that are crucial for their activity. The *N. crassa ras2* gene, a *rasB* homolog, is important for regulation of hyphal apical growth, cell wall biosynthesis, and conidia formation. The expression of the dominant-active allele of Ras2 in *U. maydis* induced filament formation (682), while *ras2* deletion resulted in a reduction in dimorphism (from yeast to filamentous growth) and a rounded-cell phenotype and rendered the fungi nonpathogenic (683, 684).

In *U. maydis*, Ras2 has an important role in pheromone perception and pathogenicity by interacting with Ubc2, an ortholog of yeast STE50p (a scaffold protein involved in mating and pathogenicity) (684). Studies in yeast have demonstrated that Ras2p antagonizes Ras1p in several cellular processes, including cellular levels of cAMP, entry into stationary phase, and resistance to certain stress conditions (677). In the insect pathogen *Beauveria bassiana*, Ras1 and Ras2 regulate not only hyphal growth and conidiation but also virulence to target pests, as well as tolerance to oxidation, cell wall disturbance, and UV irradiation (685).

T. reesei RAS1 (TR_120150, TA_301172, and TV_60928) and RAS2 (TR_110960, TA_323248, and TV_54919) play overlapping roles in polarized cell growth, branch emergence, and regulation of cAMP. However, RAS1 and RAS2 have specific functions, and while RAS1 is involved in conidiation, RAS2 regulates lytic enzyme expression, particularly cellulases. RAS2 is involved in the transcriptional regulation of *xyr1* through a mechanism independent of the carbon source (20). The transcription factor XYR1 (TR_122208, TA_58714, and TV_78601) is an activator in the regulation of cellulase gene expression (686). XYR1 was identified by epistatic analysis as one of the major targets of TrRAS2. The presence of additional transcriptional regulators of cellulases acting downstream of TrRAS2, however, cannot be excluded (20).

In *T. reesei*, RAS1 and RAS2 play important roles in the control of cAMP concentration, as they do in other species ((687, 688), suggesting that these proteins are likely components of the cAMP-PKA signaling pathway. Indeed, *T. reesei* RAS1 and RAS2 deletion mutants share phenotypes with those observed in deletion mutants lacking diverse components of the cAMP-PKA pathway, including defects in cell growth, cAMP synthesis, and lytic enzyme production (53, 613, 615–619, 642, 689). Actually, it has been proposed that *T. reesei* RAS1 or RAS2 could interact with CDC42 to regulate the process of filamentous growth (20). In addition, it has been suggested that, like other systems, RAS1 and RAS2 could act on a MAP kinase cascade to regulate filamentous growth through the control of cell elongation and cytokinesis (20). Besides Ras1 and Ras2, in fungi there are novel Ras family members for which there are no data reported so far. These members are called here RAS3 and include TV_37024, TR_107035, and TA_300322 (Fig. 13).

A further member of the Ras family of GTPases is Rsr1. In *T. reesei*, *T. atroviride*, and *T. virens*, only one homolog in each species has been described (Fig. 13) (TR_76880, TA_300901, and TV_54051). In other systems, the corresponding orthologous proteins encoded by Rsr1 are distributed uniformly throughout the plasma membrane and become highly concentrated at the division sites and the sites of polarized growth, including the bud tips (690, 691). The role of Rsr1 is to recruit components of the polarisome, as in *A. gossypii* (692) and *C. albicans* (693). In *S. cerevisiae*, Rsr1p is hypothesized to affect cell polarity via a direct

interaction with Cdc42p (694). In yeast it is most likely that Rsr1 also participates in the recruitment of components of the polarisome. In *Trichoderma*, this protein could have important roles during polarized growth and during light induction when there is a change in the polarization in response to light.

Rheb (Ras homolog enriched in brain) represents another Ras subfamily member which plays critical roles in activation of mammalian target of rapamycin (mTOR), and it is involved in the activation of protein synthesis and cell growth (695). The activity of Rheb is regulated by TSC2 (TR_1683, TA_288880, and TV_33609), a GTPase-activating protein (GAP). Rheb is conserved in a wide variety of organisms, and in *Trichoderma* there is a single member of this subfamily (RHE2; TR_66480, TA_129536, and TV_81407). Rhb1 (a Rheb homolog) is involved in the cell wall integrity pathway (695), while the deletion of Rheb homologs results in a significant reduction in virulence in *A. fumigatus* compared to the wild type and enhances sensitivity to rapamycin in *C. albicans*. Orthologs of Tsc proteins are present in other fungi, like *S. pombe*, *A. fumigatus*, and *Trichoderma* spp., but not in *S. cerevisiae*, with *rhb1* and *tsc2* being involved in nitrogen starvation-induced filamentation (695). In *Trichoderma* spp., production of lytic enzymes (i.e., proteases, chitinases, glucanases, cellulase, pectinase, etc.) is regulated by either nitrogen or carbon catabolite repression (5, 332, 696). Thus, Rheb proteins in *T. reesei*, *T. atroviride*, and *T. virens* represent good candidates to be key components in the regulation of perception of nutrients by their interaction with TOR kinase.

Era-like GTPases. Era (*E. coli* RHO) is a membrane-associated GTP binding protein with a crucial role in ribosome assembly and is essential for cell growth in *Escherichia coli*. An ortholog of this essential GTPase, Eral1 (Era G-protein-like 1), exists in higher eukaryotes, and its absence has been linked to apoptosis (697, 698). In fungi, the novel Era-like GTPase Erl1 has been identified in *M. oryzae*. Erl1 is dispensable for saprophytic growth, appressorium formation, and production of leaf lesions, and its loss reduced the growth of invasive hyphae. Deletion of *ERL1* significantly reduced root browning when *M. oryzae* was inoculated on the root (699). The gene *GIN-N* from the arbuscular mycorrhizal fungi *Glomus intraradices*, which is presumed to play a role in establishing compatibility with the plant, complemented the defect of *ERL1* deletion in root disease when expressed under control of the *ERL1* promoter, suggesting that this protein is involved in mechanisms for root colonization that are conserved between symbiotic and pathogenic fungi (699).

A search of the *T. virens* genome for Era-like GTPases revealed TV_18372, encoding a 223-amino-acid protein with domains G1 to G4. This protein model was not annotated by the automated pipeline as a small GTPase. We recently found that TV_18372 has an additional intron at the 5′ region and a longer 3′ end, which in total produces a 200-residue-longer protein than that reported in the JGI database (M. F. Nieto-Jacobo, C. Brown, and A. Mendoza-Mendoza, unpublished data). In *T. reesei* there is an ortholog of TV_18372 (TR_31210) and, surprisingly, in the endophytic fungus *T. atroviride* there are three putative paralogs to Era-like proteins (TA_131157, TA_137421, and TA_227291). A phylogenetic analysis showed that *T. reesei* and *T. virens* are derived, compared to that in *T. atroviride* (Fig. 13). Thus, during evolution the two additional Era-like proteins were likely lost in *T. virens* and *T. reesei*. This suggests that Era1-like proteins in *T. atroviride* might have additional important roles during plant root colonization or specific roles for *T. atroviride*. In *T. atroviride* the transcripts of Era-like protein-encoding genes TA_131157 and TA_137421 were reduced up to 2.7- to 3.0-fold 60 min after mechanical injury (15). TR_31210 does not have any transcriptional change when *T. reesei* is grown on cellulose in light or darkness.

The RAN subfamily. Ran has been implicated in a large number of processes, including nucleocytoplasmic transport, RNA synthesis, processing, and export, cell cycle checkpoint control, mitotic spindle assembly, and nuclear envelope assembly. The Ran GTPase regulates the association and dissociation of receptors and cargos as well as the transport direction through the nuclear pore. All receptors bind to Ran exclusively in its GTP-bound state, and this event is restricted to the nuclear compartment. Ran is only slightly related to the other RAS proteins, and it differs in that it lacks cysteine residues at its C terminus and is therefore not subject to prenylation. In addition, it has no lipid modification at the C terminus, so that Ran is not anchored in the membrane. Instead, Ran has an acidic C terminus (700). Ran homologs have been found in fungi, plants, and vertebrate and invertebrate animals, forming a well-conserved family whose genes encode proteins about 80% identical to each other. Ran proteins from *T. atroviride*, *T. virens*, and *T. reesei* share a homology of 98% (TR_75547, TA_297396, and TV_82957) and are closely related to other fungal Ran proteins. These proteins maintain nuclear and cytoplasmic compartmentalization in eukaryotic cells by asymmetric localization of the Ran guanine nucleotide exchange factor (RanGEF/RCC1) in the nucleus and Ran activating protein1 (RanGAP1) in the cytoplasm. The balance between the opposing activities of RanGEF/RCC1 and RanGAP1 generate a gradient of Ran-GTP across the nuclear envelope to control the directionality of nucleocytoplasmic transport. In other organisms, Ran proteins are localized in the nuclear envelope and in perinuclear structures, throughout the cell, and in the nuclear envelope, closely associated with nuclear pore complexes (701).

The only known RanGAPs, RanGAP1 in higher eukaryotes or Rna1p in yeast, are crescent-shaped proteins formed by 11 leucine-rich repeats, which bears no resemblance to RasGAP or RhoGAP (702) and localizes exclusively to the cytosol. The RanGEF RCC1 (for regulator of chromosome condensation 1) in higher eukaryotes, is a strictly nuclear protein that appears to be associated with chromatin. *T. reesei*, *T. atroviride*, and *T. virens*, like all species reported to date, contain only one predicted protein homolog of RanGAP1 (TR_1683, TA_288880, and TV_33609) but three putative RanGEF/RCC1 proteins (see Table S1 in the supplemental material); all of them contain the RCC domain, but none of them has been functionally characterized yet.

The RAB/YPT subfamily. Secretion of metabolites and lytic enzymes is an important biological mechanism in *Trichoderma* spp. Small GTPases of the Rab/Ypt and ARF branches play central roles in the secretory pathway. Rab proteins (Ras homologs from brain) are evolutionarily conserved small GTPases that control intracellular traffic events from yeast to mammalian cells, acting as pivotal components of the membrane trafficking machinery. Distinct Rab proteins recruit diverse specific effectors and locate to specific endomembrane compartments, and genomic studies suggest that Rab gene diversity correlates with endomembrane system complexity (703). An increased number of Rab isoforms is associated with greater complexity within the endomembrane system. In *S. cerevisiae* and *S. pombe* 11 and 7 Rab/Ypt proteins have been described, respectively; animals have nearly 70, the closely related

unicellular amoebas can have more than 100 such proteins, and multicellular plants have nearly 60. The set of Rab subfamilies common to fungi is composed of five different proteins: Ypt1p, Sec4p, Ypt3p, Ypt51p, and Ypt7p (see Table S1 in the supplemental material). In *T. atroviride*, *T. virens*, and *T. reesei*, there are two copies of Ypt51p (TR_122593, TA_132347, and TV_85002; TR_123015, TA_298602, and TV_183364), whereas for the other components, only one member was detected. In *S. cerevisiae*, Ypt1p (TR_80898, TA_151229, and TV_83505) localizes to the ER and cis-Golgi complex and works in the early steps of the secretory pathway, mediating ER-Golgi transport; Ypt3p, like its ortholog Rab11p (TR_5209, TA_152713, and TV_111539), is involved in deep endocytic recycling; Ypt51p mediates Golgi-endosome and plasma membrane-endosome transport; Ypt6p (TR_2451, TA_298994, and TV_56772) is involved in retrograde Golgi complex-ER and intra-Golgi complex transport; Ypt7p (TR_60331, TA_312376, and TV_73492) mediates vacuole fusion; and Sec4p/Rab8p/Ypt2p (TR_121223, TA_300859, and TV_28189) mediates the delivery of *trans*-Golgi complex-derived vesicles into the bud, representing a form of polarized transport reminiscent of that mediated by its putative ortholog, Rab8. Rab family proteins in 26 fungal genomes have been identified and annotated (704). Although Rab proteins have been identified in several fungal species, studies in fungi are limited. The *T. atroviride-Rhizoctonia* interaction induces expression of RAB11 (TA_152713), but no differences were reported with respect to the other Rab members, which suggests that RAB11 plays a role during fungus-fungus interactions (355). In *U. maydis* a homolog of human Rab4, which is absent in yeast, is involved in endosome-based membrane recycling necessary for extended hyphal growth (705). Homologs of yeast Ypt6p and Ypt7p, activator proteins of Rab-like small GTPases, were found to be mutated in the hypersecretory *T. reesei* mutant strain RUT-C30 (ATCC 56765), along with diverse genes involved in vesicle trafficking, vacuolar sorting, and Golgi complex association (706). Due to the involvement of ypt6p in *S. cerevisiae* in early Golgi complex function and ribosome biosynthesis (707) and the functions of the RAB/YPT subfamily in other posttranslational events, it is likely that TR_108962 contributes to the high secretory performance of RUT-C30.

The ARF/SAR subfamily. Arf (ADP-ribosylation factor) proteins are ubiquitous, eukaryotic regulators of virtually every step of vesicular membrane traffic. The most common function of the Arf proteins is to promote the formation of complete, cargo-containing transport vesicles at different sites in the cell (708). Activation of Arf1 is absolutely required to maintain Golgi complex structure and function in eukaryotic cells; the conserved, signature catalytic domains in the GEFs and GAPs are what facilitated the identification of these Arf regulators in all organisms from yeast to humans (709).

In *S. cerevisiae*, Gcs1p (TR_48835, TA_294523, and TV_48541) activates the intrinsic activity of Arf1 p (TR_45604, TA_297344, and TV_111493), Arf2p (interestingly, this protein is not present in *T. reesei*, *T. atroviride*, or *T. virens* [Fig. 13]), and Arl1p (TR_102659, TA_297671, and TV_61477). Gcs1p thus appears to be the most important ARF-GAP in yeast, and it is involved in several routes of intracellular vesicle traffic. It has functions in both exocytosis and endocytosis, is important for maintenance of mitochondrial morphology, for formation of the prospore membrane in sporulation, and for proper actin cytoskeletal organization by stimulating actin polymerization. Strains with mutations in the *C. albicans* gene *AGE3* (orthologous to *Gcs1*) showed defects in filamentation and were almost completely deficient in invasive filamentous growth ability. The defect in sustaining filament elongation is probably caused by the failure of *age3*Δ cells to polarize the actin cytoskeleton and possibly of inefficient endocytosis (710). ARF GTPases and their regulators were identified in *T. reesei*, *T. atroviride*, and *T. virens* (Fig. 13; see also Table S1 in the supplemental material). To date, there are no reports regarding their function in *Trichoderma*. However, together with the GTPases of the Rab/Ypt subfamily, they represent target proteins to study mechanisms of secretion or polarized secretory activities.

Miro-like GTPases. The Miro-like GTPases are essential regulators of mitochondrial morphogenesis and trafficking along microtubules, acting as calcium-dependent sensors in the control of mitochondrial motility. They are now considered a separate subfamily of the Ras superfamily. An additional and significant difference between the Miro proteins and Rho GTPases is the absence of a CAAX box in Miro (711). The yeast Miro GTPase, Gem1p (TR_4721, TA_293509, and TV_67465) localizes to the ER-mitochondrion interface and influences the size and distribution of mitochondria. Thus, Miro GTPases are proposed to serve as regulators of the ER-mitochondrion connection (712).

All Miro GTPase proteins, including Gem1p, contain two GTPase domains (GTPase I and II) that flank two bipartite Ca^{2+} binding EF-hand motifs (EF-I and -II). Because the C termini of these proteins are tail anchored in the outer mitochondrial membrane, all four domains are exposed to the cytoplasm. Genetic studies indicate that these domains are important for the function of *Drosophila* Miro, mammalian Miro, and yeast Gem1p (713). Miro1/Gem1p is present in *T. atroviride*, *T. virens*, and *T. reesei* (Fig. 13; see also Table S1 in the supplemental material) and is closely related to other fungal Miro proteins. Taking into account that cellular organelles need to communicate in order to coordinate homeostasis of the compartmentalized eukaryotic cell, it will be interesting to elucidate the role of this GTPase in the control of cell homeostasis and survival of *Trichoderma* in response to external stimuli. Interestingly, the Miro1/Gem1p-encoding gene TR_4721 from *T. reesei* is partially repressed during constant light in the phosducin-like protein PhLP1 deletion strain (350), suggesting a positive cross-regulation of these two proteins.

Spg1/TEM1 GTPase. The *S. pombe* septation initiation network (SIN), is a Spg1-GTPase-mediated protein kinase cascade that triggers actomyosin ring constriction, septation, and cell division. In fission yeast, the GTPase Spg1 (septum-promoting GTPase; TR_106603, TA_29072, and TV_138117) is an essential gene constitutively localized to spindle-pole bodies (SPB); in its GTP-bound form, Spg1 binds the Cdc7 protein kinase (TR_62169, TV_51517, and TA_153112) and causes it to translocate to SPB. Together, the GTPase Spg1 and protein kinase Cdc7 play a central role in regulating the onset of septation and cytokinesis (714). In the budding yeast *S. cerevisiae*, a Ras-like GTPase signaling cascade known as the mitotic exit network (MEN) promotes exit from mitosis. To ensure the accurate execution of mitosis, MEN activity is coordinated with other cellular events and is restricted to anaphase. The MEN GTPase, Tem1p (Spg1), is thought to be the central switch in MEN regulation. MEN/SIN achieve the equal distribution of genetic material between mother and daughter cells through a kinase cascade that triggers the dephosphorylation and consequent inactivation of mitotic CDK1 (cyclin-dependent

kinase 1; TR_70266, TV_36994, and TA_127794) by the serine-threonine phosphatase CDC14 (TR_27406, TV_61598, and TA_91339) (715, 716).

Sequence homology places SPG1, TEM1, and related sequences from fungi, Mycetozoa, Diplomonadida, and plants in a new subfamily which forms a monophyletic grouping sister to the monomeric G-proteins. Inspection of eukaryotic genomes reveals putative counterparts for several components of the yeast SIN and MEN pathways. The core elements of the SIN Spg1p/Tem1p proteins were only observed in the fungi, plant, and Mycetozoa clades. In contrast, metazoan cells do not use the core elements of the SIN or MEN pathways in order to coordinate the termination of cell division with cytokinesis (717).

Conclusions. All families of Ras GTPases and their corresponding regulators reported to date are present in *T. reesei*, *T. atroviride*, and *T. virens*. Two novel Rho subfamilies were identified in this work: Rho6 and Rho7. While Rho6 members are present in other filamentous fungi, Rho7 seems to be exclusive for *T. virens*. Moreover, an expanded number of ERA-like GTPases was observed in *T. atroviride*, which could suggest an important role of these proteins during root colonization, as occurs in *M. oryzae*. Also, a reduction in the number of Rab GTPase activators and consequently Rab GTPases could be more active in *T. reesei* and might be associated with the unusual hypersecretion activity in that fungus.

Protein Kinases

Phosphorylation and dephosphorylation by protein kinases (PKs) and protein phosphatases (PPs) are processes at the core of the information highways in fungi, as in other eukaryotes. By (de) phosphorylation of their targets on one or multiple sites, they can modulate biological activity, subcellular localization, half-life, posttranslational modifications, or interactions with other proteins (718). In many eukaryotic genomes, genes for kinases constitute the largest group of genes and have evolved to contain diverse additional domains which enable them to exert a wide variety of functions. According to this modular organization with diverse domain combinations, which serves as a guideline to their functional properties, protein kinases are assigned to different subgroups (719, 720).

Functions of kinases span virtually every physiological aspect of fungi by acting in signal transduction, metabolism, regulation of circadian rhythms, and many other processes. The numbers of serine/threonine (S/T) kinases range around 100 or more in fungi, fruit flies, and humans (719, 721). The *N. crassa* genome contains 107 protein kinase genes, and a recent study showed that 9 of them are essential, and for about 70%, a phenotype was detected upon deletion (722), hence emphasizing the importance of this gene group.

Within the genus *Trichoderma*, a gene encoding a homolog of *S. cerevisiae* YPK1, which is important for normal growth (723), was the first to be characterized in *T. reesei* (TR_111799, TA_283660, and TV_231045) and it was named *pkt1* (724), followed soon by protein kinase C (*pkc1*; TR_2526, TA_233660, and TV_76466) (725, 726), which was the first PKC to be isolated from a filamentous fungus. In recent years, the focus of research on kinases was mainly on their function in mycoparasitism and biocontrol; recently the focus has also been on response of light (summarized in reference 13).

The representation of the different kinase families in *T. reesei* is similar to that of other ascomycete and basidiomycete genomes (719), and the analysis presented here shows comparable numbers of protein kinases in the *T. atroviride* and *T. virens* genomes, albeit with interesting differences, as detailed below. As expected from what is known from other fungal genomes, no tyrosine kinases (TK) of the class that is so central to mammalian signal transduction were detected.

Protein kinases are classified into different groups (http://www.compbio.dundee.ac.uk/kinomer/index.html). Thereby, the cyclic nucleotide-dependent family (protein kinases A and G), the protein kinase C family, the ribosomal S6 family, and the beta-adrenergic receptor kinase are included in the AGC group. Kinases belonging to the CAMK group are characterized by calcium/calmodulin modulation of their activity. Cyclin-dependent kinases (CDKs), MAP kinases, and glycogen synthase kinases (GSKs) are in the CMGC group of kinases. Furthermore, the STE group also includes protein kinases involved in MAP kinase cascades. Several of the protein kinase families include genes encoding known conserved signal transducer proteins. For example, although the MAPKs belong to the CMGC group, kinases upstream of the MAPK pathways belong to the STE group and casein kinases belong to the CK1 (casein kinase 1) and CMGC families. However, if thematically close, we discuss groups of kinases together here (such as MAPK pathways or casein kinases). For the families, sequences of genes from *T. reesei*, *T. atroviride*, and *T. virens* (TR, TA, and TV, respectively), together with known representatives of other fungi, were combined and used to generate a phylogenetic tree. These phylogenetic trees, supported by bidirectional BLAST analysis between potential homologs, were used as a basis for functional assignments if characterized homologs of other fungi were available. Moreover, in some cases, phylogeny supported the presence of expansions in a given kinase family compared to closely related fungi. Most of the genes, as expected, appeared in the trees as closely related TA/TV/TR groups. The groups were identified or confirmed by BLAST searches of the respective genome databases as well as NCBI and Swiss-Prot databases. Protein IDs and classifications of annotated kinases are summarized in Table S1 in the supplemental material.

Histidine kinases and two-component phosphorelay systems. The two-component phosphorelays are major signal transduction pathways in bacteria and eukaryotes, but they have not been detected in animals (reviewed in reference 727). They are composed of hybrid sensor kinases, a histidine phosphotransferase (HPt), and response regulators. They sense and transmit signals from the environment and subsequently activate, for example, the HOG1/osmolarity and stress-sensing pathway (728, 729). Hence, the MAPKKKs TR_57513, TA_295042, and TV_195699 are likely targets of the two-component phosphorelay system. Consequently, these signaling systems have functions in stress response, fungicide resistance, and development. Interestingly, it was shown that several components of the *N. crassa* two-component phosphorelay system are clock controlled (730). According to the classification system established in reference 731, *T. atroviride*, *T. virens*, and *T. reesei* have histidine kinases belonging to classes I, II, III, IV, V, VI, VIII, IX, X, and XI (see Fig. S26 and Table S1 in the supplemental material).

In contrast to *N. crassa* and *A. nidulans* (728), *T. atroviride*, *T. virens*, and *T. reesei* contain only one phytochrome, which is related to *N. crassa* PHY-1 (class VIII; TR_77764, TV_190601, and TA_319399). However, class I histidine kinases are expanded in *T. atroviride*, *T. virens*, and *T. reesei* (607) with two members

(TR_53349, TA_49350, and TV_30731; TR_59384, TA_32890, and TV_150797) instead of one, as in *N. crassa* (NCU09520). As there is no function known for homologs of these genes and gene expression analysis did not reveal any significant regulation (732, 733) in *N. crassa* (http://bioinfo.townsend.yale.edu/index.jsp) or *T. reesei* (350, 351), the relevance of this obvious expansion remains to be determined.

Additionally, the genomes of *T. atroviride* and *T. virens* contain histidine kinases of group II, with one member in *T. virens* (TV_46906) and even two in *T. atroviride* (TA_133533 and TA_128614). No member of this group is present in *N. crassa* or *T. reesei*. The function of the homolog in *Gibberella moniliformis*, HHK3, is not yet known, as its deletion did not impact growth, conidiation, or sexual development (731).

All three *Trichoderma* species contain one histidine phosphotransferase gene (*hpt1*; TR_123344, TV_57588, and TA_88984). Interestingly, the *N. crassa* HPt homolog is indirectly regulated by the circadian clock (734). *T. reesei*, *T. atroviride*, and *T. virens* also have three response regulator (RR) proteins related to SSK1, SKN7, and RIM15 or RRG-1, RRG-2, and STK-12 in *N. crassa* (see Table S1 in the supplemental material). No ortholog to *Cochliobolus heterostrophus* REC1 (735, 736) was detected. In *C. heterostrophus* and *G. zeae*, functions of RRs were studied. For *rim15*, only minor growth effects were detected upon deletion, while SSK1 affects sexual development and SSK1 and SKN7 are responsible for stress resistance (735). Comparable functions were found for *N. crassa* RRs (737, 738).

CMGC family. The CMGC group includes MAP kinases (see also above), CDKs, and some apparent relatives (CDK-like), including glycogen synthase kinase (GSK), one of the two classes of casein kinases (CKII), and PRP4. One GSK in yeast, the GSK homolog Rim11p, has an essential role in the entry into meiosis, while another GSK, *S. cerevisiae* Mck1p, is needed for growth at high or low temperatures and transmits stress signals (739).The GSK3 homolog of *Drosophila*, SHAGGY (SGG), has a central role in the circadian clock, phosphorylating TIM and thus influencing the nuclear localization of the TIM/PER heterodimer (740). The *N. crassa* glycogen synthase kinase GSK-3 (encoded by NCU04185) impacts the circadian clock by regulation of the abundance of the photoreceptor complex WCC on a posttranscriptional level. Thereby, GSK-3 binds and phosphorylates WC-1 and WC-2, hence influencing accumulation of the complex (741).

Although the circadian clock has not yet been studied in *Trichoderma* spp., there is ample evidence for its presence (12, 14), and a similar function of the GSK-3 homologs (TR_74400, TV_73199, and TA_297064) is likely. Rhythmic conidiation can be induced in *Trichoderma* by periodic light treatment, but there is also evidence for a clock-dependent variation in sensitivity to light (742, 743).

Other members of the CMGC family have very central roles in the biology of the cell, and these may be superimposed on additional signal transduction functions. The pre-mRNA processing 4 (PRP4) protein kinase has homologs in *T. atroviride*, *T. virens*, and *T. reesei* (TR_21306, TA_143987, and TV_1166) and is involved in pre-mRNA splicing (744). PRP4 also acts as a spindle checkpoint protein (745). In yeast, IME2p (inducer of meiosis 2; related to TR_50071, TA_142127, and TV_47348) controls several steps in meiosis (746, 747). The *Ustilago maydis* IME2 ortholog, CRK1, is also needed for appropriate response to environmental stimuli (748). *T. atroviride*, *T. virens*, and *T. reesei* have one representative each of the cyclin-dependent protein kinases with homology to the CDK7/KIN28, CDK1, CDK8, CDK9, and CDK5 classes. There are likely members of the DYRK family and some possible CDC2-related kinases that might have cell cycle-related functions (see Table S1 in the supplemental material).

STE kinases. All three *Trichoderma* species contain 14 STE kinases each (see Table S1 in the supplemental material). This group includes upstream kinases belonging to MAP kinase cascades, including STE20/PAK (MAPKKK kinases. MAPK cascades are unique to eukaryotes, act in series, and are among the best-characterized signal transduction molecules in fungi (749, 750). These cascades act via a three-step mechanism comprising a MAPKKK, a MAPKK, and a MAPK, which itself requires activation by phosphorylation (751). In *T. reesei*, *T. atroviride*, and *T. virens*, all three pathways common in filamentous fungi are represented, i.e., the stress response pathway, the cell integrity pathway, and the pheromone/pathogenicity pathway (13, 607). Especially the MAP kinases (see "CMGC kinases") have been studied in detail and were found to be involved in regulatory processes important for mycoparasitism and induction of systemic resistance in plants, growth, regulation of hydrophobin expression, and development, as well as osmotic and oxidative stress responses (summarized in reference 5).

Each of the three MAPKKKs is on a different phylogenetic branch (data not shown), suggesting more specificity than might have been considered previously in the upstream activation of the different MAPK pathways. It will be interesting to see, by biochemical or protein-interaction techniques, which MAPK modules are actually present in the fungal cell and how the flow of different signals is compartmentalized by the products of these genes acting in different combinations.

Intriguingly, in *N. crassa*, the photoreceptor complex WCC binds to the *os-4* promoter in response to light and influences OS-2 phosphorylation rhythms (734). Hence, an influence of light and the circadian clock on the *Trichoderma* TMK3/HOG1 (TR_45018, TA_301235, and TV_83666) pathway would not be without precedent. This hypothesis is supported by the finding that *T. reesei tmk3* transcript abundance is upregulated in response to light (78).

Six PAK/STE20-related kinases were identified. There is a homolog (TV_32107, TA_231437, and TR_52021) of *A. nidulans* SepH; this kinase is required for cytokinesis/septation (752).

Casein kinases. Casein kinases of class I (CKI) are active and expressed constitutively. Their impact on downstream targets is regulated by additional kinases and phosphatases, which prime the target for CKI. Casein kinases of class II can also catalyze calmodulin phosphorylation and thereby modulate its activity. In higher eukaryotes, CKII is known to be essential for carbon metabolism and use of GTP almost as efficiently as ATP as a phosphate donor (753).

Casein kinases of class II belong to the CMGC kinases as mentioned earlier, but due to their function related to that of class I CKs, they are discussed together here (Fig. 14).

T. reesei, *T. atroviride*, and *T. virens* contain generally one casein kinase I homolog of *N. crassa* HHP1/CK1a (NCU00685; TR_109876, TA_262904, and TV_57006) and one homolog of CK1b (NCU04005; TR_79171, TA_243093, and TV_86249). *T. reesei*, *T. atroviride*, and *T. virens* contain an additional gene related to CK1b, now named CK1c. *T. reesei* also contains an additional homolog to CK1a (CK1d; TR_55049). Hence, the group of

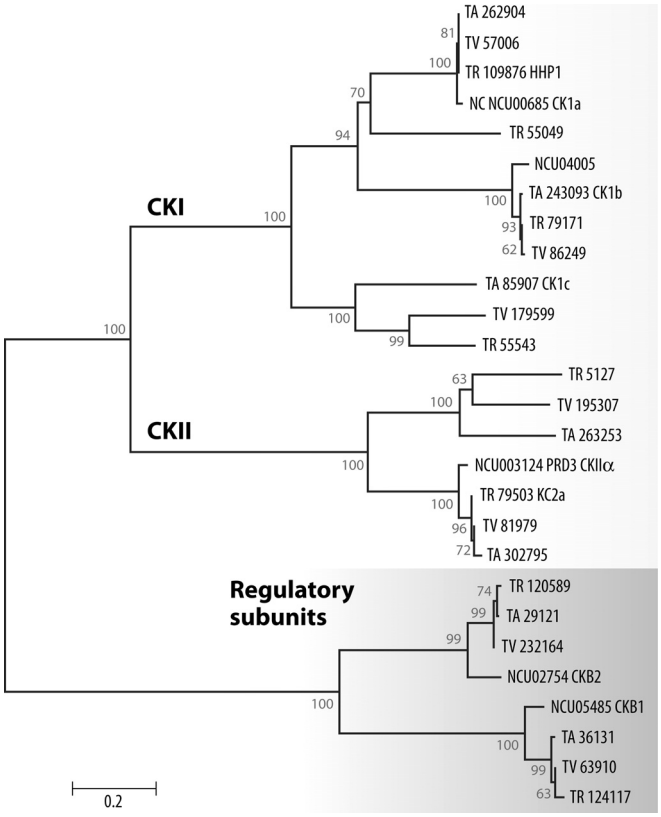

FIG 14 Phylogenetic analysis of casein kinase I and II homologs of *T. atroviride* (TA), *T. virens* (TV), and *T. reesei* (TR), along with *N. crassa* (NCU). Sequences were aligned using Clustal X, and phylogenetic analysis was performed with MEGA4 using the minimum evolution algorithm with 500 bootstrap cycles.

class I casein kinases is expanded in the three *Trichoderma* species compared to *N. crassa*, indicating an increased relevance in their physiology (Fig. 14). The *A. nidulans* homolog of CK1a, CkiA, was found to be essential and involved in amino acid utilization and resistance to toxic amino acid analogs, which is likely due to a mislocalization of amino acid transporters (754). Amino acid utilization and metabolism were shown to be connected to regulation of plant cell wall degradation in *T. reesei* and *N. crassa* (351, 415, 755–757). Consequently, an involvement of casein kinases of class I in regulation of plant cell wall-degrading enzyme production via adjustment of amino acid metabolism warrants further investigation.

In fungi, casein kinases have been thoroughly studied with respect to their function in circadian rhythms and light response, predominantly in *N. crassa* (758). With respect to casein kinases II, *T. reesei*, *T. atroviride*, and *T. virens* contain homologs of the three casein kinase genes of *N. crassa* (one catalytic CKII alpha-subunit and two regulatory CKII beta-subunits). Interestingly, all three contain, in addition, a second homolog to the *N. crassa* catalytic CKII alpha-subunit PRD3 (TR_5127, TV_195307, and TA_263253) (Fig. 14). In *N. crassa*, this CKII is involved in temperature compensation of the clock (759), circadian regulation of gene expression, growth, and conidiation (760). Interestingly, TR_5127 transcript abundance appears to remain at background levels upon growth on cellulose in light or darkness (350, 351).

AGC kinases. Two of the best known kinases, cyclic nucleotide (PKA) and calcium-phospholipid-dependent (PKC) kinases belong to this family.

Protein kinase A was shown to regulate light responses in *T. atroviride* (52). PKAc1 (TR_57399, TA_164264, and TV_184045), which is considered the major PKA catalytic subunit, impacts growth and sexual and asexual development, as well as cellulase regulation in *T. reesei* (642). *T. reesei*, *T. atroviride*, and *T. virens* also encode a second PKA catalytic subunit (PKAc2; TR_65873, TA_52331, and TV_214212), but its function has not been studied so far. However, in contrast to the severe phenotype of strains containing mutants for *pkac1*, homologs of this second PKA catalytic subunit do not show striking phenotypes (761), except for an alteration in growth of aerial hyphae (722).

Interestingly, *T. atroviride*, *T. virens*, and *T. reesei* contain an expansion in proteins related to *S. cerevisiae* YPK1p. They have one YPK1-like kinase (TA_283660, TV_231045, and TR_111799) that is closely related to *N. crassa* YPK-1/GAD-8 (NCU07280) and another one in a sister clade in the phylogenetic tree (TA_140330, TV_181871, and TR_122015). Both these clades are related to *A. fumigatus* YPK1 as well as *S. cerevisiae* YPK1 and YPK2. Additionally, there appears to be an additional clade with YPK1-type kinases, with one *T. reesei* member, one *T. atroviride* member, and three *T. virens* members (TR_53776, TA_341683, and TV_14525 as well as TV_2353 and TV_125663). In yeast, these kinases are essential for proliferation and growth. YPK1 acts as a downstream kinase in the sphingolipid signaling pathway and is important for endocytosis and cell wall integrity. Moreover, YPK1-like kinases are substrates of the TORC2 complex. Accordingly, the *N. crassa* and *A. nidulans* homologs were found to be essential as well (722, 762). Misregulation of *ypkA* using conditional promoters in *A. nidulans* revealed a function of this gene in growth, sporulation, and branching. Investigation of the targets of YpkA also showed an influence on genes involved in carbohydrate metabolism, glucose sensing, and polysaccharide degradation (762).

The first characterized PKC of fungi, *T. reesei* protein kinase C (PKC1), is homologous to *N. crassa* KPC1 (NCU06544) and is conserved in *T. atroviride* and *T. virens* (TR_2526, TA_233660, and TV_76466). *T. reesei* PKC1 is stimulated by phospholipids and phorbol esters but is calcium independent (725, 726). Its enzymatic properties are consistent with those of *S. pombe* PKCs (763), but considerably different from those of *S. cerevisiae* (764) and *C. albicans* PKCs (765). However, extensive phenotypic characterization has not been done for *Trichoderma* spp., other than a recent report, which showed that deletion of *pkc1* does not cause a severe growth or sporulation phenotype (766). In *N. crassa*, PKC1 influences circadian rhythmicity and light response due to phosphorylation of the photoreceptor WC-1 (767, 768). *A. nidulans* PkcA is involved in regulation of penicillin biosynthesis (769). Interestingly, in contrast to *T. reesei*, deletion of *pkcA* in *A. nidulans* is lethal. This difference might be due to an increased abundance of genes of these related families. For *C. neoformans*, regulation of PKC1 by the sphingolipid pathway has been shown (770). A second Pkc protein detected in the genome of *A. nidulans* (769; http://www.aspergillusgenome.org/), termed PkcB, turned out to be a YPK1 homolog, of which *T. atroviride*, *T. virens*, and *T. reesei* have particularly many.

Other members of the AGC family include homologs (TA_151447, TV_73792, and TR_78909) of the NDR-type kinase COT1. The *N. crassa* homolog of COT1 interacts with MAPK

signaling to regulate growth, hyphal fusion, and sexual development (771). Moreoever, *cot-1* is responsive to light.

CAMK kinases. Kinases of the CAMK group are predominantly activated and modulated by binding of calcium or calmodulin to a small C-terminal domain of their catalytic domain. Regulation by specific phospholipids as well as by autophosphorylation was observed (772). In *N. crassa*, their functions include regulation of growth and asexual and sexual development, as well as stress responses (722). For example, CAMK-1 is involved in regulation of growth and development, but also in circadian conidiation and phosphorylation of the circadian clock protein Frequency (FRQ) (773). *T. reesei*, *T. atroviride*, and *T. virens* genomes encode homologs to all *N. crassa* CAMK-type kinases (see Table S1 in the supplemental material). In some cases, expansions were detected in one or more *Trichoderma* spp. *N. crassa* NCU02814 (PRD-4 [period-4]) has one homolog in *T. reesei* (TR_121061) but two homologs in *T. atroviride* (TA_148272 and TA_252945) and *T. virens* (TR_175355 and TV_219049). These genes are related to mammalian checkpoint kinase 2 (CHK2). *N. crassa* PRD-4 is involved in regulation of circadian rhythmicity and temperature compensation of the clock, as well as resetting of the clock by DNA-damaging agents (774). Also, homologs to *N. crassa* CAMK-1 show an expansion, with one homolog in *T. atroviride* (TA_301592) and two homologs in *T. reesei* (TR_76522 and TR_71078) and *T. virens* (TV_87272 and TV_51284).

These interesting extensions in genes involved in regulation of the circadian clock in *Trichoderma* spp. suggest higher redundancy and hence potentially a more robust mechanism for this process, which warrants further analysis.

RIO kinases. RIO kinases (named after "right open reading frame," which refers to one of two divergently transcribed genes) are classified as atypical protein kinases. Their two yeast homologs are involved in regulation of cell cycle progression and chromosome maintenance. In *N. crassa*, two homologs are present (NCU07722 and NCU08767), one of which is involved in ribosome biogenesis. In both cases, deletion is lethal (722). Both genes have homologs in *T. atroviride*, *T. virens*, and *T. reesei*: TV_214757, TR_81007, and TA28378 are related to RIO1, TA_149115, TR_55803, and TV_179564 are more closely related to RIO2, and all contain RIO1 domains (PF01163).

TOR kinases/PIKK family. The TOR pathway is named after two yeast phosphatidylinositol kinases (PIKK) which were found to be targets of the antifungal agent rapamycin (775). TOR paralogs are present in many fungi, and in *F. fujikuroi*, TOR kinase is involved in nitrogen regulation of secondary metabolism, transcriptional control, ribosome biogenesis, and carbon metabolism (776). The Tor1p homologs of *T. reesei*, *T. atroviride*, and *T. virens* are TA_293155, TV_56056, and TR_121610. Further members of the PIKK superfamily, belonging to the ATM (ataxia telangiectasia mutated) domain subgroup are TA_181231, TV_161348, and TR_66128 and TA_152582, TV_157717, and TR_66928. Members of the PIKK subclass PI3K (phosphoinositide 3-kinase, class III) are TV_43914, TR_66913, and TA_157648. The function of the latter kinases is not yet known. The number of genes encoding these kinases is comparable to other closely related fungi (719).

Analysis of transcript profiles of *Trichoderma* kinases. Evaluation of transcript levels of kinase genes for *T. reesei* upon growth on cellulose (350) showed high-level transcription for two steps of the pheromone response/filamentation MAPK pathway containing homologs of the MAPKK Ste7p (TR_75872) and the MAPK Kss1p (TMK1; TR_121537). The corresponding MAPKKK (TR_4945) is moderately transcribed. Additionally, the casein kinase 1a homolog TR_109876 and the casein kinase IIa homolog TR_79503 are also strongly transcribed under these conditions. In accordance with the known function of the two casein kinases in *N. crassa* circadian rhythmicity and light response (758), the GSK-3 homolog that also shows light-dependent functions (TR_74400) is highly expressed. A homolog of NpkA, which is involved in cell cycle progression and damage response in *A. nidulans* (TR_124172) and another CMGC kinase (TR_44330) are highly expressed. Of the calcium/calmodulin-dependent kinases, only TR_64125 showed a strong upregulation upon deletion of *gng1* or *phlp1*. However, as no homologs of this gene have been characterized, the relevance of this regulation remains to be investigated.

For *T. atroviride*, we detected a response to mycelial injury by increased transcript levels for the class I histidine kinase TA_32890, which shows an expansion in *Trichoderma* spp. Moreover, the NpkA homolog of *T. atroviride* (TA_54757), the CAMK kinase TA_36309, and an AGC kinase (TA_298242) are upregulated upon injury (15).

Conclusions. In general, the numbers of different kinase families in *T. reesei*, *T. atroviride*, and *T. virens* are comparable to those in other fungi. However, for almost every kinase family, expansions were detected in one or more *Trichoderma* spp. Examples are the class I histidine kinases, the function of which is not yet known, with two *Trichoderma* homologs instead of one in *N. crassa* and the class I histidine kinases, with one member in *T. virens* and two in *T. atroviride*, but none in *T. reesei* or *N. crassa*.

Also, casein kinases I and II show an expansion in *T. reesei*, *T. atroviride*, and *T. virens*. These kinases have functions in amino acid metabolism as well as circadian and light-dependent gene regulation. Moreover, *T. reesei*, *T. atroviride*, and *T. virens* have many YPK1-like kinases, which are involved in proliferation and growth, in the sphingolipid signaling pathway, and endocytosis, which make them essential kinases in *N. crassa* and *A. nidulans*.

Although especially in the group of protein kinases the numbers of several groups differ in *T. reesei*, *T. atroviride*, and *T. virens*, the relevance of these differences for the specific lifestyles of these fungi can hardly be predicted based on current data and known functions of protein kinases.

Protein Phosphatases

Although not many fungal phosphatases have been characterized, genome analyses of different organisms have allowed for identification of some PPs and inferences regarding their functions (777). PP activity in *Neurospora* was first reported in 1973 (778). More recently, the biochemical and genetic analyses of *Neurospora* phosphatases have enabled elucidation of the functions of these polypeptides (71, 779–789). More recently, protein phosphatases from different fungi, such as *S. cerevisiae*, *Aspergillus* spp., and *Fusarium* spp., have been described (790).

Classification of protein phosphatases. PPs were first classified on the basis of substrate specificity (791) as serine/threonine, tyrosine, dual-specificity, or histidine PPs (Fig. 15). More recent classifications are based, besides substrate specificity, on sequence homology and structural characteristics. These classifications divide protein phosphatases in two major groups: serine/threonine protein phosphatases (PSP) and protein tyrosine phosphatases (PTP) (792, 793). In the genomes of *T. atroviride*, *T. virens*, and *T.*

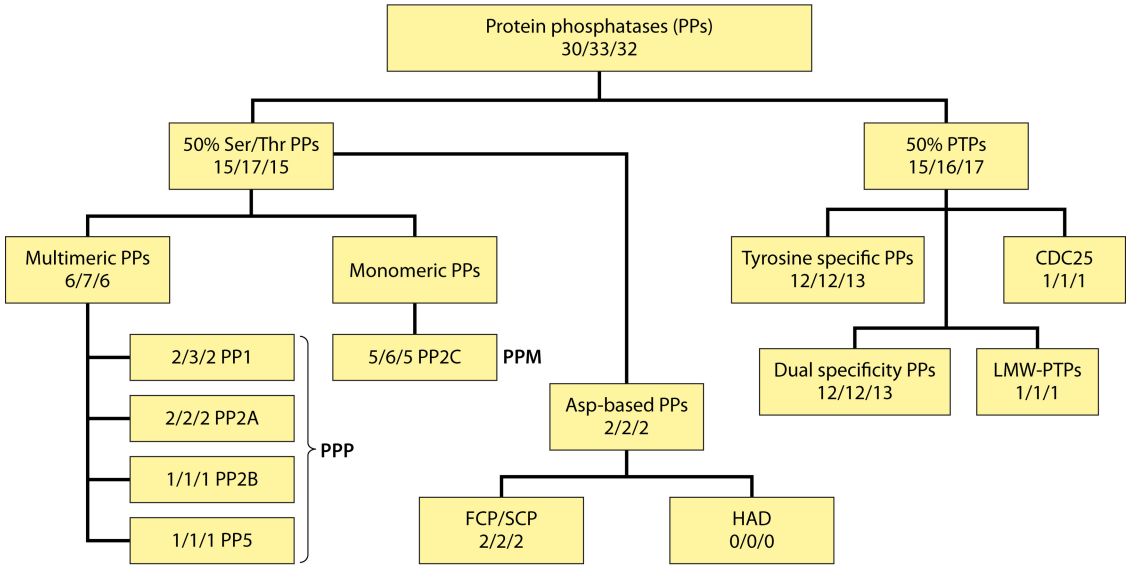

FIG 15 Classification of protein phosphatases based on substrate specificity and structure. The numbers indicate the numbers of genes encoding protein phosphatases in the following order: *T. reesei*/*T. virens*/*T. atroviride*. In this classification only the phosphatase catalytic subunits/units are included. PP, protein phosphatase; PTP, protein tyrosine phosphatase; Ser/Thr, serine-threonine; FCP/SCP TFIIF-associating component of RNA polymerase II CTD phosphatase/small CTD phosphatase; HAD, haloacid dehalogenase; PPM, protein phosphatase M sequence family; PPP, protein phosphatase P sequence family; LMW-PTP, low-molecular-weight protein tyrosine phosphatases.

reesei, 33 protein phosphatases have been annotated. Around 50% of them are PSPs and 50% are PTPs. In addition to catalytic subunits, 6 regulatory subunits for serine/threonine phosphatases were detected. Furthermore, some other proteins which regulate the activity of protein phosphatases were described, including a phosphotyrosyl phosphatase activator, PTPA, and a phosphatase inhibitor. Out of 33 genes encoding protein phosphatases in *Trichoderma* spp., 30 were found to be homologous to *N. crassa* protein phosphatase-encoding genes. However, for two genes encoding predicted protein phosphatases, phylogenetic and BLAST analysis revealed that they have no homologs in *N. crassa* and were found to have a higher similarity to homologous genes in *Giberella zeae* PH-1. Yet another gene showed higher similarity to the homologous gene in *A. nidulans*. The total number of genes encoding protein phosphatases varies between the different *Trichoderma* spp. In the genome of *T. reesei*, 30 genes encoding protein phosphatases were found, while in *T. virens* 33 genes were identified and 32 were identified for *T. atroviride*.

Serine/threonine protein phosphatases. The Ser/Thr protein phosphatases are classified into protein phosphatase 1 (PP1), protein phosphatase 2 (PP2), and protein phosphatase 5 (PP5) groups, depending on differential sensitivities to small-molecule inhibitors. Moreover, PP2 proteins are subdivided according to their metal ion requirements: the protein phosphatase 2B (PP2B) group requires Ca^{2+}, and PP2Cs require Mg^{2+}, while protein phosphatases 2A (PP2As) have no ion requirement (794). More recently, a new two-family gene classification has been proposed, comprising the PPP family, including PP1 (PPZ), PP2A, PP2B, and PP5 groups, based on their sequence similarities. Members of the PPP family are considered multimeric proteins due to the fact that their catalytic subunits are usually associated with a great variety of regulatory subunits (793). However, PP2C sequences lack this high sequence similarity to the protein phosphatase P (PPP) family. Therefore, PP2C and other Mg^{2+}-dependent Ser/Thr phosphatases (STs) were included in the protein phosphatase M (PPM) sequence family (795–797). Those families differ in amino acid sequences and three-dimensional atomic structures. Members of the PPM family do not have regulatory subunits, in contrast with members of the PPP family, but harbor instead additional domains and conserved sequence motifs that can help to determine substrate specificities (793). Nevertheless, members of the PPP and PPM families have similar structural folds (798), suggesting a common mechanism of catalysis.

Moreover, within the family of protein serine/threonine phosphatases, the aspartate-based phosphatases represent the third group. The FCP/SCP (TFIIF-associating component of RNA polymerase II CTD phosphatase/small CTD phosphatase) and haloacid dehalogenase (HAD) classes comprise this third group of protein phosphatases; they use an aspartate-based catalysis mechanism (799, 800). In *T. reesei*, *T. atroviride*, and *T. virens*, 2 protein phosphatases belonging to the FCP/SCP class were found (TR_122050, TV_157255, and TA_165307; TR_28199, TV_30939, and TA_175017).

In *T. reesei*, *T. atroviride*, and *T. virens*, from the 33 protein phosphatases identified there are 3 PP1s (PPZ), two PP2As, one PP2B, six PP2Cs, and one PP5 (see Fig. S27 and Table S1 in the supplemental material). Furthermore, there are PSPs that are not yet classified within the PPP subfamily. Regarding sequence homology, there is one PP2C-like protein phosphatase-encoding gene (TR_21256, TA_50094, and TV_40568/TV_84520) and one PP1 protein phosphatase-encoding gene (TR_120722, TA_301856, and TV_193217/TV_111355) that are homologous to two different proteins in *T. virens*. Interestingly, the PP5-encoding gene in *T. reesei* (TR_52144) shows a 2-fold positive regulation by the heterotrimeric G-protein beta-subunit GNB1 in light and darkness, as well as by the phosducin-like protein PhLP1 in light (350). Additionally, the transcript levels of a gene encoding a PP2C-related phosphatase (TR_74030) are doubled in the Δ*gnb1*

and Δ*phlp1* strains in light compared to the wild type, indicating that this gene is negatively regulated by GNB1 and PhLP1 in light (350). As GNB1, GNG1, and PhLP1 are components of the heterotrimeric G-protein signaling pathway in *T. reesei*, there is likely relevance for the regulation of these phosphatases to nutrient or pheromone signaling.

Protein tyrosine phosphatases. An evolutionary origin and catalytic mechanism distinct from the Ser/Thr PPs has been observed for protein tyrosine phosphatases. Within this group are the specific tyrosine protein phosphatases (PTPs), dual-specificity protein phosphatases (DSPs), low-molecular-weight protein tyrosine phosphatases (LMW-PTPs), and the cdc25 phosphatases (CDC25). The Tyr phosphatases are specific for phosphorylated Tyr residues, whereas dual-specificity phosphatases act at both Tyr and Ser/Thr residues. Nonetheless, both phosphatase types constitute a common evolutionary family. The common feature is a catalytic core motif with a conserved Cys residue, which acts as a nucleophile, displacing the phosphate group from the substrate and forming a phosphorylcysteinyl intermediate (801). A positionally conserved Asp participates in the removal of the phosphate group (802). The low-M_r protein Tyr phosphatases constitute an evolutionarily distinct group, the members of which have converged on a similar catalytic mechanism (801, 803). The cdc25 phosphatases were first described in yeast as having essential roles in mitotic entry (804).

In *T. atroviride*, *T. virens*, and *T. reesei*, 17 PTPs were identified (see Table S1 in the supplemental material), although in many cases it was not entirely clear whether some of these proteins exhibited tyrosine phosphatase or dual-specificity phosphatase activity. Within the PTPs identified, there are two DSPs (TR_27406, TV_61598, and TA_91339; TV_192739 and TA_185900), an M-phase-inducer phosphatase (TR_53004, TV_178424, and TA_155131), which may play a key role in mitosis and may be classified as a cdc phosphatase (ortholog to cdc25/SPAC24H6.05 in *Schizosaccharomyces pombe*) (805–807), and a LMW-PTP (TR_23417, TV_79380, and TA_302924) that is possibly involved in cell growth regulation (808, 809). Transcript levels of the gene encoding the M-phase-inducer phosphatase in *T. atroviride* (TA_155131) decrease roughly 20-fold upon self-confrontation versus growth alone (355). Additionally, the analysis of the transcriptome data for the injury response in *T. atroviride* (15) shows higher transcription levels of two PTPs (TA_80848 and TA_47283) after injury. Concerning the degree of homology between *T. reesei*, *T. atroviride*, and *T. virens*, in general all PTPs and DSP show sequence similarity. Nevertheless, 6 PTPs show a lower degree of identity among *T. atroviride*, *T. virens*, and *T. reesei* (<50%). Moreover, it is important to mention that *T. atroviride* contains one additional homolog (TA_217798) of the PTP homologous to *N. crassa* NCU02257. In *N. crassa*, this gene was found to show decreased transcript levels in response to light (810). Additionally, in contrast to *T. atroviride* and *T. virens* (TV_192739 and TA_185900), *T. reesei* lacks a homolog of NCU05049.

Protein phosphatase regulatory subunits. In addition to the structural features of the catalytic subunits, PP regulatory subunits influence phosphatase specificity and function (785). As an example, in *S. cerevisiae* 25 proteins were demonstrated to regulate phosphatase function (811). In *T. atroviride*, *T. virens*, and *T. reesei* 6 regulatory subunits were identified, 4 of them for PP2A, which is a heterotrimeric enzyme (see Table S1 in the supplemental material). One of the regulatory subunits is PR55 (TR_120545, TV_82802, and TA_51365), a form of the third subunit of the PP2A and which may act as a substrate recognition unit or target the correct subcellular localization of the enzyme (812). Another regulatory subunit is B56 (TR_77135, TV_35847, and TA_126920), which is associated with a family of B subunits that regulates the recognition of different substrates by the enzyme PP2A (813). There is also a protein phosphatase annotated SIT4-phosphatase-associated protein (TR_35316, TV_112554, and TA_272735), where SIT4 is the catalytic subunit of a type 2A-related protein phosphatase (814). A PP2A-associated protein characterized as a TAP42-like protein (TR_74861, TV_179542, and TA_294385) is suggested to negatively regulate the TOR signaling pathway, which activates cell growth in response to nutrients (815). There is a calcineurin regulatory subunit for PP2B (TR_52130, TV_85975, and TA_254295). Finally, a further putative phosphatase regulatory subunit identified (TA_250560, TV_51891, and TR_123502 [related to NCU08779]) may be involved in glycogen metabolism (816). Interestingly, these proteins comprise carbohydrate binding modules of family 21. The sequence homology of these regulatory subunits between *T. atroviride*, *T. virens*, and *T. reesei* is high.

Besides regulatory subunits, also proteins regulating the activity of protein phosphatases are important for their function in signal transduction. Two phosphotyrosyl phosphatase activators (PTPA; also known as PP2A phosphatase activator; TR_120498, TV_183029, and TA_297400 and TR_79850, TV_208703, and TA_136266) and a phosphatase inhibitor (TR_119724, TV_19035, and TA_297757) were identified in *T. atroviride*, *T. virens*, and *T. reesei* (see Table S1 in the supplemental material).

Metabolic pathways regulating the detection of different carbon sources and hydrolytic enzyme production include protein phosphatases as regulators (817). Seven protein phosphatases were found to influence cellulase and hemicellulase production in *A. nidulans*. Most of them caused reduced endocellulase activity when deleted. Homologous genes were found in *T. reesei*, *T. atroviride*, and *T. virens*, with likely similar functions. Moreover, three *A. nidulans* protein phosphatases had a role in the cell cycle: the low-molecular-weight phosphotyrosine phosphatase LptA (TR_23417, TV_79380, and TA_302924), the PTP/DSP CdcA (TR_27406, TV_61598, and TA_91339), and the PSP SitA (TR_48910, TV_82688, and TA_299066). Additionally, a role in MAPK regulation was detected for the two *A. nidulans* protein phosphatases PtcA (PP2C type; TR_58587, TV_181508, and TA_128623) and PtpA (PTP type; TR_25159, TV_20663, and TA_80848) (817).

In *S. cerevisiae*, the protein Ppg1p, which is homologous to the *A. nidulans* PP2A-related phosphatase Ppg1A (TR_56872, TV_76450, and TA_81292), is required for glycogen accumulation. This protein is also associated with *S. cerevisiae* Tap42p and Sit4p (related to the PSPs TR_48910, TV_82688, and TA_299066), which are involved in TOR signaling (817).

Conclusions. The genome of *T. reesei* harbors 30 genes encoding catalytic subunits of protein phosphatases, a slightly decreased number compared to *T. virens* (33 genes) and *T. atroviride* (32 genes). Regarding STPs, there are a PP2C-like phosphatase (TR_21256, TA_50094, and TV_40568/TV_84520) potentially involved in regulation of growth and development (818) and a PP1 protein phosphatase (TR_120722, TA_301856, and TV_193217/TV_111355), which may contribute to circadian rhythmicity ex-

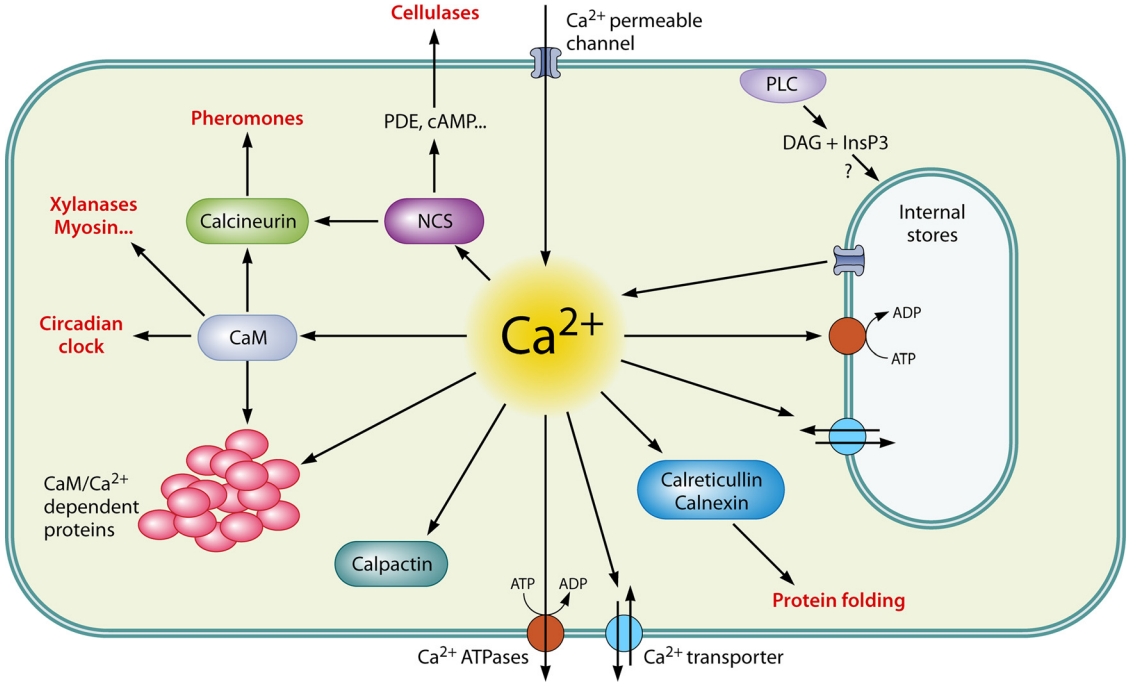

FIG 16 Schematic representation of calcium signaling. The homeostasis of the calcium level in the cytoplasm is maintained by calcium-permeable channels, transporters, and ATPases. Calcium is required by a large number of proteins, which in turn regulate, among other things, asexual and sexual development, the circadian clock, xylanases and cellulases, and protein folding.

panded in *T. virens*. Concerning PTPs, there are two DSPs that have been identified (TR_27406, TV_61598, and TA_91339; TV_192739 and TA_185900) for *T. virens* and *T. atroviride*, but only one for *T. reesei*. Characterized homologs of these phosphatases (818–820) suggest again a function in growth and development. Additionally, *T. atroviride* harbors one additional homolog (TA_217798) of the PTP related to *N. crassa* NCU02257, for which the transcript levels in response to light were decreased (810). Furthermore, in contrast to genes encoding PTPs in *T. atroviride* and *T. virens* (TV_192739 and TA_185900), *T. reesei* lacks a homolog of yeast Sdp1p, which couples oxidative stress and substrate recognition (821).

Calcium Signaling

Calcium is ubiquitous and can be found almost everywhere on earth, even in the sea as $CaCO_3$ (822). The homeostasis of Ca^{2+} in the cell is essential for all living organisms (823), and its intracellular levels reflect environmental changes (824). In fungi, calcium is involved in growth and tip orientation, metabolism, circadian clock regulation, pathogenicity, and differentiation processes (773, 825–830). Levels of calcium also affect cellulase production in *Trichoderma* (831). Here we discuss genes involved in calcium entry and release in the cytoplasm (with calcium-permeable channels, calcium ATPases, and transporters) and the subsequent signaling pathway dependent on the level of calcium (Fig. 16).

Calcium-permeable channels. Different mechanisms guarantee the entry and release of Ca^{2+} from extra- and intracellular compartments. Among other mechanisms, calcium-permeable channels allow for the exchange of calcium ions across cell membranes into the cytosol.

Genomes of *T. reesei*, *T. virens*, and *T. atroviride*, respectively, comprise homologs of the yeast calcium-permeable channels Cch1p, Mid1p, and Yvc1p (832). Yvc1p is a homolog of the TRP (transient receptor potential) family, which are receptor-operated Ca^{2+} entry channels (833). The Mid1p (TR_37060, TA_157896, and TV_73324) homolog in *Trichoderma* spp. is considered an atypical calcium-permeable channel due to only one transmembrane domain, which is supposedly part of the signal sequence and therefore likely separates after transferring the protein to the correct locus. In yeast it was shown that the first hydrophobic region (amino acids 2 to 22; H1) of Mid1p is responsible for delivery of the protein to the plasma membrane (834) and that Mid1p interacts with Cch1p (TR_23028, TA_222101, and TV_218840), with both proteins members of the same calcium signaling pathway (835). The Cch1p-Mid1p calcium-permeable channel is essential for stress tolerance (836) and mating (837). The stretch-activated component of the Cch1p-Mid1p calcium-permeable channel, Mid1p, is induced by the mating α-factor and regulates calcium ion influx (837). All three Ca^{2+}-permeable channels of *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 in the supplemental material) share high similarities (identities of 86%, 87%, and 81%, respectively).

Calcium ATPases and calcium exchangers. Ca^{2+}-permeable channels are necessary for Ca^{2+} transport into the cytoplasm. To maintain the homeostasis of ions in the cell, the surplus of Ca^{2+} has to be released. Ca^{2+} ATPases and exchangers are required for transport of the Ca^{2+} ions out of the cytolplasm across the plasma membranes and in subcellular organelles. Ca^{2+} ATPases use ATP as the energy source to actively transport calcium ions across membranes. In contrast, exchangers couple the transport of Ca^{2+} to the transport of other ions, such as H^+ or Na^+ (838, 839).

Three types of ATPases are known (840): SERCA (sarcoplasmic/endoplasmic reticulum Ca^{2+}), PMCA (plasma membrane

Ca^{2+}), and Na^+/K^+ ATPases in animals. PMR1 is another P-type ATPase and was shown to be different from SERCA and PMCA ATPases with respect to substrate affinity, location (Golgi complex), and inhibitors (841). Ca^{2+} ATPases belong to the type II P-type ATPases due to the low atomic mass of Ca^{2+} (842). In *N. crassa*, 6 ATPases were isolated and characterized (843): NCA1, NCA2, NCA3, PMR1, ENA1, and PH7. Phylogenetic analysis revealed that NCA1 shares high similarity with SERCA-type ATPases, NCA2 and NCA3 belong to the PMCA ATPases, and PMR1 belongs to the group of PMR1 ATPases. Recently, it was shown that NCA2 has a role in transferring calcium out of the cell and enables *N. crassa* to tolerate higher levels of Ca^{2+} (844). ENA1 and PH-7 are potential Na^+ ATPases. More recently, four more ATPases were identified in the genome of *N. crassa* (832), but only one (NCU04898) shares similarity with a P-type ATPase, which was shown to be involved in the Ca^{2+} signaling pathway of *S. cerevisiae* (Spf1p) (845). One of the four additional ATPases of *N. crassa* (NCU03818) is a flippase, which was not shown to be involved in Ca^{2+} signaling, and two of them are cation ATPases (NCU07966 and NCU10143), for which it is not certain whether they even transport Ca^{2+}. In *M. oryzae*, 12 P-type ATPases have been identified (846).

The genome of *T. reesei* comprises 10 different calcium (or Na^+) ATPases, whereas *T. atroviride* has only 8 and *T. virens* has 11 Ca^{2+} ATPases. *T. reesei* and *T. virens* have one additional homolog of the PMCA ATPases (TR_58952 and TV_33876) and *T. virens* has one homolog of the putative Na^+ ATPase ENA-1 in addition (TV_67662). A homolog of the cation ATPase NCU07966 is missing in *T. atroviride*.

Phylogenetic analysis results (see Fig. S28 in the supplemental material) suggest that groups with NCU04898 and NCU10143 do not belong to either the group of known SERCA-like and PMCA ATPases or to the group of PMR1 type ATPases. Interestingly, the clade with ENA-1 contains two *T. virens* homologs and one *T. reesei* homolog, but no *T. atroviride* homolog. For PMCA ATPases, there is one clear homolog in *T. reesei*, *T. atroviride*, and *T. virens* (TR_75347, TA_133801, and TV_210318) that is related to both NCA-2 and NCA-3. Additionally, we found two more homologs in *T. reesei* (TR_62362 and TR_58952) and two in *T. virens* (TV_69284 and TV_33876), but only one additional homolog for *T. atroviride* (TA_322548).

NCU07966 and its homologs (TR_81536, TA_128193, and TV_34827) are probably Na^+ ATPases, because of their relation to ENA-1.

The genome of *T. virens* comprises more calcium transporters than *T. reesei*, and *T. atroviride* has fewer than *T. reesei*. Interestingly, the genome of *T. virens* comprises three homologs (TV_188970, TV_229481, and TV_84930) of calcium transporters, which are in the immediate genomic vicinity of each other. It will be interesting to learn whether the higher number of calcium transporters in *T. virens* contributes to a greater potential as a biocontrol agent. Biocontrol activity of a yeast strain was found to be enhanced in the presence of Ca^{2+} ions, and these ions have inhibitory effects on germination and metabolism of *Botrytis cinerea* (847). The suspected cause might be a quick and efficient transport system for Ca^{2+} ions that can maintain normal levels of Ca^{2+}.

Calcium signaling pathway. One of the main components in the Ca^{2+} signaling pathway is represented by the enzyme phospholipase C (PLC) and its homologs. After binding of hormones and other growth-specific substances to receptors at the plasma membrane, PLC is activated and catalyzes the hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate ($InsP_3$) diacylglycerol. $InsP_3$ in turn acts as a second messenger in the connection between the different Ca^{2+} receptors for release or entry of calcium in the cytosol (848). Interestingly, in *T. atroviride*, *T. virens*, and *T. reesei* no $InsP_3$ receptor was found, similar to *N. crassa* (849). Despite the fact that PLCs of eukaryotes comprise two domains of high similarity between the species (the catalytic X-box and Y-box domains), the proteins are highly variable between different strains of *Neurospora*, which was originally isolated in different parts of the world (849). A phospholipase C homolog and thus the calcium flux of *M. oryzae* is important for fungal development and pathogenicity (850). *T. reesei*, *T. virens*, and *T. atroviride* have 5 homologs of phospholipase C each in their genomes (see Table S1 in the supplemental material).

Calmodulin (CaM) plays a key role in calcium signaling and comprises two pairs of EF-hand motifs, each of which consist of two α-helices and a 12-amino-acid calcium binding loop. When Ca^{2+} binds to calmodulin, a conformational change leads to the exposure of hydrophobic residues on one side of the enzyme and subsequent energy release. In that conformation, calmodulin is able to bind and interact with other target enzymes. In yeast, for example, 11 more targets for CaM other than calcineurin were identified (851). The calmodulin/calcineurin signaling pathway is required for appropriate stress responses, mitosis, growth (852–854), and other pathways, like toxin production (855). In *T. reesei*, calmodulin was shown to be essential for secretion of xylanases (839). Calcineurin is one of the target proteins of CaM in fungi (856) and functions as a Ca^{2+}/calmodulin-dependent serine/threonine protein phosphatase. The protein is a heterodimer, consisting of a catalytic subunit A and a regulatory subunit B (857). It was shown to be essential in the pheromone response of *S. cerevisiae* (858). Since *T. reesei* is able to reproduce sexually (859), the calcineurin homolog is a promising target for investigation with respect to sexual development in *T. reesei*. While *T. virens* and *T. reesei* have one homolog of CAM1 (TR_ 80447 and TV_111915), the genome of *T. atroviride* comprises two homologs (TA_297616 and TA_213443).

Calmodulin has many target proteins involved in the calcium/calmodulin-dependent signaling pathway, e.g., calmodulin-dependent kinases (see the "Protein kinases" section), such as CAMK-1, as shown in *N. crassa*. CAMK-1 is involved in the circadian clock feedback loop, because of its ability to phosphorylate FRQ (773). A homolog of CAMK-1 was found in the *T. reesei* genome and designated CMK2 (TR_71078, TA_301502, and TV_87272) (23). Many genes encoding homologs of these Ca/CaM-dependent proteins could be detected in genomes of *T. reesei* (9 genes), *T. virens* (12 genes), and *T. atroviride* (9 genes). *T. virens* again seems to have the most Ca/CaM binding proteins compared to the other two species.

In *T. reesei* three of nine Ca/CaM binding proteins were expressed at a very basal level (TR_71078, TR_22381, and TR_121061) in microarray experiments, where the strain QM9414 was grown on cellulose as the carbon source and in constant light or darkness (350). Although CAMK-1 of *N. crassa* was shown to have a function in the circadian rhythm, none of the related proteins was significantly regulated in response to constant light in *T. reesei* with the exception of TR_76522 (351). In mutant

strains deleted for photoreceptors (Δ*blr1* and Δ*blr2*), no change in expression of calcium/calmodulin-dependent kinase TR_22381 was observed, but deletion of the gene encoding the light regulatory protein ENVOY (Δ*env1*) led to a more-than-10-fold upregulation of its expression level (351), suggesting that this kinase is involved in regulation of the light signaling pathway in *T. reesei*.

A very important member of the group of calcium binding proteins is calreticulin. Calnexin and calreticulin are two proteins that share high similarities over long stretches and carry an ER signal sequence. Both of them act as molecular chaperons and bind to glycoproteins in the ER (860). Furthermore, it has been shown that calreticulin acts as a chaperone also on nonglycosylated proteins *in vitro* (861). One member of this gene family could be identified in each of the three *Trichoderma* genomes; this suggests a function of calcium signaling in protein folding.

Another important Ca^{2+}/calmodulin binding protein is the neuronal calcium sensor-like protein (NCS; TR_75001, TA_301289, and TV_77402). NCS is a 4 EF-hand-containing Ca^{2+} binding protein shown to interact with many target proteins, for example, calcineurin and 3′,5′-cyclic nucleotide phosphodiesterase (PDE), and together with calmodulin it interacts with nitric oxide synthase (862). In *T. reesei* it was suggested that ENVOY and PDE are interconnected and in concert they regulate the light-dependent G-protein signaling pathway with cellulase production as an output pathway (619). The NCS of *N. crassa* shows functions in growth, calcium stress, and UV tolerance (863). In the fission yeast *Schizosaccharomyces pombe*, NCS is required for nutrition-independent sexual development and is involved in sporulation (864). An NCS disruption mutant of the filamentous fungus *M. grisea* showed growth defects under acidic conditions and at high Ca^{2+} concentrations (865). In *Dictyostelium discoideum* there is good evidence that this factor regulates entry into development, depending on the availability of nutrients (866).

Unconventional myosins are mechanoenzymes and are known to bind calmodulin, which can considerably change their enzymatic activity (867, 868). We detected homologs for a myosin light chain (Cdc4 from *S. pombe*) (869) in *T. atroviride*, *T. virens*, and *T. reesei* (TR_65659, TA_46760, and TV_82840). Calpactins (calcium and actin binding), or annexins, are Ca^{2+}-dependent negatively charged phospholipid binding proteins (870). Two different calpactins are known: calpactin I and calpactin II. Both contain the 38-kDa calpactin heavy chain, but calpactin I has a light chain in addition (871, 872). Two homologs of the calcium binding calpactin heavy chain were detected in each of the *Trichoderma* genomes (TR_21646 and TR_29619, TA_144672 and TA_50457, and TV_88587 and TV_224689).

Conclusions. Three Ca^{2+}-permeable channels for entry of Ca^{2+} into the cytoplasm were found in each of the *Trichoderma* species, sharing high similarities. The genome of *T. virens* comprised more homologs for calcium (or Na^{+}) ATPases (11) than the genome of *T. atroviride* (8) or *T. reesei* (10) for homeostasis of Ca levels in the cell. Compared to *N. crassa* (4 homologs) and *S. cerevisiae* (1 homolog), an increased number of homologs of phospholipase C in each of the *Trichoderma* genomes was found (5 homologs), which may be responsible for efficient Ca^{2+} flux, as PLC is one of the main components of the Ca^{2+} signaling pathway. The relevance of these alterations in the genome content remains to be determined.

Photobiology

During evolution, nearly all forms of life on earth have been exposed to different levels of electromagnetic radiation and are dependent on transformations of the energy contained in the radiation emitted by the sun. While plants are characterized by their capacity to capture energy from sunlight and use it to synthesize sugars from carbon dioxide and water (photosynthesis), fungi are characterized for not using light energy in this way (873).

Many organisms, however, use sunlight not as a source of energy but to obtain information from their environment. The use of light either as energy or as information source depends on the interaction of light with the molecular system of an organism. The energy contained in light can dissipate thermally, it can initiate photochemical reactions, or it can be returned as light (fluorescence or phosphorescence). Photochemical reactions lead to physiological responses in photosensitive organisms.

Radiation emitted by the sun comprises a broad spectrum from very long radio waves to very short gamma rays. Sunlight ranging from UV to infrared (IR) regulates several biological processes, including circadian rhythms, photomorphogenesis, phototropisms, and synthesis of pigments, among others. Radiation of shorter wavelengths, containing more energy, corresponds to UV, which can initiate photochemical reactions. Among the molecules that can be affected by UV, DNA is perhaps the most important, since the result of one of such reaction can result in a change transmitted to the next generation as a mutation if the change is not repaired before DNA replication. Additionally, visible light can indirectly give rise to ROS as a product of photosensitive reactions through energy transfer from a molecule that can be activated by light, such as flavin or porphyrin. It is in this way that blue light is potentially harmful (674, 874).

Flavins such as riboflavin (vitamin B_2), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and porphyrins, such as a heme group, absorb light in the visible range. Solutions of these compounds appear colored to our eyes, defining pigments or chromophores. Consequently, all biological responses to visible light must be initiated by chromophores such as these.

Trichoderma spp. have served as model organisms for photobiology for decades (12). In 1951, for the first time it was described that in nutrient-rich medium in the dark *Trichoderma* grows indefinitely as mycelium and that a pulse of light applied to the mycelium leads to the formation of mature conidia, forming a ring at the actively growing front of a colony (875). The action spectrum of photoconidiation, which indicates the wavelength of light that is most effectively used in this specific physiological response, showed the characteristic shape attributed to what at that time was considered a "cryptochrome," including a sharp peak in the near-UV range of 350 to 380 nm, and a wider peak in the blue range, with a maximum at 440 to 450 nm (876, 877). These action spectra are consistent with the absorption spectra of some flavoproteins. Accordingly, the operation of cryptochromes in *Trichoderma* was suggested as a hypothesis corroborated by the presence of genes encoding potential flavoproteins that could act as photoreceptors in *T. reesei*, *T. atroviride*, and *T. virens* (607, 878, 879).

In *T. reesei*, 248 genes (2.7%) were found to be light regulated upon growth on cellulose, as they were significantly enriched in functions of carbohydrate metabolism, oxidoreductase activity, and sulfate transport, but there were also genes involved in devel-

opment in this group (350). These data corresponded well to earlier data for *T. atroviride* (880). In a recent analysis using high-throughput sequencing with *T. atroviride*, 401 light-regulated genes were identified. Interestingly, 39 out of the 178 light-induced genes are related to different stress responses, with 17 related to oxidative stress (1313). This set of genes includes key elements, such as components of the Tmk3 (p38/Hog1) MAP kinase cascade related to these osmotic and oxidative stresses. In addition, key elements involved in the nucleotide excision repair and mismatch repair systems are induced, as well as *phr-1* (Esquivel-Naranjo et al., unpublished). Within the set of light-regulated genes, at least 10 transcription factors were found. Two of them are orthologs of the *N. crassa* short aerial hyphae transcription factors (*sah1* and *sah3*), which as suggested by their names are involved in the correct development of aerial hyphae, a prerequisite in *Trichoderma* for the formation of conidiophores.

Photoreceptors. Two proteins, BLR1 and BLR2 (TA_229937, TV_81343, and TR_121962, and TA_42429, TV_31745, and TR_22699, respectively), were shown to be responsible for almost all blue light responses observed in *T. atroviride* (878). These *blr* genes encode proteins homologous to the *N. crassa* WC (White Collar) proteins (881), are essential for photoconidiation and gene expression regulated by blue light, and are important for mycelial growth (878, 880). In *Trichoderma* spp., BLR1 and BLR2 are PAS domain proteins with a GATA-type DNA binding domain. BLR1 has three PAS domains; the first belongs to a specialized sensory domain called LOV (Light-Oxygen-Voltage), similar to that initially described in plant phototropins. The LOV domain is a module sensitive to light broadly conserved in proteins sensing light, oxygen, and voltages from bacteria, fungi, and plants (12). The BLR1 LOV domain has all amino acids necessary to interact with the chromophore FAD, including a cysteine, which forms a photoadduct with the flavin. BLR2 has only one PAS domain, which is presumably used to interact with other proteins. Based on the structure of the BLR proteins and the phenotype observed in *blr1* and *blr2* gene replacement mutants, it is likely that the corresponding proteins form a blue light photoreceptor complex in *Trichoderma* spp. and that this complex acts as transcription factor (878, 879), similar to that described for *N. crassa* WC1/WC2 proteins (882, 883). More recently, comparable results for strains containing mutants for the *T. reesei* homologs of the *blr* genes were reported, which showed that such mutations also influence cellulase gene expression (879). In fact, overexpression of BLR2, which has no LOV domain, caused an increment in photosensitivity, a property directly associated with photoreceptors (884). Investigation of the influence of BLR1 and BLR2 on sexual development in *T. reesei* revealed an involvement in regulation of the pheromone system, albeit these photoreceptors are not essential for mating (885).

Although BLR1 and BLR2 form a blue/UV-A receptor, these proteins also have functions independent of light. The BLR proteins are required for conidiation induced by sudden carbon deprivation and by cAMP addition in the dark (52, 878). Proteins belonging to the White Collar family, including the BLRs, are undoubtedly the main photoreceptors in fungi that regulate practically all known light responses.

In addition to the putative *Trichoderma* BLR1 photoreceptor, ENV1 (ENVOY1), another blue light photoreceptor, has been described. ENV1 (TR_81609, TA_150699, and TV_73856) was discovered in *T. reesei* after a screening for novel signaling factors involved in regulation of production of cellulases (886, 887). ENV1 is a small protein that contains a PAS/LOV domain considered central for its function. It is an ortholog of VIVID from *N. crassa*, a blue light photoreceptor that functions downstream of the WC proteins to negatively regulate the responses initiated by the WC proteins. In *N. crassa* most, if not all, blue light-regulated genes are subjected to photoadaptation, which depends on the presence of a functional VIVID protein (888–890). Similarly, ENV1 is a negative regulator of the BLR1/2 light input involved in photoadaptation; however, it does not functionally complement a vivid mutant (626, 922). The *T. atroviride* ortholog of *env1* is 60% identical and 75% similar to that of *T. reesei*. In agreement with the cross talk between light and carbon source signaling discovered in *T. atroviride*, ENV1 appears to modulate the impact of light on cellulase production (52, 878, 879, 886, 887). In darkness, *env1* transcription is at low basal levels, but upon exposure of the fungus to light there is a strong induction within minutes, resulting in a 50- to 500-fold increase in transcript abundance in both *T. atroviride* and *T. reesei*. Such a response to light is, however, mediated by the BLR photoreceptor complex (879). In *T. reesei*, lack of *env1* causes a severe growth phenotype in light (885, 886), which is considered to be at least in part due to its effect on cAMP levels (619). This assumption is supported by the finding that a major portion of the regulatory targets of ENV1 (351) overlap with those of adenylate cyclase (ACY1) (620). With respect to development, ENV1 is involved in regulation of asexual development, and it is essential for female fertility in light (885, 891). The latter function is attributed to a strong deregulation of the pheromone system in *env1* mutants, which is obvious in light but not in darkness (885). Recently, it has been shown that a cysteine at position 96 in ENV1 is responsible for integration of stress responses, which contrasts with VVD. Interestingly, this cysteine is only conserved in Hypocreales, and hence an evolutionary relevance for C96 is likely (892).

The signaling function of ENV1 must involve additional, light-dependent auxiliary components, since overexpression of ENV1 in the dark does not result in gene regulation patterns similar to growth in light, where *env1* is induced (78).

Transcriptome analysis of light signaling via BLR1, BLR2, and ENV1 revealed carbon metabolic pathways, including the pentose phosphate pathway, as major targets in *T. reesei* (351), and comparable results were obtained for *N. crassa* (755). In both fungi, individual regulatory targets have been detected for BLR1, BLR2, and ENV1, indicating that they do not always act as a complex. In *T. atroviride*, 70 light-regulated genes identified appear to be *blr* independent, providing support to the existence of additional, functional, light receptors (1313). The fact that light responses have been observed even in Δ*blr* mutants indicates that *Trichoderma* spp. have additional genes encoding functional photoreceptors. In this sense, there are a CPD photolyase (PHR1; TA_302457, TV_50747, and TR_107680), cryptochrome DASH (TA_285589, TV_50684, and TR_59726), a cryptochrome/6-4 photolyase (CRY1; TA_86846, TV_37166, and TR_77473), and a phytochrome (PHY1; TA_319399, TV_190601, and TR_77764) as potential candidates.

The *N. crassa* genome has one CPD photolyase and a cryptochrome DASH, and *A. nidulans* has only one CPD photolyase but it has a cryptochrome-type role, as described for PHR1 in *T. atroviride* (77). The cryptochromes/6-4 photolyases of *Trichoderma* (CRY1 in *T. atroviride* and *T. reesei*) form part of the animal sub-

family of chryptochromes and 6-4 photolyases and have a COOH-terminal extension of 79 amino acids, similar to animal cryptochromes and the recently described 6-4 photolyase of *Cercospora zeae-maydis*, with roles in development and secondary metabolism in addition to their photolyase activity. *cry1* gene expression increases in response to exposure to blue light in both *T. atroviride* and *T. reesei* in a BLR-dependent fashion, and the corresponding proteins show photoreactivation activity (80, 1313). Nevertheless, it has been suggested that at least in *T. reesei* CRY1 may have additional regulatory functions (80). Another notable difference is that the *Trichoderma* DASH cryptochromes have a longer COOH terminus than those described in animals, plants, bacteria, and other fungi. *T. atroviride* Cry DASH has a shorter extension of 391 amino acids than *T. virens* and *T. reesei*, with 710 and 661 amino acids, respectively. However, BLAST analyses of the COOH-terminal extension against the NR database of the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed no homology, suggesting that the *Trichoderma* cryptochromes form a subdivision with novel features within the DASH-cryptochromes.

Although responses to red and far-red light have been documented for some fungi, it is only in *A. nidulans* that the function of a phytochrome (FphA) has been demonstrated. It is involved in repression of sexual and stimulation of asexual reproduction under red light (893). In *T. atroviride*, red light provokes a reduction in mycelial growth and also has an impact on the transcriptional regulation of some genes, indicating the participation of a phytochrome in these responses (878, 880). As in many fungal genomes, *T. reesei*, *T. atroviride*, and *T. virens* have a phytochrome with all features contained in the phytochromes PHYT1 and FphA as described for *N. crassa* and *A. nidulans*, except that the putative second phytochrome (PHYT2) of *N. crassa* does not contain a conserved PAS domain in its N terminus.

Surprisingly, only the *T. atroviride* genome contains a gene encoding a putative opsin (TA_210598) with homology to opsins NCU01735.3 (46% identity) and *ops-1* (27% identity) from *N. crassa*, among others. The opsin has seven transmembrane spans typical of GPCRs associated with G-proteins and could represent a link between light perception, the cAMP signaling pathway, and carbon metabolism. However, *T. atroviride* strains with mutations in the putative opsin-type photoreceptor show no obvious phenotype (1313).

Light and rhythms. To adapt to their environment, organisms have evolved endogenous cellular timekeepers that control a number of daily physiological and molecular rhythms in most eukaryotic and some prokaryotic organisms. These timekeepers are usually named circadian clocks (894). While in *N. crassa* the two photoreceptors WC-1 and WC-2 and also FRQ have a crucial function in circadian rhythmicity (895), the presence of circadian rhythms or their regulation by homologs of WC-1, WC-2, and FRQ has not yet been shown for *T. reesei*, *T. atroviride*, or *T. virens*, albeit rhythmic conidiation was observed for *Trichoderma pleuroticola* (14). Given the ubiquity of circadian regulation in countless organisms, it would be surprising if this mechanism was not operative in other *Trichoderma* spp. as well. Thus, it is likely that rhythmicity is present in *Trichoderma*, but it is not obvious in terms of conidiation cycles.

Although multiple efforts to elucidate circadian clocks in *Trichoderma* spp. have been made, little is known about such phenomena in these organisms. Most *Trichoderma* species require light for conidiation. Colonies growing in the dark were induced by light/dark cycles of 8, 16, 24, and 48 h, determining that conidiation occurred and corresponded to the interval of illumination. When *Trichoderma* spp. are returned to the dark after a pulse of light, conidiation is observed only at what was the colony perimeter at the time of the pulse. In *T. viride*, these results led to the conclusion that conidiation is not rhythmic but can be synchronized by a light pulse (742). Similarly, under continuous light, conidiation is not rhythmic; however, banding patterns are formed under light/dark cycles (886). Interestingly, a *Trichoderma* mutant (B119) that conidiates rhythmically in the dark has been described, and composition of the growth medium influences the period length of conidiation. As in the wild-type strains, application of light induced a ring of conidia, but it also delayed the reappearance of the dark banding pattern (743). Nevertheless, similar to what has been described for *frq* in *N. crassa*, the *frq* genes (TA_131340 and TR_121670) of *T. atroviride* and *T. reesei* are regulated by light, and such regulation depends on functional *blr1* and *blr2* genes (351, 894, 1313). An ortholog of *frq* has also been found in *T. virens* (TV_20727). Stability of FRQ is regulated by phosphorylation-mediated degradation via the ubiquitin pathway. The ubiquitin ligase FWD-1 plays an important role in this process in *N. crassa* (896). FWD1 homologs are also present in *T. reesei*, *T. atroviride*, and *T. virens* (TR_79756, TA_239314, and TV_182528). In *T. reesei*, deletion of *fwd1* did not cause a discernible phenotype (766). Moreover, the RNA helicase FRH regulates stability of FRQ (897), and homologs of this factor are present in *T. reesei*, *T. atroviride*, and *T. virens* (TR_49059, TA_53649, and TV_39829). However, the role of these factors in regulation of circadian clocks has not been described in *Trichoderma* spp.

Multiple lines of evidence have suggested that the circadian clock is constituted by multiple molecular feedback oscillators that generate robust rhythms. Nevertheless, the nature of potential circadian oscillators besides the core oscillator mechanism is not understood. Recently, an oscillator mechanism that drives rhythmic spore development in the absence of the well-characterized FRQ/WCC oscillator, and in constant light, conditions under which the FRQ/WCC oscillator (FWO) is not functional, was uncovered in *N. crassa* (898). While this novel oscillator does not require the FWO, it requires the cryptochrome and defines the CDO (Cry-dependent oscillator). The blue-light photoreceptors, VIVID and Cry, compensate for each other, and for WC-1, in CDO light responses, but WC-1 is still necessary for circadian light entrainment. By analogy, the *Trichoderma* light receptors ENV1 and CRY1 could constitute a similar oscillator. In agreement with this proposal, it has been found that both ENV1 and CRY1 influence light-regulated gene expression (80, 351).

VELVET family proteins. Since the discovery of VELVET (VeA) and, subsequently, further members of the VELVET family in *A. nidulans*, homologs in other fungi have been studied extensively and found to be crucial regulators of light-dependent development (899).

Orthologs of VeA are present in *T. reesei*, *T. atroviride*, and *T. virens* (VEL1; TR_122284, TA_42972, and TV_164251), along with further members of the VELVET family (VEL2, TR_40551, TA_175759, and TV_167298; VEL3, TR_102737, TA_298198, and TV_61392), but no VosA homolog is present in these species (534).

So far, the function has been studied only for VEL1 in *T. reesei* and *T. virens*. The *T. reesei* VEL1 protein shows the presence of the conserved VELVET domain (Pfam11754) and a nuclear localiza-

tion signal located in the C-terminal quarter of the protein sequence. As with *A. nidulans* VeA, the *T. reesei* VEL1 protein also contains a potential PEST region (a sequence rich in proline, serine, threonine, and glutamine indicative of a short half-life). The *T. virens* and *T. atroviride* orthologs of VEL1 are highly similar (about 80% overall similarity) and contain also a VELVET domain and a nuclear localization signal.

Recently, deletion of *vel1* in *T. reesei* was shown to cause complete and light-independent loss of conidiation (534). VEL1 was also found to be essential for cellulase gene expression (346, 534). In *T. reesei*, during growth in darkness, the *vel1* transcript accumulates to higher levels than under illumination, and no correlation was observed between *vel1* mRNA levels and the onset of sporulation (346). In contrast, in *T. virens* it has been shown that when exposed to light a slight increase in the expression of *veA* can be observed (538). With respect to sexual development in *T. reesei*, VEL1 is essential for mating in constant darkness and for female fertility in light. VEL1 regulates the pheromone system as well as mating partner communication via secondary metabolite production (534).

Strains with mutations in *T. virens veA* are defective in induction of genes that encode enzymes involved in secondary metabolism and are unable to synthesize gliotoxin, which correlates with low expression levels of *gliP*, the NRPS-encoding gene responsible for gliotoxin production (538).

In *A. nidulans*, VeA interacts with the phytochrome-White Collar light regulator complex and is a member of the VELVET complex VelB/VeA/LaeA. This complex coordinates the light signal with fungal development and secondary metabolism (900, 901). In *T. reesei*, the expression of cellulases and hemicellulases depends on the function of the methyltransferase LAE1, an ortholog of LaeA (129). Consistently, VEL1 is also essential for cellulase gene expression, suggesting that their regulation by LAE1 occurs via the VELVET complex (346).

Light regulation and metabolism. Light was shown to influence growth on diverse carbon sources in *T. atroviride*, with BLR-1 being crucial for carbon source selectivity and the oxidative stress response to light (639). Furthermore, as mentioned earlier, light has been shown to have a pronounced influence on GH gene expression in *Trichoderma*. Of particular industrial interest is the regulation of cellulase production, which led to in-depth studies. In this regard, transcriptomic analysis of *T. reesei* growing on medium with microcrystalline cellulose for 72 h in constant light and constant darkness showed that 248 genes were differentially regulated (2.7%). Among these genes, phosducin-like protein 1 (PhLPp1) was clearly induced by light after 60 min of exposure. Deletion of *env1* led to high expression of *phlp1* in response to light, whereas no considerable differences were observed in *blr1* and *blr2* mutant backgrounds. These results point to a major role of ENV1 in the regulation of *phlp1* by light and allowed us to postulate a possible role of PhLP1 in light responses through GNB1 and GNG1 (350, 619). Genome-wide transcriptional analysis by growing the three mutants and the wild-type strain on cellulose as the carbon source, under constant light or darkness, showed that the three genes are essential for regulation of all light-responsive genes. From the 628 genes positively regulated by PhLP1, GNB1, and GNG1 in light, the predominant functional group was the glycoside hydrolases, with 21 target genes. Along with regulatory targets of BLR1, BLR2, and ENV1, 78% of glycoside hydrolase genes, including members of all but one family represented in *T. reesei*, were shown to be light sensitive, either in wild-type or in mutant strains (350, 351). ENV1 and PhLP1 were found to be the most likely of several interconnected regulators coordinating the light response with metabolism (620).

Conclusions. Photobiology of *Trichoderma* has turned out to involve not only regulation of carbon metabolism but also general stress responses, apparently not directly related to light, although this is not exclusive to *Trichoderma*, since this has also been shown for at least two *Aspergillus* spp.

Genome sequencing of *Trichoderma* species also allowed detection of potential differences in light perception systems, since apparently only *T. atroviride* has an opsin, although there is no evidence yet of its role as a photoreceptor. In addition, recent evidence suggests that other putative photoreceptors may at least influence the responses triggered through the main blue light photoreceptor complex. Furthermore, ENV1, the main protein involved in adaptation to light, appears to play additional roles to those described for its ortholog in *Neurospora* (VIVID), some of which may be independent of BLR1/2, and the latter appear to play roles separately and in some cases roles that are light independent.

A major aspect of the influence of light in the behavior of most organisms is resetting of the circadian clock. In this sense, all three *Trichoderma* genomes sequenced revealed the presence of all known regulators of this input.

Heat Shock Proteins

Heat shock proteins (HSPs) belong to a family whose members are induced during stress situations for the cell. Unlike the name would suggest, a sublethal heat shock is not the only stress factor that triggers their expression but it is also one of the most investigated stress factors.

The function of HSPs can be defined as molecular chaperones. They exhibit a function in localization, disaggregation, stabilization, and degradation. Whenever a protein is not in its native conformation (due to denaturation caused by stress, incomplete folding, or wrong localization), HSPs bind to and interact with these proteins (902). They display a function, among other things, in helping polypeptides to unfold in order to get through the membrane of a required compartment, or they target proteins for degradation when refolding is not possible anymore. HSPs prevent aggregation of partially folded proteins or participate in disaggregation of unwanted protein aggregates. Furthermore, HSPs are required for stabilization of intermediate structures of incorrectly or partially folded polypeptides. All these functions are very important for an appropriate response to stress, but not all heat shock proteins are induced by stress factors like high temperature, high concentration of ions, or toxic substances. Others are expressed constitutively; these are named cognate heat shock proteins (HSC). Members of the HSP70 and HSP90 families reside in the ER in order to assist with protein folding (903). HSPs assist in stabilizing the polypeptides and prevent them from aggregation until the information can be read. HSPs often work in a complex with other chaperones, cochaperones, or nucleotide exchange factors.

In *A. fumigatus*, heat shock proteins have been suggested to regulate metabolic genes, such as genes of the tricarboxylicacid cycle and carbohydrate metabolism (904). Especially for fungi, which cannot move to other locations, mechanisms to handle high temperatures or toxic substances are very important or even

essential. Therefore, coping with environmental changes often leads to an onset of heat shock protein expression. An investigation of expression patterns in *T. atroviride* in contact with a prey during mycoparasitism showed upregulation of different genes encoding heat shock proteins, such as HSP23, HSP70, HSP90, and HSP104 (25). Often more than one family of heat shock proteins is used for correct folding of nascent polypeptides or refolding of peptides. A complex interplay between the different family members, acting as cochaperones for each other, takes place.

Heat shock proteins can be classified into different groups due to their sequence homology and molecular weight (902). We used the following groups, according to their molecular weights and functions: HSP70, HSP40 (J-domain protein), NEF (nucleotide exchange factor), HSP90 and associated proteins, cyclophilin and FKBP, HSP60 and HSP10, sHSP (small heat shock proteins), HSP104/Clp, and HSF (heat shock transcription factor).

HSP70. Proteins of the HSP70 family have an ATPase domain at their amino terminus and a peptide (substrate) binding pocket, which consists of antiparallel beta-strands and an alpha-helix as the lid at their carboxyl terminus (903, 905, 906). The ATPase function of these proteins acts to regulate the interaction with the substrate. A mechanism of HSP70s that triggers unfolding of aggregates and pulling of proteins across membranes is named entropic pulling (907). The basis of this mechanism is the energy release by ATP hydrolysis, and thereby an increase of freedom of the movement of the HSP70, when the substrate is unfolded. In *N. crassa* there are 11 members of the HSP70 family (71), and in *S. cerevisiae* 14 different members were discovered (908). In the published genome of *T. reesei* 10 different members of the HSP70 family can be identified, whereas the genome of *T. atroviride* comprises 13 genes of this family, and even in *T. virens* 15 homologs can be found (see Table S1 in the supplemental material). The gene products of *hsp70-1* and *hsp70-2* (the latter gene encodes a mitochondrial protein) of *N. crassa* exert their function preferentially in acidic milieus and accumulate after incubation at 45°C, whereas the gene product of *hsp70-3* is induced under insufficient phosphate conditions and not by heat shock (909). Members of this family were also shown to be induced during developmental changes (910). In *T. reesei* the transcription of *HSP70* was strongly induced under anaerobic conditions (911). The overexpression of the *HSP70* gene of *T. harzianum* resulted in a higher tolerance to oxidative, osmotic, and salt stress factors after heat pretreatment of the conidia (912). Furthermore, the product of the same gene under the regulation of a 35S promoter conferred more heat and stress tolerance when transferred to *Arabidopsis thaliana* (913). The fungal protein in *Arabidopsis* led to the downregulation of other HSP genes and HSF.

To exert their function as molecular chaperones, HSP70s require cochaperones and a J-domain protein (or HSP40) for stimulation of the ATP hydrolysis and substrate binding (914), as well as a NEF, which regulates the release of the substrate by catalyzing the exchange of ADP for ATP and therefore enables correct refolding of the polypeptide (915).

HSP40. HSP40s are also called J-domain proteins, due to a conserved 70-amino-acid J-domain (IPR001623). The function of HSP40s is based on the ability of the J-domain to stimulate ATPase activity of a HSP70 protein and on the binding of nascent polypeptides, which prevents the polypeptide aggregation but enables the presentation to the HSP70 (915–918). The J-domain consists of four alpha-helices, and helix II and III form a coiled-coil motif surrounding a hydrophobic core. Residues from these helices and from the loop between them form the interaction surface for HSP70s. Three residues in the interloop are responsible for the ATPase stimulation (914).

HSP40s also play a role in interaction with other species and possibly in a defense response. A HSP40 was identified in *T. atroviride* when proteins of *Trichoderma* spp. alone were compared to proteins from *Trichoderma* spp. in interaction with *R. solani* and bean roots (919). Due to the high number of predicted *hsp40* genes (13 each) in the genomes of *T. reesei*, *T. virens*, and *T. atroviride*, many different combinations with members of the HSP70 family are possible, and therefore many different functions may be involved.

A special role of an HSP40/J-domain protein found in *S. cerevisiae*. The 55-amino-acid region, enriched in glycines and phenylalanines, of the highly conserved J-protein Sis1p is important for maintenance of the prion-form [RNQ^+] for the protein and cell growth (920).

NEFs. The diversity of HSP70 function is not only dependent on the different *hsp70* genes themselves and the J proteins, but also on the various types of NEFs which interact with them. NEFs can be divided into three different groups: NEFs, which act as exchange factors only (ADP for ATP), the Bag family of NEFs, and HSP110-NEF (915).

(i) NEFs acting as exchange factors. The group of NEFs which only have a function as nucleotide exchange factors consists of two different types: GrpE homologs and HSP binding proteins (HSPB) (915). GrpE is a NEF, originally isolated from *E. coli*, where it builds a complex with DnaK (HSP70) and DnaJ (HSP40) for a functional chaperone (921). More recently, homologs were also isolated from mitochondria of *S. cerevisiae* and *N. crassa* and shown to interact with the mitochondrial HSP70 (922–924). Fes1p of *S. cerevisiae* is a HSP binding protein which is located in the cytosol and was shown to inhibit the J-protein-mediated activation of ATPase activity of Ssa1p (HSP70) of *S. cerevisiae* (925). In contrast, the homolog Sls1p is located in the ER and activates the ATPase activity of Kar2p (HSP70) (926). A *grpE* homolog (TR_73783, TA_281055, and TV_71772) and an *hspb/fes1* homolog (TR_119924, TA_297068, and TV_185457) can be found in the genomes of *T. reesei*, *T. atroviride*, and *T. virens*.

(ii) Bag family NEFs. The name Bag comes from the originally identified function of these enzymes and stands for Bcl-2-associated athanogene (927). BAG-1 binds to the HSC/HSP70 proteins and inhibits their chaperone activity (928). Members of the BAG family have a conserved interaction domain with HSP70/HSC70 in common, which is called the Bag domain. Residues in the alpha-helices 2 and 3 of BAG-1 were shown to build the interaction surface for HSP70 (929). Due to the binding of the Bag domain to HSP70, nucleotide release is induced (930). In *S. cerevisiae* there is one member of the *bag* family, named Snl1p (931), consistent with there also being one member identified in the genomes of *T. reesei*, *T. virens*, and *T. atroviride* (TR_52349, TA_315041, and TV_46351).

HSP110 NEFs. A more distantly related homolog of HSP70, Sse1p of *S. cerevisiae*, is a member of the mammalian HSP110 family and acts as a NEF for cytosolic HSP70s (932, 933). Members of the HSP110 family vary in length in the HSP70 typical carboxy-terminal peptide binding domain and have a more divergent ATPase domain than HSP70s. Sse1p stimulates the nucleo-

tide release and therefore has a positive effect on HSP70 functions (933).

HSP88 of *N. crassa* is a member of the mammalian HSP110 family. This protein was copurified with HSP30 and HSP70, which suggests a role for HSP110 in a complex with HSP30 (934). In *T. reesei*, *T. virens*, and *T. atroviride*, one homolog each of *hsp110* could be identified (TR_82534, TA_298484, and TV_58765).

HSP90 and associated proteins. The function of members of the HSP90 family is not only to prevent incorrect or unfolded proteins from aggregation, but also to stabilize unstable but already folded proteins by binding them (935). HSP90 works subsequently to HSP70 in folding of signaling proteins like steroid hormone receptors and protein kinases. HSP90 functions as a homodimer. Each subunit consists of three units: an N-domain with a pocket for ATP binding, which is linked to a C-terminal dimerization domain by an M-domain (middle domain) (936). HSP90 exerts its function in a complex with other cochaperones and is regulated by dephosphorylation by tetratricopeptide repeat domain (TPR)-containing protein PP5/Ppt1 and acetylation (937, 938).

The function of HSP90 is dependent on ATPase activity, which is part of a complex process (936, 939, 940). After binding of ATP by the N-domain pocket of the "open" HSP90, a segment of this domain closes the pocket, which releases another fragment of that domain. This fragment now binds to the same fragment of the other subunit of the dimer, which leads to a conformational change that results in a twisted, "closed" dimer. When ATP is hydrolyzed and ADP and P_i are released, the dimer opens again and can undergo another cycle. But not only the ATPase cycle is necessary for HSP90 function: also, the interaction with diverse cochaperones, many of which contain TPRs, is essential. Sti1p of *S. cerevisiae* (HOP of mammalians; a TPR-containing protein) was shown to interact with HSP90 and is required for steroid hormone receptor assembly (941). The proposed model suggests that the protein substrate is transferred from HSP70 to HSP90 via Sti1p. Sti1p binds as a dimer to the HSP90 dimer and is an inhibitor of ATPase activity (942, 943). CDC37 functions in a similar manner, but it recruits protein kinases as client proteins and inhibits ATPase activity (944, 945). The mammalian CHIP (carboxyl terminus of HSC70-interacting protein) is an E3 ubiquitin ligase, which interacts by its TPR domain with HSP70 and HSP90 and exerts its ubiquitylating activity by the U-box domain (946–948). Cpr6p, another TPR-containing cochaperone and cyclophilin, can activate the ATPase activity by releasing Sti1p from HSP90 (943). In *S. cerevisiae*, a homolog of Sti1p, Cns1p (cyclophilin seven suppressor), was found in a complex with HSP90 and Cpr7p (949). The cochaperone P23 binds to a subunit of the HSP90 dimer and also inhibits ATPase activity (950). Due to inhibition of ATPase activity, the HSP90 chaperone remains in the closed conformation and is "locked" for new substrates or releasing old ones. In contrast to that, the cofactors Aha1p and its shorter homolog Hch1p in yeast, which bind to the M-domain, are the only known activators of ATPase activity (951). Deletion of the *hsp90* gene in *C. albicans* is lethal, which suggests a single gene for *hsp90* in the genome and an essential function (952). Moreover, its expression is induced upon heat shock. This is also true for both HSP90 in *Blastocladiella emersonii*, except for the fact that there are two homologs, HSP90A and HSP90B, which are also upregulated during germination and sporulation (953), and that HSP90B is located in the ER. There is one homolog of *hsp90* in *N. crassa* (71) and also in *T. reesei*, *T. atroviride*, and *T. virens* (TR_123114, TA_297563, and TV_89650).

Cyclophilin and FKBP. Cyclophilin, FKBP, and parvulins are three families of enzymes with the common feature that they have peptidyl-prolyl *cis-trans* isomerase (PPIase) activity, which is important for correct folding of proteins and oligomerization of domains (954). Cyclophilin and FKBP were both shown to bind to HSP90 and are able to act in a complex with HSP90 and HSP70 as chaperones (955, 956). Recent studies showed that immunophilins can also function as chaperones independent of their PPIase activity (957). FKBP stands for FK506 binding proteins, which have many functions in the cell; the most prominent, and in mammals most important one, is to act as a receptor for immunosuppressors (954). An example of an FKBP of fungi is the FKBP22 of *N. crassa*, which is a chaperone in the ER. It has a dimerization domain at the C terminus and a domain for PPIase and chaperone activity at the N terminus (955). FKBP2 of *S. cerevisiae*, localized in the ER, is a homolog of the mammalian FKBP13 and was shown to be enhanced upon heat shock (958). In the genomes of *T. reesei* and *T. atroviride*, three genes were discovered, which featured characteristics of FKBP-encoding genes. In *T. virens*, only two genes were detected (see Table S1 in the supplemental material).

The most prominent cyclophilins, cyclophilin A of mammals, was identified because of its binding to an immunosuppressive drug (959). Cyclophilins share a domain of about 109 amino acids, called the cyclophilin-like domain (CLD), which represents the PPIase domain specific for cyclophilins. In addition, they also have other domains specific for localization or function (960). The major cytosolic cyclophilin Cyp1p of *S. cerevisiae* was shown to be induced by heat shock due to a heat shock response element in the promoter region (961). All eight cyclophilin homologs of *S. cerevisiae*, located in the cytosol, ER, mitochondria, and nucleus, were shown not to be essential (960). In contrast, a cyclophilin A mutant of *C. neoformans* is inviable at 39°C (681). In *S. cerevisiae*, the cyclophilin Cpr7p and HSP90 act synergistically in negative regulation of the heat shock response (962). The genomes of *S. pombe* and *N. crassa* each comprise nine members of the cyclophilin group (71, 963). In *T. atroviride*, *T. virens*, and *T. reesei*, 11 genes in each genome encoding potential cyclophilins were identified (see Table S1 in the supplemental material).

HSP60 and HSP10 chaperonins. Chaperonins can be divided into two subgroups with sequence similarities of about 15 to 25% between them (964, 965). HSP60s of group I originally derive from bacteria (GroEL of *E. coli*), and nowadays they are also found in organelles of eukaryotes, such as the mitochondrial matrix of fungi. This group of HSP60s require the cochaperonin HSP10, such as GroES in *E. coli* (966). HSP60 and HSP10 of *S. cerevisiae* and humans were shown to be homologous to GroEL and GroES of *E. coli* (967–969). HSP60 and HSP10 act together as cochaperonins in the mitochondrial matrix of yeast (970). Proteins cannot pass the matrix if they are already in their mature conformation, and so they need chaperones to be unfolded and refolded again. The HSP60 homolog of *N. crassa* is composed of twin rings, and each of them comprises seven identical subunits which have a molecular mass of about 60 kDa (971). The client polypeptides immigrate in the open cylindrical oligomer and bind to the cavity in the center of the ring structures for correct folding in an ATP-dependent manner. The cochaperonin (HSP10) is like a lid and catalyzes the hydrolyzation of ATP and therefore the release of the

client peptide (972–974). The partially folded protein is rebound to GroEL, and the cycle restarts until the protein is folded completely (975). HSP10 itself also consists of seven subunits; each of the subunits has a molecular mass of about 10 kDa (976). HSP10 was shown to be imported into the mitochondrial matrix without cleavage and is essential (977). *S. cerevisiae* cells with depleted *hsp60* stop growing, and its expression is increased at 42°C (978). *T. reesei*, *T. atroviride*, and *T. virens* genomes comprise one gene each encoding a potential mitochondrial HSP60 (TR_119731, TA_297734, and TV_79041) and HSP10 (TR_106067, TA_258186, and TV_230640).

The second group of chaperonins is located in the eukaryotic cytoplasm and in archea. In contrast to the first group, members of this group do not require a cochaperonin; they have their own built-in lid (965). The oligomers are named CCT (chaperonin containing TCP1) or TRiC (TCP1 ring complex). The ring of the TRiC consists of eight different subunits, each encoded by an essential gene (979). All eight subunits could be identified in each of the genomes of *T. atroviride*, *T. virens*, and *T. reesei* (see Table S1 in the supplemental material).

sHSPs. Small heat shock proteins (sHSPs) prevent the aggregation of unfolded proteins and are required for disaggregation of aggregated proteins (980, 981). In addition, they are proposed to act as a kind of reservoir for potential refoldable proteins, which is subsequently done by HSP70s (982). They have molecular masses between 15 and 42 kDa and are able to build oligomeric structures up to 50 subunits (982). The common feature of all sHSPs is a conserved region of about 90 residues, called the alpha-crystallin domain at the C-terminal end, flanked by a C-terminal extension, and a hydrophobic region at the N-terminal end. The alpha-crystalline domain and the hydrophobic region merge with oligomers, building a big ring structure, with the variable C-terminal extension as a kind of a lid, which can regulate the access to the chaperone complex (983).

The small heat shock protein HSP24 of *T. harzianum* was shown to enhance tolerance to salt, heat, and drought in an *HSP24*-carrying transgenic mutant of *S. cerevisiae* (984). Another sHSP, HSP23 of *T. virens* T59, was investigated for its response to high and low temperatures, and under ethanol addition the expression of *hsp23* was enhanced (985). The same gene was transferred in the biocontrol strain *T. harzianum* T34, which led to higher biomass production in the mutant strains than in the wild-type T34 strain and enhanced thermotolerance. The small heat shock protein HSP30 of *T. reesei* (TR_46285) and the heat shock protein HSP98 of the CLPA family were enhanced under hypoxic conditions and strongly induced under anaerobic conditions (986). HSP30 of *N. crassa* was shown not to be essential under high-temperature stress conditions but to be important for carbon utilization in high temperatures (987). In the genome of *T. reesei*, three genes for potential small heat shock proteins could be identified in total; in *T. atroviride* there were four genes, and in *T. virens* five genes were present (see Table S1 in the supplemental material). All of them are homologs to *N. crassa* HSP30.

HSP104/Clp. HSP104 belongs to the Clp/HSP100 family of the AAA+ superfamily (ATPase associated with various cellular activities) (988). Unlike other members of the Clp family, HSP104 is not associated with proteases, and like other chaperones, it does not have the ability to prevent denatured proteins from aggregating, but it has a function in reactivation and resolubilization of protein aggregates after heat treatment (989, 990). For refolding of denatured proteins from the aggregated state, HSP104 requires help from an HSP70 and an HSP40 protein in order to build a machine for protein refolding (989, 991). HSP78 of *S. cerevisiae* is also a member of the CLP family of heat shock proteins. It was identified as a close relative of *E. coli* CLPB, it is inducible by heat shock due to heat shock-regulated elements in its promoter region, and it is located in the mitochondrial matrix (992). HSP78 could also be identified as a protein which is downregulated when xylose was used as carbon source, compared to growth in glucose under either aerobic or anaerobic conditions (993). The genomes of *T. reesei*, *T. atroviride*, and *T. virens* comprise one homolog of each gene (*HSP104*, TR_80142, TA_157453, and TV_216898; *HSP78*, TR_2687, TA_157172, and TV_80583). The *hspA* gene product of the fungus *Phycomyces blakesleeanus* belongs to the CLPB/HSP100 family, which has a homolog in *N. crassa* (71) and in *T. reesei*, *T. atroviride*, and *T. virens*. *HspA* transcription is induced by blue light, and this activation could be required to handle the negative effect of light on the organism; alternatively, HSPA may play an important role in disaggregation and thus activation of regulators of the phototransduction pathway (994).

HSFs. Heat shock transcription factors are the regulators of heat shock proteins and responsible for the upregulation of HSPs under stress conditions. They bind to a conserved region upstream of the gene encoding the HSP. This conserved upstream response element, called the heat shock response element (HSE), consists of three contiguous inverted repeats of 5′-nGAAn-3′ (995–997). HSFs are constitutively expressed, but they are activated upon heat stress to bind to the three HSEs and interact as trimers and upon exposure of activator domains (995, 998). HSFs vary in size between different species, but the genes coding for HSFs share common core structures: at the amino-terminal end they have a conserved DNA binding and trimerization domain, and at the carboxy terminal is the less-conserved transactivation domain. Additionally, there are two other elements near the C-terminal end: a conserved domain for suppressing HSF trimerization, and a short conserved element (CF2) for suppressing the transactivation domain (995). HSF1 of *S. cerevisiae* was shown to be essential (999).

In contrast to higher eukaryotes, HSE binding activity is not increased upon heat shock but is constitutive in *S. pombe* and *S. cerevisiae* (1000). Two genes encoding proteins featuring an HSF-type DNA binding domain were identified in the genomes in *T. reesei*, *T. atroviride*, and *T. virens* (TR_78688, TA_153671, and TV_11553, and TR_27357, TA_174226, and TV_125026). One of them (TR_78688) is the gene with the highest transcript levels among all heat shock proteins in *T. reesei*, albeit it is not transcribed constitutively.

Conclusions. Several HSPs were shown to be upregulated in the course of mycoparasitism of *T. atroviride*, suggesting an important role of these genes. A high number of homologs (10 to 15) belonging to the group of HSP70 could be identified, with 15 members (the highest) in *T. virens*. In concert with members of the HSP40 family (13 predicted genes for each genome), a high variability in the functional area of HSP70 is suggested. Due to the presence of genes for all NEF subgroups in each genome, regulating the release of substrate, all typical components for HSP70 function are present in *T. reesei*, *T. atroviride*, and *T. virens*. sHSPs were shown to be involved in the stress response in *T. virens* and *T. reesei*. Three to five homologs of these genes were found in each of

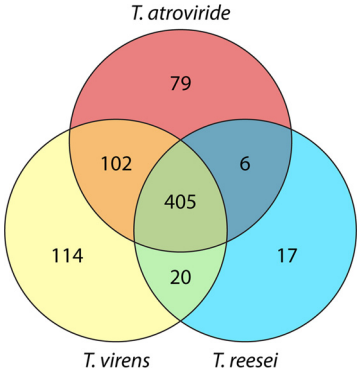

FIG 17 Distribution of transcription factor orthologs of *T. atroviride*, *T. virens*, and *T. reesei*. The Venn diagram shows the distribution found for the three species. The analysis was done using the distribution of orthologs described in reference 4.

the genomes, putatively indicating different ways of dealing with stress responses in different *Trichoderma* species.

TRANSCRIPTION FACTORS

Transcription factors (TFs) modulate gene expression control of a cell and, in many respects, their repertoire determines the life and functionality of the cell. For a better understanding of their regulatory mechanisms, it is essential to know the entire repertoire of TFs of a species (1001–1003). Here we provide a compilation and comparison of the transcription factor proteins encoded by *T. atroviride*, *T. virens*, and *T. reesei* genomes (4, 23), as well as a brief description of the largest families of transcription factors.

Identification of TFs

Our analysis for potential transcription factors (see Text S1 in the supplemental material) yielded 641 TFs for *T. virens*, 592 for *T. atroviride*, and 448 for *T. reesei*, and these belong to a total of 27 Pfam families of DNA binding domains (see Tables S1 and S2 in the supplemental material). With the purpose of identifying the orthologous transcription factors between *T. atroviride*, *T. virens*, and *T. reesei*, we used the ortholog analysis method described in reference 4. The three species of *Trichoderma* share 405 TFs (Fig. 17). Interestingly, this analysis showed an expansion of TFs that are shared between the two mycoparasitic species, *T. atroviride* and *T. virens* (102 TFs), while for *T. virens* and *T. reesei* only 20 are common and only 6 are common between *T. atroviride* and *T. reesei*. Remarkably, we found 114 TFs for *T. virens*, 79 for *T. atroviride*, and only 17 TFs for *T. reesei* without orthologs and thus unique to each species (Fig. 17; see also Tables S1 and S2 in the supplemental material), which could play an important role in the establishment of their lifestyle.

A comparison with the database FTFD (http://ftfd.snu.ac.kr/tf.php) (1001) showed five classes of TFs widely distributed in these three species of *Trichoderma*. The most abundant gene group belongs to the fungus-specific $Zn(II)_2Cys_6$ class, followed, in order of representation, by the C2H2 zinc finger, bZip, bHLH, and GATA-type classes. In addition, we found putative DNA binding domain proteins reported as transcription factors in other eukaryotic organisms, which we named "miscellaneous transcription factors" (as described in reference 71). We also found 29 TFs involved in the transcription machinery, and so we therefore considered general transcription factors (Table 2; see also Table S1 in the supplemental material).

Global Comparison with Other Fungi

The analysis of orthologs of the *Trichoderma* TFs with *F. graminearum*, *F. oxysporum*, and *A. nidulans* indicated that the mycoparasitic species *T. atroviride* and *T. viride* shared more orthologs with the fungal pathogens than the saphrophytic species *T. reesei* (see Table S2 and Fig. S29 in the supplemental material). Similarly, in the case of the comparison of *Trichoderma* TFs with the two insect pathogenic fungi *C. militaris* and *M. anisopliae*. Interestingly, about one-quarter of the *Trichoderma* TFs have no orthologs or homologs in even the relatively closely related *Fusarium* genus and are thus unique to *Trichoderma* (see Fig. S29 and Table S2).

Using the distribution of orthologs common to the three *Trichoderma* species (Fig. 17), we determined the distribution of orthologs and homologs for each group of TFs against the fungi summarized in Table 3. Of the 405 TFs common to all *Trichoderma* species, 346 have orthologs in *N. crassa*, *F. graminearum*, *F. oxysporum*, or *A. nidulans*. Among those, 214 have orthologs in all of these species; 36 were found to have orthologs only in *N. crassa*, *F. graminearum*, or *F. oxysporum*, 36 have orthologs in *F. graminearum*, *F. oxysporum*, or *A. nidulans*, 47 have orthologs only in *F. graminearum* or *F. oxysporum*, 10 have orthologs only in *A. nidulans*, and a total of 59 have no orthologs among any of the species. Similarly, homologs with the two insect-pathogenic fungi *M. anisopliae* and *C. militaris* were found: 329 TFs have homologs in *M. anisopliae* or *C. militaris*, and 76 TFs have no homologs in either of them (see Fig. S30 and Table S2 in the supplemental material). Moreover, many genes common to only two of the three *Trichoderma* spp. analyzed here have specific homologs in other fungi (see Text S2 in the supplemental material).

The $Zn(II)_2Cys_6$-Type Fungal Binuclear Cluster Family

The $Zn(II)_2Cys_6$-type binuclear cluster family proteins comprise six cysteine residues that, unlike the other zinc finger proteins,

TABLE 2 Transcription factors found in the three species of *Trichoderma*

Transcription factor family[a]	No. of TF type found in species		
	T. atroviride[b]	*T. virens*[c]	*T. reesei*[d]
$Zn(II)_2Cys_6$ type	382	422	258
C_2H_2 zinc finger	53	61	49
bZIP	28	28	22
bHLH	10	10	9
GATA-type zinc finger	8	8	8
General transcription factors	29	29	29
Miscellaneous transcription factors	82	83	73
Total	592	641	448

[a] Based on data obtained from Fungal Transcription Factor Database (http://ftfd.snu.ac.kr/tf.php).
[b] Based on data obtained from the Joint Genome Institute MycoCosm resource (http://genome.jgi-psf.org/Triat2/Triat2.home.html).
[c] Based on data obtained from the Joint Genome Institute MycoCosm resource (http://genome.jgi-psf.org/TriviGv29_8_2/TriviGv29_8_2.home.html).
[d] Based on data obtained from the Joint Genome Institute MycoCosm resource (http://genome.jgi-psf.org/Trire2/Trire2.home.html).

TABLE 3 Numbers of transcription factor orthologs and homologs with other fungi

Trichoderma species	No. of TF orthologs shared with[a]:				No. of TF homologs with best BLAST hit[b]		No. with no BLAST hit[c]
	N. crassa	*F. graminearum*	*F. oxysporum*	*A. nidulans*	*M. anisopliae*	*C. militaris*	
T. atroviride	280	382	383	315	373	347	83
T. virens	289	392	398	325	390	365	99
T. reesei	266	336	334	271	315	299	44

[a] Orthologs were identified by using the Bidirectional Best Hit (BBH) method and BLASTP, with an E value of <1e−5.
[b] Homologs were identified by using BLASTP with an E value of <1e−3.
[c] Number of TFs without orthologs to *N. crassa*, *F. graminearum*, and *F. oxysporum* and without homologs to *M. anisopliae* and *C. militaris*.

bind two zinc atoms; thus, this domain can also be found under the name zinc binuclear cluster. The zinc clusters can interact with DNA as monomers or as homo- or heterodimers. The *S. cerevisiae* transcription factor Gal4p is arguably the best-known and most-studied one. Furthermore, they are strictly fungal proteins (reviewed in references 71, 1002, and 1004).

In *T. reesei*, *T. atroviride*, and *T. virens*, the fungus-specific $Zn(II)_2Cys_6$ binuclear cluster family is the largest class of transcription factors (Table 2). We found 382 genes for *T. atroviride* and 422 for *T. virens*, but only 258 for *T. reesei*. Thus, there is a clear expansion of fungus-specific transcription factors in the two mycoparasitic species. Around 80% of these TFs have orthologs or homologs in at least another fungus used in the global comparison analysis described above. A comparison showed that 227 of these TFs are common between the three species; *T. atroviride* and *T. virens* share 83 TFs, while *T. virens and T. reesei* share only 15, and only 3 are common between *T. atroviride* and *T. reesei*. Furthermore, 97, 71, and 13 TFs are specific for *T. virens*, *T. atroviride*, and *T. reesei*, respectively (see Tables S1 and S2 in the supplemental material).

Transcription factors belonging to this class have been reported to regulate various cellular processes, including sugar and amino acid metabolism, gluconeogenesis and respiration, vitamin synthesis, cell cycle, chromatin remodeling, nitrogen utilization, peroxisome proliferation, pleiotropic drug response, and the stress response (reviewed in references 455 and 1004). For example, in *N. crassa* nitrate assimilation is controlled by the transcription factor NIT-4. Another TF belonging to this class is the *fluffy* gene, which is required for conidial development (63) and is not encoded in *T. reesei*, *T. atroviride*, or *T. virens*. In *M. oryzae*, the TFs containing fungus-specific Zn_2Cys_6 zinc finger domains represent the largest group of TFs. A systematic analysis showed that these TFs have critical biological roles in vegetative growth, conidial germination, conidiation, appressorium formation, and pathogenicity to rice and barley (1005).

Only a few of the $Zn(II)_2Cys_6$ proteins have been characterized in *Trichoderma* spp.; all efforts have been focused on studying the regulation of cellulase and xylanase genes in *T. reesei*, which offers one of the best-characterized xylanolytic and cellulolytic enzyme systems (1006–1008). The *ace2* gene (TR_78445, TA_42504, and TV_69287) encodes a transcriptional activator protein, which together with *ace1* (C2H2 zinc finger TF [see below]) is involved in the regulation of cellulase and xylanase genes of *T. reesei* (TR_78445). Deletion of *ace2* caused lower transcript levels of the cellobiohydrolase genes *cbh1/cel7a* and *cbh2/cel6a* and the endoglucanase gene *egl2*, which is necessary for efficient hydrolysis of cellulose (for more information, see references 1006 and 1009).

The xylanase regulator 1 (XYR1) transcription factor protein (TR_122208, TA_78601, and TV_58714) also has been characterized in *T. reesei*. This protein is a homolog to XlnR of *A. niger* (1010) and has been demonstrated to act as an activator of xylanase and cellulase genes (1008, 1009, 1011). A third $Zn(II)_2Cys_6$ TF has been studied in *T. reesei* (1012). The beta-glucosidase regulator (BglR), a new $Zn(II)_2Cys_6$-type transcription factor (TR_55105, TA_22871, and TV_164135), activates expression of specific genes encoding β-glucosidases (with the exception of *bgl1*, which is seemingly under the direct control of XYR1). The BglR TF shares weak homology with *amyR* of *Aspergillus oryzae*, which is considered a key regulator of starch degradation. The *Trichoderma* $Zn(II)_2Cys_6$ TF CTF1 (originally designated *Thctf1*; TR_103230, TA_145197, and TV_73221) is related to the production of secondary metabolites and the antifungal activity of *T. harzianum* (1013). The complete analysis of this TF class in the three species is given in Tables S1 and S2 in the supplemental material.

C2H2 Zinc Finger Transcription Factors

The zinc finger DNA binding domain C2H2 has an array of two cysteine and two histidine residues with a single zinc ion. This class of DNA binding domains is found in many proteins, from bacteria to humans. They are involved in the regulation of development, responses to stress, carbon metabolism, differentiation, and tumor suppression (1014, 1015). The first C2H2 protein found was the transcription factor TFIIIA in *Xenopus laevis* and was defined the canonical cysteine and histidine residues for this DNA binding domain (1016). This DNA binding domain is the most abundant in mammals. In fungi, they represent the third most represented group in their genomes (1003). In *T. reesei*, *T. atroviride*, and *T. virens*, the second most abundant DNA binding class corresponds to the C2H2 type ($CX_{2-4}CX_{12}HX_{2-6}H$). We found 53 genes for *T. atroviride*, 61 for *T. virens*, and 49 for *T. reesei* (Table 2) with this C2H2 zinc finger domain. Taking into account that *T. virens* and *T. reesei* emerged from *T. atroviride*, these findings might reflect the evolutionary forces driving the adaptation to the environment, with an expansion of transcription factors in the mycoparasitic *T. virens*, whereas *T. reesei* had the lowest number of TFs in general (4). In this context, we found 10 genes encoding this TF type exclusively in *T. virens*, 3 in *T. atroviride*, and 3 in *T. reesei*; none of those have been further studied yet. Interestingly, of the unique ones in *T. virens*, there is one with an ortholog to *N. crassa*, *F. graminearum*, *A. nidulans*, *F. oxysporum*, *M. anisopliae*, and *C. militaris*. Among the unique ones, the *T. virens* TV_217035 gene has an ortholog in *N. crassa*, *F. graminearum*, *A. nidulans*, *F. oxysporum*, *M. anisopliae*, and *C. militaris*; while the unique ones

of *T. atroviride* have 2 orthologs (TA_134223 and TA_44295) with *F. graminearum* and 1 (TA_44295) with *F. oxysporum*, *M. anisopliae*, and *C. militaris*. Among those of *T. reesei*, we found one (TR_59270) with an ortholog in *N. crassa*, *F. graminearum*, *M. anisopliae*, and *C. militaris* and 2 (TR_105458 and TR_59270) in *A. nidulans* and *F. oxysporum* (see Tables S1 and S2 in the supplemental material).

Only a few proteins with a C2H2 DNA binding domain have been characterized in *Trichoderma* spp. In *T. reesei*, *ace1* (1017) encodes a protein (TR_75418, TA_83090, and TV_88565) that has been extensively studied as a positive regulator of cellulases, and this TF is also conserved in *N. crassa*, *F. graminearum*, *F. oxysporum*, *A. nidulans*, *M. anisopliae*, and *C. militaris* (see Tables S1 and S2 in the supplemental material). The carbon catabolite repressor gene *cre1* was described in *T. reesei*; it recognizes the AGGGG motif, similar to CREA of *A. nidulans* (1018, 1019). CRE1 (TR_120117, TA_301116, and TV_149403) is essential for the control of carbon catabolite repression by glucose and is of utmost importance to industrial enzyme production, especially with respect to cellulases (1009). A truncation of CRE1 is the major defect of the *T. reesei* strain RutC30, the parental strain of numerous industrial production strains (1020). Recently, it has been shown that this very truncation is crucial for high cellulase production and causes a different phenotype than the complete deletion (1021). In *T. atroviride* (at that time described as *T. harzianum*), CRE1 controls the expression of the endochitinase *ech42* during the mycoparasitic interaction (1022). The *Trichoderma* stress response element binding protein 1 (SEB1; TR_76505, TA_135877, and TV_184820) is an ortholog of *S. cerevisiae msn2/msn4*. In *T. atroviride*, it is expressed in response to high-osmolarity stress ($CdSO_4$, sorbitol, or cold shock) and is capable of binding to the AGGGG sequence, although its direct targets are still unknown (1023). Moreover, SEB1 is involved in but not essential for glycerol dehydrogenase (*gld1*) gene expression and glycerol accumulation in *T. atroviride* during osmotic stress (1024). In *A. fumigatus*, SEB1 is involved in the response to oxidative stress and virulence, and the absence of *seb1* results in the deregulation of *Afatf1* and *Afyap1*, which have also been implicated in the response to stress (1025).

In fungi, the response to pH change is regulated by the Pal/Rim pathway, activated in neutral to alkaline pH with a downstream transcription factor effector, PacC/Rim101 (1026, 1027). The *pacC/pac1* homolog of *T. reesei*, *T. atroviride*, and *T. virens* (TR_120698, TA_78054, and TV_40391) encodes a protein with three C2H2 zinc fingers in the N-terminal region, as is the case in *N. crassa* and *Aspergillus* spp. (1028). In *T. harzianum*, PAC1 is required for growth in alkaline soil and for proper biocontrol of *R. solani* (1028). In *T. virens*, PAC1 is required for the antagonism against *Sclerotium rolfsii*, perhaps regulating genes involved in the biosynthesis of secondary metabolites and ion transport in coordination with the ability to sense pH (1029). In *T. reesei*, PAC1 is a negative regulator of cellulase gene expression (1030).

T. reesei, *T. atroviride*, and *T. virens* have many TFs known to be involved in asexual reproduction in other systems and these are shared with *N. crassa*, *A. nidulans*, *F. oxysporum*, *C. militaris*, and *M. anisopliae* (see the "Asexual development" section) (see also Table S2 in the supplemental material). Most of them have not been studied in *Trichoderma*, but they have been found to be transcriptionally active (see Table S1 in the supplemental material).

The genomes of *T. atroviride*, *T. virens*, and *T. reesei* encode an ortholog of the WCC (White Collar complex, or photoreceptor complex [see below]) that is dependent on CSP1 (conidial separation-1) of *N. crassa* (TR_120597, TA_248464, and TV_40853), a C2H2 ZnF TF that is involved in regulation of the release of spores during macroconidiation and separation of the conidial chain (1031, 1032). In *T. atroviride*, *csp1* is expressed under basal conditions with slightly increased transcript levels after mechanical injury (15). Interestingly, the absence of the gene *cp2* (encoding GHH, grainyhead-homolog transcription factor), which represents an allele of *csp-2* in *N. crassa* (1033), results in a similar phenotype to that observed for the *csp-1* mutant in *N. crassa*. CP2 (GHH in *N. crassa*) is involved in the separation of the conidial chains and remodeling of the cell wall. In *Trichoderma* spp., there are orthologs for this TF (TR_60761, TA_319089, and TV_148539). Interestingly, it is expressed in *Trichoderma* in response to mechanical damage, but at low levels (15). In *T. reesei*, however, lack of the *cp2* homolog causes no discernible phenotype (1034). The *cp2* gene is regulated by light in *A. nidulans* and *T. atroviride* (50, 1035), and so it may be a good candidate in future studies of developmental processes in fungi.

Signaling through the MAPK pathway is involved in sexual and asexual development, and targets have been subjects of extensive studies. The *S. cerevisiae* transcription factor Ste12p, which comprises a homeodomain, is activated by the Fus3p/Kss1p MAPK to regulate mating (1036). In *N. crassa*, the ortholog FUS3/Kss1 MAK-2 also activates PP-1 (the Ste12p homolog) to regulate vegetative growth and female fertility (1037). The *A. nidulans* homolog SteA is required for sexual but not for asexual development (1038). Interestingly, SteA has a C2H2 zinc finger domain, as in *N. crassa*, which is not present in Ste12p in *S. cerevisiae* (1038). STE12 has been studied in *T. atroviride* (TA_29631, TR_36543, and TV_75179) and mediates regulation of vegetative hyphal fusion and mycoparasitism by the MAP kinase TMK1 (1039).

TVK1 (MAPK1) of *T. virens*, an ortholog of the FUS3/Kss1 MAPK, acts as a positive regulator of colony growth, similar to the reported Fus3p/Kss1p/Ste12p pathway (1040), and its function may be mediated by STE12 as well. Whether the STE12 ortholog of *T. atroviride*, *T. virens*, or *T. reesei* has a function in sexual reproduction requires further studies, especially since sexual reproduction in the laboratory has been achieved only for *T. reesei* (859).

Calcium (Ca^{2+}) is an important signaling ion in all organisms; the most-studied pathway is the Ca^{2+}/calmodulin calcineurin phosphatase. The yeast Crz1p (TA_173231, TV_232155, and TR_36391) transcription factor is one of the best-studied targets of calcineurin in fungi (1041), and the nuclear localization of Crz depends on its cytosolic dephosphorylation by calcineurin. In fungi, Crz is required to cope with extracellular changes of Ca^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, Li^{+}, and K^{+} levels tested *in vitro*, and it is also required for sexual/asexual development and virulence (1042). Interestingly, Ca^{2+} can positively regulate conidiation in submerged media and stimulate formation of chlamydospores at low levels in *T. viride* (1043). Whether calcineurin/Crz regulation is required for calcium signaling remains to be tested.

bZip Transcription Factors

The bZIP domain consists of two functionally distinct regions: the basic region and the leucine zipper region. The basic region is highly conserved and undergoes sequence-specific DNA binding, and the leucine zipper region is less conserved and forms a coiled-coil structure, which confers dimerization specificity and the abil-

ity to form homo- and heterodimers, giving each transcription factor the capability of multiple functions (732, 1044, 1045). bZIP proteins belong to one of the largest and most diverse TF families found in fungi, animals, plants, and a very few virus species (reviewed in references 71 and 1002).

bZIP TFs are involved in many critical processes in a diverse range of species. In filamentous fungi, the processes known to be regulated by characterized bZIP proteins include asexual development, amino acid biosynthesis, unfolded protein response (UPR), nutrient utilization, and various stress responses (732, 1046–1049). In *T. reesei*, *T. atroviride*, and *T. virens*, the class of bZIP TFs is the third most highly represented one. Our analysis shows that 28 genes of *T. atroviride*, 28 in *T. virens*, and 22 in *T. reesei* encode bZIP TFs (Table 2). An orthology analysis showed that 24, 26, and 21 *T. atroviride*, *T. virens*, and *T. reesei* bZIP TFs have orthologs or homologs in other fungi. A comparison between the three species of *Trichoderma* showed that 19 bZIP TFs are common to all; *T. atroviride* and *T. virens* share 4 further TFs, while for *T. virens* and *T. reesei* only 2 more are common, and only 1 gene is additionally common between *T. atroviride* and *T. reesei*. Finally, 4 and 3 TFs are specific for *T. atroviride* and *T. virens*, respectively. There were no specific bZIP TFs for *T. reesei* (see Tables S1 and S2 in the supplemental material).

Only one protein belonging to this class has been studied in *Trichoderma*, the *hac1* gene product, the UPR transcription factor, in *T. reesei* (TR_46902, TA_137322, and TV_83840) (1050). HAC1 regulates the UPR pathway, which activates genes involved in multiple functions related to folding, quality control, and transport of secreted proteins (17, 1050, 1051) (see Tables S1 and S2 in the supplemental material).

bHLH Transcription Factors

bHLH TFs are present in eukaryotes and prokaryotes with relative early evolution in Metazoa (1052). There are about 130 bHLH TFs encoded in the genome of mammals and more than 140 in plants. In contrast, in fungi there are less than 16 in most reported genomes (1053, 1054). bHLH-type TFs reported in fungi are involved in interorganelle communication, metabolism, growth, and sexual/asexual development (1053). Our analysis showed that there are 10 genes in the genome of *T. atroviride* or *T. virens* and 9 in the *T. reesei* genome, with 1 bHLH motif. None of these bHLH transcription factors has been characterized in *T. reesei*, *T. atroviride*, or *T. virens*, but some orthologs have been studied in *N. crassa* and *A. nidulans*. The *nuc-1* (nuclease-1) gene of *N. crassa* (TA_173635, TV_124401, and TR_102464) is responsible for the regulation of phosphate uptake under starvation, like the *nuc-1* homolog *palcA* from *A. nidulans* (1055, 1056). As phosphate is an important nutrient, the *T. atroviride*, *T. virens*, and *T. reesei* homologs of *nuc-1/palcA* might be involved in the interaction with plants to obtain this substrate from soil. Another bHLH TF-encoding gene is *sah-2* (short aerial hyphae-2) from *N. crassa* (TA_172645, TV_212678, and TR_22774), where a *sah-2* null mutation results in decreased aerial mycelia compared to the WT strain (1057). The *anbH1* gene of *A. nidulans*, involved in negative control of penicillin production (1058), is present in *T. atroviride* (TA_168377), *T. virens* (TV_214285), and *T. reesei* (TR_4933), despite the absence of penicillin biosynthetic genes. Interestingly, a regulatable *alcAp-anbH1* transformant was found in *A. nidulans* (1058), and upon growth under inducing conditions the strain showed slower growth than the WT, pointing to regulation of genes other than those in the penicillin biosynthesis pathway. Another interesting bHLH TF-encoding gene from *A. nidulans* is *devR*, which regulates sexual and asexual development (1059). A Δ*devR* mutant develops conidiophores with metulae and phialide but does not form conidial chains; instead, conidiophores elongate and form additional conidiophores with a vesicle at the tip (1059). Although *Trichoderma* conidiophores are different from those of *A. nidulans*, regulation of conidial organization by DEVR might be conserved, as this gene is found in many fungi (1053). We found orthologs of *devR* for *T. atroviride* (TA_299973), *T. virens* (TV_35604), and for *T. reesei* (TR_122371) (see Tables S1 and S2 in the supplemental material).

GATA-Type Zinc Finger Transcription Factors

GATA1 is the founding member of the GATA-type zinc finger transcription factors, and it was found as a regulator of genes in erythroid cells (1060). The DNA sequence to which GATA1 binds is (T/A)GATA(AIG), which is recognized by the Cys-X-X-Cys-(X)17-Cys-X-X-Cys DNA binding domain of this Zn finger transcription factor (1061). This TF is present in mammals, plants, and fungi, but no GATA-type TF has been found in prokaryotes yet (1062). The genomes of *T. atroviride*, *T. virens*, and *T. reesei* encode 8 GATA TF genes, all of them with an ortholog in *N. crassa*, *A. nidulans*, and *Fusarium* spp. (Table 3; see also Table S2 in the supplemental material). The *T. atroviride sfh1* gene (TA_215727), encoding a subunit of the chromatin remodeling complex RSC with orthologs in *N. crassa*, *A. nidulans*, and *Fusarium* spp. *T. virens* (TV_61629) and *T. reesei* (TR_54598) share an SNF5 domain with all of them, but the putative DNA binding GATA domain is present only in *Trichoderma* (see Table S2). Sfh1 is a component of the RSC and required for viability, as it regulates the transition of the G_2/M-phase cell cycle in *S. cerevisiae* (181). Since GATA domains are not only involved in protein-DNA interactions but also may be involved in protein-protein interactions, it is conceivable that the domain present in the *Trichoderma* SFH1 protein is involved in interaction with other proteins, or it might reflect an increase in the number of targets.

Among the best-studied GATA factors in fungi are the orthologs of the WC (White Collar) proteins 1 and 2, two photoreceptors of *N. crassa* (881, 882). In *Trichoderma*, the orthologs of WC-1 and WC-2 were named BLR-1 and -2 (blue light regulators) (878, 879). The BLR1 protein (TR_121962, TA_229937, and TV_81343) has three PAS (Per-ARNT-Sim) domains, and BLR2 (TR_22699, TA_42429, and TV_31745) has only one, but both have the GATA domain in the C-terminal region, as in *N. crassa* and other fungi (1063). The BLR proteins of *T. atroviride* are required for induction of conidiation after a pulse of light. The BLR proteins turn on and off light-regulated genes. Proteomic analyses showed a different protein profile in the dark versus the light in the absence of *blr1* or *blr2*, suggesting that BLR1 and BLR2 can regulate genes in an independent fashion (1064). Independent regulation by BLR1 and BLR2 was also observed in *T. reesei* upon growth on cellulose (351) and for *N. crassa* WC-1 and WC-2 under similar conditions (755). Carbon source metabolism in the presence of light regulates growth and conidiation through BLR1 and BLR2 (639, 1065). In *T. reesei*, metabolism is the most enriched functional category upon light treatment, increasing hydrolase gene expression in the presence of light (351).

The GATA ZnF TF encoded by the *nit-2* gene of *N. crassa*, which

is the ortholog of the *areA* gene in *A. nidulans*, acts as a positive regulator in nitrogen deficiency (1066, 1067). *nit-2* gene expression is constitutive in the wild-type strain, although in an *N. crassa* mutant lacking *wc-2* there are lower levels of the transcript (883). Thus, nitrogen uptake regulation in light requires WCC and NIT-2. The *Trichoderma* orthologs (TA_224741, TV_113059, and TR_76817) of *nit-2* are still uncharacterized. However, the TF NMRA of *T. atroviride* (TA_35890; containing an NADP Rossman fold domain), a negative regulator of the Nit-2 ortholog AreA in *A. nidulans* (389), is induced in the presence of light (1313). Another GATA factor not yet characterized in *T. atroviride* (TA_174504), *T. virens* (TV_192933), or *T. reesei* (TR_4231) is *sre* (siderophore regulation), which is involved in the uptake of iron in *N. crassa* and *A. nidulans* (1068, 1069). In *Trichoderma* spp., siderophores are considered part of the biocontrol mechanism against phytopathogenic fungi and in the enhancement of plant growth (8, 1070). SRE might be involved in this process in *Trichoderma* as well, but this effect has not been studied yet. The GATA factors SUB-1 (submerged protoperithecia-1) and ASD-4 (ascus development-4) are involved in sexual development in *N. crassa* (1057, 1071), and their expression is controlled by the WCC (810, 883). *T. reesei*, *T. atroviride*, and *T. virens* have orthologs of *sub-1* (TA_258818, TV_214458, and TR_57735) and *asd-4* (TR_120127, TA_320532, and TV_116843) but until now neither their regulation nor their function has been established. SUB-1 is required to regulate late light-responsive genes in *N. crassa* that are important as part of the second transcriptional event of the cascade initiated by the WCC (810). However, light exposure of *T. atroviride* does not appear to cause *sub-1* or *asd-4* differential expression in wild-type or BLR photoreceptor mutants (50, 880). Additional transcription factors involved in sexual development in *N. crassa* have been identified, including ascospore maturation-1 (ASM-1) (1072), submerged protoperithecia-2 (SUB-2) (1057), female and male fertility-1 (FMF-1) (1073), and female fertility-7 (FF-7) (1057). Interestingly, our analysis allowed us to identify orthologs to these TFs in *Trichoderma*: ASM-1 (TR_74531, TA_163709, and TV_133620), SUB-2 (TA_290129 and TV_42746), FMF-1 (TR_34248, TA_28325, and TV_19768), and FF-7 (TR_71689, TA_243083, and TV_114462) (see Tables S1 and S2 in the supplemental material).

Miscellaneous Other Transcription Factors

Another very abundant class of TFs is the miscellaneous one, containing 21 different putative DNA binding classes. They include Myb binding factors, heteromeric CCAAT factors, homeobox domains, bromodomain binding factors, HMG binding factors, APSES, and MAD box binding factors, among others that are less abundant (see Tables S1 and S2 in the supplemental material). These transcription factors play important roles in the biology of eukaryotic organisms, including cell cycle regulation, morphogenesis, RNA metabolism, meiosis, mitosis, cell death, DNA repair, chromatin remodeling, and nucleosome assembly (reviewed in references 71 and 1002]). Our analysis showed that a total of 82 proteins belonging to these classes are present in *T. atroviride*, 83 in *T. virens*, and 73 in *T. reesei* (Table 2). The most abundant class includes 15 Myb proteins in *T. atroviride*, 19 in *T. virens*, and 16 in *T. reesei*, of which 14 TFs are shared in the three species, only 1 is shared between *T. atroviride* and *T. virens*, and 2 TFs are shared between *T. virens* and *T. reesei*. Moreover, 2 TFs are specific to *T. virens* (see Tables S1 and S2). Myb proteins represent a diverse and widely distributed class of eukaryotic DNA binding factors, many of which are sequence-specific transcriptional regulators. In fungi, the Myb TFs have been reported to regulate conidiophore formation in *A. nidulans* (1074). The second most abundant class includes the heteromeric CCAAT factors. We found 8 in *T. atroviride*, 8 in *T. virens*, and 7 in *T. reesei*, of which 7 TFs are shared in the three species and only 1 is shared between *T. atroviride* and *T. virens* (see Tables S1 and S2). In yeast and filamentous fungi, the CCAAT factors identified so far belong to the HAP-like group (1075–1077). In *T. reesei*, a complex HAP-like factor that binds to the CCAAT motif within the cellobiohydrolase II gene promoter (*cbh2*) has been identified; this complex contains the TFs Hap2/Hap3/Hap5 (TR_124286, TR_121080, and TR_62979, respectively). In addition, these TFs are constitutively expressed, independently of the carbon source used for growth (887, 1078). Orthology analyses showed that these TFs are conserved in *T. reesei*, *T. atroviride*, and *T. virens* (see Tables S1 and S2).

Twenty other classes of TFs were found, although with lower representation (see Table S1 in the supplemental material). Interestingly, there are factors that may have been acquired by horizontal transfer from bacteria or viruses. These transcription factors include the proteins that contain helix-turn-helix (HTH)–AraC domains, which are named after a bacterial regulator (1079), and the APSES domain, which appears to have evolved through the capture of a viral KilA-N-like precursor early in fungal evolution (1080). The function of transcription factors containing the HTH-AraC domain has not yet been proven experimentally in fungi (1002). TFs containing the APSES domain have been reported to play important roles in cellular differentiation in ascomycetes, morphogenesis and metabolism in yeast, developmental complexity in filamentous fungi, regulation of the cell cycle, and yeast-hypha transitions (reviewed in reference 1002). Our analysis showed that the three species of *Trichoderma* contain 4 genes that encode proteins with an APSES domain. Furthermore, the analysis also showed that *T. atroviride* has 4 TFs containing the HTH-AraC domain, *T. virens* has 2 TFs, and *T. reesei* has only 1 TF that contains this bacterial domain, from which only 1 is shared in the three species, 1 is shared between *T. atroviride* and *T. virens*, and 2 TFs are specific to *T. atroviride* (see Tables S1 and S2).

Conclusions. Our analysis showed that the *T. virens*, *T. atroviride*, and *T. reesei* genomes encode 641, 592, and 448 potential transcription factors, which represent about of 5% of the total predicted genes and are in accordance with many other fungal and oomycete genomes (1001). Interestingly, we found an expansion of TFs that are shared between the two mycoparasitic species, *T. atroviride* and *T. virens* (102 TFs in common), while for *T. virens* and *T. reesei* only 20 are common, and only 6 are common to *T. atroviride* and *T. reesei*. In addition, we found 114 TFs for *T. virens*, 79 for *T. atroviride*, and only 17 TFs for *T. reesei* without orthologs and that were thus unique to each species. Finally, our analysis showed that 44, 65, and 7 TFs of *T. atroviride*, *T. virens*, and *T. reesei* have no orthologs in any of the other fungal genomes analyzed and thus could be considered species specific; these may play important roles in the establishment of their lifestyles.

In general, the potential transcription factors found in the three species of *Trichoderma* are classified in 27 Pfam families of DNA binding domains. The distribution of TF families in *T. reesei*, *T. atroviride*, and *T. virens* is comparable, except for the $Zn(II)_2Cys_6$ TF family. Interestingly, the $Zn(II)_2Cys_6$ TF family is expanded in the two mycoparasitic species. Eighty-three

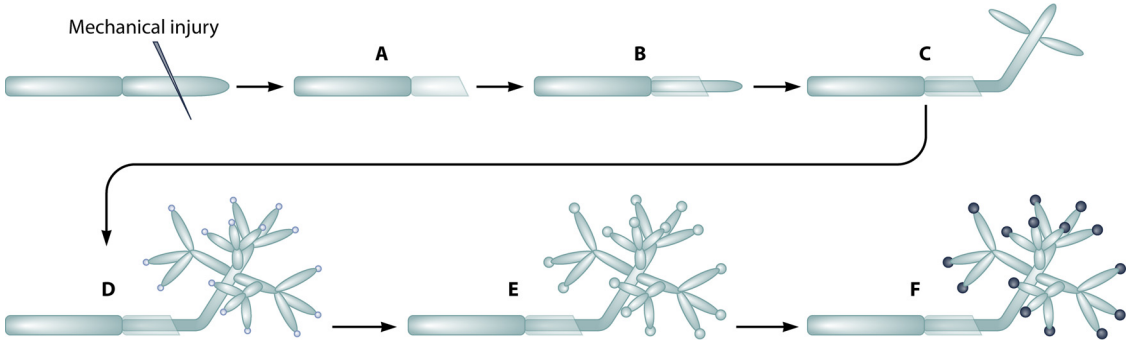

FIG 18 Morphological stages of *T. atroviride* during conidiation after mechanical injury. (A) Stage 0, sealed damage hypha; (B) stage 1, growth of a new hypha from the damaged hypha; (C) stage 2, hyphal branching; (D) stage 3, phialide formation; (E) stage 4, immature conidia emergence; (F) stage 5, conidiophores (15). (The figure was designed by E. B. Beltrán-Hernández.)

$Zn(II)_2Cys_6$ TFs are shared in *T. atroviride* and *T. virens*, while for *T. virens* and *T. reesei* only 13 are shared and only 3 are common to *T. atroviride* and *T. reesei*. In addition, 97, 71, and 13 of this TF family are specific for *T. virens*, *T. atroviride*, and *T. reesei*, respectively. This suggests that the TFs belonging to this family could play a role in the mycoparasitic lifestyle of two of these species. Additionally, our analysis of TF families showed the presence of one TF with an HTH-AraC domain only in *T. atroviride* and *T. reesei*, which is known as a bacterial DNA binding domain and suggesting that this TF may have been horizontally transferred to *Trichoderma* spp., as previously proposed (558).

DEVELOPMENT

Although *Trichoderma* (*Hypocrea*) strains in nature are often encountered in their sexual form and isolated from fruiting bodies, mating has only been achieved experimentally with *T. reesei* so far. Results from numerous taxonomic studies in Europe and worldwide indicate that not just a small number of *Trichoderma* species are capable of forming a teleomorph, but rather the majority of them do so (1081). However, especially for the economically important strains used for applications in biocontrol, sexual development has not been achieved under laboratory conditions, and the strains are considered strictly mitotic.

Asexual Development

Colonies of *Trichoderma* spp. have apically growing pluripotent hyphae that extend away from the germination point if nutrients are not a limiting factor. In *Trichoderma*, conidiation involves the differentiation of the hyphal apex into a specialized reproductive cell called a phialide, which undergoes successive mitotic divisions, each resulting in a new specialized daughter cell (the conidia). In the active growing front of a photocompetent colony, light induces a succession of developmental changes leading to the formation of conidia. In addition, mechanical injury induces conidiation at the injured sites (15, 878). The size of conidia is less than 5 μm long and wide and it is globose, subglobose, ellipsoidal, or oblong shaped. The color of conidia varies from colorless to dark green, and the adornment can be smooth, warted, or tuberculate (1082–1084). The morphological and physiological responses of *Trichoderma* to some of the cues that induce conidiation have been extensively studied for decades. The transition from mycelium to spore after the stimulus includes 5 morphogenetic stages, starting with the emergence of aerial hyphae evident by 4 h (stage 1); after 8 h hyphal branching is observed (stage 2); after 12 h phialides develop (stage 3), continuing with emergence of hyaline conidia by 16 h (stage 4) and finally, after 24 h (stage 5), conidial pigmentation and maturation take place (742). Several differences have been observed when the cue is mechanical damage, besides the 6-h delay in the developmental process, compared to light-induced conidiation. A special case of induction of development has been discovered in *T. atroviride*, development after injury of the mycelium, *T. atroviride* seals the injured hypha, and from the sealed hypha a new one emerges an hour later, which continues with the developmental program outlined above (Fig. 18) (15).

Diverse studies on development in *Trichoderma* allowed the discovery of genes with potential use as marker genes for conidiation. Such is the case with *cmp1* (conidial multidomain protein 1; TR_72379, TV_10277, and TA_323283), expression of which is strongly upregulated during conidiation and is considered specific for conidiophores and conidia (1085). Also, *lxr1* (TR_74194, TV_110629, and TA_297930) was used as a marker gene for conidiation because its homolog is only expressed during formation of conidiospores in *A. niger* (1086) and *T. reesei*, where its gene product also accumulates in conidiospores (1087). However, microarray data revealed only a moderate increase of *lxr1* over the course of conidiation (352).

Other examples of genes useful as markers for conidiation are those encoding hydrophobins. Hydrophobins are small secreted proteins, found in the outer surface of the cell wall of hyphae and conidia. In this regard, *hfb-2c*, *hfb-6a*, and *hfb-6b* are expressed in *T. atroviride* under the conditions and time at which conidia are observed (1088). In *T. reesei*, expression of plant cell wall-degrading enzymes was found to be a major event during conidiation (352). Hence, the presence of these hydrolytic enzymes is likely to allow *Trichoderma* immediate initiation of the metabolism of cellulosic and hemicellulosic material after breaking conidia dormancy and the reinitiation of vegetative growth after a period of unfavorable conditions, giving it an advantage in its habitat.

Fungal asexual development has been widely investigated, and the genetic mechanisms controlling asexual development have been addressed most comprehensively in *A. nidulans*. In this fungus, the development of asexual spores is controlled by three main components of a singular pathway: *brlA*, *abaA*, and *wetA*. Spatial and temporal expression of these regulatory genes maintain the molecular cascade, which controls the correct activation and re-

pression of genes implicated throughout the conidiation process. BrlA is the first component in the regulatory cascade, and its transcript is controlled by several genes, including the *fluffy* genes (1089). The *abaA* gene is essential for phialide development. The *wetA* gene is activated by AbaA during the late phase of conidiation and plays a role in the impermeability and maturity of conidia. The *fluffy* genes (*fluG* and *flbA-E*) are upstream regulators of this molecular cascade, and deletion of any of the *fluffy* genes in *A. nidulans* gives a typical cotton-like colony phenotype. Additionally, *stuA* and *medA* are developmental modifiers involved in the spatial organization of the conidiophore (1090–1092). There is no ortholog of *brlA* in *T. atroviride*, *T. reesei*, or *T. virens*. However, *abaA* (TR_108775, TA_322845, and TV_61967), *wetA* (TR_4430, TA_299841, and TV_218445), and *medA* (TR_123713, TA_139437, and TV_34415) are present in these genomes and their transcripts were detected by transcriptome sequencing (RNA-seq) in *T. atroviride* exposed for 60 h to light, when conidiation is evident (50). These data suggest that these genes may be functional only at late stages of the conidiation process. In the transcriptome analyzed in response to blue light, there was evidence of transcription of most of these genes, except *flbD*, during the first couple of hours after exposure to light, but they were not differentially expressed (1093, 1313). However, in response to mechanical injury, *abaA*, *wetA*, *flbE*, and *medA* are induced and *flbC* is repressed (1093). This suggests that the pathway for conidiation in response to mechanical injury is via *flbE* in *T. atroviride*. In *A. nidulans*, *abaA*, *wetA*, *flbE*, and *medA* are expressed at different stages of conidiophore development (1091). Thus, it is possible that in *Trichoderma* we are still missing expression data at early stages of development, when upstream genes can be differentially expressed. Furthermore, under conditions leading to conidiation in *T. atroviride*, it was observed that the *flbD* transcript was undetectable (50). These data suggest that the pathway involving *flbD* may not be functional, at least not in *T. atroviride*. On the other hand, transcriptional analysis in *T. reesei* subjected to conditions that triggered conidiation showed that only *medA* was differentially expressed at late stages of conidiation (352).

Promoter analysis of the *T. atroviride abaA* ortholog indicated the presence of putative BrlA binding motifs, suggesting that possibly other C2H2 transcription factors could assume *brlA* functions (13). The lack of homologs of a key regulatory element, such as *brlA* of the *Aspergillus* conidiation genes in *T. atroviride*, *T. reesei*, or *T. virens*, suggests that specific key elements determine conidiophore development in the different fungal genera, even though there is conservation of the upstream and downstream elements.

N. crassa is used as a model for many fungal species that are important animal and plant pathogens. This fungus has three distinct sporulation pathways that lead to the production of either of two types of asexual spores, macroconidia and microconidia, or sexual spores, the ascospores (1094). Formation of macroconidia, known as macroconidiation or conidiation, has been extensively studied at the morphological and molecular levels (1057, 1095, 1096). The asexual life cycle of *N. crassa* comprises three stages: vegetative hyphae, aerial hyphae, and macroconidia. In this fungus, some *con* genes (*con-6*, *con-8*, *con-10*, and *con-13*) are specifically expressed during conidiation but are not essential for development (1097, 1098). Interestingly, *con-6* is only present in *T. reesei* (TR_34312) and *con-8* is absent, and *con-10* (TR_5084, TA_291013, and TV_92556) and *con-13* (TR_105808, TA_168477, and TV_216126) are present in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1 in the supplemental material). In addition, the circadian clock controls the timing of asexual development in *N. crassa*, and clock-controlled genes (*ccg*) have been reported to be involved, specifically, *ccg-1*, *ccg-2*, *ccg-4*, *ccg-6*, and *ccg-9* (1099). The orthologs of *N. crassa ccg-6* and *con-10* are expressed during conidiation in *T. atroviride* (50); *ccg-1* is induced in response to light, and *ccg-4*, *ccg-6*, and *ccg-9* are differentially upregulated in response to injury (15, 1093) (see Table S1). Nine orthologous genes involved in conidiation in *N. crassa* were induced according to transcriptional data for *T. atroviride* obtained by high-throughput sequencing of samples obtained upon exposure to light and mycelial injury, two conditions that induce conidiation. These genes are *spr3* (sporulation-specific homolog of the yeast CDC/10/11/12 family), *ady2* (acetate transporter), *spo14* (phospholipase D), *dtr1* (dityrosine transporter), *spo20* (meiosis-specific subunit of the t-SNARE complex), *spo75* (meiosis-specific protein), *gna3* (G-protein complex alpha-subunit GpaA/FadA), *stu1* (cell pattern formation-associated protein), and *wet1* (developmental regulatory protein WetA) (see Table S1) (25).

On the other hand, an analysis of a series of *N. crassa* knockout mutants revealed the role of several transcription factors in different stages of the conidiation process (1057) (see Table S1 in the supplemental material). Most of these knockout mutants showed short aerial hyphae and abnormalities during macroconidiation, for example, *ada-1*, *-2*, and *-6* (all development altered-1, -2, and -6), *kal-1* (kaleidoscope-1), *vad-5* (vegetative asexual development-5), *adv-1* (arrested development-1), and *fl* (*fluffy*). Strains with mutations in another set of transcription factors showed either short hyphae (short aerial hypha-1 to -3 [*sah-1* to *-3*]) or long aerial hyphae (*lah-1* to *-3*), but without abnormalities during macroconidiation. Most of these transcription factors have orthologs in *T. reesei*, *T. atroviride*, and *T. virens* (see Table S1). In *T. atroviride*, they are not differentially expressed under conditions that trigger conidiation, such as a blue light pulse or mechanical injury (15, 1093), but in *T. reesei*, *ada-2* and *bek-2* are induced after exposure to light (350). However, the fact that the *Trichoderma* genomes encode these transcription factors and the lack of strong transcriptional evidence, i.e., significant differential regulation, imply that they have roles in stages or conditions for asexual development that have not been explored yet.

In *N. crassa*, light regulates different physiological processes, including asexual reproduction or conidiation. In this regard, a group of genes with a delayed light response has been described that are in part regulated by the early light-responsive transcription factor *sub-1* (810). However, unlike in *Neurospora*, the ortholog of *sub-1* from *T. atroviride* is not induced by light, and a *sub-1* mutant strain showed a constitutive conidiation phenotype, indicating a role as a repressor in this process (J. E. Cetz-Chel, E. U. Esquivel-Naranjo, and A. Herrera-Estrella, unpublished results). In contrast, in *T. reesei* this gene is upregulated by light (350), suggesting the same role in the light response as with *Neurospora*.

Conclusions. Most of the genes reported to be involved in asexual reproduction in *N. crassa* and *A. nidulans* are encoded in the *Trichoderma* genomes, suggesting that they may play a role in this process in *Trichoderma*. Even though the signal transduction mechanisms of cues that trigger conidiation have been reasonably well studied in *Trichoderma*, the analysis of transcriptional regulators specifically involved in development has hardly been ad-

dressed. Interestingly, key regulators of this process in other fungi, such as *fl* and *blrA*, are absent in the genomes of *T. reesei*, *T. atroviride*, and *T. virens*, denoting that in this genus their function is carried out by a gene(s) not clearly related to those previously described in other systems. Here, we also list a number of genes that can be used as conidiation markers and can be used to extend our understanding of this vital process for the survival and dispersal of *Trichoderma*.

Sexual Development

Sexual development under laboratory conditions has only been achieved for *T. reesei* so far (859; for overviews, see references 1100 and 1101), although teleomorphs are known for *T. atroviride* (*Hypocrea atroviridis*) and *T. virens* (*Hypocrea virens*) as well. In *T. reesei*, sexual development is influenced by light response (885) and nutrient-sensing pathways (350, 642, 891). Analysis of the mating competence of the original isolate of *T. reesei*, QM6a, revealed that this strain is female sterile, i.e., artificial exchange of the mating type of this strain did not enable sexual development (859). Although this finding constitutes a serious drawback for industrial application of sexual development with *T. reesei*, female sterility of QM6a is now frequently used to test the influence of a given gene on male and female fertility (534, 885, 1102).

The surprisingly low number of gene duplications in *T. reesei* (23) indicates that RIP mutation may be operative. Thereby, duplicated sequences are detected and rendered unfunctional (24). However, RIP was not confirmed experimentally yet (1100) but is under investigation. Should RIP indeed be operative in *T. reesei*, one option would be deletion of RID1, the homolog of which perturbs RID in *N. crassa* (1103). RID1 has homologs in *Trichoderma* spp., with TR_37515 in *T. reesei*. In *T. atroviride* and *T. virens*, the homologs are split into two models each (TA_320162 and TA_310388; TV_221941 and TV_221942).

Of the mating-type genes that regulate mating-type-specific functions, only *mat1-2-1* of the MAT1-2 idiomorph is present in the sequenced genomes of *T. reesei*, *T. atroviride*, and *T. virens* (TR_124341, TA_33998, and TV_60622). For *T. reesei*, the MAT1-1 locus has been sequenced in the isolate CBS999.97, and this revealed three genes in this idiomorph (*mat1-1-1* [GenBank accession number ACR78244.1], *mat1-1-2* [ACR78245.1], and *mat1-1-3* [ACR78246.1]). In *T. reesei*, transcript levels of *mat1-2-1* are regulated by the photoreceptors BLR1, BLR2, and ENV1 (885) and by VEL1 (534).

The pheromone system. For induction of sexual development, sensing of a potential mating partner is a prerequisite (1104). In fungi, mainly the pheromone system fulfills this purpose, with peptide pheromone precursors and pheromone receptors (1105). The α-type peptide pheromone precursor PPG1 (TR_104292, TA_297240, and TV_67354) is comparable to its homologs in other fungi. However, *T. reesei*, *T. atroviride*, or *T. virens* does not contain characteristic **a**-type peptide pheromone precursors, but instead has h-type precursors, which share characteristics of **a**- and α-types with the consensus motif (LI)GC(TS)VM with *T. reesei* HPP1 (TR_34493) as the first representative (1106). *T. atroviride* HPP1 is shorter than its homolog in *T. reesei*, and its function remains to be defined. Nevertheless, EST support for transcription of *T. atroviride hpp1* (http://genome.jgi-psf.org/cgi-bin/browserLoad/?db=Triat2&position=contig_13:13838-14223) is available. *T. virens* HPP1 (TV_91991) shares similar characteristics with *T. reesei* HPP1, and ESTs span its genomic locus (http://genome.jgi-psf.org/cgi-bin/browserLoad/?db=TriviGv29_8_2&position=scaffold_1:2398162-2398007).

While peptide pheromone precursors are responsible for male fertility in *T. reesei* (1102), peptide pheromone receptors are required for female fertility in their cognate mating type (1102). Further factors required for female fertility in *T. reesei* upon growth in light are ENV1 and VEL1 (534, 885). As usual in heterothallic ascomycetes, *T. reesei*, *T. atroviride*, and *T. virens* have one Ste2p-type peptide pheromone receptor (HPR2; TR_64018, TA_36032, and TV_147400), which is associated with the MAT1-1 mating type, and one Ste3p-type peptide pheromone receptor (HPR1; TR_57526, TA_147894, and TV_40681), which is associated with the MAT1-2 mating type. In *T. reesei*, transcript levels of pheromone precursors and receptors are regulated by the mating type (1102), by the photoreceptors BLR1, BLR2, and ENV1 (885), by heterotrimeric G-protein pathway components GNB1, GNG1, and PhLP1 (350), and by VEL1 (534).

Meiosis-related genes. Meiosis is the most efficient process to generate diverse genetic materials during the life cycle of sexually propagating organisms. The most unique feature of meiosis is the reduction of the number of chromosomes by half. Each daughter cell that is produced will have half as many chromosomes as the parent cell. Budding yeast *S. cerevisiae* and fission yeast *S. pombe* undergo meiotic sporulation to generate tetrads with four ascospores per ascus. Most ascomycetes, including *N. crassa* and *Sordaria macrospora*, undergo sporic meiosis to generate octads with 8 ascospores per ascus. These 8 ascospores are produced by two rounds of meiotic nuclear division followed by one round of postmeiotic mitotic division (1104, 1107). Interestingly, in *T. reesei* (teleomorph *Hypocrea jecorina*), sexual development results in fertilized perithecia embedded in stromata containing decahexads with 16 ascospores per ascus (859). Recently, we showed that the 16 ascospores are generated by one round of meiosis followed by two rounds of postmeiotic mitosis (1108).

Previous studies, particularly in *S. cerevisiae*, *S. pombe*, and *S. macrospora*, have provided important insights into mechanisms of meiosis, including meiotic entry, premeiotic DNA replication, DNA recombination, chromosome morphogenesis, meiotic nuclear divisions, and ascospore formation. Here we have searched and compared *Trichoderma* genes that are conserved in sporic meiosis of other fungi, including *S. cerevisiae*, fission yeast *S. pombe*, *S. macrospora*, and *N. crassa*. Although DNA-based phylogenetic analyses have inferred two other pantropical *Hypocrea* ascomycetes as the sexual stage to two *Trichoderma* species, *T. atroviride* and *T. virens*, respectively (1109, 1110), sexual mating of these two *Trichoderma* species has not been achieved under laboratory conditions. By understanding the mechanism of sporic meiosis in these *Trichoderma* species, we may broaden their industrial and agricultural usages.

In *S. cerevisiae*, sporic meiosis was divided into three major phases: early, middle, and late phases (1111, 1112). Accordingly, meiotic genes were assigned into the three phases according to the time of gene expression. The early phase initiates from the point when cells decide to undergo meiosis. Subcellular events in this phase include the entry into the premeiotic DNA synthesis, synapsis of homologous chromosomes, and meiotic DNA recombination. Following the early phase, two rounds of meiotic nuclear division (MI and MII), and the formation of a prospore membrane occurs in the middle phase. The late phase spans from the completion of prospore membrane closure to the maturation of

ascospores, including chromatin compaction, restoration of vegetative cytoplasmic organization, and spore wall assembly. The functions of key players involved in these three sporic meiosis phases are described along with the corresponding putative *Trichoderma* orthologs as follows.

(i) Early meiotic gene regulators. The expression of early genes in *S. cerevisiae* depends on the activation of Ime1p, a master transcription factor for meiosis. The transcriptional expression of *IME1* is positively and negatively regulated by Ime4p and Rme1p, respectively (1113, 1114). In **a**/α diploid cells, the inhibition of the repression of Rme1p leads to the expression of Ime1p through Ime4p. The *IME1* RNA level is also regulated by Mck1p and a set of Rim proteins (Rim1p/Rim101p, Rim8p, Rim9p, and Rim13p) through distinct pathways (1115). However, there is no *IME1* ortholog identified in *T. atroviride*, *T. reesei*, and *T. virens*, and many other organisms (1116). Moreover, *IME4*, *RME1*, and *MCK1* orthologs do not exist in the three *Trichoderma* species. Rim101p (TR_120698, TA_78054, and TV_40291) is a transcription factor containing three C2H2 zinc fingers and a C-terminal acidic region, which is functional in the conserved pH-sensing pathway. Rim8p (TR_56605, TA_172689, and TV_113483), Rim9p (TR_21415, TA_34860, and TV_27893), and Rim13p (TR_70560, TA_158516, and TV_78188) activate Rim101p by proteolytic cleavage of the C-terminal acidic region in response to alkaline pH (1117). Although these *RIM* genes are present in *Trichoderma*, their function in *Trichoderma* meiosis, due to the absence of *IME1*, needs to be further examined. Our results suggested that *IME1* is unique to *S. cerevisiae*, and other organisms may adopt another gene or have evolved with a new gene to trigger meiotic gene expression.

Ime2p (TR_50071, TA_142127, and TV_47348), the downstream target of Ime1p, is required for induction or full level expression of a part of early meiotic genes. In addition, Rim11p (TR_74400, TA_297064, and TV_73199), Rim15p (TR_120605, TA_320942, and TV_183763), and Ume6p (TR_62199, TA_54121, and TV_193568) also positively regulate *IME2* expression in meiotic cells (1118). Ume6p originally was identified as a negative regulator of meiotic genes. The interaction of Ime1p and Ume6p, however, activates transcription of *IME2*, other early genes, and the mid-meiosis transcription factor gene *NDT80* (TR_28781, TA_225495, and TV_191114), respectively (1118–1120). *IME2*, *UME6*, *RIM11*, *RIM15*, and *NDT80* homologs are present in *T. atroviride*, *T. reesei*, and *T. virens* (see Table S1 in the supplemental material), suggesting that *IME2* transcription and Ume6p-associated gene expression may be operated in a modified mechanism to adapt the loss of *IME1*. Notably, the human pathogen *Candida lusitaniae* is able to undergo mating and meiosis despite lacking the ortholog of *IME1*, as was found for many other organisms, including *T. reesei*. *C. lusitaniae* utilizes the orthologs of *IME2* and *STE12*, the master regulator of *S. cerevisiae* mating, to initiate a complex sexual cell cycle (1121). The ortholog of *IME2* in *N. crassa* is not involved in meiosis, but rather regulates the formation of female reproductive structures (1122). Our search results also indicated that many negative regulators of early meiotic genes are present in *Trichoderma* species. Sin3p (TR_79441, TA_254023, and TV_33123) and Rpd3p (TR_48386, TA_163610, and TV_114500), for instance, are considered transcriptional modulators that negatively regulate gene expression in the same pathway (1123, 1124). Sin3p has been shown to repress the meiotic early genes, such as *IME2*, *SPO11* (see below), and *SPO16* (no homologs in *T. reesei*, *T. atroviride*, or *T. virens*) (1125). Other gene products, *SSN2* (TR_22783, TA_223394, and TV_112408), *SSN3* (TR_81720, TA_322994, and TV_31744), and *SSN8* (TR_5196, TR_40849, and TR_83013) that lower the RNA level of early meiotic genes in nonmeiotic cells (1125) are also present in the three *Trichoderma* species.

Trichoderma also contains some orthologs of fission yeast *S. pombe* meiotic regulatory genes, such as *ste11*, *mei2*, *cdc10*, *res2*, *pab2*, and *mmi1*, but not *mei3* and *mei4* (see Table S1 in the supplemental material). Ste11p, a transcription factor, initiates two distinct meiotic pathways that are mediated by Rep1p and Mei2p, respectively (1126). Mei2p is an RNA-binding protein required for premeiotic DNA synthesis and meiosis I (1127). Rep1p and Cdc10p form a transcriptional activator heterocomplex to control the expression of several *rec* (recombination) genes (1128). The Res2p-Cdc10p complex is required for premeiotic DNA synthesis and meiotic division (1129, 1130). Pab2p and Mmi1p are involved in the RNA decay pathway in which meiotic mRNA transcripts are eliminated during vegetative growth or an untimely meiosis (1131, 1132).

(ii) DNA recombination and chromosome morphogenesis during meiotic prophase. *(a) Generation of the double-strand break.* In most sexually reproducing organisms, meiotic recombination is initiated by developmentally programmed DSBs. In *S. cerevisiae*, the formation of meiotic DSBs is mediated by Spo11p, an evolutionarily conserved endonuclease. Several accessory factors are also required for DSB formation, including meiosis-specific factors for expression (Mei4p, Rec102p, Rec104p, Rec114p), splicing (Mer2p), or nuclear localization (Ski8p). Spo11p and Ski8p are physically associated with DSB sites throughout the genome of the chromatin loops and can interact directly with the Rec102p/Rec104p subcomplex. Mei4p, Rec114p, and Mer2p form a distinct subcomplex that can interact with the Rec102p/Rec104p subcomplex via Rec104p. Specific posttranslational modification of Mer2p is required for its interaction with Rec114p and Xrs2p, respectively (1133).

Although the presence of an evolutionarily constrained catalytic motif in Spo11p allows ready identification of orthologs throughout eukaryotic lineages, the overall conservation is still fairly low. For example, the *S. cerevisiae* Spo11p and its ortholog Rec12p in *S. pombe* have only 31% amino acid identity (1134). Thus, orthologs of *SPO11* in *T. atroviride*, *T. reesei*, and *T. virens* were not identified previously or annotated in currently released genome database versions. Using *S. pombe* Rec12 (but not *S. cerevisiae* Spo11p) as a query to search the assembled genomic sequences released by JGI, we successfully identified the Spo11 orthologs in *T. reesei*, *T. atroviride*, and *T. virens* (TA_166457 and TV_48006) with weak homology (approximate E value = 7E−08). There is no gene model in version 2 of the *T. reesei* genome database at this locus. In version 1, protein model 17833 is related to Spo11 and was correctly annotated. The annotated *Trichoderma SPO11* genes encode protein sequences with canonical start and stop codons. Notably, *S. macrospora* Ski8 (TR_61570, TA_148471, TV_74530) was also identified and shown to be required for the localization of Spo11 to meiotic chromosomes and for generating the DSBs during meiosis (1135). In spite of very low homology, Ski8 homologs were also found in *T. atroviride*, *T. reesei*, and *T. virens*. In contrast, other DSB proteins were not identified in *T. reesei*, *T. atroviride*, or *T. virens*. This is consistent with earlier reports that identification of orthologs of

other DSB proteins from *S. cerevisiae* was very difficult (Mei4p and Rec114p) or thus far has been impossible (Mer2p, Rec102p, and Rec104p) in other organisms, because they are among the most rapidly diverging of all cellular proteins (1133, 1136).

(b) Removal of Spo11 from DNA and DNA end resection. Repair of DSBs by homologous recombination requires resection of 5′-termini to generate 3′-ssDNA tails. Meiotic DSB termini are blocked by bound transesterase Spo11. Endonucleolytic removal of budding yeast Spo11p and fission yeast Rec12 requires the nuclease activity of the Mre11-Rad50-Xrs2 (MRX) complex and its functional partner, budding yeast Sae2p or fission yeast Ctp1 (1137, 1138). *S. cerevisiae* MRX has a unique function in promoting Spo11p-generated DSBs. Moreover, MRX has additional important roles in the resection of mitotic DSBs and activation of the DNA damage checkpoint (1139, 1140). Due to their general functions for repairing mitotic and meiotic DSBs, it is not surprising that these proteins are conserved in *Trichoderma* species. *T. atroviride*, *T. reesei*, and *T. virens* orthologs of Mre11p (TR_70084, TA_134195, and TV_164171) and Rad50p (TR_64204; no gene catalogue model, TA_252710 and TV_143603) were identified with high homology, while the Xsr2 (TR_64498, TA_296803, and TV_33005) ortholog was found through BLAST searches with Human Nbs1 rather than *S. cerevisiae* Xsr2p. However, the gene encoding homologs of the functional partner, budding yeast Sae2p or fission yeast Ctp1, was not found in *Trichoderma*.

After Spo11p removal from the cleaved DNA strands, two exonuclease activities in *S. cerevisiae* are required for generation of 3′ ssDNA tails. First, Mre11p nicks the strand to be resected up to 300 nucleotides from the 5′-terminus of the DSB. Second, this nick enables resection in a bidirectional manner, using Exo1p in the 5′ → 3′ direction away from the DSB and Mre11p in the 3′ → 5′ direction toward the DSB end (1141). Exo1p (TR_60709, TA_319288, and TV_37141) orthologs also exist in *T. atroviride*, *T. reesei*, and *T. virens*. Together, our search results suggest that *T. reesei* meiosis likely utilizes the conserved mechanisms for DSB resection.

(c) Homologous pairing and strand invasion reactions. In *S. cerevisiae*, the long 3′ ssDNA tails serve as the substrate for assembly of filaments of the RecA family strand exchange proteins or recombinases. Thereby, Dmc1p is meiosis specific (1142) and Rad51p (TR_30700 [no gene catalog model in v2.0], TA_161197, and TV_112066) is ubiquitously expressed (1143). These presynaptic nucleoprotein filaments engage in the search for a homologous template, with a strong preference toward homologous chromosomes rather than the sister chromatid, and then catalyze DNA strand exchange to create a displacement loop (D loop) (1144). Neither Rad51p nor Dmc1p acts alone to promote homologous pairing and strand exchange reactions. In *S. cerevisiae*, the accessory factors of Dmc1p include a Hop1p-Mnd1p complex, Mei5p-Sae3p complex, and Rhd54p. The accessory factors of Rad51p are Rad52p, a Rad55p-Rad57p complex, Rad54p, Rhd54p, and Hed1p (1145). Hed1p, a meiosis-specific protein, inhibits Rad51's recombinase activity (1146). The presence of Hed1p converts Rad51p from a recombinase to a recombination accessory factor. Recently, it was reported that budding yeast Dmc1p, not Rad51p, catalyzes the homology search and strand exchange for most, if not all, meiotic recombination events. Rad51p mainly functions as a Dmc1p accessory factor (1147). Hed1p homologs were not detected in *T. reesei*, *T. atroviride*, or *T. virens*.

In *S. pombe*, DSBs at hot spots are repaired primarily with the sister, independent of Dmc1p, whereas DSBs in DSB-poor (cold) regions are repaired primarily with the homolog dependent on Dmc1p (1148).

Searching homologs of Dmc1p and Rad51p, the same protein was identified in *T. atroviride*, *T. reesei*, and *T. virens*. The protein was then validated as the bona fide Rad51p ortholog by reciprocal best hit, indicating that Dmc1p does not exist in *T. atroviride*, *T. reesei*, and *T. virens*. Interestingly, *N. crassa* and *S. macrospora* also lack Dmc1p homologs (71, 1149). Consistently, several Rad51p accessory factors (e.g., Rad52p, Rad54p, Rad57p), but not Dmc1p accessory factors, were identified in *T. reesei*, *T. atroviride*, or *T. virens*.

(d) Crossover recombination and chromosome synapsis. Dmc1p- or Rad51p-catalyzed D-loop products are ultimately repaired into either crossovers with exchange of chromosome arms or noncrossover products. These two pathways appear to differentiate shortly after the initial strand exchange. Along the crossover pathway, two major types of joint molecules have been identified: singe-end invasions, in which one DSB has undergone strand exchange with a template chromosome, and Holliday junctions, in which both DSB ends have been engaged (1150). Double Holliday junctions are a prominent intermediate in *S. cerevisiae* meiosis, where they form preferentially between homologs rather than between sister chromatids (1151). In sharp contrast, single Holliday junctions (sHJs) are the predominant intermediates in *S. pombe* meiosis, and sHJs arise preferentially between sister chromatids rather than between homologs (1152).

In many organisms, crossover recombination takes place in the context of the synaptonemal complex (SC), a proteinaceous structure that juxtaposes homologous chromosomes. The SC consists of a central region and two dense lateral elements. The lateral element constitutes the rod-like homolog axis, which is called an axial element prior to synapsis. In *S. cerevisiae*, sister chromatid cohesin components (Rec8p, Pds5p), topoisomereae II (Top2p), and two other meiosis-specific proteins (Red1p, Hop1p) are structural components of axial elements. ZMM proteins (also known as the synapsis initiation complex [SIC]) play crucial roles in the linkage between recombination and SC assembly. *S. cerevisiae* ZMM proteins were classified into three subgroups based on functional criteria (1153): subgroup I includes highly conserved proteins that exhibit sequence similarities with proteins involved in mitotic DNA metabolism, and Mer3p DNA helicase can unwind various double-strand substrates. Msh4p and Msh5p are meiosis-specific homologs of bacterial DNA mismatch repair protein. Msh4p and Msh5p heterodimers perform mostly meiotic functions and are not required for standard mismatch repair. In *S. macrospora*, Mer3 plays a role in avoidance of entanglement in chromosome alignment while Msh4/Msh5 is required to establish the correct alignment distance (1154). Subgroup II is represented by *S. cerevisiae* Zip1p, the major structural component in the transverse filament of the SC detected by electron microscopy (1155). The terminal globular domain of Zip1p can interact with conjugates and/or polymeric chains of SUMO (1156). Orthologs of Zip1p have been identified in several model organisms that localize to the SC and share structural similarities with Zip1p, including a closely related *S. macrospora* protein Sme4 (1157). Subgroup III includes several proteins (e.g., Zip2p-4p, Spo16p) required for SC initiation or elongation (1153). Zip3p is an E3 ligase of SUMO (1156). Zip1p, Zip3p, and Red1p all can bind to SUMO polymeric chains and/or SUMO conjugates. It was pro-

posed that Zip1 and Red1 directly sandwich the SUMO chains to mediate SC assembly (1158). SC does not form during meiotic prophase in *S. pombe*. However, structures resembling the axial elements of SCs, the so-called linear elements, appear in *S. pombe*. Structural components of linear elements include Rec10p (a distant homolog of *S. cerevisiae* Red1p), Hop1p, Rec25p, Rec4p, Rec27p, and Pmt3p (*S. pombe*'s SUMO protein) (1159). Detailed structural components of SCs (including the central elements and recombination nodules) in *N. crassa* were observed previously by using a nuclear spreading protocol with electron microscopy (1160, 1161); however, the components of *N. crassa* SCs are still unknown.

Our search results indicated that *T. reesei*, *T. atroviride*, and *T. virens* possess all genes encoding cohesion, condensin, Pds5, and Top2 (see Table S1 in the supplemental material). These proteins are functional in establishment and maintenance of chromosome condensation. Orthologs of subgroup I ZMM proteins (Mer3p [TR_65494, and TA_91197, and TV_190180], Msh4p [TR_56515, TA_261202, and TV_194557], and Msh5p [TR_110535, TA_127936, and TV_218842) also exist in *T. atroviride*, *T. reesei*, and *T. virens*. In contrast, orthologs of SC components in *S. cerevisiae* (Red1p, Hop1p, Mek1p, Zip1p-4) and orthologs of linear elements (Rec10p, Rec25p, Rec24p, and Rec27p) in *S. pombe* were not found in *T. reesei*, *T. atroviride*, and *T. virens*. Notably, the SC structure and a central elemental protein Sme4 were demonstrated in *S. macrospora*. Although the orthologs of Sme4 are not shown in our results, a gene encoding the potential ortholog of the *S. macrospora* SC central element protein Sme4 was identified in *T. reesei* (TR_109189) (1157) based on domain similarity. Accordingly, the potential orthologs of Sme4 in *T. atroviride* and *T. virens* are TA_275534 and TV_67475. The relevance of the absence of SC or linear elements in *T. atroviride*, *T. reesei*, and *T. virens* needs further examination.

Finally, *S. cerevisiae* Mer1p and Nam8p are required for splicing of Mer3p pre-mRNA. Such posttranscriptional regulation may not be evolutionarily conserved, since orthologs of Mer1p and Nam8p were not found in *T. atroviride*, *T. reesei*, and *T. virens*.

(e) Coupling DSB repair and chromosome synapsis with meiosis progression. Programmed DSB repair and SC assembly are coupled to cell cycle progression by a surveillance mechanism, named the pachytene checkpoint, which delays meiosis I until DSB repair and SC assembly are achieved. This checkpoint mechanism prevents chromosome missegregation that would lead to the production of aneuploid gametes. In *S. cerevisiae*, the pachytene checkpoint is mediated by the mitotic DNA damage checkpoint (Mec1p and Tel1p), axial element components (Hop1p and Red1p), and a meiosis-specific pachytene checkpoint protein, Pch2p. Mec1p and Tel1p are the budding yeast homologs of mammalian DNA damage sensor kinases ATR and ATM, respectively. Recruitment of Tel1p/ATM and its binding partner, Tel2p, to the MRX complex at DSBs via the C terminus of yeast Xrs2p or its mammalian homolog Nbs1, respectively, is critical for Tel1p/ATM-initiated signaling events that trigger cell cycle arrest and DNA repair. Mec1p/ATR is recruited to RPA-coated ssDNA tails via its binding partner Ddc2p/ATRIP. Mec1p activation requires three additional DNA damage sensors: the yeast 9-1-1 checkpoint complex (Ddc1p-Mec3p-Rad17p), its clamp loader Rad24p-RFC complex, and Dpb11p (1162).

In response to meiotic DSBs, Red1p associates with SUMO polymeric chains (1158) and the 9-1-1 checkpoint complex (1163) to activate Mec1p and Tel1p kinases. These two checkpoint kinases then mediate Hop1p phosphorylation, which is required for chromosomal recruitment and activation of Mek1p protein kinase (1164). Mek1p phosphorylates multiple targets in meiosis, including Rad54p. Phosphorylated Rad54p acts synergistically with a meiosis-specific protein Hed1p to suppress the recombinase activity of Rad51p (1165). Accordingly, Red1p, Hop1p, and Mek1p function together to positively promote IH (interhomolog) recombination (1166–1168) and to slow down the rate of IS (intersister) recombination (1169). The higher rate of IS recombination is mediated by cohesion (Rec8p), while Red1p/Hop1p/Mek1p counteracts this effect, thereby ensuring IH bias (1170). The yeast pachytene checkpoint protein Pch2p was shown to associate with the N terminus of Xrs2p (1171). This Pch2p-Xrs2p interaction might enable Pch2p to remodel the chromosome structure adjacent to DSB sites to promote accessibility of Tel1p kinase for Hop1p phosphorylation (1171). Recent studies further revealed two additional functions of Pch2p in budding yeast meiosis: first, purified Pch2p proteins displaces Hop1p from large DNA substrates *in vitro* in an ATP-dependent manner (1172); second, Pch2p prevents Mec1p/Tel1p-mediated Hop1p phosphorylation occurring independently of Red1p (1173). Our search results revealed that some mitotic DNA damage checkpoint genes are conserved in *T. atroviride*, *T. reesei*, and *T. virens*, such as Mec1p (TR_66128, TA_181231, and TV_161348), Tel1p (TR_66928, TA_152582, and TV_157717), and Rad24p (TR_120447, TA_212293, and TV_42692). In contrast, orthologs of Rad17p, Ddc1p, Mec3p, Dpb11p, and Pch2p are not found in *Trichoderma* spp. Pch2p orthologs were also not found in several other filamentous fungi, e.g., *A. nidulans* (1174).

(f) Resolution of joint molecules. The efficient and timely resolution of DNA recombination intermediates is essential for bipolar chromosome segregation. The Mus81p-Eme1p endonuclease complex is a resolvase responsible for resolution of single Holliday junctions in *S. pombe* meiosis (1152). In *S. cerevisiae* meiosis, Mus81p-Mms4p endonuclease and Yen1p act sequentially in meiosis I and meiosis II to resolve double Holliday junctions, respectively (1175). Sgs1, a Bloom's helicase ortholog, prevents aberrant crossing over by suppressing the formation of multiple-chromatid joint molecules (1176, 1177). Sgs1 is a component of the highly conserved Sgs1p-Top3p-Rmi1p complex. Deletion of *SGS1*, *TOP3*, or *RMI1* is synthetically lethal when combined with the loss of the Mus81p-Mms4p or Slx1p-Slx4p endonucleases, which have been implicated in HJ resolution. We found that *T. reesei*, *T. atroviride*, and *T. virens* possess orthologs of Yen1p, Mus81p, Slx1p, Sgs1p, and Top3p (see Table S1 in the supplemental material), but not Slx4p and Mms4p orthologs.

Chromosome segregation. *S. cerevisiae PBP2* (Pbp1p binding protein), *MAM1* (monopolar microtubule attachment during meiosis I), and *CSM1-4* (chromosome segregation in meiosis) were identified to be associated with chromosome segregation through a screen of gene deletion strains (1178). Pbp2p is an RNA binding protein involved in the regulation of telomere position effect and telomere length (1179). Mam1p, Csm1p, and Lrs4p form a kinetochore-associated protein complex essential for monopolar attachment of microtubules (1180). Csm3p forms a heterotrimeric mediator complex with Tof1p (topoisomerase I interaction factor) and Mrc1p (mediator of replication checkpoint) at DNA replication forks (1181). Our search results indicated that orthologs of Pbp2p, Csm3p, Tof1p, and Mrc1p exist in *T. atro-*

viride, *T. reesei*, and *T. virens* (see Table S1 in the supplemental material).

The middle phase of meiotic sporulation. In *S. cerevisiae*, exit from the pachytene stage of meiosis requires the midmeiosis transcription factor Ndt80p, which promotes expression of approximately 200 genes (1182). Ndt80p binds to the MSE (middle sporulation element) in the promoters of middle sporulation-specific genes (1183). The induction of *NDT80* is facilitated by the Ime1p-Ume6p complex, while it is repressed by the Sum1p-Hst1p complex (1183, 1184). Interestingly, it was shown that Polo-like kinase (PLK, Cdc5p) is the only member of the Ndt80 transcriptome required for this critical step in meiotic progression (1185). One critical function of Cdc5p is to phosphorylate and subsequently activate Mus81p-Mms4p resolvase in meiosis I, generating the crossovers necessary for chromosome segregation (1175).

T. atroviride, *T. reesei*, and *T. virens* contain orthologs of Ndt80p, Ume6p, and Cdc5p (TR_2829, TA_226570, and TV_56894) and Hst1p, but not the Sum1p ortholog. Notably, the filamentous fungus *N. crassa* has three homologs of *NDT80*. None of the Ndt80p homologs is required for meiosis, and even the triple mutant is unaffected. In contrast, two Ndt80p homologs of *N. crassa*, VIB-1 and FSD-1, exhibit a synergistic effect on the timing of female reproductive structure (protoperithecia) formation and ascospore maturation (1122), and *vib-1* is involved not only in regulation of programmed cell death but also in regulation of plant cell wall degradation (1186, 1187). Moreover, mutations in the *vib-1* homolog of *A. nidulans* (*xprG*) result in decreased extracellular protease production in response to carbon and nitrogen starvation (1188). CaNdt80, the *C. albicans* homolog of Ndt80p, is an important transcription modulator to various stress response genes, including cell separation, drug resistance, nitric oxide inactivation, germ tube formation, hyphal growth, and virulence (1189). The identified *Trichoderma* NDT80 orthologs (TR_28781, TA_225495, and TV_191114) are phylogenetically close to *N. crassa* FSD-1. It will be interesting to further investigate the roles of *Trichoderma* NDT80 orthologs in meiosis, perithecium formation, and stress responses.

B-type cyclins and the anaphase-promoting complex. In *S. cerevisiae*, key meiotic events, such as DNA replication, recombination, and the meiotic division, are regulated by B-type cyclin-dependent kinase (Clb-CDKs). Clb5p-CDK and Clb6p-CDK are required for initiation of premeiotic DNA replication and recombination (1190, 1191). The major mitotic cyclin Clb2p is not expressed during meiosis. Instead, Clb1p, Clb3p, and Clb4p promote progression through the meiotic division (1192). Clb3p-CDK activity is restricted to meiosis II through translation control, while Clb1p-CDK activity is restricted to meiosis I and Clb4p-CDK activity is restricted to metaphase II by posttranslational modification (1193). Following the expression of Ndt80p, the anaphase-promoting complex (APC) promotes degradation of the ubiquitinated Clbs and other substrates. The role of APC in meiosis is implicated in the transition from metaphase I to anaphase I and in the exit from metaphase II (1194). The activities of APC are modulated by three activators (Cdc20p, Cdh1p, and Ama1p) (1194). Ama1p is meiosis specific and required for the destruction of Clb1p (1195). Our search data indicate that orthologs of several APC components and three activators are present in *Trichoderma*. Out of six Clbs, only orthologs of Clb2p (TR_57421, TA_301423, and TV_183741) and Clb4p (TR_69437, TA_173601, and TV_34612) are found in *T. atroviride*, *T. reesei*, and *T. virens*.

Prospore membrane. After meiosis II, budding yeast and fission yeast form prospore membranes and forespore membranes, respectively to delimit spores. The detailed processes have been reviewed recently (1112). The prospore membrane in budding yeast is generated from a sporulation-specific structure termed the meiotic II outer plaque (MOP) which is organized on SPBs (spindle pole bodies) embedded in the nuclear envelope during meiosis. MOP is formed by recruiting sporulation-specific proteins onto the sides of SPBs that are exposed to cytoplasm. MOP serves as a vesicle docking complex, allowing vesicle fusion to form a membrane cap. The membrane cap is expanded to form a membrane sac by additional vesicle fusion before completion of the formation of the prospore membrane. This vesicle fusion requires a SNARE complex that is formed by Sso1p, Snc1/2p, and Spo20p (1196, 1197) and other upstream proteins required for vegetative growth. Spo20p, a sporulation-specific protein, directs the complex to function on the prospore membrane rather than plasma membrane (1198). However, the meiotic SNARE components (Spo20p and Sso1p) were not found in *T. atroviride*, *T. reesei*, and *T. virens*. On the other hand, a few layers of outer plaque and a growing forespore membrane similar to the prospore membrane of *S. cerevisiae* were also observed in *S. pombe*. Mutated proteins of *S. pombe* SPB components (*spo2*, *spo13*, and *spo15*) showed defects in the formation of the forespore membrane (1199). It was proposed that the forespore membrane of *S. pombe* is generated in an operation mode analogous to that for the *S. cerevisiae* prospore membrane (1200). However, the numbers of conserved proteins between the two yeasts are surprisingly low. The major components of *S. cerevisiae* MOP include Spo74p, Mpc54p, Spo21p, and Ady4p, but the components of the outer plaque are still largely unknown in *S. pombe*. In filamentous fungi, spindle plaques were observed from the interphase of meiosis II. The function of the spindle plaques in the formation of spore wall membranes is obscure, because the spore wall membrane is formed around the ascus cytoplasm during interphase II rather than being generated from the spindle plaques. Ascospore delimitation, therefore, is thought to be carried out through membrane invagination (1201–1203). In that context, it may not be surprising that the MOP components were not found in *T. atroviride*, *T. reesei*, and *T. virens*.

Associated with the growing prospore membrane, septins assemble into a bar or a sheet that appears along the nuclear-proximal side of the prospore membrane, while a leading edge complex forms a ring structure along the opening of the growing prospore membrane sac in *S. cerevisiae*. Septins are also functional in the proper localization of proteins involved in cytokinesis and signal transduction in vegetative cells. However, their role in sporulation is still unclear. Septin filaments are organized by two tetramers that linearly assemble Cdc3p, Cdc10p, Cdc11p, and Cdc12p in vegetative cells (1204). Two sporulation-specific septins, Spr3p and Spr28p, replace Cdc11p and Cdc12p to compose the tetramers during the formation of the prospore membrane (1205). A Gip1p-Glc7p phosphatase complex is required for the proper localization of septin bars to the prospore membrane (1206). On the other hand, the membrane shape is controlled by the leading edge complex and gene products (Sma2p, Spo1p, and Spo19p) that regulate the membrane curvature in *S. cerevisiae* (1207). Three components of the leading edge complex have been

identified: Ady3p, Ssp1p, and Don1p (1208–1210). Ady3p is thought to play a role in segregation of mitochondria to inherit a part of mitochondria from mother cells (asci) (1211). Coordinated with the cytokinesis machinery, the proteolysis of SspI results in membrane closure, and the leading edge complex disassembles prior to membrane closure. Consistent with the absence of MOP, the potential orthologs of the two sporulation-specific septins and components of the leading edge complex and membrane curvature were not found in *T. atroviride*, *T. reesei*, and *T. virens*. As described above, ultrastructural images of ascospore delimitation in other filamentous ascomycetes suggest that the wall membrane of ascospores is derived from the invagination of a preformed membrane around ascus cytoplasm (1201–1203). Combined with the results of ortholog identification, these data imply that *Trichoderma* species may not form MOP and a leading edge complex to form the ascospore membrane and to delimit ascospores. Instead, *Trichoderma* species may apply a strategy similar to that of other filamentous ascomycetes.

The late phase of sporic meiosis. After the membrane closure, sporulation transits to the late phase. Spore wall assembly is the most discernible event in this phase. Meanwhile, spores are also preparing for returning to vegetative conditions. In the cytoplasm, the MOP complex disappears, and septin bars disassemble and redistribute in cytoplasm. In the nucleus, chromatin compaction was observed.

The prospore membrane is a double membrane unit. After the closure of the prospore membrane, the cell wall starts to synthesize and accumulate in the luminal space between the two layers of the prospore membrane. The spore wall of *S. cerevisiae* consists of four layers. The four layers of the spore wall are deposited outward from the inner membrane in the order of mannan, β-1,3-glucan, chitosan, and dityrosine (1206). The first two layers are also present in the vegetative cell wall in a reversed order, while chitosan and dityrosine are unique components of spore walls. The synthesis and regulation of the spore wall, however, are still largely not understood. In addition to localization of septin bars, Gip1p is thought to be involved in a signaling pathway that initiates the formation of the first mannan layer (1206). *FKS1*, *FKS2*, and *FKS3*, encoding predicted catalytic subunits of a beta-glucan synthase, are responsible for the synthesis of the β-glucan layer. *FKS2* is highly expressed upon sporulation. Secretion of Fks2p to prospore membrane requires Sps1p, while its activity is negatively regulated by Smk1p (1212, 1213). In addition, three sporulation-specific proteins, Spo73p, Spo77p, and Ssp2p, also indirectly mediate β-glucan layer assembly with unknown functions (1214–1216). The chitosan synthesis in spore wall requires chitin synthase Chs3p and two deacetylases Cda1p and Cda2p. Osw1p and an acyltransferase Mum3p were inferred to have a role in assembly of the chitosan layer (1214). The dityrosine layer contains diamino acid *N*,*N*-bisformyl-dityrosine that is synthesized through the activity of Dit1p and Dit2p (1217). The synthesized product is then exported by Dtr1p to the surface of chitosan for polymerization (1218).

The cell wall of *Trichoderma viride* mycelia was found to contain β-(1-3) glucan, and β-(1-6) glucan. Instead of chitin, *T. viride* conidia contain melanin with these glucan polymers (1219, 1220). However, the cell wall composition of *Trichoderma* ascospores is still unknown. *T. reesei*, *T. atroviride* and *T. virens* have orthologs of *SPS1* (TR_57016, TA_32174, TV_159566, and TV_207728), *FKS1* (TR_78176, TA_140247, and TV_90713), *CHS3* (TR_58188, TA_248556, and TV_87952), and *CDA1* (TR_69490, TA_292288, and TV_49798), which supports the fact that *T. reesei*, *T. atroviride* and *T. virens* have the abilities of glucan and chitosan synthesis. Notably, the orthologs of *DIT1* (TR_69881, TA_35063, and TV_28177) and *DIT2* (TR_56966, TA_129592, and TV_28177) are present in *T. atroviride*, *T. reesei*, and *T. virens*, indicating *Trichoderma* may also synthesize *N*,*N*-bisformyl-dityrosine.

Transcriptional evidence of putative meiotic genes. Currently, transcriptomic data generated from *Trichoderma* sexual developmental stages are not available. The expression of several putative meiotic genes has been inferred in various nonmeiotic processes by genome-wide microarray analysis, high-throughput RNA-seq analyses (15), or EST analysis (25), including mycoparasitism (22, 355), injury responses, biocontrol, conidium development (352), light responses (e.g., *T. reesei* wild-type strain and the deletion mutants of G-protein subunit genes under 24-h light or 24-h dark conditions) (350). Also notable, induction of gene expression under certain conditions may not help interpret their roles. A genome-wide transcriptional comparison between *T. reesei* sexual development and vegetative growth will clarify if these putative meiotic genes are involved in meiotic sporulation.

Conclusions. By adopting the *S. cerevisiae* meiotic sporulation mechanism as a model, we found that the presence and the absence of yeast meiotic sporulation genes in *T. atroviride*, *T. reesei*, and *T. virens* are largely similar patterns. Since meiotic sporulation of *T. reesei* has been confirmed experimentally, this suggests that the sequenced *T. atroviride* and *T. virens* strains are potentially competent to undergo sexual development and meiotic sporulation. In general, a set of *S. cerevisiae* and *S. pombe* core meiotic genes (1221) is present in *Trichoderma*. Several genes involved in key meiotic steps are partially conserved in *Trichoderma*. This reiterates the notion that meiotic genes are rapidly evolving during speciation (1133, 1136). It also implies that new genes or remote homologs carry out the functions of these absent proteins during meiosis, e.g., *Sme4* in *S. macrospora*. Some conserved genes may gain additional functions (e.g., *Mer3* and *Msh4* in *S. macrospora*). while some play divergent roles from orthologs in budding yeast (e.g., *Ndt80* in *N. crassa*). These preliminary results implicate different molecular interactions that carry out each key meiotic process in filamentous fungi, including *Trichoderma*. Moreover, the prospore membrane of yeasts and the ascospore membrane of filamentous fungi are generated through dissimilar operation mechanisms. Membrane fractions may also derive from unrelated origins. The genes involved in the formation of the prospore membrane, therefore, are not present in *T. atroviride*, *T. reesei*, and *T. virens*. Genes for depositing the spore cell wall are partially conserved in *T. atroviride*, *T. reesei*, and *T. virens*, though one may argue that their functions focus on vegetative cells rather than on formation of ascospores. Further biochemical assays on cell wall components of *Trichoderma* ascospores and characterizations of functional genes will uncover their roles in cell wall formation. Finally, both the biological significance and molecular mechanisms underlying postmeiotic mitosis in all filamentous fungi (including *Trichoderma*) are essentially unknown, and further study will help uncover a new understanding of fungal sexual reproduction and evolution.

GENES RELATED TO COMPETITION AND DEFENSE (BIOCONTROL)

Most plants are colonized by fungi that cause no disease symptoms. These fungi, called endophytes, improve plant growth and promote disease resistance as well as abiotic stress tolerance, while the fungi themselves obtain nutrients from this association (1222). Several strains of *Trichoderma* establish an endophytic relationship with roots of economically important crops, such as maize, tomato, cacao, and cucumber, resulting in crop improvement (513, 1223–1228). Despite directly benefiting from this symbiosis, plants still react to colonization from endophytes (1222) by activating their innate immune system (1224, 1225).

The innate immune system has evolved to recognize common features of microorganisms, which are known as microbe-associated molecular patterns (MAMPs). MAMPs include molecules such as chitin from fungi and the flagellin protein from bacteria (1229). Biochemically diverse MAMPs have been identified in *Trichoderma* (29, 338, 1224), including the cerato-platanin protein SM1/EPL1 (TV_110852, TR_82662, and TA_302952) (29, 1230), ethylene-inducing xylanase (EIX; TR_123818, TV_72838, and TA_82355) (1231), and Swollenin protein (ACB05430) from *T. asperellum* (TR_123992, TV_49838, and TA_80187). SM1 is induced during plant interactions but is also expressed without the presence of a plant and promotes the expression of pathogenesis-related genes and hypersensitive reactions (29). EIX has a dual role during plant colonization: the first role involves lytic enzyme activity, while the second involves the induction of systemic resistance as seen in specific cultivars of tobacco and tomato (1231–1234). Moreover, hydrophobins were shown to be involved in *Trichoderma*-plant interactions (1235, 1236). In theory, endophytes should modulate the immune response of the plant as they must overcome the plant MAMP immune responses in order to establish successfully in the intercellular space (apoplast) or into restricted cellular areas, as in the case of mycorrhizae. This modulation is achieved by reprogramming the cells surrounding the fungal colonization area to accommodate the fungus and to maintain host cell integrity for long-term symbiotic interactions (29, 513, 1224–1226, 1237).

Effectors, in contrast to MAMPs, are strictly inducible molecules in both pathogenic, and presumably endophytic, interactions with plants (1238). To date, they have mostly been studied in plant and animal pathogens (1239, 1240), with comparable research lacking in endophytes and other symbionts. They are secreted proteins that are quite diverse, and alter plant processes and facilitate colonization with the mechanisms by which they modulate the innate plant immune response. These mechanisms include the inhibition of chitinases and proteases, ROS scavenging, or direct modulation of the host transcriptional machinery (1241). The presence of effector-like proteins in the mutualistic root symbionts *Piriformospora indica* (1242) and *Laccaria bicolor* (1243) and in the arbuscular mycorrhizal symbiosis (1244) suggests that symbiotic microorganisms do regulate plant innate defenses in a way similar to pathogens (1237, 1245). However, the roles of these molecules in endophytic fungi, such as *Trichoderma*, which are capable of priming the plant's immune system while not being pathogenic to the plant, remain to be fully elucidated.

How effectors function by interfering with plant immunity was explained in Jones's zig-zag model (1229). This model describes the quantitative output of the plant immune system and proposes four phases of molecular recognition. In the first phase, MAMPs are recognized by plant cells displaying MAMP-triggered immunity (PTI), which is a light response that prevents colonization. In the second phase, microorganisms with plant colonization capacity release molecules that interfere with the PTI, resulting in effector-triggered susceptibility (ETS). In the third phase, if the plant recognizes the effectors, it is able to activate effector-triggered immunity (ETI). This response is faster, longer, and more intense than the PTI and often gains sufficient strength to pass the molecular threshold for the induction of hypersensitive cell death. The fourth phase occurs when pathogens no longer express the recognizable effector or when they have acquired a new one that allows them to counteract the ETI, thereby making them successful colonizers (1229). PTI involves a local response against the invader as a callose deposition, limiting the extent of fungal growth (1225). This local response may also include oxidative bursts, the production of toxic compounds, and hypersensitive reactions, which all act to help banish the invader from the plant (1225). PTI also includes induced systemic resistance (ISR), which is effective against a broad spectrum of pathogens and is dependent on the plant hormones jasmonic acid and ethylene (1237, 1245, 1246).

Common Features of Known Effector Proteins

Effectors often have at least one of several common properties. They can be small, cysteine-rich proteins, and/or contain a pathogenesis motif, a nuclear localization signal, or any domain that is atypical of secreted proteins, such as transcription factors, ubiquitin ligases, or internal repeats (1247). Effectors that are secreted into the plant apoplast tend to be small cysteine-rich proteins that contain intramolecular disulfide bridges, most likely to maintain stability in the harsh apoplastic environment. They are highly variable, but some functional classes have been identified. These include serine and cysteine protease inhibitors that target host proteases (1248, 1249), effectors that minimize the levels of ROS (1250, 1251), proteins that protect fungal cell walls against hydrolysis by plant chitinases (1252), and phytotoxic proteins. Apoplastic effectors may be involved in masking fungal detection by the plant by operating as protectors of fungal proteins (1253, 1254). LysM domain proteins lacking chitinase activity are thought to operate this way by scavenging chitin molecules, thus limiting the scope of the plant response (1253, 1254).

In contrast to bacteria and nematodes (1255–1257), little is known about how the effectors of filamentous fungal pathogens are released into the plant host cell. It has been suggested that fungal effectors are secreted at the hyphal tip using the conventional type II secretion signal (1241, 1258) (Fig. 19), but recent findings in *M. oryzae* have identified two distinct secretion systems with a specific localization site for effectors secreted into the host cell cytoplasm. The effectors are accumulated in the biotrophic interfacial complex (BIC), and apoplastic effectors that follow the conventional secretory pathway are localized in the extrainvasive hyphal membrane (EIHM) (1259). Hence, this suggests that fungi have evolved distinct secretory mechanisms to deliver effector molecules to manipulate host cell processes.

Effectors are thought to contain specific domains that facilitate translocation into the host cell. Typical signal peptides that facilitate translocation have been located in the majority of filamentous pathogen effectors identified to date (1239, 1258, 1260). Cysteine-rich domains may also play a role in translocation signaling, as translocated apoplastic effectors and host-translocated effec-

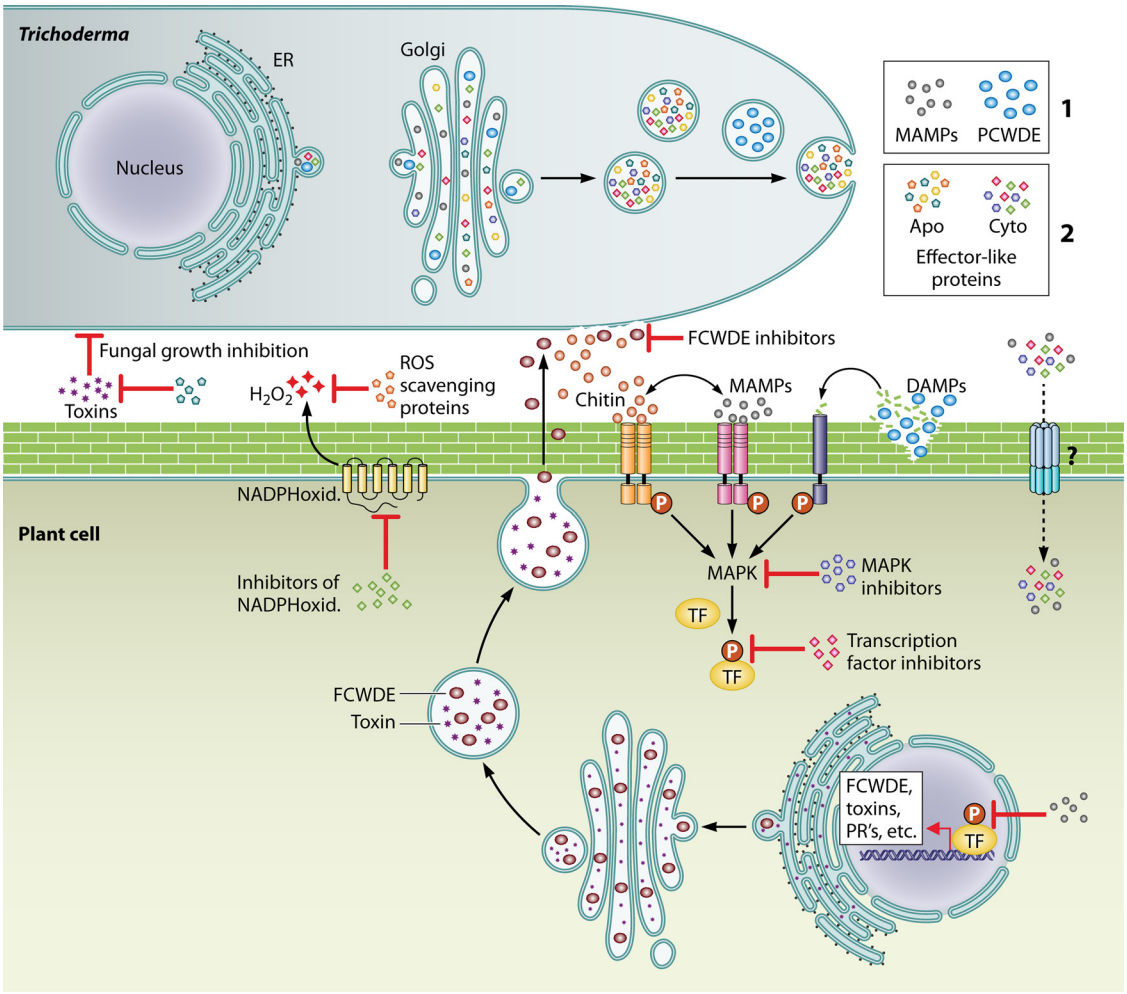

FIG 19 Secretion of effector-like proteins during plant-*Trichoderma* interactions. *Trichoderma* spp. constitutively secrete proteins which are detected by plant cells (e.g., Sm1). These proteins are called microbe-associated molecular patterns (MAMPs). Together with the release of plant cell material (called damage-associated molecular patterns [DAMPs]) by the action of plant cell wall-degrading enzymes (PCWDE) secreted by *Trichoderma*, they activate the plant immune system. Plant cells release antifungal compounds, including toxins and fungal cell wall-degrading enzymes (FCWDE). The chitin released by the action of FCWDE is recognized by a specific receptor from the plant cell and activates immune responses, including the activation of MAPK and phosphorylation (red circles) of TFs, which regulate different defense responses (including the expression of pathogenesis-related proteins [PRs], toxins, and FCWDE). *Trichoderma* spp. also secrete proteins that are inducible during plant interaction. These proteins, here called effector-like proteins, are secreted through the type 2 secretion system. The roles of secreted proteins by *Trichoderma* during their interaction with plant roots may be to assist the fungus to evade, manipulate, and ultimately avoid the plant immune system. These proteins can act in the apoplast (Apo) or in the cytoplasm (Cyto). An effector might have different targets, including the scavenging of ROS, inhibition of FCWDEs and toxins in the apoplastic space, or MAPKs, TFs, or NADPH oxidases in the cytoplasm. Cytoplasmatic effector-like proteins may be translocated inside the plant cell by an unknown transporter or mechanism.

tors are both small cysteine-rich proteins (1247). Some fungal effectors are thought to be translocated into the host cytoplasm, with the presence of potential translocation domains (RxRL) (1261) being reported in rust fungi, although their function has not yet been confirmed (1247). In other fungi, including *Trichoderma*, the domains responsible for effector translocation have not yet been identified.

Extracellular Proteins of *Trichoderma* spp.

To identify potential effector molecules secreted by *Trichoderma* spp., potential extracellular proteins from the three *Trichoderma* spp. were determined. From this analysis (see Text S3 in the supplemental material), 10.26% of the total proteome of the three *Trichoderma* spp. (3,422 from over 33,341) represent proteins with a potential secretion signal. Of these potential secreted proteins, 2,565 sequences (74.9%) do not have any predicted transmembrane helix domain (870 sequences belong to *T. atroviride*, 979 to *T. virens*, and 716 to *T. reesei*). From these proteins, 51 sequences with a potential ER retention signal (ERrs) and 216 sequences with GPI attachment signals were identified. The final data set of proteins contains 2,525 sequences from the genomes of *T. atroviride*, *T. virens*, and *T. reesei* (Fig. 20). These results differ slightly from those reported previously (26), in which 2,662 instead of 2,525 putative extracellular proteins were reported. This difference could be due to the different algorithms used in the two studies. However, the two analyses were consistent in terms of the proportions of proteins with a signal peptide in each of the *Trichoderma* species analyzed (7.3% to 7.8% of proteins contain-

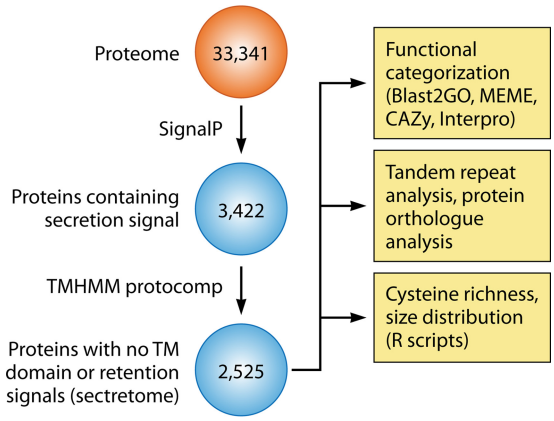

FIG 20 Diagrammatic outline of the process used to detect and analyze putative small secreted proteins. The initial proteome for *T. atroviride*, *T. reesei*, and *T. virens* was obtained from the JGI Genome database. This data set was examined with SignalP4 to detect signal peptide regions. Protein models containing secretion signals were then analyzed to detect transmembrane domains and cellular retention signals, using TMHMM and Protcomp. The predicted secretome was then analyzed for functional groups, domains, motifs, and homology to known lytic enzymes (Blast2GO, MEME, CAZy, Interpro). Tandem repeat analysis was performed with XSTREAM, and orthologs of secreted proteins in the main data set were identified. Cysteine richness and the size distribution of the secretome were analyzed using custom-designed R scripts.

ing a signal peptide in the present analysis, in contrast to 7.6% to 8.2% in the analysis reported previously [26]).

Orthologs of the 2,525 proteins with signal peptides in *T. atroviride*, *T. virens*, and *T. reesei* were identified. Known lytic enzymes and CAZomes were excluded from the summary, as they form a distinct subclass of small secreted proteins with their primary function being cell wall modification or nutrition. Candidate proteins were included on the basis of either unknown function with effector-like attributes (e.g., cysteine rich) or by homology to proteins with known functions that appear likely to be capable of altering or regulating the fungus-host interaction. These are comprised of a large number of functionalities, including CFEM proteins, transcriptional regulators, killer toxins, ligases, and tandem repeat proteins (see Table S1 in the supplemental material).

An unexpectedly high number (661 proteins) of orthologous proteins lacking the signal peptide in one or two *Trichoderma* species were identified in this analysis. In total, 258 orthologs had no signal peptide in *T. atroviride*, 221 in *T. virens*, and 182 in *T. reesei*. These differences could be due to misannotation of the genomes during automatic gene modeling or to alternative splicing from some of these genes. For example, TV_213200 lacks a secretion signal but contains a thaumatin domain (IPR001938) that is highly similar to the putatively secreted thaumatin-like TV_70385, yet it has no known apparent secretion signal. Another important aspect to consider is that this analysis focused on the presence/absence of signal peptide (the classical T2 extracellular secretion system), and the nonclassical secretory proteins could have been missed.

The distribution of secreted proteins among species appears to be relatively similar. However, *T. atroviride* has a larger extracellular data set, which could be related to its mycoparasitic lifestyle. On the other hand, *T. reesei* has a slightly smaller extracellular protein set, which may be the result of a smaller genome or its predominant saprophytic activity. Analysis of the size distributions of these proteins revealed that the majority of secreted proteins have a size of between 100 and 500 amino acids. Slightly over half of all secreted proteins were larger than the 300-amino-acid limit that we assigned for small secreted proteins. The largest putatively secreted protein was 3,241 amino acids. This may indicate that the suggested size limits for putative effector proteins should serve as a guideline only.

SMALL SECRETED PROTEINS

T. reesei, *T. atroviride*, and *T. virens* contain 825 extracellular proteins containing fewer than 300 amino acids. Of the 825 proteins, 523 had no homology to proteins with known functions in the nonredundant (nr) sequence database of NCBI (BLASTp search). Of these 523 small extracellular proteins, 208 sequences were specific to the genus *Trichoderma*. From these 825 proteins, 173 contain over 5% cysteine residues (Fig. 20; see also Text S3 in the supplemental material).

T. atroviride in particular had a considerable number of proteins with low homology to known proteins in the nr database of NCBI. Thirty-one of the 64 small cysteine-rich proteins (SCRPs) analyzed had only 1 hit with BLASTp. Interestingly, 13 of these were from *T. atroviride*, 11 from *T. virens*, and 7 from *T. reesei*, indicating that *T. atroviride* potentially has a greater range of novel cysteine rich-effectors than the other two species (see Table S1 in the supplemental material).

To detect putative effector proteins, the initial data set from the JGI was screened using SignalP4 to identify proteins containing a known secretion signal. This list was then sorted by size, and proteins under 300 amino acids were separated. For the purpose of this study, 300 amino acids was the cutoff for "small secreted proteins." The small protein data set was then analyzed using Blast2Go. Proteins that were unlikely to have effector activity were removed from the data set. This included ribosomal proteins, lytic enzymes (e.g., glucanases, proteases), and enzymes involved in known biochemical pathways (e.g., synthases, DNA repair proteins, and cytochromes). BLAST results were screened to find the best hits among organisms other than *Trichoderma*. Interpro scans identified 12 Killer-toxin like proteins in the genomes of *T. atroviride* (5 genes), *T. virens* (4 genes), and *T. reesei* (3 genes) (see Table S1 in the supplemental material). This type of protein is commonly found in pathogens, and its presence in an endophytic strain is potentially intriguing. Additionally, 28 protein-encoding genes containing a CFEM domain but no transmembrane domain were identified in the data set (see Table S1 in the supplemental material). These proteins have been previously found in a number of fungi, including several pathogens, where they are potentially associated with pathogenesis. Fungal proteins exhibiting the 8-cysteine-containing CFEM domain may function as cell surface receptors or signal transducers, or as adhesion molecules in host-pathogen interactions (1262). Other proteins of interest that are not directly involved in pathogenesis include transcription factors, such as zinc-6 finger proteins, necrosis-inducing peptides, thaumatin, pathogenesis-related proteins, ubiquitin ligases, etc. (see Table S1). The proteins in Table S1 were compared to known transcriptome data only available for *T. atroviride* and *T. reesei*, in an attempt to predict whether they had been expressed under any conditions. The existing transcriptome data were based on different growth conditions, including the interaction with *R. solani*, a plant pathogen that is a host for mycoparasitic *Trichoderma*. The majority of the proteins in Table S1 were expressed in *T. atroviride*

and *T. reesei*. Many of the genes are differentially regulated in mutants affected for G-protein signaling components in *T. reesei*, including the G-protein beta-subunit GNB1, the G-protein gamma-subunit GNG1, and the phosducin-like protein PhLP1, considered a cochaperone for the G-protein beta-gamma folding involved in regulation of the expression of glycosidases in this fungus (350).

Hydrophobins and Cerato-Platanin Proteins

Hydrophobins are small cysteine-rich surface-active amphipathic proteins. Their main functions are as structural components conferring hydrophobicity to fungal surfaces during fungal growth or during interaction with hydrophobic surfaces, such as plant cells (1263, 1264). Based on the solubility of the aggregates they form, hydropathy patterns, and solubility characteristics, hydrophobins are classified in two groups, class I and II. Recently, a novel subclass of hydrophobins was proposed for *Trichoderma* (27). The 10 *T. atroviride* hydrophobin genes are expressed in response to various conditions, including exposure to light pulses, starvation, and injury, but do not show mycoparasitism-specific regulation patterns (1088). Moreover, *T. reesei hfb1* (TR_73173, TA_258206, and TV_60531) and *hfb2* (TR_119989, TA_258295, and TV_91466) are regulated in response to light and by the G-protein alpha-subunit GNA1 (617). Regulation of *hfb1* and *hfb2* is dependent on the carbon source (M. Schmoll and G. Gremel, unpublished data). *Trichoderma* spp. produce a much wider range of class II hydrophobins than do other ascomycetes, and some of these function during mycoparasitism to attach to the hyphae of other fungi (see Table S1 in the supplemental material) (1265). For example, the hydrophobin-like TasHyd1 protein, isolated from *T. asperellum*, plays an important role in root attachment and colonization (1236). One of the biological functions of hydrophobins is the recognition and adhesion to host surfaces, where they positively influence root colonization ability (337, 1236) and act as elicitors of the plant defense response (1235, 1266). Moreover, hydrophobins have an important role in plant-fungus recognition in *M. grisea* (1267) and *T. asperellum* (1236). A new role of hydrophobins has been hypothesized in that they might function as protection from detection by the plant (1236, 1268), masking the fungal surface during its growth inside the plant tissues.

The cerato-platinin (CP) family of proteins are small, secreted, cysteine-rich proteins that are widely represented in several fungal models for species with different lifestyles (1269, 1270). Despite the fact that CPs are highly represented in fungal genomes, their primary function is not entirely clear, but some species have been demonstrated to be involved as virulence factors or elicitors during plant-pathogenic and symbiotic interactions, respectively. For example, during the mutualistic *T. virens*-plant interaction, fungal CP elicitors Sm1 (TR_82662, TA_302952, and TV_110852) and Sm2 (TR_123955, TA_88590, and TV_111830) are secreted during the first stages of root sensing and induce systemic disease resistance, conferring resistance to phytopathogens (28, 29, 1271, 1272). In contrast, CP virulence factor BcSpl1 from *Botritys cinerea* has a phytotoxic function in inducing necrosis on tobacco leaves (1273). Furthermore, CPs have also been recently classified as MAMPs/PAMPs for their role during host plant colonization (1270).

Tandem Repeat Proteins

Proteins with tandem repeats, such as transcription activator-like (TAL) effectors, comprising ~34 amino acid tandem repeats, may be important effector proteins in fungi. TAL effectors of plant-pathogenic bacteria represent a new class of DNA binding proteins (1274) that regulate gene expression in the host plant, and they have been shown to bind to DNA in the host cell during *Xanthomonas*-plant interactions (1274). In our analysis, 55 separate tandem repeat motifs longer than 15 residues were identified among 98 total tandem repeat proteins (see Table S1 in the supplemental material). *T. virens* has the largest number (38) of tandem repeat proteins, and *T. atroviride* and *T. reesei* have 30 each. Tandem repeat proteins were found to be 65 to 1,500 amino acids in length, again with the majority of proteins containing 100 to 500 amino acids. They comprise 15.4% of the proteins identified as secreted. The percentage of tandem repeats across proteomes of *T. atroviride*, *T. virens*, and *T. reesei* is slightly lower at 12.4%. The number of tandem repeat proteins was remarkably similar between species, regardless of the smaller secretome size of *T. reesei*. From 16 *Trichoderma*-specific tandem repeats identified in this analysis, 2 were exclusively present in *T. virens* and 1 in *T. reesei*, and they might be involved in specific processes in the life cycles of these fungi (Table S1 in the supplemental material). Among these groups of tandem repeat proteins, ankyrin repeat domains were detected in TA_224344 and TV_132539. No orthologs with tandem repeats and ankyrin domains along with secretion signals were found in *T. reesei*, albeit TR_67795 (TV_112704) does not contain a secretion signal but the gene does encode a protein with tandem repeats and ankyrin domains.

Repeat sequence proteins have the potential to act as effectors, allowing delivery of multiple copies of an active domain to be transmitted into the host in a single protein. In *U. maydis*, short repeat sites were identified which may have the potential to serve as cleavage sites, allowing for subsequent modification of the protein into an active form or shorter peptides (1275). This ability bears the potential to mask effectors until they are delivered into the target regions of the host, or simply to generate a higher copy number of an effector molecule, and they may also allow for targeting and regulation of effector molecules. Regardless of their purpose, the fact that 15.4% of the secretome in *T. reesei*, *T. atroviride*, and *T. virens* (98 total sequences with tandem repeats longer than 15 residues; see Table S1 in the supplemental material) is comprised of tandem repeat proteins suggests that these are worthy of further investigation. Several fungal cell wall proteins, including those responsible for evasion of chitinases during plant colonization (LysM) contain tandem repeats. In addition, some tandem repeat proteins, such as Msb2 in *U. maydis* (1276) and *F. oxysporum* (1277), are involved in the perception of the host cell and in the transmission of plant signals required for successful plant colonization.

Proteins with Known Effector Motifs

Approximately 100 proteins with domains reported to be involved in pathogenicity (1247) were detected in *T. reesei*, *T. atroviride*, and *T. virens*. These included 28 CFEM repeat proteins (IPR011018), an 8-cysteine-containing domain protein present exclusively in fungi, proteins from the CAP protein family (cysteine-rich secretory proteins, antigen 5, and pathogenesis-related protein 1) as well as diverse protease inhibitors and LysM domain proteins (see Table S1 in the supplemental material).

Manual and bioinformatics searches with perl and R scripts (see Text S2 in the supplemental material) for known effector motifs revealed the presence of several RxLR-like motifs, such as RxxL, RxxL-dEER, WxxxE, KRKR, and Lx2Rx4L motifs, in the genomes of *T. atroviride*, *T. virens*, and *T. reesei*. These motifs are commonly found in oomycetes and bacterial effector systems, comprising part of the translocation signal in the former and the type III secretion system in the latter (1278). However, it was observed that many of the proteins with this motif are lytic enzymes or proteins involved in transport of sugars. A KDxK motif was also found in 30 proteins, often as a repeat sequence. According to the Eukaryote Linear Motif (ELM) resource (http://elm.eu.org/elms/browse_elms.html), KR-rich motifs are potential phosphorylation or localization motifs and thus may be relevant to effector movement or targeting in the host cell. Previous reports have indicated that these act as nuclear localization signals in bacterial effectors (1279). Eight proteins were found that had no homology to any known sequences, with a further 142 proteins having only one hit match using BLASTp.

Proteinase inhibitors. In oomycetes, three types of apoplastic effectors have been described: inhibitors of host enzymes, proteins involved in cell wall/plasma membrane adhesion (1280), and toxins that lead to host death (1281). Additionally, in filamentous fungi there are proteins involved in the protection of hyphae from host attack (1253, 1254). Surprisingly, although proteinase inhibitors are common in oomycetes (between 18 and 43 different proteins) (1281), *T. atroviride*, *T. virens*, and *T. reesei* contain only a few examples of these proteins. *T. atroviride* and *T. virens* have three putative proteinase inhibitors, whereas *T. reesei* contains only two (see Table S1 in the supplemental material).

The proteinase inhibitors mentioned above contain domains that are homologous to known proteins. These domains were identified by using InterProScan, which provides a functional analysis of proteins by classifying them into families (1282). Three clear groups of protease inhibitors were identifiable in the data set. Members of the first group (TR_111915, TV_92793, and TA_50405) all contain Kazal domains (Interpro [IPR]domain IPR011497). The second group (TV_10277, TA_323283, and TR_72379) contains a proteinase inhibitor I7 (IPR000737), proteinase/amylase inhibitor (IPR011052), or adhesin (IPR018871) domains, while those in the third group (TV_177054 and TA_300122) contain a proteinase inhibitor (IPR009020) domain. From the second group, the gene TR_72379 is highly expressed under various axenic conditions and is highly expressed (up to 10-fold) in the G-protein gamma-subunit GNG1 deletion mutant grown in constant darkness, suggesting an important role under those conditions. This makes it seem more likely that the corresponding gene has a role in differentiation, rather than as an effector; however, experimental evidence is needed to support this hypothesis.

The Kazal domain (IPR011497) is a commonly found serine proteinase inhibitor domain that often occurs as a tandem repeat. These proteins specifically inhibit S1 serine proteases by either lock-and-key or conformational change mechanisms. The proteinase inhibitor I7 (IPR000737) domain has previously been detected in squash and acts as a serine protease inhibitor. Other examples of proteins containing this domain have been identified as trypsin and elastase inhibitors from plants (1283). It is notable that these inhibitors have not previously been detected in fungi (http://www.ebi.ac.uk/interpro/entry/IPR000737). The proteinase/amylase inhibitor (IPR0011052) is the active inhibitor domain and is also similar to carboxypeptidase inhibitors. The adhesin (IPR018871) domain is found in fungal adhesins, which aid in attachment to the cell surface. This suggests that the inhibitor remains bound to the cell wall, which was further supported by the ProtComp localization and GPI anchor analysis results. The proteinase inhibitor (IPR009020) domain of group three is a protease propeptide inhibitor domain. These inhibitors are found in many organisms, where they inhibit a wide range of enzymes. They regulate protein folding and activity of peptidases in such a way as to block the substrate from the active site.

The limited number of proteinase inhibitors detectable in *T. atroviride*, *T. virens*, and *T. reesei* suggests that either protease inhibitors have a broad-spectrum effect or inhibition of plant proteases is not a major mechanism behind the ability of *Trichoderma* to survive as a plant endophyte.

Necrosis and ethylene-inducing peptides. Necrosis and ethylene inducing peptides (NEP) and NEP-like proteins (NPL) are toxins identified in many different microorganisms, including fungi, oomycetes, and bacteria (1284). NEPs elevate internal K^+, H^+, and Ca^{2+} levels and activate MAPKs. They promote the accumulation of reactive oxygen species, pathogenesis-related proteins, and the production of ethylene, as well as callose deposition and localized cell death (1284). Interestingly, these proteins seem to elicit necrosis and ethylene production only in dicotyledonous plants and are inactive in monocots (1285–1288). NPL-encoding genes, however, are also present in pathogens of monocots that do not elicit necrosis, suggesting additional roles (1289). Despite their diverse distribution across taxa, most of the NPLs share a fold characterized by a heptapeptide (GHRHDWE) motif (1285–1288), although it was shown that only 36% (12 of 33 proteins) of NLPs from *Phytophthora* possessed a full complement of the amino acids from this domain (1290). *T. reesei*, *T. atroviride*, and *T. virens* have four genes for a putative NEP-like protein domain (IPR008701) (see Fig. S31 in the supplemental material). Although they have variations in the heptapeptide sequence motif (GH[R/K][H/S/Y/N]DWE), the proteins still contain the residues required for the full activity of NEPs reported in other fungi (1288).

T. virens contains three NEP-like proteins that show significant similarity to NPL proteins: TV_46830 has a significant E value of 1.9E−61, while TV_52963 and TV_50664 have lower values (2.30E−05 and 3.20E−07, respectively) (see Table S1 in the supplemental material). In addition, an Interpro analysis with all of them showed the complete domain (see Fig. S31 in the supplemental material). Although these latter two proteins share identity to NEP-like proteins, they lack a signal peptide. This could be due to a misannotation (TV_52963) in the genome of *T. virens* or due to differences in the secretion mechanisms (TV_50664) in different *Trichoderma* species, as has previously been suggested for other effectors from filamentous pathogens (1241, 1258). For example, our phylogenetic analysis showed that TA_302472 is closely related to TV_50664 (see Table S1 and Fig. S31 in the supplemental material). While TA_302472 contains a signal peptide, its ortholog in *T. virens* lacks it. The NEP TV_46830 showed homology to XP_001804225.1 from *Phaeosphaeria nodorum*, which is a major pathogen of wheat. Interestingly, although the NLPs are proteins that usually comprise an N-terminal secretion signal peptide followed by the semiconserved heptapeptide motif called here the NEP-like motif (1284, 1290), the proteins TV_46830 and XP_001804225.1 have an additional domain be-

tween the signal peptide and the NEP-like motif that is unrelated to other proteins or domains (see Fig. S31). Remarkably, the NEP-like proteins share the heptapeptide motif, with acyl thioesterases. Indeed, there are additional proteins in *T. reesei*, *T. atroviride*, and *T. virens* with heptapeptide motifs, but they are shorter than those found in the characterized NEPs (see Fig. S31). Several NLP toxins from *Phytophthora sojae* lack necrosis-inducing activity, which suggests there may be additional roles for these proteins in fungi (1290). From these genes, TR_50664 is highly expressed in *T. reesei* when the G-protein beta-subunit is deleted. The levels of expression of this protein might suggest a putative role in normal growth instead and induction by plant host cells. Another possibility is that the genes regulated by these signaling cascades modulate plant responses, as has been shown in other models, such as *Verticillium dahliae*, *Fusarium verticillioides*, and *G. zeae* (1291–1293).

PR proteins: TLPs. Thaumatin-like proteins (TLPs; IPR001938) are pathogenesis-related (PR) proteins of family 5 (1294). They form a group of proteins with diverse properties, including being antifungal, and some have a sweet taste (1294). TLPs are induced in plants in response to various biotic and abiotic stresses and have been thought to play a role in plant defense systems, particularly in systemically acquired resistance (1295). TLPs are known to accumulate in plants in response to infection by a pathogen, and some are known to have antifungal properties (1294, 1296–1299). Some TLPs hydrolyze crude fungal walls, and one barley TL enzyme even lyses fungal spores (1295). TLPs are present in *T. atroviride*, *T. virens*, and *T. reesei*. Interestingly, a secretion signal in these proteins was found only in *T. atroviride* (TA_ 306146, TA_224184, and TA_32968) and in *T. virens* (TV_65931 and TV_70385) but not in the TLP ortholog of *T. reesei* (TR_109835) (see Table S1 in the supplemental material). However, additional proteins with a thaumatin domain (IPR001938), which lack a signal peptide, are present in *T. atroviride* (TA_233988) and *T. virens* (TV_213200) (see Table S1). This could be due to the presence of different mechanisms of secretion, as was mentioned above, or maybe the N termini for some of the proteins analyzed here are missing or in the wrong frame during the annotation.

Xylanase inhibitor proteins (XIP) are considered potential defense molecules, which prevent cell wall degradation by hydrolytic fungal enzymes (1297). The presence of thaumatin-like xylanase inhibitors (TLX) and chitinase-like xylanase inhibitor proteins was recently reported in plants (1296). Possibly, TLPs from *Trichoderma* act as potential effector proteins by inhibiting plant chitinases activated during root penetration and colonization.

The cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins (CAPs) are widely distributed in all kingdoms, including plants and fungi (1300–1302). *T. reesei*, *T. atroviride*, and *T. virens* contain three members of the CAP superfamily (see Table S1 in the supplemental material). Members of the CAP superfamily are involved in multiple processes, including the regulation of extracellular matrix and branching morphogenesis, potentially as either proteases or protease inhibitors. Proteins of the PR-1 family are synthesized during plant-pathogen interactions and belong to the CAP superfamily (1302). In *T. reesei*, some of the encoded proteins (TR_81331, TR_102441, TR_33029, and TR_102441) are expressed when the fungus is grown on cellulose (350). On the other hand, the remaining CAP proteins are either not expressed (TA_272020, TV_213853, TV_220308, and TV_193212) during mycoparasitism (22) and sporulation (352) or are expressed at very low levels (TA_299424 and TA_218718). In general terms, the CAP proteins from *Trichoderma* share identity with some toxins that could be involved in the inhibition of the plant immune response.

***Trichoderma* LysM-like putative effectors.** Chitin is an *N*-acetyl-D-glucosamine (GlcNAc) homopolymer found as a primary structural component in the cell wall of fungi (1303). As plants do not contain chitin, this molecule is recognized as a non-self component, and its presence activates host immunity (1253, 1254, 1304). In fungi, diverse effector proteins have been described that prevent triggering host plant immunity when cell wall chitin oligosaccharides of fungi are released into the apoplast. These effectors contain a diverse number of LysM domains (IPR002482) that bind chitin. A lectin-like LysM protein from *Cladosporium fulvum* was found to inhibit chitin oligosaccharide-triggered and PRR-mediated activation of host immunity in tomato (1254). It was recently shown that *Mycosphaerella graminicola* (1304) and *M. oryzae* (1305) use the same strategy to avoid eliciting plant immune reactions. *T. atroviride* and *T. virens* each contain 10 potential LysM effectors, but *T. reesei* only has 3 (1253). In this analysis, 21 proteins with a LysM domain and without any chitinase activity were identified, and they were distributed in the following manner: 8 in *T. atroviride* (TA_179670, TA_297859, TA_299633, TA_300200, TA_31285, TA_43321, and TA_85797), 9 in *T. virens* (TV_124493, TV_128337, TV_128781, TV_149422, TV_200487, TV_201746, TV_215015, TV_215780, and TV_66683), and 4 in *T. reesei* (TR_105336, TR_121579, TR_123663, and TR_54723) (see Table S1 and Fig. S32 in the supplemental material). Interestingly, it was reported recently that TAL6 (TA_297859) is implicated in the inhibition of conidial germination in *T. atroviride* (1306). However, this finding does not exclude a role of these molecules in plant or fungi interactions and needs to be tested. Of the 21 LysM proteins, only 6 are expressed under axenic conditions; 3 of them (TR_121579, TR_123663, and TR_105336) have significant expression values (RPKMs above 1,500) but the other 3 have very low values that need to be corroborated (TA_299633, TA_297859, and TR_54723). Interestingly, the bioinformatics analysis in *Trichoderma* indicated that the LysM proteins form a separate group that is distant from those previously reported as authentic effectors.

Putative cytoplasmic effectors. Intriguingly, 103 effectors with motifs similar to RxRL from *Phytophthora* spp. were identified in *Trichoderma*. In the three species analyzed, 103 putative secreted proteins containing this sequence were identified. Some of these proteins were recognized as potential transcription factors, protein kinases, protein phosphatases, and proteins involved in the ubiquitination process (see Table S1 in the supplemental material). Members of these families are of interest since, if transported into the plant cell, they could reprogram its gene expression directly (via transcription factors) or indirectly (via signaling and protein stability).

Another group of proteins which have effector activities in bacteria and eukaryotes are ubiquitin ligases (1307). For example, the effector protein AvrPtoB E3 ligase specifically ubiquitinates the protein kinase Fen and consequently promotes its degradation in a proteasome-dependent manner. This degradation leads to disease susceptibility in Fen-expressing tomato lines (1308). The *T. reesei*, *T. atroviride*, and *T. virens* genomes encode six ubiquitin-

ligase-like proteins containing signal peptides and lacking transmembrane domains, as well as domains related to ubiquitination. Five of them are E3 ubiquitin ligases (TA_130535, TA_42133, TR_62333, TV_210292, and TV_62551). Ubiquitination and phosphorylation are the most common posttranslational modifications; both are involved in a number of cellular processes, including cellular differentiation and proliferation (1309). An example is the F-box protein TA_130535, belonging to the E3 ligase family. This family also includes proteins containing either a HECT (homologous to the E6-AP carboxyl terminus) domain or a RING (really interesting new gene) domain (1310), and they are responsible for substrate specificity in the ubiquitin cascade. In the TV_210292 sequence, a UBX (IPR001012) domain is present that is found in ubiquitin regulatory proteins, along with an UAS (IPR006577) domain that has an unknown function found in FAF1 proteins (FAS-associated factor 1) and a UBA-like (IPR009060) domain that is commonly found in diverse proteins involved in the ubiquitination process. All these motifs are present in proteins involved in the ubiquitin/proteasome pathway, DNA excision repair, and cell signaling via protein kinases. In our analysis, we found that some paralogs of the E3 ubiquitin ligases mentioned above do not contain a signal peptide (see Table S1 in the supplemental material).

An additional set of effector proteins includes transcription factor-like proteins containing a secretion signal or those that are released by other means to the host cells. For example, in *Xanthomonas*, the AvrBs3 effector protein is injected into the plant cell by the type III secretion system and localizes to the plant cell nucleus. Hypertrophy of plant mesophyll cells results through the induction of the expression of Upa20, a master regulator of cell size, which encodes a transcription factor containing a basic helix-loop-helix domain (1311). In this analysis, 10 transcription factors belonging to the zinc finger transcription factor family were identified (see Table S1 in the supplemental material). Of these 10 transcription factors, only TR_121794 and TR_72993 are expressed under the conditions evaluated in this work (growth on cellulose in light or darkness) (350). Another type of transcription factors (TR_81990, TA_292251, and TV_154011) contain a forkhead-associated domain and are similar to TOS4 and PLM2 from *S. cerevisiae*. In yeast, these proteins are involved in the response to DNA damage (1312).

Fungus-plant interactions are often accompanied by ROS production by both fungus and plant. ROS produce oxidative stress but also act as signals. Secreted antioxidant enzymes can help the fungal endophyte cope with ROS and reduce or prevent their activity from damaging the fungus. Over 20 of the proteins in our data set have domains suggesting ROS-related enzymatic activity (see Table S1 in the supplemental material). For example, each of the three species has a peroxidase-catalase; TR_70803 and TA_88379 have 91% and 85% identity, respectively, to TV_140278, suggesting conserved function. There are also 15 with oxidase activity, 5 with chloroperoxidase activity (TR_112640, TR_123079, TA_280794, TV_51248, and TV_58307), and 1 with a thioredoxin domain (TV_71032). The latter seems to be secreted and not maintained in the endoplasmic reticulum. This, of course, cannot be confirmed by sequence similarity alone but raises the intriguing and testable prediction that these effectors might degrade a ROS signal either inside or outside the plant cells.

Conclusions. *T. reesei*, *T. atroviride*, and *T. virens* produce a large array of secreted proteins. Around 8% of the proteome is composed of secreted proteins, which include lytic enzymes and proteins with potential effector activity. Intriguingly, however, previous studies have shown that the differences in secretome size do not necessarily correspond in any simple way to the endophytic or pathogenic behavior of species with effector proteins. Numerous *Trichoderma* proteins show no homology to any protein, based on the nr database of NCBI. The low homology to known proteins is common for effector proteins, and consequently these are potentially good targets for future studies on the endophytic behavior of *Trichoderma* spp.

T. atroviride has a large number of extracellular proteins, especially compared to *T. reesei*, which may reflect the primary lifestyles of these fungi (mycoparasite/endophyte versus saprophyte). Sixty-four small cysteine-rich proteins (putative effectors) were identified, of which 31 were unique to these species; the majority belonged to *T. atroviride*. *T. atroviride* also contained 5 of the 12 killer toxins identified in this study. *T. virens* had the highest content of LysM proteins, potentially enhancing its ability to mask itself from detection by host plants. In general, *T. atroviride* and *T. virens* contain more diverse and numerous proteins related to host-plant interactions, while *T. reesei*'s protein diversity was limited, potentially by the smaller genome size. This was most likely related to the particular niche of each fungus and may affect the abilities of more genetically diverse *Trichoderma* spp. to colonize wide varieties of habitats and hosts.

GENERAL CONSIDERATIONS, HIGHLIGHTS, AND OUTLOOK

Trichoderma spp. are among the most important fungi currently used in biotechnology and agriculture. With its high efficiency in expressing both homologous and heterologous genes, *T. reesei* clearly is the biotechnological workhorse of the genus. This status more or less also dictates conditions of analysis of gene expression and regulation. In the case of *T. reesei*, most studies are based on submerged liquid cultures, like shake flask cultivation, batch or continuous fermentation with medium related to plant cell walls or inducing plant cell wall-degrading enzymes. Due to the high expression levels of these enzymes, the respective promoters are most useful for protein production, and hence these conditions are crucial for the focus of studies on *T. reesei*.

In contrast, for *T. atroviride* and *T. virens*, two potent biocontrol organisms, research is centered on the ability to antagonize or kill plant-pathogenic fungi and to initiate plant immunity (ISR). Therefore, entirely different growth conditions for analysis are required. In order to answer questions as to the mechanism of antagonism, these *Trichoderma* spp. have to face a potential plant pathogen, and ideally also the presence of the threatened plant is required to simulate the situation in the field. However, this very complex interaction of three partners is very difficult to study experimentally, and most reports deal with selected aspects of this interaction, such as the direct effect of the presence of a pathogen. The reaction of *Trichoderma* in this case was often tested in plate assays, which present very artificial conditions in terms of the nutrient and spacial situation. Another example would be an analysis of the systemic response of a plant in the presence of *Trichoderma* in hydroponic culture, which is much closer to nature but still different from field conditions.

In summary, the available studies on all species analyzed in this genome annotation analysis are mostly based on very artificial and quite diverse conditions. This is either due to the research focus of the species or due to experimental limitations and the complexity

TABLE 4 Functions of unique genes in *T. atroviride* and *T. virens*[a]

Gene group product(s) or purpose	No. of genes unique to *T. atroviride* or *T. virens*[b]	No. of genes found in both *T. atroviride* and *T. virens*	% overlap	No. of genes of group in:	
				T. atroviride	*T. virens*
RAS GTPases	3	0	0	2	1
Heat shock proteins	**10**	**1**	**10**	**4**	**7**
ABC transporters	**17**	**2**	**12**	**4**	**15**
Secondary metabolism	**50**	**6**	**12**	**25**	**31**
Calcium signaling	**8**	**1**	**13**	**2**	**7**
Glycoside transferases	19	3	16	11	11
CBM containing	15	3	20	8	10
Effector proteins	**81**	**19**	**23**	**47**	**53**
Sulfur metabolism	4	1	25	3	2
General substrate transporters	4	1	25	2	3
Protein phosphatases	4	1	25	2	3
Carbohydrate esterases	74	22	30	41	55
Zn_2Cys_6 TFs	193	61	32	117	137
Glycoside hydrolases	118	38	32	74	82
Polysaccharide lyase	3	1	33	3	1
HTH-type TFs	3	1	33	3	1
Myb TFs	3	1	33	1	3
Protein kinases	6	2	33	3	5
GPCRs	30	10	33	18	22
C2H2 zinc finger TFs	17	6	35	7	16
bZIP TFs	11	4	36	8	7
Fungal TF type	58	22	38	37	43
Development	5	2	40	4	3
Sugar transporter	26	11	42	16	21
Chromatin and histone modification	13	6	46	13	6
Homeobox TFs	5	3	60	4	4
Lipid metabolism	**11**	**7**	**64**	**9**	**9**
HMG TFs	3	2	67	2	3
Genome integrity	**10**	**7**	**70**	**10**	**7**
Oligopeptide transporter	1	1	100	1	1
bHLH TFs	1	1	100	1	1
Heteromeric CCAAT factors	1	1	100	1	1
Total	807	247	31[c]	483	571

[a] Data shown in boldface indicate gene groups considered particularly promising for investigation of their relevance for biocontrol and mycoparasitism due to their function and/or overlap of genes between species.
[b] Genes present in *T. atroviride* or *T. virens* but not found in *T. reesei*.
[c] Average percentage for all gene groups.

of the natural situation, which makes identification of the natural causes and prerequisites for a given lifestyle problematic. Moreover, the aims of the research done with *T. atroviride* and *T. virens* versus *T. reesei* are almost entirely different, which consequently makes comparisons based on transcriptome data between these species very difficult and rather speculative. Only very few studies are available that compare the three species under similar conditions. Considering the now-available genome annotation and transcriptome data, efforts should be increased to compare the different lifestyles using *T. reesei* versus *T. atroviride* and/or *T. virens*.

However, these very differences also led to a much broader view on the genus *Trichoderma* and on the potential of the individual species than one single application or research focus would have. The extensive analysis done for the genome annotation presented in this study clearly reflect this benefit but also the limitations in comparison resulting from fundamentally different experimental designs. By bringing together aspects of both biotechnology and fungi-plant pathogen interactions, a basis is formed for broader studies.

Characteristics of Unique Genes

Previously, it was shown that *T. virens* and *T. reesei* are derived from *T. atroviride* and that *T. reesei* apparently has lost the genome content required for efficient mycoparasitism (4). Consequently, every shortage of genome content in *T. reesei* versus *T. atroviride* or *T. virens* may be related to this loss. Our analysis indeed indicated a more narrow inventory of genes for several gene groups. Therefore, we screened our annotations for the presence of genes in *T. atroviride* or *T. virens* but not in *T. reesei*. In contrast to previous studies, our bidirectional BLAST analyses along with phylogenetic analyses now allow us to determine overlaps of homologs present in *T. atroviride* and *T. virens* and to distinguish them from genes present in only one of the two mycoparasitic species.

Among the annotated genes, we found 807 genes present in *T. atroviride* or *T. virens* but not in *T. reesei* (Table 4). A total of 483 of them were from *T. atroviride* and 571 were from *T. virens*, with an overlap for homologous genes of only 247. Consequently, less than one-third of the genes not present in *T. reesei* are shared

between *T. atroviride* and *T. virens* (see Table S3 in the supplemental material). Albeit surprising, this result is in agreement with earlier data, indicating that these three species apply different strategies of attack against other fungi (22). Of the 50 unique secondary metabolite genes, only 6 (12%) overlap between *T. atroviride* and *T. virens*, supporting the hypothesis of different chemicals applied in antagonism. A similar phenomenon applies to ABC transporters (12%) or effector proteins (23%). However, also the unique genes encoding heat shock proteins, glycosyl transferases, or such involved in calcium signaling show only little overlap (Table 4). Hence these genes are promising candidates for investigation of the individual strategies of *T. atroviride* and *T. virens* in biocontrol.

Concerning the genome content relevant for the enhanced mycoparasitic abilities of *T. atroviride* and *T. virens* compared to *T. reesei*, the overlapping unique genes in *T. atroviride* and *T. virens* are more interesting. In this respect, particularly genes encoding effector proteins, Zn_2Cys_6 transcription factors, carbohydrate esterases, and glycoside hydrolases are prevalent (Table 4). Nevertheless, also G-protein-coupled receptors, sugar transporters, and genes involved in lipid metabolism or genome integrity show considerable overlap among unique genes and may be responsible for important differences in sensing and reaction.

Previously, it was also speculated that chromatin rearrangement may play a role in regulation of the mycoparasitic response (22). The 13 genes involved in this process that are unique to *T. atroviride* and *T. virens* (6 genes overlap) may be promising candidates for a better understanding of this involvement.

Screening for genes unique to *T. reesei* among our annotated genes revealed only 51 genes with annotation (Table S1 in the supplemental material). Additionally, many of them show very low expression levels (350). They include genes encoding CAZymes, 2 PKSs, 5 transporters, 17 transcription factors, 1 casein kinase I, 2 GPCRs, 3 genes involved in conidiation. and 13 effector proteins. Consequently, the function of the majority of the 577 genes unique to *T. reesei* (4) remains unknown and likely represents a machinery needed under conditions not relevant to current applications of *Trichoderma* or to studies in fungal biology. However, these genes may also belong to gene groups unique to some fungi with intriguing functions that may open up new fields of research.

Characteristic Gene Content in Selected Groups

The initial comparison of *T. reesei*, *T. atroviride* and *T. virens* also showed that every species has their unique genes and especially chitinases and chitosanases of *T. atroviride* and *T. virens* were associated with their enhanced mycoparasitic abilities (4). Here we tackled the aspect of carbohydrate degradation preferences in the three species, which may reflect altered necessities between saprotrophic and mycoparasitic lifestyle. However, the genome content of these three species did not indicate any significant shift between preferred cleavage of alpha versus beta linkages or mono- versus oligosaccharides. Still, it remains to be shown if specific regulation patterns under similar conditions are responsible for succeeding with the respective lifestyle.

Recently, several studies showed evidence for an involvement of chromatin remodeling in regulation of diverse processes in *Trichoderma* spp. Moreover, differences in the regulatory output of chromatin rearrangements were suggested for different species. Our analysis showed that especially histone acetyl transferases, histone methyltransferases, and histone deacetylases with numerous gene expansions in not only *T. atroviride* but also *T. virens*, compared to *T. reesei*, are promising candidates for investigation of the relevance of chromatin rearrangements for the different lifestyles of these fungi.

Considering overall gene numbers for a gene category along with unique genes for *T. reesei*, *T. atroviride*, and/or *T. virens*, genes involved in environmental signaling represent another important target for further studies toward understanding differences in these fungi. In many cases, deletion of the same gene has a severe phenotype in one species but hardly any phenotype in another, even within *Trichoderma* spp. Examples are G-protein alpha-subunits or the light regulatory protein ENV1 (for details see above). Thirty G-protein-coupled receptors are unique to *T. atroviride* or *T. virens*, with only 10 homologs among them. All of them belong to the group of PTH11-like receptors, which represent a tremendous and diversified potential for recognition of competitors in nature, especially because in *T. reesei* only 2 GPCRs of this group are unique, which emphasizes the relevance for *T. atroviride* and *T. virens*.

In summary, this work aimed to provide a basis for analysis of "omics" data not only regarding *T. reesei*, *T. atroviride*, and *T. virens* but also other *Trichoderma* spp., either already sequenced or yet to be sequenced. Additionally, this extensive, manual annotation will tremendously speed up and improve annotation of other species of the genus *Trichoderma* and beyond. By providing detailed lists on annotations along with homologs and paralogs, this study provides important support for hypothesis generation in further studies, including high-throughput studies on selected gene groups. To the best of our knowledge, this combined *Trichoderma* annotation is currently the largest and most detailed annotation report available for fungi.

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We declare we have no conflicts of interest.

REFERENCES

1. **Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG.** 2014. Biotechnology and biology of *Trichoderma*. Elsevier, Oxford, United Kingdom.

2. **Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley CM.** 2013. *Trichoderma* research in the genome era. Annu Rev Phytopathol **51:**105–129. http://dx.doi.org/10.1146/annurev-phyto-082712-102353.
3. **Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M.** 2013. *Trichoderma*: biology and applications. CAB International, Oxfordshire, United Kingdom.
4. **Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, Zeilinger S, Casas-Flores S, Horwitz BA, Mukherjee PK, Mukherjee M, Kredics L, Alcaraz LD, Aerts A, Antal Z, Atanasova L, Cervantes-Badillo MG, Challacombe J, Chertkov O, McCluskey K, Coulpier F, Deshpande N, von Dohren H, Ebbole DJ, Esquivel-Naranjo EU, Fekete E, Flipphi M, Glaser F, Gomez-Rodriguez EY, Gruber S, Han C, Henrissat B, Hermosa R, Hernandez-Onate M, Karaffa L, Kosti I, Le Crom S, Lindquist E, Lucas S, Lubeck M, Lubeck PS, Margeot A, Metz B, Misra M, Nevalainen H, Omann M, Packer N, Perrone G, Uresti-Rivera EE, Salamov A, Schmoll M, Seiboth B, Shapiro H, Sukno S, Tamayo-Ramos JA, Tisch D, Wiest A, Wilkinson HH, Zhang M, Coutinho PM, Kenerley CM, Monte E, Baker SE, Grigoriev IV.** 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Genome Biol **12:**R40. http://dx.doi.org/10.1186/gb-2011-12-4-r40.
5. **Schmoll M, Seiboth B, Druzhinina I, Kubicek CP.** 2014. Genomics analysis of biocontrol species and industrial enzyme producers from the genus *Trichoderma*, p 233–266. *In* Esser K, Nowrousian M (ed), The Mycota XIII. Springer, Berlin, Germany.
6. **Schuster A, Schmoll M.** 2010. Biology and biotechnology of *Trichoderma*. Appl Microbiol Biotechnol **87:**787–799. http://dx.doi.org/10.1007/s00253-010-2632-1.
7. **Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP.** 2011. *Trichoderma*: the genomics of opportunistic success. Nat Rev Microbiol **9:**749–759. http://dx.doi.org/10.1038/nrmicro2637.
8. **Benitez T, Rincon AM, Limon MC, Codon AC.** 2004. Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol **7:**249–260.
9. **Harman GE.** 2011. Multifunctional fungal plant symbionts: new tools to enhance plant growth and productivity. New Phytol **189:**647–649. http://dx.doi.org/10.1111/j.1469-8137.2010.03614.x.
10. **Howell CR.** 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis **87:**4–10. http://dx.doi.org/10.1094/PDIS.2003.87.1.4.
11. **Hermosa R, Viterbo A, Chet I, Monte E.** 2012. Plant-beneficial effects of *Trichoderma* and of its genes. Microbiology **158:**17–25. http://dx.doi.org/10.1099/mic.0.052274-0.
12. **Schmoll M, Esquivel-Naranjo EU, Herrera-Estrella A.** 2010. *Trichoderma* in the light of day-physiology and development. Fungal Genet Biol **47:**909–916. http://dx.doi.org/10.1016/j.fgb.2010.04.010.
13. **Carreras-Villaseñor N, Sanchez-Arreguin JA, Herrera-Estrella AH.** 2012. *Trichoderma*: sensing the environment for survival and dispersal. Microbiology **158:**3–16. http://dx.doi.org/10.1099/mic.0.052688-0.
14. **Steyaert JM, Weld RJ, Loguercio LL, Stewart A.** 2010. Rhythmic conidiation in the blue-light fungus *Trichoderma pleuroticola*. Fungal Biol **114:**219–223. http://dx.doi.org/10.1016/j.funbio.2010.01.001.
15. **Hernandez-Oñate MA, Esquivel-Naranjo EU, Mendoza-Mendoza A, Stewart A, Herrera-Estrella AH.** 2012. An injury-response mechanism conserved across kingdoms determines entry of the fungus *Trichoderma atroviride* into development. Proc Natl Acad Sci U S A **109:**14918–14923. http://dx.doi.org/10.1073/pnas.1209396109.
16. **Schmoll M, Kubicek CP.** 2003. Regulation of *Trichoderma* cellulase formation: lessons in molecular biology from an industrial fungus. A review. Acta Microbiol Immunol Hung **50:**125–145. http://dx.doi.org/10.1556/AMicr.50.2003.2-3.3.
17. **Saloheimo M, Pakula TM.** 2012. The cargo and the transport system: secreted proteins and protein secretion in *Trichoderma reesei* (*Hypocrea jecorina*). Microbiology **158:**46–57. http://dx.doi.org/10.1099/mic.0.053132-0.
18. **Mukherjee PK, Horwitz BA, Kenerley CM.** 2012. Secondary metabolism in *Trichoderma*: a genomic perspective. Microbiology **158:**35–45. http://dx.doi.org/10.1099/mic.0.053629-0.
19. **Komon-Zelazowska M, Neuhof T, Dieckmann R, von Dohren H, Herrera-Estrella A, Kubicek CP, Druzhinina IS.** 2007. Formation of atroviridin by *Hypocrea atroviridis* is conidiation associated and positively regulated by blue light and the G protein GNA3. Eukaryot Cell **6:**2332–2342. http://dx.doi.org/10.1128/EC.00143-07.
20. **Zhang J, Zhang Y, Zhong Y, Qu Y, Wang T.** 2012. Ras GTPases modulate morphogenesis, sporulation and cellulase gene expression in the cellulolytic fungus *Trichoderma reesei*. PLoS One **7:**e48786. http://dx.doi.org/10.1371/journal.pone.0048786.
21. **Atanasova L, Druzhinina I, Jaklitsch WM.** 2013. Two hundred *Trichoderma* species recognized on the basis of molecular phylogeny, p 10–42. *In* Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (ed), *Trichoderma*: biology and applications. CAB International, Oxfordshire, United Kingdom.
22. **Atanasova L, Le Crom S, Gruber S, Coulpier F, Seidl-Seiboth V, Kubicek CP, Druzhinina IS.** 2013. Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. BMC Genomics **14:**121. http://dx.doi.org/10.1186/1471-2164-14-121.
23. **Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EG, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, de Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barbote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M, Brettin TS.** 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat Biotechnol **26:**553–560. http://dx.doi.org/10.1038/nbt1403.
24. **Galagan JE, Selker EU.** 2004. RIP: the evolutionary cost of genome defense. Trends Genet **20:**417–423. http://dx.doi.org/10.1016/j.tig.2004.07.007.
25. **Seidl V, Song L, Lindquist E, Gruber S, Koptchinskiy A, Zeilinger S, Schmoll M, Martinez P, Sun J, Grigoriev I, Herrera-Estrella A, Baker SE, Kubicek CP.** 2009. Transcriptomic response of the mycoparasitic fungus *Trichoderma atroviride* to the presence of a fungal prey. BMC Genomics **10:**567. http://dx.doi.org/10.1186/1471-2164-10-567.
26. **Druzhinina IS, Shelest E, Kubicek CP.** 2012. Novel traits of *Trichoderma* predicted through the analysis of its secretome. FEMS Microbiol Lett **337:**1–9. http://dx.doi.org/10.1111/j.1574-6968.2012.02665.x.
27. **Seidl-Seiboth V, Gruber S, Sezerman U, Schwecke T, Albayrak A, Neuhof T, von Dohren H, Baker SE, Kubicek CP.** 2011. Novel hydrophobins from *Trichoderma* define a new hydrophobin subclass: protein properties, evolution, regulation and processing. J Mol Evol **72:**339–351. http://dx.doi.org/10.1007/s00239-011-9438-3.
28. **Seidl V, Marchetti M, Schandl R, Allmaier G, Kubicek CP.** 2006. Epl1, the major secreted protein of *Hypocrea atroviridis* on glucose, is a member of a strongly conserved protein family comprising plant defense response elicitors. FEBS J **273:**4346–4359. http://dx.doi.org/10.1111/j.1742-4658.2006.05435.x.
29. **Djonovic S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM.** 2007. A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol **145:**875–889. http://dx.doi.org/10.1104/pp.107.103689.
30. **Krwawicz J, Arczewska KD, Speina E, Maciejewska A, Grzesiuk E.** 2007. Bacterial DNA repair genes and their eukaryotic homologues: 1. Mutations in genes involved in base excision repair (BER) and DNA-end processors and their implication in mutagenesis and human disease. Acta Biochim Pol **54:**413–434.
31. **Arczewska KD, Kusmierek JT.** 2007. Bacterial DNA repair genes and their eukaryotic homologues. 2. Role of bacterial mutator gene homologues in human disease. Overview of nucleotide pool sanitization and mismatch repair systems. Acta Biochim Pol **54:**435–457.
32. **Nieminuszczy J, Grzesiuk E.** 2007. Bacterial DNA repair genes and their eukaryotic homologues. 3. AlkB dioxygenase and Ada methyltransferase in the direct repair of alkylated DNA. Acta Biochim Pol **54:**459–468.
33. **Nowosielska A.** 2007. Bacterial DNA repair genes and their eukaryotic homologues. 5. The role of recombination in DNA repair and genome stability. Acta Biochim Pol **54:**483–494.
34. **Maddukuri L, Dudzinska D, Tudek B.** 2007. Bacterial DNA repair genes and their eukaryotic homologues. 4. The role of nucleotide excision DNA repair (NER) system in mammalian cells. Acta Biochim Pol **54:**469–482.

35. **Fu Y, Pastushok L, Xiao W.** 2008. DNA damage-induced gene expression in *Saccharomyces cerevisiae*. FEMS Microbiol Rev **32:**908–926. http://dx.doi.org/10.1111/j.1574-6976.2008.00126.x.
36. **Dang Y, Yang Q, Xue Z, Liu Y.** 2011. RNA interference in fungi: pathways, functions, and applications. Eukaryot Cell **10:**1148–1155. http://dx.doi.org/10.1128/EC.05109-11.
37. **Ninomiya Y, Suzuki K, Ishii C, Inoue H.** 2004. Highly efficient gene replacements in *Neurospora* strains deficient for nonhomologous end-joining. Proc Natl Acad Sci U S A **101:**12248–12253. http://dx.doi.org/10.1073/pnas.0402780101.
38. **Carthew RW, Sontheimer EJ.** 2009. Origins and mechanisms of miRNAs and siRNAs. Cell **136:**642–655. http://dx.doi.org/10.1016/j.cell.2009.01.035.
39. **Shiu PK, Raju NB, Zickler D, Metzenberg RL.** 2001. Meiotic silencing by unpaired DNA. Cell **107:**905–916. http://dx.doi.org/10.1016/S0092-8674(01)00609-2.
40. **Cerutti H, Casas-Mollano JA.** 2006. On the origin and functions of RNA-mediated silencing: from protists to man. Curr Genet **50:**81–99. http://dx.doi.org/10.1007/s00294-006-0078-x.
41. **Ghildiyal M, Zamore PD.** 2009. Small silencing RNAs: an expanding universe. Nat Rev Genet **10:**94–108. http://dx.doi.org/10.1038/nrg2504.
42. **Romano N, Macino G.** 1992. Quelling: transient inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. Mol Microbiol **6:**3343–3353. http://dx.doi.org/10.1111/j.1365-2958.1992.tb02202.x.
43. **Cogoni C.** 2001. Homology-dependent gene silencing mechanisms in fungi. Annu Rev Microbiol **55:**381–406. http://dx.doi.org/10.1146/annurev.micro.55.1.381.
44. **Segers GC, Zhang X, Deng F, Sun Q, Nuss DL.** 2007. Evidence that RNA silencing functions as an antiviral defense mechanism in fungi. Proc Natl Acad Sci U S A **104:**12902–12906. http://dx.doi.org/10.1073/pnas.0702500104.
45. **Hammond TM, Andrewski MD, Roossinck MJ, Keller NP.** 2008. *Aspergillus* mycoviruses are targets and suppressors of RNA silencing. Eukaryot Cell **7:**350–357. http://dx.doi.org/10.1128/EC.00356-07.
46. **Nakayashiki H, Kadotani N, Mayama S.** 2006. Evolution and diversification of RNA silencing proteins in fungi. J Mol Evol **63:**127–135. http://dx.doi.org/10.1007/s00239-005-0257-2.
47. **Tabach Y, Billi AC, Hayes GD, Newman MA, Zuk O, Gabel H, Kamath R, Yacoby K, Chapman B, Garcia SM, Borowsky M, Kim JK, Ruvkun G.** 2013. Identification of small RNA pathway genes using patterns of phylogenetic conservation and divergence. Nature **493:**694–698. http://dx.doi.org/10.1038/nature11779.
48. **Hammond TM, Bok JW, Andrewski MD, Reyes-Dominguez Y, Scazzocchio C, Keller NP.** 2008. RNA silencing gene truncation in the filamentous fungus *Aspergillus nidulans*. Eukaryot Cell **7:**339–349. http://dx.doi.org/10.1128/EC.00355-07.
49. **Sun Q, Choi GH, Nuss DL.** 2009. A single Argonaute gene is required for induction of RNA silencing antiviral defense and promotes viral RNA recombination. Proc Natl Acad Sci U S A **106:**17927–17932. http://dx.doi.org/10.1073/pnas.0907552106.
50. **Carreras-Villaseñor N, Esquivel-Naranjo EU, Villalobos-Escobedo JM, Abreu-Goodger C, Herrera-Estrella A.** 2013. The RNAi machinery regulates growth and development in the filamentous fungus *Trichoderma atroviride*. Mol Microbiol **89:**96–112. http://dx.doi.org/10.1111/mmi.12261.
51. **Cardoza RE, Vizcaino JA, Hermosa MR, Sousa S, Gonzalez FJ, Llobell A, Monte E, Gutierrez S.** 2006. Cloning and characterization of the *erg1* gene of *Trichoderma harzianum*: effect of the *erg1* silencing on ergosterol biosynthesis and resistance to terbinafine. Fungal Genet Biol **43:**164–178. http://dx.doi.org/10.1016/j.fgb.2005.11.002.
52. **Casas-Flores S, Rios-Momberg M, Rosales-Saavedra T, Martinez-Hernandez P, Olmedo-Monfil V, Herrera-Estrella A.** 2006. Cross talk between a fungal blue-light perception system and the cyclic AMP signaling pathway. Eukaryot Cell **5:**499–506. http://dx.doi.org/10.1128/EC.5.3.499-506.2006.
53. **Rocha-Ramirez V, Omero C, Chet I, Horwitz BA, Herrera-Estrella A.** 2002. *Trichoderma atroviride* G-protein alpha-subunit gene *tga1* is involved in mycoparasitic coiling and conidiation. Eukaryot Cell **1:**594–605. http://dx.doi.org/10.1128/EC.1.4.594-605.2002.
54. **Brunner K, Omann M, Pucher ME, Delic M, Lehner SM, Domnanich P, Kratochwill K, Druzhinina I, Denk D, Zeilinger S.** 2008. *Trichoderma* G protein-coupled receptors: functional characterisation of a cAMP receptor-like protein from *Trichoderma atroviride*. Curr Genet **54:**283–299. http://dx.doi.org/10.1007/s00294-008-0217-7.
55. **Kang K, Zhong J, Jiang L, Liu G, Gou CY, Wu Q, Wang Y, Luo J, Gou D.** 2013. Identification of microRNA-Like RNAs in the filamentous fungus *Trichoderma reesei* by solexa sequencing. PLoS One **8:**e76288. http://dx.doi.org/10.1371/journal.pone.0076288.
56. **Jiang N, Yang Y, Janbon G, Pan J, Zhu X.** 2012. Identification and functional demonstration of miRNAs in the fungus *Cryptococcus neoformans*. PLoS One **7:**e52734. http://dx.doi.org/10.1371/journal.pone.0052734.
57. **Zhou J, Fu Y, Xie J, Li B, Jiang D, Li G, Cheng J.** 2012. Identification of microRNA-like RNAs in a plant-pathogenic fungus *Sclerotinia sclerotiorum* by high-throughput sequencing. Mol Genet Genomics **287:**275–282. http://dx.doi.org/10.1007/s00438-012-0678-8.
58. **Zhou Q, Wang Z, Zhang J, Meng H, Huang B.** 2012. Genome-wide identification and profiling of microRNA-like RNAs from *Metarhizium anisopliae* during development. Fungal Biol **116:**1156–1162. http://dx.doi.org/10.1016/j.funbio.2012.09.001.
59. **Nunes CC, Gowda M, Sailsbery J, Xue M, Chen F, Brown DE, Oh Y, Mitchell TK, Dean RA.** 2011. Diverse and tissue-enriched small RNAs in the plant-pathogenic fungus, *Magnaporthe oryzae*. BMC Genomics **12:**288. http://dx.doi.org/10.1186/1471-2164-12-288.
60. **Lee HC, Li L, Gu W, Xue Z, Crosthwaite SK, Pertsemlidis A, Lewis ZA, Freitag M, Selker EU, Mello CC, Liu Y.** 2010. Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. Mol Cell **38:**803–814. http://dx.doi.org/10.1016/j.molcel.2010.04.005.
61. **Nicolas FE, Moxon S, de Haro JP, Calo S, Grigoriev IV, Torres-Martinez S, Moulton V, Ruiz-Vazquez RM, Dalmay T.** 2010. Endogenous short RNAs generated by Dicer 2 and RNA-dependent RNA polymerase 1 regulate mRNAs in the basal fungus *Mucor circinelloides*. Nucleic Acids Res **38:**5535–5541. http://dx.doi.org/10.1093/nar/gkq301.
62. **Halic M, Moazed D.** 2010. Dicer-independent primal RNAs trigger RNAi and heterochromatin formation. Cell **140:**504–516. http://dx.doi.org/10.1016/j.cell.2010.01.019.
63. **Wang X, Hsueh YP, Li W, Floyd A, Skalsky R, Heitman J.** 2010. Sex-induced silencing defends the genome of *Cryptococcus neoformans* via RNAi. Genes Dev **24:**2566–2582. http://dx.doi.org/10.1101/gad.1970910.
64. **Djupedal I, Kos-Braun IC, Mosher RA, Soderholm N, Simmer F, Hardcastle TJ, Fender A, Heidrich N, Kagansky A, Bayne E, Wagner EG, Baulcombe DC, Allshire RC, Ekwall K.** 2009. Analysis of small RNA in fission yeast; centromeric siRNAs are potentially generated through a structured RNA. EMBO J **28:**3832–3844. http://dx.doi.org/10.1038/emboj.2009.351.
65. **Drinnenberg IA, Weinberg DE, Xie KT, Mower JP, Wolfe KH, Fink GR, Bartel DP.** 2009. RNAi in budding yeast. Science **326:**544–550. http://dx.doi.org/10.1126/science.1176945.
66. **Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M, Bamford DH, Liu Y.** 2009. qiRNA is a new type of small interfering RNA induced by DNA damage. Nature **459:**274–277. http://dx.doi.org/10.1038/nature08041.
67. **Reinhart BJ, Bartel DP.** 2002. Small RNAs correspond to centromere heterochromatic repeats. Science **297:**1831. http://dx.doi.org/10.1126/science.1077183.
68. **Eisen JA, Hanawalt PC.** 1999. A phylogenomic study of DNA repair genes, proteins, and processes. Mutat Res **435:**171–213. http://dx.doi.org/10.1016/S0921-8777(99)00050-6.
69. **Aravind L, Walker DR, Koonin EV.** 1999. Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res **27:**1223–1242. http://dx.doi.org/10.1093/nar/27.5.1223.
70. **Goldman GH, Kafer E.** 2004. *Aspergillus nidulans* as a model system to characterize the DNA damage response in eukaryotes. Fungal Genet Biol **41:**428–442. http://dx.doi.org/10.1016/j.fgb.2003.12.001.
71. **Borkovich KA, Alex LA, Yarden O, Freitag M, Turner GE, Read ND, Seiler S, Bell-Pedersen D, Paietta J, Plesofsky N, Plamann M, Goodrich-Tanrikulu M, Schulte U, Mannhaupt G, Nargang FE, Radford A, Selitrennikoff C, Galagan JE, Dunlap JC, Loros JJ, Catcheside D, Inoue H, Aramayo R, Polymenis M, Selker EU, Sachs MS, Marzluf GA, Paulsen I, Davis R, Ebbole DJ, Zelter A, Kalkman ER, O'Rourke R, Bowring F, Yeadon J, Ishii C, Suzuki K, Sakai W, Pratt R.** 2004. Lessons from the genome sequence of *Neurospora crassa*: tracing the

path from genomic blueprint to multicellular organism. Microbiol Mol Biol Rev **68:**1–108. http://dx.doi.org/10.1128/MMBR.68.1.1-108.2004.
72. **Goldman GH, McGuire SL, Harris SD.** 2002. The DNA damage response in filamentous fungi. Fungal Genet Biol **35:**183–195. http://dx.doi.org/10.1006/fgbi.2002.1344.
73. **Sinha RP, Häder DP.** 2002. UV-induced DNA damage and repair: a review. Photochem Photobiol Sci **1:**225–236. http://dx.doi.org/10.1039/b201230h.
74. **Selby CP, Sancar A.** 2006. A cryptochrome/photolyase class of enzymes with single-stranded DNA-specific photolyase activity. Proc Natl Acad Sci U S A **103:**17696–17700. http://dx.doi.org/10.1073/pnas.0607993103.
75. **Pokorny R, Klar T, Hennecke U, Carell T, Batschauer A, Essen LO.** 2008. Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc Natl Acad Sci U S A **105:**21023–21027. http://dx.doi.org/10.1073/pnas.0805830106.
76. **Berrocal-Tito G, Sametz-Baron L, Eichenberg K, Horwitz BA, Herrera-Estrella A.** 1999. Rapid blue light regulation of a *Trichoderma harzianum* photolyase gene. J Biol Chem **274:**14288–14294. http://dx.doi.org/10.1074/jbc.274.20.14288.
77. **Berrocal-Tito GM, Esquivel-Naranjo EU, Horwitz BA, Herrera-Estrella A.** 2007. *Trichoderma atroviride* PHR1, a fungal photolyase responsible for DNA repair, autoregulates its own photoinduction. Eukaryot Cell **6:**1682–1692. http://dx.doi.org/10.1128/EC.00208-06.
78. **Schuster A, Kubicek CP, Friedl MA, Druzhinina IS, Schmoll M.** 2007. Impact of light on *Hypocrea jecorina* and the multiple cellular roles of ENVOY in this process. BMC Genomics **8:**449. http://dx.doi.org/10.1186/1471-2164-8-449.
79. **Bluhm BH, Dunkle LD.** 2008. PHL1 of *Cercospora zeae-maydis* encodes a member of the photolyase/cryptochrome family involved in UV protection and fungal development. Fungal Genet Biol **45:**1364–1372. http://dx.doi.org/10.1016/j.fgb.2008.07.005.
80. **Guzmán-Moreno J, Flores-Martínez A, Brieba L. G, Herrera-Estrella A.** 2014. The *Trichoderma reesei* Cry1 protein is a member of the cryptochrome/photolyase family with 6-4 photoproduct repair activity. PLoS One **9:**e100625. http://dx.doi.org/10.1371/journal.pone.0100625.
81. **Sancar GB, Smith FW.** 1989. Interactions between yeast photolyase and nucleotide excision repair proteins in *Saccharomyces cerevisiae* and *Escherichia coli*. Mol Cell Biol **9:**4767–4776. http://dx.doi.org/10.1128/MCB.9.11.4767.
82. **Bayram O, Biesemann C, Krappmann S, Galland P, Braus GH.** 2008. More than a repair enzyme: *Aspergillus nidulans* photolyase-like CryA is a regulator of sexual development. Mol Biol Cell **19:**3254–3262. http://dx.doi.org/10.1091/mbc.E08-01-0061.
83. **Sedgwick B, Robins P, Totty N, Lindahl T.** 1988. Functional domains and methyl acceptor sites of the *Escherichia coli* Ada protein. J Biol Chem **263:**4430–4433.
84. **He C, Wei H, Verdine GL.** 2003. Converting the sacrificial DNA repair protein N-ada into a catalytic methyl phosphotriester repair enzyme. J Am Chem Soc **125:**1450–1451. http://dx.doi.org/10.1021/ja028046a.
85. **Xiao W, Derfler B, Chen J, Samson L.** 1991. Primary sequence and biological functions of a *Saccharomyces cerevisiae* O^{6}-methylguanine/O^{4}-methylthymine DNA repair methyltransferase gene. EMBO J **10:**2179–2186.
86. **Lin Y, Zhao T, Jian X, Farooqui Z, Qu X, He C, Dinner AR, Scherer NF.** 2009. Using the bias from flow to elucidate single DNA repair protein sliding and interactions with DNA. Biophys J **96:**1911–1917. http://dx.doi.org/10.1016/j.bpj.2008.11.021.
87. **Sancar A.** 1996. DNA excision repair. Annu Rev Biochem **65:**43–81. http://dx.doi.org/10.1146/annurev.bi.65.070196.000355.
88. **Wood RD.** 1996. DNA repair in eukaryotes. Annu Rev Biochem **65:**135–167. http://dx.doi.org/10.1146/annurev.bi.65.070196.001031.
89. **Friedberg EC, Bardwell AJ, Bardwell L, Feaver WJ, Kornberg RD, Svejstrup JQ, Tomkinson AE, Wang Z.** 1995. Nucleotide excision repair in the yeast *Saccharomyces cerevisiae*: its relationship to specialized mitotic recombination and RNA polymerase II basal transcription. Philos Trans R Soc Lond B Biol Sci **347:**63–68. http://dx.doi.org/10.1098/rstb.1995.0010.
90. **Sung JS, Demple B.** 2006. Roles of base excision repair subpathways in correcting oxidized abasic sites in DNA. FEBS J **273:**1620–1629. http://dx.doi.org/10.1111/j.1742-4658.2006.05192.x.
91. **Parsons JL, Dianova II, Allinson SL, Dianov GL.** 2005. Poly(ADP-ribose) polymerase-1 protects excessive DNA strand breaks from deterioration during repair in human cell extracts. FEBS J **272:**2012–2021. http://dx.doi.org/10.1111/j.1742-4658.2005.04628.x.
92. **Wood RD, Shivji MK.** 1997. Which DNA polymerases are used for DNA-repair in eukaryotes? Carcinogenesis **18:**605–610. http://dx.doi.org/10.1093/carcin/18.4.605.
93. **Leibeling D, Laspe P, Emmert S.** 2006. Nucleotide excision repair and cancer. J Mol Histol **37:**225–238. http://dx.doi.org/10.1007/s10735-006-9041-x.
94. **Hanawalt PC.** 2002. Subpathways of nucleotide excision repair and their regulation. Oncogene **21:**8949–8956. http://dx.doi.org/10.1038/sj.onc.1206096.
95. **Gillet LC, Scharer OD.** 2006. Molecular mechanisms of mammalian global genome nucleotide excision repair. Chem Rev **106:**253–276. http://dx.doi.org/10.1021/cr040483f.
96. **Saxowsky TT, Doetsch PW.** 2006. RNA polymerase encounters with DNA damage: transcription-coupled repair or transcriptional mutagenesis? Chem Rev **106:**474–488. http://dx.doi.org/10.1021/cr040466q.
97. **Schofield MJ, Hsieh P.** 2003. DNA mismatch repair: molecular mechanisms and biological function. Annu Rev Microbiol **57:**579–608. http://dx.doi.org/10.1146/annurev.micro.57.030502.090847.
98. **McCulloch SD, Gu L, Li GM.** 2003. Bi-directional processing of DNA loops by mismatch repair-dependent and -independent pathways in human cells. J Biol Chem **278:**3891–3896. http://dx.doi.org/10.1074/jbc.M210687200.
99. **Schmutte C, Sadoff MM, Shim KS, Acharya S, Fishel R.** 2001. The interaction of DNA mismatch repair proteins with human exonuclease I. J Biol Chem **276:**33011–33018. http://dx.doi.org/10.1074/jbc.M102670200.
100. **Lee SD, Alani E.** 2006. Analysis of interactions between mismatch repair initiation factors and the replication processivity factor PCNA. J Mol Biol **355:**175–184. http://dx.doi.org/10.1016/j.jmb.2005.10.059.
101. **Wyman C, Kanaar R.** 2006. DNA double-strand break repair: all's well that ends well. Annu Rev Genet **40:**363–383. http://dx.doi.org/10.1146/annurev.genet.40.110405.090451.
102. **Fleck O, Nielsen O.** 2004. DNA repair. J Cell Sci **117:**515–517. http://dx.doi.org/10.1242/jcs.00952.
103. **Gaillard PH, Noguchi E, Shanahan P, Russell P.** 2003. The endogenous Mus81-Eme1 complex resolves Holliday junctions by a nick and counternick mechanism. Mol Cell **12:**747–759. http://dx.doi.org/10.1016/S1097-2765(03)00342-3.
104. **Gottlieb TM, Jackson SP.** 1993. The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. Cell **72:**131–142. http://dx.doi.org/10.1016/0092-8674(93)90057-W.
105. **Karlsson KH, Stenerlow B.** 2007. Extensive ssDNA end formation at DNA double-strand breaks in non-homologous end-joining deficient cells during the S phase. BMC Mol Biol **8:**97. http://dx.doi.org/10.1186/1471-2199-8-97.
106. **Krappmann S.** 2007. Gene targeting in filamentous fungi: the benefits of impaired repair. Fungal Biol Rev **21:**25–29. http://dx.doi.org/10.1016/j.fbr.2007.02.004.
107. **Guangtao Z, Hartl L, Schuster A, Polak S, Schmoll M, Wang T, Seidl V, Seiboth B.** 2009. Gene targeting in a nonhomologous end joining deficient *Hypocrea jecorina*. J Biotechnol **139:**146–151. http://dx.doi.org/10.1016/j.jbiotec.2008.10.007.
108. **Catalano V, Vergara M, Hauzenberger JR, Seiboth B, Sarrocco S, Vannacci G, Kubicek CP, Seidl-Seiboth V.** 2011. Use of a non-homologous end-joining-deficient strain (delta-*ku70*) of the biocontrol fungus *Trichoderma virens* to investigate the function of the laccase gene *lcc1* in sclerotia degradation. Curr Genet **57:**13–23. http://dx.doi.org/10.1007/s00294-010-0322-2.
109. **Steiger MG, Vitikainen M, Uskonen P, Brunner K, Adam G, Pakula T, Penttila M, Saloheimo M, Mach RL, Mach-Aigner AR.** 2011. Transformation system for *Hypocrea jecorina* (*Trichoderma reesei*) that favors homologous integration and employs reusable bidirectionally selectable markers. Appl Environ Microbiol **77:**114–121. http://dx.doi.org/10.1128/AEM.02100-10.
110. **Snow ET, Foote RS, Mitra S.** 1984. Kinetics of incorporation of O6-methyldeoxyguanosine monophosphate during in vitro DNA synthesis. Biochemistry **23:**4289–4294. http://dx.doi.org/10.1021/bi00314a006.
111. **Purmal AA, Kow YW, Wallace SS.** 1994. 5-Hydroxypyrimidine deoxynucleoside triphosphates are more efficiently incorporated into DNA by exonuclease-free Klenow fragment than 8-oxopurine deoxynucleoside triphosphates. Nucleic Acids Res **22:**3930–3935. http://dx.doi.org/10.1093/nar/22.19.3930.

112. **Miller H, Prasad R, Wilson SH, Johnson F, Grollman AP.** 2000. 8-oxodGTP incorporation by DNA polymerase beta is modified by active-site residue Asn279. Biochemistry **39:**1029–1033. http://dx.doi.org/10.1021/bi991789x.
113. **Imoto S, Patro JN, Jiang YL, Oka N, Greenberg MM.** 2006. Synthesis, DNA polymerase incorporation, and enzymatic phosphate hydrolysis of formamidopyrimidine nucleoside triphosphates. J Am Chem Soc **128:**14606–14611. http://dx.doi.org/10.1021/ja065525r.
114. **Inoue M, Kamiya H, Fujikawa K, Ootsuyama Y, Murata-Kamiya N, Osaki T, Yasumoto K, Kasai H.** 1998. Induction of chromosomal gene mutations in *Escherichia coli* by direct incorporation of oxidatively damaged nucleotides. New evaluation method for mutagenesis by damaged DNA precursors in vivo. J Biol Chem **273:**11069–11074.
115. **Satou K, Yamada M, Nohmi T, Harashima H, Kamiya H.** 2005. Mutagenesis induced by oxidized DNA precursors: roles of Y family DNA polymerases in *Escherichia coli*. Chem Res Toxicol **18:**1271–1278. http://dx.doi.org/10.1021/tx050046b.
116. **Ishibashi T, Hayakawa H, Sekiguchi M.** 2003. A novel mechanism for preventing mutations caused by oxidation of guanine nucleotides. EMBO Rep **4:**479–483. http://dx.doi.org/10.1038/sj.embor.embor838.
117. **Guillet M, Van Der Kemp PA, Boiteux S.** 2006. dUTPase activity is critical to maintain genetic stability in *Saccharomyces cerevisiae*. Nucleic Acids Res **34:**2056–2066. http://dx.doi.org/10.1093/nar/gkl139.
118. **Koivisto P, Duncan T, Lindahl T, Sedgwick B.** 2003. Minimal methylated substrate and extended substrate range of *Escherichia coli* AlkB protein, a 1-methyladenine-DNA dioxygenase. J Biol Chem **278:**44348–44354. http://dx.doi.org/10.1074/jbc.M307361200.
119. **Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S.** 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature **419:**135–141. http://dx.doi.org/10.1038/nature00991.
120. **Luijsterburg MS, van Attikum H.** 2011. Chromatin and the DNA damage response: the cancer connection. Mol Oncol **5:**349–367. http://dx.doi.org/10.1016/j.molonc.2011.06.001.
121. **Wakabayashi M, Ishii C, Hatakeyama S, Inoue H, Tanaka S.** 2010. ATM and ATR homologes of *Neurospora crassa* are essential for normal cell growth and maintenance of chromosome integrity. Fungal Genet Biol **47:**809–817. http://dx.doi.org/10.1016/j.fgb.2010.05.010.
122. **Pearce AK, Humphrey TC.** 2001. Integrating stress-response and cell-cycle checkpoint pathways. Trends Cell Biol **11:**426–433. http://dx.doi.org/10.1016/S0962-8924(01)02119-5.
123. **Day AM, Veal EA.** 2010. Hydrogen peroxide-sensitive cysteines in the StyI MAPK regulate the transcriptional response to oxidative stress. J Biol Chem **285:**7505–7516. http://dx.doi.org/10.1074/jbc.M109.040840.
124. **Montero-Barrientos M, Hermosa R, Cardoza RE, Gutierrez S, Monte E.** 2011. Functional analysis of the *Trichoderma harzianum nox1* gene, encoding an NADPH oxidase, relates production of reactive oxygen species to specific biocontrol activity against *Pythium ultimum*. Appl Environ Microbiol **77:**3009–3016. http://dx.doi.org/10.1128/AEM.02486-10.
125. **Wang JS, Groopman JD.** 1999. DNA damage by mycotoxins. Mutat Res **424:**167–181. http://dx.doi.org/10.1016/S0027-5107(99)00017-2.
126. **Fox EM, Howlett BJ.** 2008. Secondary metabolism: regulation and role in fungal biology. Curr Opin Microbiol **11:**481–487. http://dx.doi.org/10.1016/j.mib.2008.10.007.
127. **Grimaldi B, Coiro P, Filetici P, Berge E, Dobosy JR, Freitag M, Selker EU, Ballario P.** 2006. The *Neurospora crassa* White Collar-1 dependent blue light response requires acetylation of histone H3 lysine 14 by NGF-1. Mol Biol Cell **17:**4576–4583. http://dx.doi.org/10.1091/mbc.E06-03-0232.
128. **Zeilinger S, Schmoll M, Pail M, Mach RL, Kubicek CP.** 2003. Nucleosome transactions on the *Hypocrea jecorina* (*Trichoderma reesei*) cellulase promoter *cbh2* associated with cellulase induction. Mol Genet Genomics **270:**46–55. http://dx.doi.org/10.1007/s00438-003-0895-2.
129. **Seiboth B, Karimi RA, Phatale PA, Linke R, Hartl L, Sauer DG, Smith KM, Baker SE, Freitag M, Kubicek CP.** 2012. The putative protein methyltransferase LAE1 controls cellulase gene expression in *Trichoderma reesei*. Mol Microbiol **84:**1150–1164. http://dx.doi.org/10.1111/j.1365-2958.2012.08083.x.
130. **Baxevanis AD, Landsman D.** 1996. Histone Sequence Database: a compilation of highly-conserved nucleoprotein sequences. Nucleic Acids Res **24:**245–247. http://dx.doi.org/10.1093/nar/24.1.245.
131. **Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ.** 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature **389:**251–260. http://dx.doi.org/10.1038/38444.
132. **Arents G, Moudrianakis EN.** 1993. Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosomal DNA. Proc Natl Acad Sci U S A **90:**10489–10493. http://dx.doi.org/10.1073/pnas.90.22.10489.
133. **Thatcher TH, Gorovsky MA.** 1994. Phylogenetic analysis of the core histones H2A, H2B, H3, and H4. Nucleic Acids Res **22:**174–179. http://dx.doi.org/10.1093/nar/22.2.174.
134. **Talbert PB, Henikoff S.** 2010. Histone variants: ancient wrap artists of the epigenome. Nat Rev Mol Cell Biol **11:**264–275. http://dx.doi.org/10.1038/nrm2861.
135. **Meluh PB, Koshland D.** 1997. Budding yeast centromere composition and assembly as revealed by in vivo cross-linking. Genes Dev **11:**3401–3412. http://dx.doi.org/10.1101/gad.11.24.3401.
136. **Blower MD, Karpen GH.** 2001. The role of *Drosophila* CID in kinetochore formation, cell-cycle progression and heterochromatin interactions. Nat Cell Biol **3:**730–739. http://dx.doi.org/10.1038/35087045.
137. **Van Hooser AA, Mancini MA, Allis CD, Sullivan KF, Brinkley BR.** 1999. The mammalian centromere: structural domains and the attenuation of chromatin modeling. FASEB J **13**(Suppl 2)**:**S216–S220.
138. **Stoler S, Keith KC, Curnick KE, Fitzgerald-Hayes M.** 1995. A mutation in CSE4, an essential gene encoding a novel chromatin-associated protein in yeast, causes chromosome nondisjunction and cell cycle arrest at mitosis. Genes Dev **9:**573–586. http://dx.doi.org/10.1101/gad.9.5.573.
139. **Folco HD, Pidoux AL, Urano T, Allshire RC.** 2008. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. Science **319:**94–97. http://dx.doi.org/10.1126/science.1150944.
140. **Wieland G, Orthaus S, Ohndorf S, Diekmann S, Hemmerich P.** 2004. Functional complementation of human centromere protein A (CENP-A) by Cse4p from Saccharomyces cerevisiae. Mol Cell Biol **24:**6620–6630. http://dx.doi.org/10.1128/MCB.24.15.6620-6630.2004.
141. **Rangasamy D, Berven L, Ridgway P, Tremethick DJ.** 2003. Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J **22:**1599–1607. http://dx.doi.org/10.1093/emboj/cdg160.
142. **Li B, Pattenden SG, Lee D, Gutierrez J, Chen J, Seidel C, Gerton J, Workman JL.** 2005. Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. Proc Natl Acad Sci U S A **102:**18385–18390. http://dx.doi.org/10.1073/pnas.0507975102.
143. **Hild M, Paro R.** 2003. Anti-silencing from the core: a histone H2A variant protects euchromatin. Nat Cell Biol **5:**278–280. http://dx.doi.org/10.1038/ncb0403-278.
144. **Billon P, Coté J.** 2012. Precise deposition of histone H2A.Z in chromatin for genome expression and maintainance. Biochim Biophys Acta **1819:**290–302. http://dx.doi.org/10.1016/j.bbagrm.2011.10.004.
145. **Hays SM, Swanson J, Selker EU.** 2002. Identification and characterization of the genes encoding the core histones and histone variants of *Neurospora crassa*. Genetics **160:**961–973.
146. **De Koning L, Corpet A, Haber JE, Almouzni G.** 2007. Histone chaperones: an escort network regulating histone traffic. Nat Struct Mol Biol **14:**997–1007. http://dx.doi.org/10.1038/nsmb1318.
147. **Loyola A, Almouzni G.** 2004. Histone chaperones, a supporting role in the limelight. Biochim Biophys Acta **1677:**3–11. http://dx.doi.org/10.1016/j.bbaexp.2003.09.012.
148. **Hondele M, Ladurner AG.** 2011. The chaperone-histone partnership: for the greater good of histone traffic and chromatin plasticity. Curr Opin Struct Biol **21:**698–708. http://dx.doi.org/10.1016/j.sbi.2011.10.003.
149. **Harata M, Oma Y, Mizuno S, Jiang YW, Stillman DJ, Wintersberger U.** 1999. The nuclear actin-related protein of *Saccharomyces cerevisiae*, Act3p/Arp4, interacts with core histones. Mol Biol Cell **10:**2595–2605. http://dx.doi.org/10.1091/mbc.10.8.2595.
150. **Polo SE, Roche D, Almouzni G.** 2006. New histone incorporation marks sites of UV repair in human cells. Cell **127:**481–493. http://dx.doi.org/10.1016/j.cell.2006.08.049.
151. **Loyola A, Almouzni G.** 2007. Marking histone H3 variants: how, when and why? Trends Biochem Sci **32:**425–433. http://dx.doi.org/10.1016/j.tibs.2007.08.004.
152. **Park YJ, Luger K.** 2008. Histone chaperones in nucleosome eviction

and histone exchange. Curr Opin Struct Biol **18:**282–289. http://dx.doi.org/10.1016/j.sbi.2008.04.003.
153. **Agez M, Chen J, Guerois R, van Heijenoort C, Thuret JY, Mann C, Ochsenbein F.** 2007. Structure of the histone chaperone ASF1 bound to the histone H3 C-terminal helix and functional insights. Structure **15:** 191–199. http://dx.doi.org/10.1016/j.str.2007.01.002.
154. **Natsume R, Eitoku M, Akai Y, Sano N, Horikoshi M, Senda T.** 2007. Structure and function of the histone chaperone CIA/ASF1 complexed with histones H3 and H4. Nature **446:**338–341. http://dx.doi.org/10.1038/nature05613.
155. **Mousson F, Lautrette A, Thuret JY, Agez M, Courbeyrette R, Amigues B, Becker E, Neumann JM, Guerois R, Mann C, Ochsenbein F.** 2005. Structural basis for the interaction of Asf1 with histone H3 and its functional implications. Proc Natl Acad Sci U S A **102:**5975–5980. http://dx.doi.org/10.1073/pnas.0500149102.
156. **Dalal Y, Bui M.** 2010. Down the rabbit hole of centromere assembly and dynamics. Curr Opin Cell Biol **22:**392–402. http://dx.doi.org/10.1016/j.ceb.2010.02.005.
157. **Bjerling P, Ekwall K.** 2002. Centromere domain organization and histone modifications. Braz J Med Biol Res **35:**499–507. http://dx.doi.org/10.1590/S0100-879X2002000500001.
158. **Fitzgerald-Hayes M, Clarke L, Carbon J.** 1982. Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. Cell **29:**235–244. http://dx.doi.org/10.1016/0092-8674(82)90108-8.
159. **Cheeseman IM, Drubin DG, Barnes G.** 2002. Simple centromere, complex kinetochore: linking spindle microtubules and centromeric DNA in budding yeast. J Cell Biol **157:**199–203. http://dx.doi.org/10.1083/jcb.200201052.
160. **Meraldi P, McAinsh AD, Rheinbay E, Sorger PK.** 2006. Phylogenetic and structural analysis of centromeric DNA and kinetochore proteins. Genome Biol **7:**R23. http://dx.doi.org/10.1186/gb-2006-7-3-r23.
161. **Sullivan KF, Hechenberger M, Masri K.** 1994. Human CENP-A contains a histone H3 related histone fold domain that is required for targeting to the centromere. J Cell Biol **127:**581–592. http://dx.doi.org/10.1083/jcb.127.3.581.
162. **Iwahara J, Kigawa T, Kitagawa K, Masumoto H, Okazaki T, Yokoyama S.** 1998. A helix-turn-helix structure unit in human centromere protein B (CENP-B). EMBO J **17:**827–837. http://dx.doi.org/10.1093/emboj/17.3.827.
163. **Cleveland DW, Mao Y, Sullivan KF.** 2003. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell **112:**407–421. http://dx.doi.org/10.1016/S0092-8674(03)00115-6.
164. **Irelan JT, Gutkin GI, Clarke L.** 2001. Functional redundancies, distinct localizations and interactions among three fission yeast homologues of centromere protein-B. Genetics **157:**1191–1203.
165. **Smith KM, Galazka JM, Phatale PA, Connolly LR, Freitag M.** 2012. Centromeres of filamentous fungi. Chromosome Res **20:**635–656. http://dx.doi.org/10.1007/s10577-012-9290-3.
166. **Wigge PA, Kilmartin JV.** 2001. The Ndc80p complex from *Saccharomyces cerevisiae* contains conserved centromere components and has a function in chromosome segregation. J Cell Biol **152:**349–360. http://dx.doi.org/10.1083/jcb.152.2.349.
167. **Ciferri C, Pasqualato S, Screpanti E, Varetti G, Santaguida S, Dos Reis G, Maiolica A, Polka J, De Luca JG, De Wulf P, Salek M, Rappsilber J, Moores CA, Salmon ED, Musacchio A.** 2008. Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. Cell **133:**427–439. http://dx.doi.org/10.1016/j.cell.2008.03.020.
168. **Wei RR, Sorger PK, Harrison SC.** 2005. Molecular organization of the Ndc80 complex, an essential kinetochore component. Proc Natl Acad Sci U S A **102:**5363–5367. http://dx.doi.org/10.1073/pnas.0501168102.
169. **Miranda JJ, De Wulf P, Sorger PK, Harrison SC.** 2005. The yeast DASH complex forms closed rings on microtubules. Nat Struct Mol Biol **12:**138–143. http://dx.doi.org/10.1038/nsmb896.
170. **Goshima G, Saitoh S, Yanagida M.** 1999. Proper metaphase spindle length is determined by centromere proteins Mis12 and Mis6 required for faithful chromosome segregation. Genes Dev **13:**1664–1677. http://dx.doi.org/10.1101/gad.13.13.1664.
171. **Aravind L, Koonin EV.** 1998. The HORMA domain: a common structural denominator in mitotic checkpoints, chromosome synapsis and DNA repair. Trends Biochem Sci **23:**284–286.
172. **He X, Patterson TE, Sazer S.** 1997. The *Schizosaccharomyces pombe* spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. Proc Natl Acad Sci U S A **94:**7965–7970. http://dx.doi.org/10.1073/pnas.94.15.7965.
173. **Rudner AD, Murray AW.** 1996. The spindle assembly checkpoint. Curr Opin Cell Biol **8:**773–780. http://dx.doi.org/10.1016/S0955-0674(96)80077-9.
174. **Sansam CG, Roberts CW.** 2006. Epigenetics and cancer: altered chromatin remodeling via Snf5 loss leads to aberrant cell cycle regulation. Cell Cycle **5:**621–624. http://dx.doi.org/10.4161/cc.5.6.2579.
175. **Duerr H, Flaus A, Owen-Hughes T, Hopfner KP.** 2006. Snf2 family ATPases and DExx box helicases: differences and unifying concepts from high-resolution crystal structures. Nucleic Acids Res **34:**4160–4167. http://dx.doi.org/10.1093/nar/gkl540.
176. **Muchardt C, Yaniv M.** 2001. When the SWI/SNF complex remodels.the cell cycle. Oncogene **20:**3067–3075. http://dx.doi.org/10.1038/sj.onc.1204331.
177. **Flaus A, Martin DM, Barton GJ, Owen-Hughes T.** 2006. Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Res **34:**2887–2905. http://dx.doi.org/10.1093/nar/gkl295.
178. **Holstege FC, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA.** 1998. Dissecting the regulatory circuitry of a eukaryotic genome. Cell **95:**717–728. http://dx.doi.org/10.1016/S0092-8674(00)81641-4.
179. **Sudarsanam P, Iyer VR, Brown PO, Winston F.** 2000. Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A **97:**3364–3369. http://dx.doi.org/10.1073/pnas.97.7.3364.
180. **Geng F, Cao Y, Laurent BC.** 2001. Essential roles of Snf5p in Snf-Swi chromatin remodeling in vivo. Mol Cell Biol **21:**4311–4320. http://dx.doi.org/10.1128/MCB.21.13.4311-4320.2001.
181. **Cao Y, Cairns BR, Kornberg RD, Laurent BC.** 1997. Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. Mol Cell Biol **17:**3323–3334. http://dx.doi.org/10.1128/MCB.17.6.3323.
182. **Reeves R.** 2010. Nuclear functions of the HMG proteins. Biochim Biophys Acta **1799:**3–14. http://dx.doi.org/10.1016/j.bbagrm.2009.09.001.
183. **Bustin M.** 2001. Revised nomenclature for high mobility group (HMG) chromosomal proteins. Trends Biochem Sci **26:**152–153.
184. **Bourachot B, Yaniv M, Muchardt C.** 1999. The activity of mammalian brm/SNF2α is dependent on a high-mobility-group protein I/Y-like DNA binding domain. Mol Cell Biol **19:**3931–3939. http://dx.doi.org/10.1128/MCB.19.6.3931.
185. **Zhang Q, Wang Y.** 2010. HMG modifications and nuclear function. Biochim Biophys Acta **1799:**28–36. http://dx.doi.org/10.1016/j.bbagrm.2009.11.009.
186. **Stros M.** 2010. HMGB proteins: interactions with DNA and chromatin. Biochim Biophys Acta **1799:**101–113. http://dx.doi.org/10.1016/j.bbagrm.2009.09.008.
187. **Workman JL, Kingston RE.** 1998. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem **67:** 545–579. http://dx.doi.org/10.1146/annurev.biochem.67.1.545.
188. **Sobel RE, Cook RG, Perry CA, Annunziato AT, Allis CD.** 1995. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. Proc Natl Acad Sci U S A **92:**1237–1241. http://dx.doi.org/10.1073/pnas.92.4.1237.
189. **Howe L, Auston D, Grant P, John S, Cook RG, Workman JL, Pillus L.** 2001. Histone H3 specific acetyltransferases are essential for cell cycle progression. Genes Dev **15:**3144–3154. http://dx.doi.org/10.1101/gad.931401.
190. **Suka N, Luo K, Grunstein M.** 2002. Sir2p and Sas2p opposingly regulate acetylation of yeast histone H4 lysine16 and spreading of heterochromatin. Nat Genet **32:**378–383. http://dx.doi.org/10.1038/ng1017.
191. **Eriksson PR, Ganguli D, Clark DJ.** 2011. Spt10 and Swi4 control the timing of histone H2A/H2B gene activation in budding yeast. Mol Cell Biol **31:**557–572. http://dx.doi.org/10.1128/MCB.00909-10.
192. **Dollard C, Ricupero-Hovasse SL, Natsoulis G, Boeke JD, Winston F.** 1994. SPT10 and SPT21 are required for transcription of particular histone genes in *Saccharomyces cerevisiae*. Mol Cell Biol **14:**5223–5228. http://dx.doi.org/10.1128/MCB.14.8.5223.
193. **Driscoll R, Hudson A, Jackson SP.** 2007. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science **315:** 649–652. http://dx.doi.org/10.1126/science.1135862.
194. **Han J, Zhou H, Horazdovsky B, Zhang K, Xu RM, Zhang Z.** 2007.

Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. Science **315:**653–655. http://dx.doi.org/10.1126/science.1133234.
195. **Ohkawa N, Sugisaki S, Tokunaga E, Fujitani K, Hayasaka T, Setou M, Inokuchi K.** 2008. N-acetyltransferase ARD1-NAT1 regulates neuronal dendritic development. Genes Cells **13:**1171–1183.
196. **Starheim KK, Arnesen T, Gromyko D, Ryningen A, Varhaug JE, Lillehaug JR.** 2008. Identification of the human N(alpha)-acetyltransferase complex B (hNatB): a complex important for cell-cycle progression. Biochem J **415:**325–331. http://dx.doi.org/10.1042/BJ20080658.
197. **Tercero JC, Dinman JD, Wickner RB.** 1993. Yeast MAK3 N-acetyltransferase recognizes the N-terminal four amino acids of the major coat protein (gag) of the L-A double-stranded RNA virus. J Bacteriol **175:**3192–3194.
198. **Haynes SR, Dollard C, Winston F, Beck S, Trowsdale J, Dawid IB.** 1992. The bromodomain: a conserved sequence found in human, Drosophila and yeast proteins. Nucleic Acids Res **20:**2603. http://dx.doi.org/10.1093/nar/20.10.2603.
199. **Sterner DE, Grant PA, Roberts SM, Duggan LJ, Belotserkovskaya R, Pacella LA, Winston F, Workman JL, Berger SL.** 1999. Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol Cell Biol **19:**86–98. http://dx.doi.org/10.1128/MCB.19.1.86.
200. **Jenuwein T, Allis CD.** 2001. Translating the histone code. Science **293:**1074–1080. http://dx.doi.org/10.1126/science.1063127.
201. **Florence B, Faller DV.** 2001. You bet-cha: a novel family of transcriptional regulators. Front Biosci **6:**D1008–D1018. http://dx.doi.org/10.2741/Florence.
202. **Matangkasombut O, Buratowski RM, Swilling NW, Buratowski S.** 2000. Bromodomain factor 1 corresponds to a missing piece of yeast TFIID. Genes Dev **14:**951–962.
203. **Durant M, Pugh BF.** 2006. Genome-wide relationships between TAF1 and histone acetyltransferases in *Saccharomyces cerevisiae*. Mol Cell Biol **26:**2791–2802. http://dx.doi.org/10.1128/MCB.26.7.2791-2802.2006.
204. **Wu PY, Winston F.** 2002. Analysis of Spt7 function in the *Saccharomyces cerevisiae* SAGA coactivator complex. Mol Cell Biol **22:**5367–5379. http://dx.doi.org/10.1128/MCB.22.15.5367-5379.2002.
205. **Kasten M, Szerlong H, Erdjument-Bromage H, Tempst P, Werner M, Cairns BR.** 2004. Tandem bromodomains in the chromatin remodeler RSC recognize acetylated histone H3 Lys14. EMBO J **23:**1348–1359. http://dx.doi.org/10.1038/sj.emboj.7600143.
206. **Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M.** 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. Nature **418:**387–391. http://dx.doi.org/10.1038/nature00935.
207. **Mellor J.** 2006. It takes a PHD to read the histone code. Cell **126:**22–24. http://dx.doi.org/10.1016/j.cell.2006.06.028.
208. **Li H, Ilin S, Wang W, Duncan EM, Wysocka J, Allis CD, Patel DJ.** 2006. Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. Nature **442:**91–95.
209. **Pena PV, Davrazou F, Shi X, Walter KL, Verkhusha VV, Gozani O, Zhao R, Kutateladze TG.** 2006. Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. Nature **442:**100–103.
210. **Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, Kauer M, Tackett AJ, Chait BT, Badenhorst P, Wu C, Allis CD.** 2006. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature **442:**86–90.
211. **Martin DG, Baetz K, Shi X, Walter KL, MacDonald VE, Wlodarski MJ, Gozani O, Hieter P, Howe L.** 2006. The Yng1p plant homeodomain finger is a methyl-histone binding module that recognizes lysine 4-methylated histone H3. Mol Cell Biol **26:**7871–7879. http://dx.doi.org/10.1128/MCB.00573-06.
212. **Galarneau L, Nourani A, Boudreault AA, Zhang Y, Heliot L, Allard S, Savard J, Lane WS, Stillman DJ, Cote J.** 2000. Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. Mol Cell **5:**927–937. http://dx.doi.org/10.1016/S1097-2765(00)80258-0.
213. **Gildea JJ, Lopez R, Shearn A.** 2000. A screen for new trithorax group genes identified little imaginal discs, the *Drosophila melanogaster* homologue of human retinoblastoma binding protein 2. Genetics **156:**645–663.
214. **Boyer LA, Latek RR, Peterson CL.** 2004. The SANT domain: a unique histone-tail-binding module? Nat Rev Mol Cell Biol **5:**158–163. http://dx.doi.org/10.1038/nrm1314.
215. **Aasland R, Stewart AF, Gibson T.** 1996. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. Trends Biochem Sci **21:**87–88.
216. **Boyer LA, Langer MR, Crowley KA, Tan S, Denu JM, Peterson CL.** 2002. Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. Mol Cell **10:**935–942. http://dx.doi.org/10.1016/S1097-2765(02)00634-2.
217. **Yu J, Li Y, Ishizuka T, Guenther MG, Lazar MA.** 2003. A SANT motif in the SMRT corepressor interprets the histone code and promotes histone deacetylation. EMBO J **22:**3403–3410. http://dx.doi.org/10.1093/emboj/cdg326.
218. **Hebbes TR, Thorne AW, Crane-Robinson C.** 1988. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J **7:**1395–1402.
219. **Chen ZJ, Pikaard CS.** 1997. Epigenetic silencing of RNA polymerase I transcription: a role for DNA methylation and histone modification in nucleolar dominance. Genes Dev **11:**2124–2136. http://dx.doi.org/10.1101/gad.11.16.2124.
220. **Chua YL, Watson LA, Gray JC.** 2003. The transcriptional enhancer of the pea plastocyanin gene associates with the nuclear matrix and regulates gene expression through histone acetylation. Plant Cell **15:**1468–1479. http://dx.doi.org/10.1105/tpc.011825.
221. **Fuchs J, Demidov D, Houben A, Schubert I.** 2006. Chromosomal histone modification patterns–from conservation to diversity. Trends Plant Sci **11:**199–208. http://dx.doi.org/10.1016/j.tplants.2006.02.008.
222. **Lusser A, Brosch G, Loidl A, Haas H, Loidl P.** 1997. Identification of maize histone deacetylase HD2 as an acidic nucleolar phosphoprotein. Science **277:**88–91. http://dx.doi.org/10.1126/science.277.5322.88.
223. **Wu J, Grunstein M.** 2000. 25 years after the nucleosome model: chromatin modifications. Trends Biochem Sci **25:**619–623. http://dx.doi.org/10.1016/S0968-0004(00)01718-7.
224. **Dangl M, Brosch G, Haas H, Loidl P, Lusser A.** 2001. Comparative analysis of HD2 type histone deacetylases in higher plants. Planta **213:**280–285. http://dx.doi.org/10.1007/s004250000506.
225. **Loidl P.** 2004. A plant dialect of the histone language. Trends Plant Sci **9:**84–90.
226. **Pandey R, Muller A, Napoli CA, Selinger DA, Pikaard CS, Richards EJ, Bender J, Mount DW, Jorgensen RA.** 2002. Analysis of histone acetyltransferase and histone deacetylase families of *Arabidopsis thaliana* suggests functional diversification of chromatin modification among multicellular eukaryotes. Nucleic Acids Res **30:**5036–5055. http://dx.doi.org/10.1093/nar/gkf660.
227. **Gregoretti IV, Lee YM, Goodson HV.** 2004. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. J Mol Biol **338:**17–31. http://dx.doi.org/10.1016/j.jmb.2004.02.006.
228. **Brosch G, Loidl P, Graessle S.** 2008. Histone modifications and chromatin dynamics: a focus on filamentous fungi. FEMS Microbiol Rev **32:**409–439. http://dx.doi.org/10.1111/j.1574-6976.2007.00100.x.
229. **Carmen AA, Griffin PR, Calaycay JR, Rundlett SE, Suka Y, Grunstein M.** 1999. Yeast HOS3 forms a novel trichostatin A-insensitive homodimer with intrinsic histone deacetylase activity. Proc Natl Acad Sci U S A **96:**12356–12361. http://dx.doi.org/10.1073/pnas.96.22.12356.
230. **Srikantha T, Tsai L, Daniels K, Klar AJ, Soll DR.** 2001. The histone deacetylase genes HDA1 and RPD3 play distinct roles in regulation of high-frequency phenotypic switching in *Candida albicans*. J Bacteriol **183:**4614–4625. http://dx.doi.org/10.1128/JB.183.15.4614-4625.2001.
231. **Trojer P, Brandtner EM, Brosch G, Loidl P, Galehr J, Linzmaier R, Haas H, Mair K, Tribus M, Graessle S.** 2003. Histone deacetylases in

fungi: novel members, new facts. Nucleic Acids Res **31:**3971–3981. http://dx.doi.org/10.1093/nar/gkg473.
232. **Ekwall K.** 2005. Genome-wide analysis of HDAC function. Trends Genet **21:**608–615. http://dx.doi.org/10.1016/j.tig.2005.08.009.
233. **Frye RA.** 1999. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res Commun **260:**273–279. http://dx.doi.org/10.1006/bbrc.1999.0897.
234. **Finkel T, Deng CX, Mostoslavsky R.** 2009. Recent progress in the biology and physiology of sirtuins. Nature **460:**587–591. http://dx.doi.org/10.1038/nature08197.
235. **Haigis MC, Guarente LP.** 2006. Mammalian sirtuins-emerging roles in physiology, aging, and calorie restriction. Genes Dev **20:**2913–2921. http://dx.doi.org/10.1101/gad.1467506.
236. **Michan S, Sinclair D.** 2007. Sirtuins in mammals: insights into their biological function. Biochem J **404:**1–13. http://dx.doi.org/10.1042/BJ20070140.
237. **Rajendran R, Garva R, Krstic-Demonacos M, Demonacos C.** 2011. Sirtuins: molecular traffic lights in the crossroad of oxidative stress, chromatin remodeling, and transcription. J Biomed Biotechnol **2011:** 368276.
238. **Imai S, Armstrong CM, Kaeberlein M, Guarente L.** 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature **403:**795–800. http://dx.doi.org/10.1038/35001622.
239. **Xu F, Zhang Q, Zhang K, Xie W, Grunstein M.** 2007. Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. Mol Cell **27:**890–900. http://dx.doi.org/10.1016/j.molcel.2007.07.021.
240. **Rine J, Herskowitz I.** 1987. Four genes responsible for a position effect on expression from HML and HMR in Saccharomyces cerevisiae. Genetics **116:**9–22.
241. **Gottlieb S, Esposito RE.** 1989. A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. Cell **56:**771–776. http://dx.doi.org/10.1016/0092-8674(89)90681-8.
242. **Smith JS, Boeke JD.** 1997. An unusual form of transcriptional silencing in yeast ribosomal DNA. Genes Dev **11:**241–254. http://dx.doi.org/10.1101/gad.11.2.241.
243. **Frye RA.** 2000. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun **273:**793–798. http://dx.doi.org/10.1006/bbrc.2000.3000.
244. **Smith KM, Kothe GO, Matsen CB, Khlafallah TK, Adhvaryu KK, Hemphill M, Freitag M, Motamedi MR, Selker EU.** 2008. The fungus *Neurospora crassa* displays telomeric silencing mediated by multiple sirtuins and by methylation of histone H3 lysine 9. Epigenetics Chromatin **1:**5. http://dx.doi.org/10.1186/1756-8935-1-5.
245. **Pal S, Sif S.** 2007. Interplay between chromatin remodelers and protein arginine methyltransferases. J Cell Physiol **213:**306–315. http://dx.doi.org/10.1002/jcp.21180.
246. **Nguyen AT, Zhang Y.** 2011. The diverse functions of Dot1 and H3K79 methylation. Genes Dev **25:**1345–1358. http://dx.doi.org/10.1101/gad.2057811.
247. **Cedar H, Bergman Y.** 2009. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet **10:**295–304. http://dx.doi.org/10.1038/nrg2540.
248. **Qian C, Zhou MM.** 2006. SET domain protein lysine methyltransferases: structure, specificity and catalysis. Cell Mol Life Sci **63:**2755–2763. http://dx.doi.org/10.1007/s00018-006-6274-5.
249. **Cheng X, Zhang X.** 2007. Structural dynamics of protein lysine methylation and demethylation. Mutat Res **618:**102–115. http://dx.doi.org/10.1016/j.mrfmmm.2006.05.041.
250. **Zhang X, Tamaru H, Khan SI, Horton JR, Keefe LJ, Selker EU, Cheng X.** 2002. Structure of the *Neurospora* SET domain protein DIM-5, a histone H3 lysine methyltransferase. Cell **111:**117–127. http://dx.doi.org/10.1016/S0092-8674(02)00999-6.
251. **Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, Zhang Y.** 2002. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol **12:**1052–1058. http://dx.doi.org/10.1016/S0960-9822(02)00901-6.
252. **Lacoste N, Utley RT, Hunter JM, Poirier GG, Cote J.** 2002. Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. J Biol Chem **277:**30421–30424. http://dx.doi.org/10.1074/jbc.C200366200.
253. **Bachand F.** 2007. Protein arginine methyltransferases: from unicellular eukaryotes to humans. Eukaryot Cell **6:**889–898. http://dx.doi.org/10.1128/EC.00099-07.
254. **Gary JD, Lin WJ, Yang MC, Herschman HR, Clarke S.** 1996. The predominant protein-arginine methyltransferase from *Saccharomyces cerevisiae*. J Biol Chem **271:**12585–12594. http://dx.doi.org/10.1074/jbc.271.21.12585.
255. **Pawlak MR, Scherer CA, Chen J, Roshon MJ, Ruley HE.** 2000. Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable. Mol Cell Biol **20:**4859–4869. http://dx.doi.org/10.1128/MCB.20.13.4859-4869.2000.
256. **Yu MC, Lamming DW, Eskin JA, Sinclair DA, Silver PA.** 2006. The role of protein arginine methylation in the formation of silent chromatin. Genes Dev **20:**3249–3254. http://dx.doi.org/10.1101/gad.1495206.
257. **Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P, Sif S.** 2004. Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol **24:**9630–9645. http://dx.doi.org/10.1128/MCB.24.21.9630-9645.2004.
258. **Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y.** 2004. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell **119:**941–953. http://dx.doi.org/10.1016/j.cell.2004.12.012.
259. **Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y.** 2006. Histone demethylation by a family of JmjC domain-containing proteins. Nature **439:**811–816.
260. **Klose RJ, Kallin EM, Zhang Y.** 2006. JmjC-domain-containing proteins and histone demethylation. Nat Rev Genet **7:**715–727.
261. **Mosammaparast N, Shi Y.** 2010. Reversal of histone methylation: biochemical and molecular mechanisms of histone demethylases. Annu Rev Biochem **79:**155–179. http://dx.doi.org/10.1146/annurev.biochem.78.070907.103946.
262. **Chen X, Hu Y, Zhou DX.** 2011. Epigenetic gene regulation by plant Jumonji group of histone demethylase. Biochim Biophys Acta **1809:** 421–426. http://dx.doi.org/10.1016/j.bbagrm.2011.03.004.
263. **Benevolenskaya EV.** 2007. Histone H3K4 demethylases are essential in development and differentiation. Biochem Cell Biol **85:**435–443. http://dx.doi.org/10.1139/O07-057.
264. **Doenecke D.** 2014. Chromatin dynamics from S-phase to mitosis: contributions of histone modifications. Cell Tissue Res **356:**467–475. http://dx.doi.org/10.1007/s00441-014-1873-1.
265. **Sawicka A, Seiser C.** 2012. Histone H3 phosphorylation: a versatile chromatin modification for different occasions. Biochimie **94:**2193–2201. http://dx.doi.org/10.1016/j.biochi.2012.04.018.
266. **Dai J, Sultan S, Taylor SS, Higgins JM.** 2005. The kinase haspin is required for mitotic histone H3 Thr 3 phosphorylation and normal metaphase chromosome alignment. Genes Dev **19:**472–488. http://dx.doi.org/10.1101/gad.1267105.
267. **Hendzel MJ, Wei Y, Mancini MA, Van Hooser A, Ranalli T, Brinkley BR, Bazett-Jones DP, Allis CD.** 1997. Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G_2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. Chromosoma **106:**348–360. http://dx.doi.org/10.1007/s004120050256.
268. **Lo WS, Duggan L, Emre NC, Belotserkovskya R, Lane WS, Shiekhattar R, Berger SL.** 2001. Snf1: a histone kinase that works in concert with the histone acetyltransferase Gcn5 to regulate transcription. Science **293:**1142–1146. http://dx.doi.org/10.1126/science.1062322.
269. **Sawicka A, Seiser C.** 2014. Sensing core histone phosphorylation: a matter of perfect timing. Biochim Biophys Acta **1839:**711–718. http://dx.doi.org/10.1016/j.bbagrm.2014.04.013.
270. **Giet R, Glover DM.** 2001. *Drosophila* aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. J Cell Biol **152:**669–682. http://dx.doi.org/10.1083/jcb.152.4.669.
271. **Higgins JM.** 2010. Haspin: a newly discovered regulator of mitotic chromosome behavior. Chromosoma **119:**137–147. http://dx.doi.org/10.1007/s00412-009-0250-4.
272. **Hsu JY, Sun ZW, Li X, Reuben M, Tatchell K, Bishop DK, Grushcow JM, Brame CJ, Caldwell JA, Hunt DF, Lin R, Smith MM, Allis CD.** 2000. Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora

kinase and Glc7/PP1 phosphatase in budding yeast and nematodes. Cell **102:**279–291. http://dx.doi.org/10.1016/S0092-8674(00)00034-9.
273. **Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD.** 2005. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature **438:**1116–1122. http://dx.doi.org/10.1038/nature04219.
274. **De Souza CP, Osmani AH, Wu LP, Spotts JL, Osmani SA.** 2000. Mitotic histone H3 phosphorylation by the NIMA kinase in Aspergillus nidulans. Cell **102:**293–302. http://dx.doi.org/10.1016/S0092-8674(00)00035-0.
275. **Happel N, Stoldt S, Schmidt B, Doenecke D.** 2009. M phase-specific phosphorylation of histone H1.5 at threonine 10 by GSK-3. J Mol Biol **386:**339–350. http://dx.doi.org/10.1016/j.jmb.2008.12.047.
276. **Abate G, Bastonini E, Braun KA, Verdone L, Young ET, Caserta M.** 2012. Snf1/AMPK regulates Gcn5 occupancy, H3 acetylation and chromatin remodelling at S. cerevisiae ADY2 promoter. Biochim Biophys Acta **1819:**419–427. http://dx.doi.org/10.1016/j.bbagrm.2012.01.009.
277. **Lo WS, Gamache ER, Henry KW, Yang D, Pillus L, Berger SL.** 2005. Histone H3 phosphorylation can promote TBP recruitment through distinct promoter-specific mechanisms. EMBO J **24:**997–1008. http://dx.doi.org/10.1038/sj.emboj.7600577.
278. **Cziferszky A, Seiboth B, Kubicek CP.** 2003. The Snf1 kinase of the filamentous fungus *Hypocrea jecorina* phosphorylates regulation-relevant serine residues in the yeast carbon catabolite repressor Mig1 but not in the filamentous fungal counterpart Cre1. Fungal Genet Biol **40:**166–175. http://dx.doi.org/10.1016/S1087-1845(03)00082-3.
279. **Robzyk K, Recht J, Osley MA.** 2000. Rad6-dependent ubiquitination of histone H2B in yeast. Science **287:**501–504. http://dx.doi.org/10.1126/science.287.5452.501.
280. **Weake VM, Workman JL.** 2008. Histone ubiquitination: triggering gene activity. Mol Cell **29:**653–663. http://dx.doi.org/10.1016/j.molcel.2008.02.014.
281. **Wright DE, Wang CY, Kao CF.** 2012. Histone ubiquitylation and chromatin dynamics. Front Biosci **17:**1051–1078. http://dx.doi.org/10.2741/3973.
282. **Hammond-Martel I, Yu H, el Affar, B.** 2012. Roles of ubiquitin signaling in transcription regulation. Cell Signal **24:**410–421. http://dx.doi.org/10.1016/j.cellsig.2011.10.009.
283. **Wood A, Krogan NJ, Dover J, Schneider J, Heidt J, Boateng MA, Dean K, Golshani A, Zhang Y, Greenblatt JF, Johnston M, Shilatifard A.** 2003. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell **11:**267–274. http://dx.doi.org/10.1016/S1097-2765(02)00802-X.
284. **Soshi T, Sakuraba Y, Kafer E, Inoue H.** 1996. The *mus*-8 gene of *Neurospora crassa* encodes a structural and functional homolog of the Rad6 protein of *Saccharomyces cerevisiae*. Curr Genet **30:**224–231. http://dx.doi.org/10.1007/s002940050125.
285. **Melchior F.** 2000. SUMO-nonclassical ubiquitin. Annu Rev Cell Dev Biol **16:**591–626. http://dx.doi.org/10.1146/annurev.cellbio.16.1.591.
286. **Shiio Y, Eisenman RN.** 2003. Histone sumoylation is associated with transcriptional repression. Proc Natl Acad Sci U S A **100:**13225–13230. http://dx.doi.org/10.1073/pnas.1735528100.
287. **Zhao X, Blobel G.** 2005. A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc Natl Acad Sci U S A **102:**4777–4782. http://dx.doi.org/10.1073/pnas.0500537102.
288. **Johnson ES, Gupta AA.** 2001. An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell **106:**735–744. http://dx.doi.org/10.1016/S0092-8674(01)00491-3.
289. **Takahashi Y, Kikuchi Y.** 2005. Yeast PIAS-type Ull1/Siz1 is composed of SUMO ligase and regulatory domains. J Biol Chem **280:**35822–35828. http://dx.doi.org/10.1074/jbc.M506794200.
290. **Nathan D, Ingvarsdottir K, Sterner DE, Bylebyl GR, Dokmanovic M, Dorsey JA, Whelan KA, Krsmanovic M, Lane WS, Meluh PB, Johnson ES, Berger SL.** 2006. Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic interplay with positive-acting histone modifications. Genes Dev **20:**966–976. http://dx.doi.org/10.1101/gad.1404206.
291. **Fahie K, Hu P, Swatkoski S, Cotter RJ, Zhang Y, Wolberger C.** 2009. Side chain specificity of ADP-ribosylation by a sirtuin. FEBS J **276:**7159–7176. http://dx.doi.org/10.1111/j.1742-4658.2009.07427.x.
292. **Hottiger MO, Boothby M, Koch-Nolte F, Luscher B, Martin NM, Plummer R, Wang ZQ, Ziegler M.** 2011. Progress in the function and regulation of ADP-ribosylation. Sci Signal **4:**mr5.
293. **Karras GI, Kustatscher G, Buhecha HR, Allen MD, Pugieux C, Sait F, Bycroft M, Ladurner AG.** 2005. The macro domain is an ADP-ribose binding module. EMBO J **24:**1911–1920. http://dx.doi.org/10.1038/sj.emboj.7600664.
294. **Messner S, Altmeyer M, Zhao H, Pozivil A, Roschitzki B, Gehrig P, Rutishauser D, Huang D, Caflisch A, Hottiger MO.** 2010. PARP1 ADP-ribosylates lysine residues of the core histone tails. Nucleic Acids Res **38:**6350–6362. http://dx.doi.org/10.1093/nar/gkq463.
295. **Hassa PO, Haenni SS, Elser M, Hottiger MO.** 2006. Nuclear ADP-ribosylation reactions in mammalian cells: where are we today and where are we going? Microbiol Mol Biol Rev **70:**789–829. http://dx.doi.org/10.1128/MMBR.00040-05.
296. **Schreiber V, Dantzer F, Ame JC, de Murcia G.** 2006. Poly(ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol **7:**517–528. http://dx.doi.org/10.1038/nrm1963.
297. **Mehrotra P, Riley JP, Patel R, Li F, Voss L, Goenka S.** 2011. PARP-14 functions as a transcriptional switch for Stat6-dependent gene activation. J Biol Chem **286:**1767–1776. http://dx.doi.org/10.1074/jbc.M110.157768.
298. **Hottiger MO, Hassa PO, Luscher B, Schuler H, Koch-Nolte F.** 2010. Toward a unified nomenclature for mammalian ADP-ribosyltransferases. Trends Biochem Sci **35:**208–219. http://dx.doi.org/10.1016/j.tibs.2009.12.003.
299. **Ladurner AG.** 2003. Inactivating chromosomes: a macro domain that minimizes transcription. Mol Cell **12:**1–3. http://dx.doi.org/10.1016/S1097-2765(03)00284-3.
300. **Pehrson JR, Fried VA.** 1992. MacroH2A, a core histone containing a large nonhistone region. Science **257:**1398–1400. http://dx.doi.org/10.1126/science.1529340.
301. **Hottiger MO.** 2011. ADP-ribosylation of histones by ARTD1: an additional module of the histone code? FEBS Lett **585:**1595–1599. http://dx.doi.org/10.1016/j.febslet.2011.03.031.
302. **Rolli V, O'Farrell M, Menissier-de Murcia J, de Murcia G.** 1997. Random mutagenesis of the poly(ADP-ribose) polymerase catalytic domain reveals amino acids involved in polymer branching. Biochemistry **36:**12147–12154. http://dx.doi.org/10.1021/bi971055p.
303. **Benjamin RC, Gill DM.** 1980. Poly(ADP-ribose) synthesis in vitro programmed by damaged DNA. A comparison of DNA molecules containing different types of strand breaks. J Biol Chem **255:**10502–10508.
304. **Menissier-de Murcia J, Molinete M, Gradwohl G, Simonin F, de Murcia G.** 1989. Zinc-binding domain of poly(ADP-ribose)polymerase participates in the recognition of single strand breaks on DNA. J Mol Biol **210:**229–233. http://dx.doi.org/10.1016/0022-2836(89)90302-1.
305. **Alvarez-Gonzalez R, Watkins TA, Gill PK, Reed JL, Mendoza-Alvarez H.** 1999. Regulatory mechanisms of poly(ADP-ribose) polymerase. Mol Cell Biochem **193:**19–22. http://dx.doi.org/10.1023/A:1006979220009.
306. **Wacker DA, Ruhl DD, Balagamwala EH, Hope KM, Zhang T, Kraus WL.** 2007. The DNA binding and catalytic domains of poly(ADP-ribose) polymerase 1 cooperate in the regulation of chromatin structure and transcription. Mol Cell Biol **27:**7475–7485. http://dx.doi.org/10.1128/MCB.01314-07.
307. **Citarelli M, Teotia S, Lamb RS.** 2010. Evolutionary history of the poly(ADP-ribose) polymerase gene family in eukaryotes. BMC Evol Biol **10:**308. http://dx.doi.org/10.1186/1471-2148-10-308.
308. **Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B.** 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res **42:**D490–D495. http://dx.doi.org/10.1093/nar/gkt1178.
309. **Boraston AB, Bolam DN, Gilbert HJ, Davies GJ.** 2004. Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J **382:**769–781. http://dx.doi.org/10.1042/BJ20040892.
310. **Guillen D, Sanchez S, Rodriguez-Sanoja R.** 2010. Carbohydrate-binding domains: multiplicity of biological roles. Appl Microbiol Biotechnol **85:**1241–1249. http://dx.doi.org/10.1007/s00253-009-2331-y.
311. **Levasseur A, Drula E, Lombard V, Coutinho PM, Henrissat B.** 2013. Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnol Biofuels **6:**41. http://dx.doi.org/10.1186/1754-6834-6-41.
312. **Terwisscha van Scheltinga AC, Armand S, Kalk KH, Isogai A, Henrissat B, Dijkstra BW.** 1995. Stereochemistry of chitin hydrolysis by a plant chitinase/lysozyme and X-ray structure of a complex with allosamidin: evidence for substrate assisted catalysis. Biochemistry **34:**15619–15623. http://dx.doi.org/10.1021/bi00048a003.
313. **Lairson LL, Henrissat B, Davies GJ, Withers SG.** 2008. Glycosyltrans-

ferases: structures, functions, and mechanisms. Annu Rev Biochem **77:** 521–555. http://dx.doi.org/10.1146/annurev.biochem.76.061005.092322.
314. **Gebler J, Gilkes NR, Claeyssens M, Wilson DB, Beguin P, Wakarchuk WW, Kilburn DG, Miller RC, Jr, Warren RA, Withers SG.** 1992. Stereoselective hydrolysis catalyzed by related beta-1,4-glucanases and beta-1,4-xylanases. J Biol Chem **267:**12559–12561.
315. **Beeson WT, Phillips CM, Cate JH, Marletta MA.** 2012. Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monooxygenases. J Am Chem Soc **134:**890–892. http://dx.doi.org/10.1021/ja210657t.
316. **Book AJ, Yennamalli RM, Takasuka TE, Currie CR, Phillips GN, Jr, Fox BG.** 2014. Evolution of substrate specificity in bacterial AA10 lytic polysaccharide monooxygenases. Biotechnol Biofuels **7:**109. http://dx.doi.org/10.1186/1754-6834-7-109.
317. **Forsberg Z, Rohr AK, Mekasha S, Andersson KK, Eijsink VG, Vaaje-Kolstad G, Sorlie M.** 2014. Comparative study of two chitin-active and two cellulose-active AA10-type lytic polysaccharide monooxygenases. Biochemistry **53:**1647–1656. http://dx.doi.org/10.1021/bi5000433.
318. **Hemsworth GR, Davies GJ, Walton PH.** 2013. Recent insights into copper-containing lytic polysaccharide mono-oxygenases. Curr Opin Struct Biol **23:**660–668. http://dx.doi.org/10.1016/j.sbi.2013.05.006.
319. **Quinlan RJ, Sweeney MD, Lo Leggio L, Otten H, Poulsen JC, Johansen KS, Krogh KB, Jorgensen CI, Tovborg M, Anthonsen A, Tryfona T, Walter CP, Dupree P, Xu F, Davies GJ, Walton PH.** 2011. Insights into the oxidative degradation of cellulose by a copper metalloenzyme that exploits biomass components. Proc Natl Acad Sci U S A **108:** 15079–15084. http://dx.doi.org/10.1073/pnas.1105776108.
320. **Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sorlie M, Eijsink VG.** 2010. An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. Science **330:**219–222. http://dx.doi.org/10.1126/science.1192231.
321. **Phillips CM, Beeson WT, Cate JH, Marletta MA.** 2011. Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by *Neurospora crassa*. ACS Chem Biol **6:**1399–1406. http://dx.doi.org/10.1021/cb200351y.
322. **Hemsworth GR, Henrissat B, Davies GJ, Walton PH.** 2014. Discovery and characterization of a new family of lytic polysaccharide monooxygenases. Nat Chem Biol **10:**122–126.
323. **Vu VV, Beeson WT, Span EA, Farquhar ER, Marletta MA.** 2014. A family of starch-active polysaccharide monooxygenases. Proc Natl Acad Sci U S A **111:**13822–13827. http://dx.doi.org/10.1073/pnas.1408090111.
324. **Harris PV, Welner D, McFarland KC, Re E, Navarro Poulsen JC, Brown K, Salbo R, Ding H, Vlasenko E, Merino S, Xu F, Cherry J, Larsen S, Lo Leggio L.** 2010. Stimulation of lignocellulosic biomass hydrolysis by proteins of glycoside hydrolase family 61: structure and function of a large, enigmatic family. Biochemistry **49:**3305–3316. http://dx.doi.org/10.1021/bi100009p.
325. **Schnellmann J, Zeltins A, Blaak H, Schrempf H.** 1994. The novel lectin-like protein CHB1 is encoded by a chitin-inducible *Streptomyces olivaceoviridis* gene and binds specifically to crystalline alpha-chitin of fungi and other organisms. Mol Microbiol **13:**807–819. http://dx.doi.org/10.1111/j.1365-2958.1994.tb00473.x.
326. **Vaaje-Kolstad G, Horn SJ, van Aalten DM, Synstad B, Eijsink VG.** 2005. The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. J Biol Chem **280:**28492–284927. http://dx.doi.org/10.1074/jbc.M504468200.
327. **Yin YB, Mao XZ, Yang JC, Chen X, Mao FL, Xu Y.** 2012. dbCAN: a web resource for automated carbohydrate-active enzyme annotation. Nucleic Acids Res **40:**W445–W451. http://dx.doi.org/10.1093/nar/gks479.
328. **Foreman PK, Brown D, Dankmeyer L, Dean R, Diener S, Dunn-Coleman NS, Goedegebuur F, Houfek TD, England GJ, Kelley AS, Meerman HJ, Mitchell T, Mitchinson C, Olivares HA, Teunissen PJ, Yao J, Ward M.** 2003. Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus *Trichoderma reesei*. J Biol Chem **278:**31988–31997. http://dx.doi.org/10.1074/jbc.M304750200.
329. **Aro N, Pakula T, Penttila M.** 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol Rev **29:** 719–739. http://dx.doi.org/10.1016/j.femsre.2004.11.006.
330. **Kubicek CP, Mikus M, Schuster A, Schmoll M, Seiboth B.** 2009. Metabolic engineering strategies for improvement of cellulase production by *Hypocrea jecorina*. Biotechnol Biofuels **2:**19. http://dx.doi.org/10.1186/1754-6834-2-19.
331. **Stricker AR, Mach RL, de Graaff LH.** 2008. Regulation of transcription of cellulases- and hemicellulases-encoding genes in *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*). Appl Microbiol Biotechnol **78:** 211–220. http://dx.doi.org/10.1007/s00253-007-1322-0.
332. **Gruber S, Seidl-Seiboth V.** 2012. Self versus non-self: fungal cell wall degradation in *Trichoderma*. Microbiology **158:**26–34. http://dx.doi.org/10.1099/mic.0.052613-0.
333. **Nummi M, Niku-Paavola ML, Lappalainen A, Enari TM, Raunio V.** 1983. Cellobiohydrolase from *Trichoderma reesei*. Biochem J **215:**677–683. http://dx.doi.org/10.1042/bj2150677.
334. **Merino ST, Cherry J.** 2007. Progress and challenges in enzyme development for biomass utilization. Adv Biochem Eng Biotechnol **108:**95–120.
335. **Gruber S, Kubicek CP, Seidl-Seiboth V.** 2011. Differential regulation of orthologous chitinase genes in mycoparasitic *Trichoderma* species. Appl Environ Microbiol **77:**7217–7226. http://dx.doi.org/10.1128/AEM.06027-11.
336. **Stals I, Samyn B, Sergeant K, White T, Hoorelbeke K, Coorevits A, Devreese B, Claeyssens M, Piens K.** 2010. Identification of a gene coding for a deglycosylating enzyme in Hypocrea jecorina. FEMS Microbiol Lett **303:**9–17. http://dx.doi.org/10.1111/j.1574-6968.2009.01849.x.
337. **Dubey MK, Ubhayasekera W, Sandgren M, Jensen DF, Karlsson M.** 2012. Disruption of the *Eng18B* ENGase gene in the fungal biocontrol agent *Trichoderma atroviride* affects growth, conidiation and antagonistic ability. PLoS One **7:**e36152. http://dx.doi.org/10.1371/journal.pone.0036152.
338. **Djonovic S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM.** 2006. Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol Plant Microbe Interact **19:**838–853. http://dx.doi.org/10.1094/MPMI-19-0838.
339. **Seidl-Seiboth V, Ihrmark K, Druzhinina I, Karlsson M.** 2014. Molecular evolution of *Trichoderma* chitinases, p 67–80. *In* Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG (ed), Biotechnology and biology of *Trichoderma*, vol 1. Elsevier, Oxford, United Kingdom.
340. **Häkkinen M, Arvas M, Oja M, Aro N, Penttila M, Saloheimo M, Pakula TM.** 2012. Re-annotation of the CAZy genes of *Trichoderma reesei* and transcription in the presence of lignocellulosic substrates. Microb Cell Fact **11:**134. http://dx.doi.org/10.1186/1475-2859-11-134.
341. **Dos Santos Castro L, Pedersoli WR, Antonieto AC, Steindorff AS, Silva-Rocha R, Martinez-Rossi NM, Rossi A, Brown NA, Goldman GH, Faca VM, Persinoti GF, Silva RN.** 2014. Comparative metabolism of cellulose, sophorose and glucose in *Trichoderma reesei* using high-throughput genomic and proteomic analyses. Biotechnol Biofuels **7:**41. http://dx.doi.org/10.1186/1754-6834-7-41.
342. **Chen X, Luo Y, Yu H, Sun Y, Wu H, Song S, Hu S, Dong Z.** 2014. Transcriptional profiling of biomass degradation-related genes during *Trichoderma reesei* growth on different carbon sources. J Biotechnol **173:**59–64. http://dx.doi.org/10.1016/j.jbiotec.2014.01.011.
343. **Ries L, Pullan ST, Delmas S, Malla S, Blythe MJ, Archer DB.** 2013. Genome-wide transcriptional response of *Trichoderma reesei* to lignocellulose using RNA sequencing and comparison with *Aspergillus niger*. BMC Genomics **14:**541. http://dx.doi.org/10.1186/1471-2164-14-541.
344. **Seiboth B, Herold S, Kubicek CP.** 2012. Metabolic engineering of inducer formation for cellulase and hemicellulase gene expression in *Trichoderma reesei*. Subcell Biochem **64:**367–390. http://dx.doi.org/10.1007/978-94-007-5055-5_18.
345. **Seiboth B, Ivanova C, Seidl-Seiboth V.** 2011. *Trichoderma reesei*: a fungal enzyme producer for cellulosic biofuels, p 309–340. *In* dos Santos Bernardes MA (ed), Biofuel production: recent developments and prospects. Intech, Rijeka, Croatia.
346. **Karimi Aghcheh R, Nemeth Z, Atanasova L, Fekete E, Paholcsek M, Sandor E, Aquino B, Druzhinina IS, Karaffa L, Kubicek CP.** 2014. The VELVET A orthologue VEL1 of *Trichoderma reesei* regulates fungal development and is essential for cellulase gene expression. PLoS One **9:**e112799. http://dx.doi.org/10.1371/journal.pone.0112799.
347. **Bischof R, Fourtis L, Limbeck A, Gamauf C, Seiboth B, Kubicek CP.** 2013. Comparative analysis of the *Trichoderma reesei* transcriptome during growth on the cellulase inducing substrates wheat straw and lactose. Biotechnol Biofuels **6:**127. http://dx.doi.org/10.1186/1754-6834-6-127.
348. **Portnoy T, Margeot A, Linke R, Atanasova L, Fekete E, Sandor E,**

Hartl L, Karaffa L, Druzhinina IS, Seiboth B, Le Crom S, Kubicek CP. 2011. The CRE1 carbon catabolite repressor of the fungus *Trichoderma reesei*: a master regulator of carbon assimilation. BMC Genomics **12:**269. http://dx.doi.org/10.1186/1471-2164-12-269.

349. **Seidl V, Huemer B, Seiboth B, Kubicek CP.** 2005. A complete survey of *Trichoderma* chitinases reveals three distinct subgroups of family 18 chitinases. FEBS J **272:**5923–5939. http://dx.doi.org/10.1111/j.1742-4658.2005.04994.x.
350. **Tisch D, Kubicek CP, Schmoll M.** 2011. The phosducin-like protein PhLP1 impacts regulation of glycoside hydrolases and light response in *Trichoderma reesei*. BMC Genomics **12:**613. http://dx.doi.org/10.1186/1471-2164-12-613.
351. **Tisch D, Schmoll M.** 2013. Targets of light signalling in *Trichoderma reesei*. BMC Genomics **14:**657. http://dx.doi.org/10.1186/1471-2164-14-657.
352. **Metz B, Seidl-Seiboth V, Haarmann T, Kopchinskiy A, Lorenz P, Seiboth B, Kubicek CP.** 2011. Expression of biomass-degrading enzymes is a major event during conidium cevelopment in *Trichoderma reesei*. Eukaryot Cell **10:**1527–1535. http://dx.doi.org/10.1128/EC.05014-11.
353. **Kubicek CP, Mühlbauer G, Grotz M, John E, Kubicek-Pranz EM.** 1988. Properties of the conidial-bound cellulase system of *Trichoderma reesei*. J Gen Microbiol **134:**1215–1222.
354. **Messner R, Gruber F, Kubicek CP.** 1988. Differential regulation of synthesis of multiple forms of specific endoglucanases by *Trichoderma reesei* QM9414. J Bacteriol **170:**3689–3693.
355. **Reithner B, Ibarra-Laclette E, Mach RL, Herrera-Estrella A.** 2011. Identification of mycoparasitism-related genes in *Trichoderma atroviride*. Appl Environ Microbiol **77:**4361–4370. http://dx.doi.org/10.1128/AEM.00129-11.
356. **Vizcaino JA, Redondo J, Suarez MB, Cardoza RE, Hermosa R, Gonzalez FJ, Rey M, Monte E.** 2007. Generation, annotation, and analysis of ESTs from four different *Trichoderma* strains grown under conditions related to biocontrol. Appl Microbiol Biotechnol **75:**853–862. http://dx.doi.org/10.1007/s00253-007-0885-0.
357. **Marzluf GA.** 1997. Genetic regulation of nitrogen metabolism in the fungi. Microbiol Mol Biol Rev **61:**17–32.
358. **Arst HN, Jr, Scazzocchio C.** 1985. Formal genetics and molecular biology of the control of gene expression in *Aspergillus nidulans*, p 309–343. *In* Bennett J, Lasure L (ed), Gene manipulations in fungi. Academic Press, Inc., Orlando, FL.
359. **Cove DJ.** 1979. Genetic studies of nitrate assimilation in *Aspergillus nidulans*. Biol Rev Camb Philos Soc **54:**291–327. http://dx.doi.org/10.1111/j.1469-185X.1979.tb01014.x.
360. **Marzluf GA.** 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol Rev **45:**437–461.
361. **Hofman-Bang J.** 1999. Nitrogen catabolite repression in *Saccharomyces cerevisiae*. Mol Biotechnol **12:**35–73. http://dx.doi.org/10.1385/MB:12:1:35.
362. **ter Schure EG, van Riel NA, Verrips CT.** 2000. The role of ammonia metabolism in nitrogen catabolite repression in *Saccharomyces cerevisiae*. FEMS Microbiol Rev **24:**67–83.
363. **Wiame JM, Grenson M, Arst HN, Jr.** 1985. Nitrogen catabolite repression in yeasts and filamentous fungi. Adv Microb Physiol **26:**1–88. http://dx.doi.org/10.1016/S0065-2911(08)60394-X.
364. **Olsson S.** 1999. Nutrient translocation and electrical signaling, p 25–48. *In* Gow NARR, GM, Gadd GM (ed), The fungal colony. Cambridge University Press, Cambridge, United Kingdom.
365. **Watkinson S.** 1999. Metabolism and hyphal differentiation in large basidiomycete colonies, p 126–156. *In* Gow NAR, Robson GD, Gadd GM (ed), The fungal colony. Cambridge University Press, Cambridge, United Kingdom.
366. **Steyaert JM, Weld RJ, Stewart A.** 2010. Isolate-specific conidiation in *Trichoderma* in response to different nitrogen sources. Fungal Biol **114:**179–188. http://dx.doi.org/10.1016/j.funbio.2009.12.002.
367. **Olmedo-Monfil V, Mendoza-Mendoza A, Gomez I, Cortes C, Herrera-Estrella A.** 2002. Multiple environmental signals determine the transcriptional activation of the mycoparasitism related gene *prb1* in *Trichoderma atroviride*. Mol Genet Genomics **267:**703–712. http://dx.doi.org/10.1007/s00438-002-0703-4.
368. **Carsolio C, Gutierrez A, Jimenez B, Van Montagu M, Herrera-Estrella A.** 1994. Characterization of *ech-42*, a *Trichoderma harzianum* endochitinase gene expressed during mycoparasitism. Proc Natl Acad Sci U S A **91:**10903–10907. http://dx.doi.org/10.1073/pnas.91.23.10903.
369. **de las Mercedes Dana M, Limon MC, Mejias R, Mach RL, Benitez T, Pintor-Toro JA, Kubicek CP.** 2001. Regulation of chitinase 33 (*chit33*) gene expression in *Trichoderma harzianum*. Curr Genet **38:**335–342. http://dx.doi.org/10.1007/s002940000169.
370. **Mach RL, Peterbauer CK, Payer K, Jaksits S, Woo SL, Zeilinger S, Kullnig CM, Lorito M, Kubicek CP.** 1999. Expression of two major chitinase genes of *Trichoderma atroviride* (*T. harzianum* P1) is triggered by different regulatory signals. Appl Environ Microbiol **65:**1858–1863.
371. **Crawford NM, Arst HN, Jr.** 1993. The molecular genetics of nitrate assimilation in fungi and plants. Annu Rev Genet **27:**115–146. http://dx.doi.org/10.1146/annurev.ge.27.120193.000555.
372. **Fu YH, Marzluf GA.** 1987. Molecular cloning and analysis of the regulation of *nit-3*, the structural gene for nitrate reductase in *Neurospora crassa*. Proc Natl Acad Sci U S A **84:**8243–8247. http://dx.doi.org/10.1073/pnas.84.23.8243.
373. **Johnstone IL, McCabe PC, Greaves P, Gurr SJ, Cole GE, Brow MA, Unkles SE, Clutterbuck AJ, Kinghorn JR, Innis MA.** 1990. Isolation and characterisation of the *crnA-niiA-niaD* gene cluster for nitrate assimilation in *Aspergillus nidulans*. Gene **90:**181–192. http://dx.doi.org/10.1016/0378-1119(90)90178-T.
374. **Premakumar R, Sorger GJ, Gooden D.** 1979. Nitrogen metabolite repression of nitrate reductase in *Neurospora crassa*. J Bacteriol **137:**1119–1126.
375. **Garrett RH.** 1972. The induction of nitrite reductase in *Neurospora crassa*. Biochim Biophys Acta **264:**481–489. http://dx.doi.org/10.1016/0304-4165(72)90011-6.
376. **Campbell WH, Kinghorn KR.** 1990. Functional domains of assimilatory nitrate reductases and nitrite reductases. Trends Biochem Sci **15:**315–319. http://dx.doi.org/10.1016/0968-0004(90)90021-3.
377. **Brownlee AG, Arst HN, Jr.** 1983. Nitrate uptake in *Aspergillus nidulans* and involvement of the third gene of the nitrate assimilation gene cluster. J Bacteriol **155:**1138–1146.
378. **Tomsett AB, Cove DJ.** 1979. Deletion mapping of the *niiA niaD* gene region of *Aspergillus nidulans*. Genet Res **34:**19–32. http://dx.doi.org/10.1017/S001667230001925X.
379. **Unkles SE, Hawker KL, Grieve C, Campbell EI, Montague P, Kinghorn JR.** 1991. *crnA* encodes a nitrate transporter in *Aspergillus nidulans*. Proc Natl Acad Sci U S A **88:**204–208. http://dx.doi.org/10.1073/pnas.88.1.204.
380. **Hurlburt BK, Garrett RH.** 1988. Nitrate assimilation in *Neurospora crassa:* enzymatic and immunoblot analysis of wild-type and nit mutant protein products in nitrate-induced and glutamine-repressed cultures. Mol Gen Genet **211:**35–40. http://dx.doi.org/10.1007/BF00338390.
381. **Okamoto PM, Fu YH, Marzluf GA.** 1991. *nit-3*, the structural gene of nitrate reductase in *Neurospora crassa*: nucleotide sequence and regulation of mRNA synthesis and turnover. Mol Gen Genet **227:**213–223.
382. **Gao-Rubinelli F, Marzluf GA.** 2004. Identification and characterization of a nitrate transporter gene in *Neurospora crassa*. Biochem Genet **42:**21–34. http://dx.doi.org/10.1023/B:BIGI.0000012141.51114.23.
383. **Sorger GJ.** 1965. Simultaneous induction and repression of nitrate reductase and Tpnh cytochrome reductase in *Neurospora crassa*. Biochim Biophys Acta **99:**234–245. http://dx.doi.org/10.1016/S0926-6593(65)80120-5.
384. **Sorger GJ, Giles NH.** 1965. Genetic control of nitrate reductase in *Neurospora crassa*. Genetics **52:**777–788.
385. **Burger G, Strauss J, Scazzocchio C, Lang BF.** 1991. *nirA*, the pathway-specific regulatory gene of nitrate assimilation in *Aspergillus nidulans*, encodes a putative GAL4-type zinc finger protein and contains four introns in highly conserved regions. Mol Cell Biol **11:**5746–5755. http://dx.doi.org/10.1128/MCB.11.11.5746.
386. **Mo X, Marzluf GA.** 2003. Cooperative action of the NIT2 and NIT4 transcription factors upon gene expression in *Neurospora crassa*. Curr Genet **42:**260–267.
387. **Narendja F, Goller SP, Wolschek M, Strauss J.** 2002. Nitrate and the GATA factor AreA are necessary for in vivo binding of NirA, the pathway-specific transcriptional activator of *Aspergillus nidulans*. Mol Microbiol **44:**573–583. http://dx.doi.org/10.1046/j.1365-2958.2002.02911.x.
388. **Young JL, Jarai G, Fu YH, Marzluf GA.** 1990. Nucleotide sequence and analysis of NMR, a negative-acting regulatory gene in the nitrogen circuit of *Neurospora crassa*. Mol Gen Genet **222:**120–128.
389. **Andrianopoulos A, Kourambas S, Sharp JA, Davis MA, Hynes MJ.**

1998. Characterization of the *Aspergillus nidulans nmrA* gene involved in nitrogen metabolite repression. J Bacteriol **180:**1973–1977.
390. **Xiao X, Fu YH, Marzluf GA.** 1995. The negative-acting NMR regulatory protein of *Neurospora crassa* binds to and inhibits the DNA-binding activity of the positive-acting nitrogen regulatory protein NIT2. Biochemistry **34:**8861–8868. http://dx.doi.org/10.1021/bi00027a038.
391. **Xiao XD, Marzluf GA.** 1993. Amino-acid substitutions in the zinc finger of NIT2, the nitrogen regulatory protein of *Neurospora crassa*, alter promoter element recognition. Curr Genet **24:**212–218. http://dx.doi.org/10.1007/BF00351794.
392. **Berger H, Pachlinger R, Morozov I, Goller S, Narendja F, Caddick M, Strauss J.** 2006. The GATA factor AreA regulates localization and in vivo binding site occupancy of the nitrate activator NirA. Mol Microbiol **59:**433–446. http://dx.doi.org/10.1111/j.1365-2958.2005.04957.x.
393. **Bernreiter A, Ramon A, Fernandez-Martinez J, Berger H, Araujo-Bazan L, Espeso EA, Pachlinger R, Gallmetzer A, Anderl I, Scazzocchio C, Strauss J.** 2007. Nuclear export of the transcription factor NirA is a regulatory checkpoint for nitrate induction in *Aspergillus nidulans*. Mol Cell Biol **27:**791–802. http://dx.doi.org/10.1128/MCB.00761-06.
394. **Lee H, Fu YH, Marzluf GA.** 1990. Nucleotide sequence and DNA recognition elements of *alc*, the structural gene which encodes allantoicase, a purine catabolic enzyme of *Neurospora crassa*. Biochemistry **29:**8779–8787. http://dx.doi.org/10.1021/bi00489a039.
395. **Reinert WR, Marzluf GA.** 1975. Genetic and metabolic control of the purine catabolic enzymes of *Neurospora crassa*. Mol Gen Genet **139:**39–55. http://dx.doi.org/10.1007/BF00267994.
396. **Suarez T, Oestreicher N, Kelly J, Ong G, Sankarsingh T, Scazzocchio C.** 1991. The *uaY* positive control gene of *Aspergillus nidulans*: fine structure, isolation of constitutive mutants and reversion patterns. Mol Gen Genet **230:**359–368. http://dx.doi.org/10.1007/BF00280292.
397. **Suarez T, Oestreicher N, Penalva MA, Scazzocchio C.** 1991. Molecular cloning of the *uaY* regulatory gene of *Aspergillus nidulans* reveals a favoured region for DNA insertions. Mol Gen Genet **230:**369–375. http://dx.doi.org/10.1007/BF00280293.
398. **Darlington AJ, Scazzocchio C.** 1967. Use of analogues and the substrate-sensitivity of mutants in analysis of purine uptake and breakdown in *Aspergillus nidulans*. J Bacteriol **93:**937–940.
399. **Diallinas G, Scazzocchio C.** 1989. A gene coding for the uric acid-xanthine permease of *Aspergillus nidulans:* inactivational cloning, characterization, and sequence of a cis-acting mutation. Genetics **122:**341–350.
400. **Valdez-Taubas J, Diallinas G, Scazzocchio C, Rosa AL.** 2000. Protein expression and subcellular localization of the general purine transporter UapC from *Aspergillus nidulans*. Fungal Genet Biol **30:**105–113. http://dx.doi.org/10.1006/fgbi.2000.1197.
401. **Gournas C, Oestreicher N, Amillis S, Diallinas G, Scazzocchio C.** 2011. Completing the purine utilisation pathway of *Aspergillus nidulans*. Fungal Genet Biol **48:**840–848. http://dx.doi.org/10.1016/j.fgb.2011.03.004.
402. **Chaleff RS.** 1974. The inducible quinate-shikimate catabolic pathway in *Neurospora crassa*: induction and regulation of enzyme synthesis. J Gen Microbiol **81:**357–372. http://dx.doi.org/10.1099/00221287-81-2-357.
403. **Chaleff RS.** 1974. The inducible quinate-shikimate catabolic pathway in *Neurospora crassa*: genetic organization. J Gen Microbiol **81:**337–355. http://dx.doi.org/10.1099/00221287-81-2-337.
404. **Liu TD, Marzluf GA.** 2004. Characterization of *pco-1*, a newly identified gene which regulates purine catabolism in *Neurospora*. Curr Genet **46:**213–227. http://dx.doi.org/10.1007/s00294-004-0530-8.
405. **Marzluf GA.** 2001. Metabolic regulation in fungi. Applied Mycol Biotechnol **1:**55–72.
406. **Griffith AB, Garrett RH.** 1988. Xanthine dehydrogenase expression in *Neurospora crassa* does not require a functional *nit-2* regulatory gene. Biochem Genet **26:**37–52. http://dx.doi.org/10.1007/BF00555487.
407. **Liaw SH, Eisenberg D.** 1994. Structural model for the reaction mechanism of glutamine synthetase, based on five crystal structures of enzyme-substrate complexes. Biochemistry **33:**675–681. http://dx.doi.org/10.1021/bi00169a007.
408. **Wedler FC, Horn BR.** 1976. Catalytic mechanisms of glutamine synthetase enzymes. Studies with analogs of possible intermediates and transition states. J Biol Chem **251:**7530–7538.
409. **Calderon J, Mora J.** 1989. Glutamine assimilation pathways in *Neurospora crassa* growing on glutamine as sole nitrogen and carbon source. J Gen Microbiol **135:**2699–2707.
410. **Mora J.** 1990. Glutamine metabolism and cycling in *Neurospora crassa*. Microbiol Rev **54:**293–304.
411. **Calderon J, Morett E, Mora J.** 1985. Omega-amidase pathway in the degradation of glutamine in *Neurospora crassa*. J Bacteriol **161:**807–809.
412. **Dunn-Coleman NS, Tomsett AB, Garrett RH.** 1981. The regulation of nitrate assimilation in *Neurospora crassa*: biochemical analysis of the *nmr-1* mutants. Mol Gen Genet **182:**234–239. http://dx.doi.org/10.1007/BF00269663.
413. **Platt A, Langdon T, Arst HN, Jr, Kirk D, Tollervey D, Sanchez JM, Caddick MX.** 1996. Nitrogen metabolite signalling involves the C terminus and the GATA domain of the *Aspergillus* transcription factor AREA and the 3′ untranslated region of its mRNA. EMBO J **15:**2791–2801.
414. **Wagner D, Wiemann P, Huss K, Brandt U, Fleissner A, Tudzynski B.** 2013. A sensing role of the glutamine synthetase in the nitrogen regulation network in *Fusarium fujikuroi*. PLoS One **8:**e80740. http://dx.doi.org/10.1371/journal.pone.0080740.
415. **Gremel G, Dorrer M, Schmoll M.** 2008. Sulphur metabolism and cellulase gene expression are connected processes in the filamentous fungus *Hypocrea jecorina* (anamorph *Trichoderma reesei*). BMC Microbiol **8:**174. http://dx.doi.org/10.1186/1471-2180-8-174.
416. **Piłsyk S, Paszewski A.** 2009. Sulfate permeases: phylogenetic diversity of sulfate transport. Acta Biochim Pol **56:**375–384.
417. **Alper SL, Sharma AK.** 2013. The SLC26 gene family of anion transporters and channels. Mol Aspects Med **34:**494–515. http://dx.doi.org/10.1016/j.mam.2012.07.009.
418. **Kertesz MA.** 2001. Bacterial transporters for sulfate and organosulfur compounds. Res Microbiol **152:**279–290. http://dx.doi.org/10.1016/S0923-2508(01)01199-8.
419. **Loughlin P, Shelden MC, Tierney ML, Howitt SM.** 2002. Structure and function of a model member of the SulP transporter family. Cell Biochem Biophys **36:**183–190. http://dx.doi.org/10.1385/CBB:36:2-3:183.
420. **Busch W, Saier MH, Jr.** 2002. The transporter classification (TC) system. Crit Rev Biochem Mol Biol **37:**287–337. http://dx.doi.org/10.1080/10409230290771528.
421. **Ketter JS, Marzluf GA.** 1988. Molecular cloning and analysis of the regulation of *cys-14*$^{+}$, a structural gene of the sulfur regulatory circuit of *Neurospora crassa*. Mol Cell Biol **8:**1504–1508. http://dx.doi.org/10.1128/MCB.8.4.1504.
422. **McGuire WG, Marzluf GA.** 1974. Developmental regulation of choline sulfatase and aryl sulfatase in *Neurospora crassa*. Arch Biochem Biophys **161:**360–368. http://dx.doi.org/10.1016/0003-9861(74)90316-6.
423. **Scott WA, Metzenberg RL.** 1970. Location of aryl sulfatase in conidia and young mycelia of *Neurospora crassa*. J Bacteriol **104:**1254–1265.
424. **Kosugi A, Koizumi Y, Yanagida F, Udaka S.** 2001. MUP1, high affinity methionine permease, is involved in cysteine uptake by *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem **65:**728–731. http://dx.doi.org/10.1271/bbb.65.728.
425. **Autry AR, Fitzgerald JW.** 1991. Potential for organic sulfur accumulation in a variety of forest soils at saturating sulfate concentrations. Biol Fertil Soils **10:**281–284. http://dx.doi.org/10.1007/BF00337379.
426. **Palmieri L, Vozza A, Agrimi G, De Marco V, Runswick MJ, Palmieri F, Walker JE.** 1999. Identification of the yeast mitochondrial transporter for oxaloacetate and sulfate. J Biol Chem **274:**22184–22190. http://dx.doi.org/10.1074/jbc.274.32.22184.
427. **Wiebers JL, Garner HR.** 1967. Homocysteine and cysteine synthetases of *Neurospora crassa*. Purification, properties, and feedback control of activity. J Biol Chem **242:**12–23.
428. **Topczewski J, Sienko M, Paszewski A.** 1997. Cloning and characterization of the *Aspergillus nidulans cysB* gene encoding cysteine synthase. Curr Genet **31:**348–356. http://dx.doi.org/10.1007/s002940050215.
429. **Grynberg M, Topczewski J, Godzik A, Paszewski A.** 2000. The *Aspergillus nidulans cysA* gene encodes a novel type of serine O-acetyltransferase which is homologous to homoserine O-acetyltransferases. Microbiology **146:**2695–2703. http://dx.doi.org/10.1099/00221287-146-10-2695.
430. **Crawford JM, Geever RF, Asch DK, Case ME.** 1992. Sequence and characterization of the *met-7* gene of *Neurospora crassa*. Gene **111:**265–266. http://dx.doi.org/10.1016/0378-1119(92)90698-O.

431. **Cossins EA, Chan PY.** 1983. Folylpolyglutamate synthetase activities of *Neurospora crassa*: nature of products formed by soluble and particulate enzymes in the wild type and polyglutamate-deficient mutants. Adv Exp Med Biol **163:**183–197. http://dx.doi.org/10.1007/978-1-4757-5241-0_15.
432. **Atkinson IJ, Nargang FE, Cossins EA.** 1995. Folylpolyglutamate synthesis in *Neurospora crassa*: transformation of polyglutamate-deficient mutants. Phytochemistry **38:**603–608. http://dx.doi.org/10.1016/0031-9422(94)00713-4.
433. **Sienko M, Natorff R, Zielinski Z, Hejduk A, Paszewski A.** 2007. Two *Aspergillus nidulans* genes encoding methylenetetrahydrofolate reductases are up-regulated by homocysteine. Fungal Genet Biol **44:**691–700. http://dx.doi.org/10.1016/j.fgb.2006.12.002.
434. **Emanuelsson O, Nielsen H, Brunak S, von Heijne G.** 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. J Mol Biol **300:**1005–1016. http://dx.doi.org/10.1006/jmbi.2000.3903.
435. **Mudd SH, Datko AH.** 1986. Methionine methyl group metabolism in lemna. Plant Physiol **81:**103–114. http://dx.doi.org/10.1104/pp.81.1.103.
436. **Kacprzak MM, Lewandowska I, Matthews RG, Paszewski A.** 2003. Transcriptional regulation of methionine synthase by homocysteine and choline in *Aspergillus nidulans*. Biochem J **376:**517–524. http://dx.doi.org/10.1042/bj20030747.
437. **Tehlivets O, Hasslacher M, Kohlwein SD.** 2004. *S*-Adenosyl-L-homocysteine hydrolase in yeast: key enzyme of methylation metabolism and coordinated regulation with phospholipid synthesis. FEBS Lett **577:**501–506. http://dx.doi.org/10.1016/j.febslet.2004.10.057.
438. **Sienko M, Natorff R, Owczarek S, Olewiecki I, Paszewski A.** 2009. *Aspergillus nidulans* genes encoding reverse transsulfuration enzymes belong to homocysteine regulon. Curr Genet **55:**561–570. http://dx.doi.org/10.1007/s00294-009-0269-3.
439. **Andersen KM, Madsen L, Prag S, Johnsen AH, Semple CA, Hendil KB, Hartmann-Petersen R.** 2009. Thioredoxin Txnl1/TRP32 is a redox-active cofactor of the 26S proteasome. J Biol Chem **284:**15246–15254. http://dx.doi.org/10.1074/jbc.M900016200.
440. **Ma YB, Zhang ZF, Shao MY, Kang KH, Shi XL, Dong YP, Li JL.** 2012. Response of sulfide:quinone oxidoreductase to sulfide exposure in the echiuran worm *Urechis unicinctus*. Mar Biotechnol (NY) **14:**245–251. http://dx.doi.org/10.1007/s10126-011-9408-1.
441. **Theissen U, Hoffmeister M, Grieshaber M, Martin W.** 2003. Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. Mol Biol Evol **20:**1564–1574. http://dx.doi.org/10.1093/molbev/msg174.
442. **Thomas D, Surdin-Kerjan Y.** 1997. Metabolism of sulfur amino acids in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev **61:**503–532.
443. **Blaiseau PL, Thomas D.** 1998. Multiple transcriptional activation complexes tether the yeast activator Met4 to DNA. EMBO J **17:**6327–6336. http://dx.doi.org/10.1093/emboj/17.21.6327.
444. **Lee TA, Jorgensen P, Bognar AL, Peyraud C, Thomas D, Tyers M.** 2010. Dissection of combinatorial control by the Met4 transcriptional complex. Mol Biol Cell **21:**456–469. http://dx.doi.org/10.1091/mbc.E09-05-0420.
445. **Paietta JV, Akins RA, Lambowitz AM, Marzluf GA.** 1987. Molecular cloning and characterization of the *cys-3* regulatory gene of *Neurospora crassa*. Mol Cell Biol **7:**2506–2511. http://dx.doi.org/10.1128/MCB.7.7.2506.
446. **Natorff R, Sienko M, Brzywczy J, Paszewski A.** 2003. The *Aspergillus nidulans metR* gene encodes a bZIP protein which activates transcription of sulphur metabolism genes. Mol Microbiol **49:**1081–1094. http://dx.doi.org/10.1046/j.1365-2958.2003.03617.x.
447. **Piłsyk S, Natorff R, Sieńko M, Skoneczny M, Paszewski A, Brzywczy J.** 2014. The *Aspergillus nidulans metZ* gene encodes a transcription factor involved in regulation of sulfur metabolism in this fungus and other Eurotiales. Curr Genet **61:**115–125. http://dx.doi.org/10.1007/s00294-014-0459-5.
448. **Kumar A, Paietta JV.** 1995. The sulfur controller-2 negative regulatory gene of *Neurospora crassa* encodes a protein with beta-transducin repeats. Proc Natl Acad Sci U S A **92:**3343–3347. http://dx.doi.org/10.1073/pnas.92.8.3343.
449. **Natorff R, Piotrowska M, Paszewski A.** 1998. The *Aspergillus nidulans* sulphur regulatory gene *sconB* encodes a protein with WD40 repeats and an F-box. Mol Gen Genet **257:**255–263. http://dx.doi.org/10.1007/s004380050646.
450. **Piotrowska M, Natorff R, Paszewski A.** 2000. *sconC*, a gene involved in the regulation of sulphur metabolism in *Aspergillus nidulans*, belongs to the SKP1 gene family. Mol Gen Genet **264:**276–282. http://dx.doi.org/10.1007/s004380000319.
451. **Sizemore ST, Paietta JV.** 2002. Cloning and characterization of scon-3+, a new member of the *Neurospora crassa* sulfur regulatory system. Eukaryot Cell **1:**875–883. http://dx.doi.org/10.1128/EC.1.6.875-883.2002.
452. **Paszewski A, Ono BI.** 1992. Biosynthesis of sulphur amino acids in *Saccharomyces cerevisiae*: regulatory roles of methionine and S-adenosylmethionine reassessed. Curr Genet **22:**273–275. http://dx.doi.org/10.1007/BF00317920.
453. **Hansen J, Johannesen PF.** 2000. Cysteine is essential for transcriptional regulation of the sulfur assimilation genes in *Saccharomyces cerevisiae*. Mol Gen Genet **263:**535–542. http://dx.doi.org/10.1007/s004380051199.
454. **Pu WT, Struhl K.** 1993. Dimerization of leucine zippers analyzed by random selection. Nucleic Acids Res **21:**4348–4355. http://dx.doi.org/10.1093/nar/21.18.4348.
455. **Yin W, Keller NP.** 2011. Transcriptional regulatory elements in fungal secondary metabolism. J Microbiol **49:**329–339. http://dx.doi.org/10.1007/s12275-011-1009-1.
456. **Anitha R, Murugesan K.** 2005. Production of gliotoxin on natural substrates by *Trichoderma virens*. J Basic Microbiol **45:**12–19. http://dx.doi.org/10.1002/jobm.200410451.
457. **Vranova E, Coman D, Gruissem W.** 2013. Network analysis of the MVA and MEP pathways for isoprenoid synthesis. Annu Rev Plant Biol **64:**665–700. http://dx.doi.org/10.1146/annurev-arplant-050312-120116.
458. **Sivasithamparam K, Ghisalberti EL.** 1998. *Trichoderma* and *Gliocladium*, p 139–191. *In* Harman GE, Kubicek CP (ed), Secondary metabolism in *Trichoderma* and *Gliocladium*, vol 1. Taylor and Francis, London, United Kingdom.
459. **Hiser L, Basson ME, Rine J.** 1994. ERG10 from *Saccharomyces cerevisiae* encodes acetoacetyl-CoA thiolase. J Biol Chem **269:**31383–31389.
460. **Servouse M, Karst F.** 1986. Regulation of early enzymes of ergosterol biosynthesis in *Saccharomyces cerevisiae*. Biochem J **240:**541–547. http://dx.doi.org/10.1042/bj2400541.
461. **Rodriguez-Vargas S, Estruch F, Randez-Gil F.** 2002. Gene expression analysis of cold and freeze stress in baker's yeast. Appl Environ Microbiol **68:**3024–3030. http://dx.doi.org/10.1128/AEM.68.6.3024-3030.2002.
462. **Servouse M, Mons N, Baillargeat JL, Karst F.** 1984. Isolation and characterization of yeast mutants blocked in mevalonic acid formation. Biochem Biophys Res Commun **123:**424–430. http://dx.doi.org/10.1016/0006-291X(84)90247-X.
463. **Dimster-Denk D, Thorsness MK, Rine J.** 1994. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in *Saccharomyces cerevisiae*. Mol Biol Cell **5:**655–665. http://dx.doi.org/10.1091/mbc.5.6.655.
464. **Gardner RG, Hampton RY.** 1999. A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes. J Biol Chem **274:**31671–31678. http://dx.doi.org/10.1074/jbc.274.44.31671.
465. **Burg JS, Espenshade PJ.** 2011. Regulation of HMG-CoA reductase in mammals and yeast. Prog Lipid Res **50:**403–410. http://dx.doi.org/10.1016/j.plipres.2011.07.002.
466. **Hampton R, Dimster-Denk D, Rine J.** 1996. The biology of HMG-CoA reductase: the pros of contra-regulation. Trends Biochem Sci **21:**140–145. http://dx.doi.org/10.1016/S0968-0004(96)80168-X.
467. **Cardoza RE, Hermosa MR, Vizcaino JA, Gonzalez F, Llobell A, Monte E, Gutierrez S.** 2007. Partial silencing of a hydroxymethylglutaryl-CoA reductase-encoding gene in *Trichoderma harzianum* CECT 2413 results in a lower level of resistance to lovastatin and lower antifungal activity. Fungal Genet Biol **44:**269–283. http://dx.doi.org/10.1016/j.fgb.2006.11.013.
468. **Imblum RL, Rodwell VW.** 1974. 3-Hydroxy-3-methylglutaryl CoA reductase and mevalonate kinase of *Neurospora crassa*. J Lipid Res **15:**211–222.
469. **Oulmouden A, Karst F.** 1990. Isolation of the ERG12 gene of *Saccharomyces cerevisiae* encoding mevalonate kinase. Gene **88:**253–257. http://dx.doi.org/10.1016/0378-1119(90)90039-T.

470. **Tsay YH, Robinson GW.** 1991. Cloning and characterization of ERG8, an essential gene of *Saccharomyces cerevisiae* that encodes phosphomevalonate kinase. Mol Cell Biol **11:**620–631. http://dx.doi.org/10.1128/MCB.11.2.620.
471. **Houten SM, Waterham HR.** 2001. Nonorthologous gene displacement of phosphomevalonate kinase. Mol Genet Metab **72:**273–276. http://dx.doi.org/10.1006/mgme.2000.3133.
472. **Chambliss KL, Slaughter CA, Schreiner R, Hoffmann GF, Gibson KM.** 1996. Molecular cloning of human phosphomevalonate kinase and identification of a consensus peroxisomal targeting sequence. J Biol Chem **271:**17330–17334. http://dx.doi.org/10.1074/jbc.271.29.17330.
473. **Bonanno JB, Edo C, Eswar N, Pieper U, Romanowski MJ, Ilyin V, Gerchman SE, Kycia H, Studier FW, Sali A, Burley SK.** 2001. Structural genomics of enzymes involved in sterol/isoprenoid biosynthesis. Proc Natl Acad Sci U S A **98:**12896–12901. http://dx.doi.org/10.1073/pnas.181466998.
474. **Krepkiy D, Miziorko HM.** 2004. Identification of active site residues in mevalonate diphosphate decarboxylase: implications for a family of phosphotransferases. Protein Sci **13:**1875–1881. http://dx.doi.org/10.1110/ps.04725204.
475. **Berges T, Guyonnet D, Karst F.** 1997. The *Saccharomyces cerevisiae* mevalonate diphosphate decarboxylase is essential for viability, and a single Leu-to-Pro mutation in a conserved sequence leads to thermosensitivity. J Bacteriol **179:**4664–4670.
476. **Cordier H, Lacombe C, Karst F, Berges T.** 1999. The *Saccharomyces cerevisiae* mevalonate diphosphate decarboxylase (erg19p) forms homodimers in vivo, and a single substitution in a structurally conserved region impairs dimerization. Curr Microbiol **38:**290–294. http://dx.doi.org/10.1007/PL00006804.
477. **Berthelot K, Estevez Y, Deffieux A, Peruch F.** 2012. Isopentenyl diphosphate isomerase: a checkpoint to isoprenoid biosynthesis. Biochimie **94:**1621–1634. http://dx.doi.org/10.1016/j.biochi.2012.03.021.
478. **Anderson MS, Muehlbacher M, Street IP, Proffitt J, Poulter CD.** 1989. Isopentenyl diphosphate:dimethylallyl diphosphate isomerase. An improved purification of the enzyme and isolation of the gene from *Saccharomyces cerevisiae*. J Biol Chem **264:**19169–19175.
479. **Mayer MP, Hahn FM, Stillman DJ, Poulter CD.** 1992. Disruption and mapping of IDI1, the gene for isopentenyl diphosphate isomerase in *Saccharomyces cerevisiae*. Yeast **8:**743–748. http://dx.doi.org/10.1002/yea.320080907.
480. **Goldstein JL, Brown MS.** 1990. Regulation of the mevalonate pathway. Nature **343:**425–430. http://dx.doi.org/10.1038/343425a0.
481. **Anderson MS, Yarger JG, Burck CL, Poulter CD.** 1989. Farnesyl diphosphate synthetase. Molecular cloning, sequence, and expression of an essential gene from *Saccharomyces cerevisiae*. J Biol Chem **264:**19176–19184.
482. **Homann V, Mende K, Arntz C, Ilardi V, Macino G, Morelli G, Bose G, Tudzynski B.** 1996. The isoprenoid pathway: cloning and characterization of fungal FPPS genes. Curr Genet **30:**232–239. http://dx.doi.org/10.1007/s002940050126.
483. **Clarke CF, Tanaka RD, Svenson K, Wamsley M, Fogelman AM, Edwards PA.** 1987. Molecular cloning and sequence of a cholesterol-repressible enzyme related to prenyltransferase in the isoprene biosynthetic pathway. Mol Cell Biol **7:**3138–3146. http://dx.doi.org/10.1128/MCB.7.9.3138.
484. **Pilsyk S, Perlinska-Lenart U, Gorka-Niec W, Graczyk S, Antosiewicz B, Zembek P, Palamarczyk G, Kruszewska JS.** 2014. Overexpression of *erg20* gene encoding farnesyl pyrophosphate synthase has contrasting effects on activity of enzymes of the dolichyl and sterol branches of mevalonate pathway in *Trichoderma reesei*. Gene **544:**114–122. http://dx.doi.org/10.1016/j.gene.2014.04.073.
485. **Daleo GR, Hopp HE, Romero PA, Pont Lezica R.** 1977. Biosynthesis of dolichol phosphate by subcellular fractions from liver. FEBS Lett **81:**411–414. http://dx.doi.org/10.1016/0014-5793(77)80566-8.
486. **Adair WL, Jr, Cafmeyer N.** 1987. Characterization of the *Saccharomyces cerevisiae* cis-prenyltransferase required for dolichyl phosphate biosynthesis. Arch Biochem Biophys **259:**589–596. http://dx.doi.org/10.1016/0003-9861(87)90525-X.
487. **Szkopinska A, Karst F, Palamarczyk G.** 1996. Products of *S. cerevisiae* cis-prenyltransferase activity in vitro. Biochimie **78:**111–116. http://dx.doi.org/10.1016/0300-9084(96)82642-3.
488. **Chojnacki T, Dallner G.** 1988. The biological role of dolichol. Biochem J **251:**1–9. http://dx.doi.org/10.1042/bj2510001.
489. **Kharel Y, Takahashi S, Yamashita S, Koyama T.** 2006. Manipulation of prenyl chain length determination mechanism of cis-prenyltransferases. FEBS J **273:**647–657. http://dx.doi.org/10.1111/j.1742-4658.2005.05097.x.
490. **Sato M, Sato K, Nishikawa S, Hirata A, Kato J, Nakano A.** 1999. The yeast RER2 gene, identified by endoplasmic reticulum protein localization mutations, encodes cis-prenyltransferase, a key enzyme in dolichol synthesis. Mol Cell Biol **19:**471–483. http://dx.doi.org/10.1128/MCB.19.1.471.
491. **Sato M, Fujisaki S, Sato K, Nishimura Y, Nakano A.** 2001. Yeast *Saccharomyces cerevisiae* has two cis-prenyltransferases with different properties and localizations. Implication for their distinct physiological roles in dolichol synthesis. Genes Cells **6:**495–506.
492. **Schenk B, Rush JS, Waechter CJ, Aebi M.** 2001. An alternative cis-isoprenyltransferase activity in yeast that produces polyisoprenols with chain lengths similar to mammalian dolichols. Glycobiology **11:**89–98. http://dx.doi.org/10.1093/glycob/11.1.89.
493. **Perlinska-Lenart U, Bankowska R, Palamarczyk G, Kruszewska JS.** 2006. Overexpression of the *Saccharomyces cerevisiae* RER2 gene in *Trichoderma reesei* affects dolichol dependent enzymes and protein glycosylation. Fungal Genet Biol **43:**422–429. http://dx.doi.org/10.1016/j.fgb.2006.01.009.
494. **Faulkner A, Chen X, Rush J, Horazdovsky B, Waechter CJ, Carman GM, Sternweis PC.** 1999. The LPP1 and DPP1 gene products account for most of the isoprenoid phosphate phosphatase activities in *Saccharomyces cerevisiae*. J Biol Chem **274:**14831–14837. http://dx.doi.org/10.1074/jbc.274.21.14831.
495. **Cantagrel V, Lefeber DJ, Ng BG, Guan Z, Silhavy JL, Bielas SL, Lehle L, Hombauer H, Adamowicz M, Swiezewska E, De Brouwer AP, Blumel P, Sykut-Cegielska J, Houliston S, Swistun D, Ali BR, Dobyns WB, Babovic-Vuksanovic D, van Bokhoven H, Wevers RA, Raetz CR, Freeze HH, Morava E, Al-Gazali L, Gleeson JG.** 2010. SRD5A3 is required for converting polyprenol to dolichol and is mutated in a congenital glycosylation disorder. Cell **142:**203–217. http://dx.doi.org/10.1016/j.cell.2010.06.001.
496. **Heller L, Orlean P, Adair WL, Jr.** 1992. *Saccharomyces cerevisiae* sec59 cells are deficient in dolichol kinase activity. Proc Natl Acad Sci U S A **89:**7013–7016. http://dx.doi.org/10.1073/pnas.89.15.7013.
497. **Orlowski J, Machula K, Janik A, Zdebska E, Palamarczyk G.** 2007. Dissecting the role of dolichol in cell wall assembly in the yeast mutants impaired in early glycosylation reactions. Yeast **24:**239–252. http://dx.doi.org/10.1002/yea.1479.
498. **Kruszewska J, Kubicek CP, Palamarczyk G.** 1994. Modulation of mannosylphosphodolichol synthase and dolichol kinase activity in *Trichoderma*, related to protein secretion. Acta Biochim Pol **41:**331–337.
499. **Suutari M.** 1995. Effect of growth temperature on lipid fatty acids of 4 fungi (*Aspergillus niger*, *Neurospora crassa*, *Penicillium chrysogenum*, and *Trichoderma reesei*). Arch Microbiol **164:**212–216. http://dx.doi.org/10.1007/BF02529973.
500. **Stahl PD, Klug MJ.** 1996. Characterization and differentiation of filamentous fungi based on fatty acid composition. Appl Environ Microbiol **62:**4136–4146.
501. **Serrano-Carreon L, Hathout Y, Bensoussan M, Belin JM.** 1992. Lipid accumulation in *Trichoderma* species. FEMS Microbiol Lett **93:**181–187. http://dx.doi.org/10.1111/j.1574-6968.1992.tb05087.x.
502. **Ratledge C.** 2002. Regulation of lipid accumulation in oleaginous micro-organisms. Biochem Soc Trans **30:**1047–1050. http://dx.doi.org/10.1042/bst0301047.
503. **Betina V, Koman V.** 1980. Changes in the lipid composition during the photo-induced conidiation of *Trichoderma viride*. Folia Microbiol (Praha) **25:**295–300. http://dx.doi.org/10.1007/BF02876608.
504. **Serrano-Carreon L, Hathout Y, Bensoussan M, Belin JM.** 1993. Metabolism of linoleic acid or mevalonate and 6-pentyl-alpha-pyrone biosynthesis by *Trichoderma* species. Appl Environ Microbiol **59:**2945–2950.
505. **Colla LM, Primaz AL, Benedetti S, Loss RA, de Lima M, Reinehr CO, Bertolin TE, Costa JA.** 2010. Selection of lipase-producing microorganisms through submerged fermentation. Z Naturforsch C **65:**483–488.
506. **Morinaga N, Maeda A, Mizuno T, Bunya M, Sugihara S, Sugihara A.**

2011. Synthesis of fatty acid sterol esters using cholesterol esterase from *Trichoderma* sp. AS59. Enzyme Microb Technol **48:**498–504. http://dx.doi.org/10.1016/j.enzmictec.2011.02.007.

507. **Del Carratore R, Gervasi PG, Contini MP, Beffy P, Maserti BE, Giovannetti G, Brondolo A, Longo V.** 2011. Expression and characterization of two new alkane-inducible cytochrome P450s from *Trichoderma harzianum*. Biotechnol Lett **33:**1201–1206. http://dx.doi.org/10.1007/s10529-011-0557-0.
508. **Bhanja A, Minde G, Magdum S, Kalyanraman V.** 2014. Comparative studies of oleaginous fungal strains (*Mucor circinelloides* and *Trichoderma reesei*) for effective wastewater treatment and bio-oil production. Biotechnol Res Int **2014:**479370.
509. **Hermosa R, Rubio MB, Cardoza RE, Nicolas C, Monte E, Gutierrez S.** 2013. The contribution of *Trichoderma* to balancing the costs of plant growth and defense. Int Microbiol **16:**69–80.
510. **Mukherjee M, Mukherjee PK, Horwitz BA, Zachow C, Berg G, Zeilinger S.** 2012. *Trichoderma*-plant-pathogen interactions: advances in genetics of biological control. Indian J Microbiol **52:**522–529. http://dx.doi.org/10.1007/s12088-012-0308-5.
511. **Brunner K, Zeilinger S, Ciliento R, Woo SL, Lorito M, Kubicek CP, Mach RL.** 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Appl Environ Microbiol **71:**3959–3965. http://dx.doi.org/10.1128/AEM.71.7.3959-3965.2005.
512. **Longa CM, Savazzini F, Tosi S, Elad Y, Pertot I.** 2009. Evaluating the survival and environmental fate of the biocontrol agent *Trichoderma atroviride* SC1 in vineyards in northern Italy. J Appl Microbiol **106:**1549–1557. http://dx.doi.org/10.1111/j.1365-2672.2008.04117.x.
513. **Chacon MR, Rodriguez-Galan O, Benitez T, Sousa S, Rey M, Llobell A, Delgado-Jarana J.** 2007. Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum*. Int Microbiol **10:**19–27.
514. **Brotman Y, Lisec J, Meret M, Chet I, Willmitzer L, Viterbo A.** 2012. Transcript and metabolite analysis of the *Trichoderma*-induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. Microbiology **158:**139–146. http://dx.doi.org/10.1099/mic.0.052621-0.
515. **Zheng ZF, Zou JT.** 2001. The initial step of the glycerolipid pathway: identification of glycerol 3-phosphate/dihydroxyacetone phosphate dual substrate acyltransferases in *Saccharomyces cerevisiae*. J Biol Chem **276:**41710–41716. http://dx.doi.org/10.1074/jbc.M104749200.
516. **Fang Z, Wang S, Du X, Shi P, Huang Z.** 2014. Phosphatidate phosphatase-1 is functionally conserved in lipid synthesis and storage from human to yeast. Acta Biol Hung **65:**481–492. http://dx.doi.org/10.1556/ABiol.65.2014.4.11.
517. **Han GS, Wu WI, Carman GM.** 2006. The *Saccharomyces cerevisiae* Lipin homolog is a Mg^{2+}-dependent phosphatidate phosphatase enzyme. J Biol Chem **281:**9210–9218. http://dx.doi.org/10.1074/jbc.M600425200.
518. **Fakas S, Konstantinou C, Carman GM.** 2011. DGK1-encoded diacylglycerol kinase activity is required for phospholipid synthesis during growth resumption from stationary phase in *Saccharomyces cerevisiae*. J Biol Chem **286:**1464–1474. http://dx.doi.org/10.1074/jbc.M110.194308.
519. **Beopoulos A, Haddouche R, Kabran P, Dulermo T, Chardot T, Nicaud JM.** 2012. Identification and characterization of DGA2, an acyltransferase of the DGAT1 acyl-CoA:diacylglycerol acyltransferase family in the oleaginous yeast *Yarrowia lipolytica*. New insights into the storage lipid metabolism of oleaginous yeasts. Appl Microbiol Biotechnol **93:**1523–1537.
520. **Oelkers P, Cromley D, Padamsee M, Billheimer JT, Sturley SL.** 2002. The DGA1 gene determines a second triglyceride synthetic pathway in yeast. J Biol Chem **277:**8877–8881. http://dx.doi.org/10.1074/jbc.M111646200.
521. **Oelkers P, Tinkelenberg A, Erdeniz N, Cromley D, Billheimer JT, Sturley SL.** 2000. A lecithin cholesterol acyltransferase-like gene mediates diacylglycerol esterification in yeast. J Biol Chem **275:**15609–15612. http://dx.doi.org/10.1074/jbc.C000144200.
522. **Hanada K.** 2003. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. Biochim Biophys Acta **1632:**16–30. http://dx.doi.org/10.1016/S1388-1981(03)00059-3.
523. **Shen H, Heacock PN, Clancey CJ, Dowhan W.** 1996. The CDS1 gene encoding CDP-diacylglycerol synthase in *Saccharomyces cerevisiae* is essential for cell growth. J Biol Chem **271:**789–795. http://dx.doi.org/10.1074/jbc.271.2.789.
524. **Carman GM, Han GS.** 2011. Regulation of phospholipid synthesis in the yeast *Saccharomyces cerevisiae*. Annu Rev Biochem **80:**859–883. http://dx.doi.org/10.1146/annurev-biochem-060409-092229.
525. **Kohlwein SD, Veenhuis M, van der Klei IJ.** 2013. Lipid droplets and peroxisomes: key players in cellular lipid homeostasis or a matter of fat–store 'em up or burn 'em down. Genetics **193:**1–50. http://dx.doi.org/10.1534/genetics.112.143362.
526. **Roche CM, Blanch HW, Clark DS, Glass NL.** 2013. Physiological role of acyl coenzyme A synthetase homologues in lipid metabolism in *Neurospora crassa*. Eukaryot Cell **12:**1244–1257. http://dx.doi.org/10.1128/EC.00079-13.
527. **Brakhage AA.** 2013. Regulation of fungal secondary metabolism. Nat Rev Microbiol **11:**21–32.
528. **Keswani C, Mishra S, Sarma BK, Singh SP, Singh HB.** 2014. Unraveling the efficient applications of secondary metabolites of various *Trichoderma* spp. Appl Microbiol Biotechnol **98:**533–544. http://dx.doi.org/10.1007/s00253-013-5344-5.
529. **Pusztahelyi T, Holb IJ, Pocsi I.** 2015. Secondary metabolites in fungus-plant interactions. Front Plant Sci **6:**573.
530. **Yim G, Wang HH, Davies J.** 2007. Antibiotics as signalling molecules. Philos Trans R Soc Lond B Biol Sci **362:**1195–1200. http://dx.doi.org/10.1098/rstb.2007.2044.
531. **Baker SE, Perrone G, Richardson NM, Gallo A, Kubicek CP.** 2012. Phylogenomic analysis of polyketide synthase-encoding genes in *Trichoderma*. Microbiology **158:**147–154. http://dx.doi.org/10.1099/mic.0.053462-0.
532. **Bushley KE, Turgeon BG.** 2010. Phylogenomics reveals subfamilies of fungal nonribosomal peptide synthetases and their evolutionary relationships. BMC Evol Biol **10:**26. http://dx.doi.org/10.1186/1471-2148-10-26.
533. **Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG.** 2003. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. Proc Natl Acad Sci U S A **100:**15670–15675. http://dx.doi.org/10.1073/pnas.2532165100.
534. **Bazafkan H, Dattenböck C, Böhmdorfer S, Tisch D, Stappler E, Schmoll M.** 2015. Mating type dependent partner sensing as mediated by VEL1 in *Trichoderma reesei*. Mol Microbiol **96:**1103–1118. http://dx.doi.org/10.1111/mmi.12993.
535. **Fekete E, Karaffa L, Karimi Aghcheh R, Nemeth Z, Fekete E, Orosz A, Paholcsek M, Stagel A, Kubicek CP.** 2014. The transcriptome of lae1 mutants of *Trichoderma reesei* cultivated at constant growth rates reveals new targets of LAE1 function. BMC Genomics **15:**447. http://dx.doi.org/10.1186/1471-2164-15-447.
536. **Karimi Aghcheh R, Druzhinina IS, Kubicek CP.** 2013. The putative protein methyltransferase LAE1 of *Trichoderma atroviride* is a key regulator of asexual development and mycoparasitism. PLoS One **8:**e67144. http://dx.doi.org/10.1371/journal.pone.0067144.
537. **Karimi-Aghcheh R, Bok JW, Phatale PA, Smith KM, Baker SE, Lichius A, Omann M, Zeilinger S, Seiboth B, Rhee C, Keller NP, Freitag M, Kubicek CP.** 2013. Functional analyses of *Trichoderma reesei* LAE1 reveal conserved and contrasting roles of this regulator. G3 (Bethesda) **3:**369–378. http://dx.doi.org/10.1534/g3.112.005140.
538. **Mukherjee PK, Kenerley CM.** 2010. Regulation of morphogenesis and biocontrol properties in *Trichoderma virens* by a VELVET protein, Vel1. Appl Environ Microbiol **76:**2345–2352. http://dx.doi.org/10.1128/AEM.02391-09.
539. **Crutcher FK, Parich A, Schuhmacher R, Mukherjee PK, Zeilinger S, Kenerley CM.** 2013. A putative terpene cyclase, *vir4*, is responsible for the biosynthesis of volatile terpene compounds in the biocontrol fungus *Trichoderma virens*. Fungal Genet Biol **56:**67–77. http://dx.doi.org/10.1016/j.fgb.2013.05.003.
540. **Atanasova L, Knox BP, Kubicek CP, Druzhinina IS, Baker SE.** 2013. The polyketide synthase gene *pks4* of *Trichoderma reesei* provides pigmentation and stress resistance. Eukaryot Cell **12:**1499–1508. http://dx.doi.org/10.1128/EC.00103-13.
541. **Degenkolb T, Karimi Aghcheh R, Dieckmann R, Neuhof T, Baker SE, Druzhinina IS, Kubicek CP, Bruckner H, von Döhren H.** 2012. The production of multiple small peptaibol families by single 14-module peptide synthetases in *Trichoderma/Hypocrea*. Chem Biodivers **9:**499–535. http://dx.doi.org/10.1002/cbdv.201100212.
542. **Stoppacher N, Neumann NK, Burgstaller L, Zeilinger S, Degenkolb T, Bruckner H, Schuhmacher R.** 2013. The comprehensive peptaibi-

otics database. Chem Biodivers **10:**734–743. http://dx.doi.org/10.1002/cbdv.201200427.
543. **Wiest A, Grzegorski D, Xu BW, Goulard C, Rebuffat S, Ebbole DJ, Bodo B, Kenerley C.** 2002. Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. J Biol Chem **277:**20862–20868. http://dx.doi.org/10.1074/jbc.M201654200.
544. **Kubicek CP, Druzhinina IS.** 2016. *Trichoderma* mycoses and mycotoxins, p 521–538. *In* Russel R, Paterson M, Lima N (ed), Molecular biology of food and water borne mycotoxigenic and mycotic fungi. CRC Press, Boca Raton, FL.
545. **Brian PW.** 1944. Production of gliotoxin by *Trichoderma viride*. Nature **154:**667–668.
546. **Brian PW, Hemming HG.** 1945. Gliotoxin, a fungistatic metabolic product of *Trichoderma viride*. Ann Appl Biol **32:**214–220. http://dx.doi.org/10.1111/j.1744-7348.1945.tb06238.x.
547. **Vargas WA, Mukherjee PK, Laughlin D, Wiest A, Moran-Diez ME, Kenerley CM.** 2014. Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. Microbiology **160:**2319–2330. http://dx.doi.org/10.1099/mic.0.079210-0.
548. **Herscovics A, Orlean P.** 1993. Glycoprotein biosynthesis in yeast. FASEB J **7:**540–550.
549. **Jin C.** 2012. Protein glycosylation in *Aspergillus fumigatus* is essential for cell wall synthesis and serves as a promising model of multicellular eukaryotic development. Int J Microbiol **2012:**21.
550. **Mora-Montes HM, Ponce-Noyola P, Villagómez-Castro JC, Gow NAR, Flores-Carreón A, López-Romero E.** 2009. Protein glycosylation in *Candida*. Future Microbiol **4:**1167–1183. http://dx.doi.org/10.2217/fmb.09.88.
551. **Gorka-Niec W, Pniewski M, Kania A, Perlinska-Lenart U, Palamarczyk G, Kruszewska JS.** 2008. Disruption of *Trichoderma reesei* gene encoding protein O-mannosyltransferase I results in a decrease of the enzyme activity and alteration of cell wall composition. Acta Biochim Pol **55:**251–259.
552. **Rush JS, Gao N, Lehrman MA, Matveev S, Waechter CJ.** 2009. Suppression of Rft1 expression does not impair the transbilayer movement of $Man_5GlcNAc_2$-P-P-dolichol in sealed microsomes from yeast. J Biol Chem **284:**19835–19842. http://dx.doi.org/10.1074/jbc.M109.000893.
553. **Lehle L, Strahl S, Tanner W.** 2006. Protein glycosylation, conserved from yeast to man: a model organism helps elucidate congenital human diseases. Angew Chem Int Ed **45:**6802–6818. http://dx.doi.org/10.1002/anie.200601645.
554. **Kornfeld R, Kornfeld S.** 1985. Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem **54:**631–664. http://dx.doi.org/10.1146/annurev.bi.54.070185.003215.
555. **Yan A, Lennarz WJ.** 2005. Unraveling the mechanism of protein N-glycosylation. J Biol Chem **280:**3121–3124.
556. **Frade-Pérez M, Hernández-Cervantes A, Flores-Carreón A, Mora-Montes HM.** 2010. Biochemical characterization of *Candida albicans* α-glucosidase I heterologously expressed in *Escherichia coli*. Antonie Van Leeuwenhoek **98:**291–298. http://dx.doi.org/10.1007/s10482-010-9437-1.
557. **Mora-Montes HM, Bates S, Netea MG, Diaz-Jimenez DF, Lopez-Romero E, Zinker S, Ponce-Noyola P, Kullberg BJ, Brown AJ, Odds FC, Flores-Carreon A, Gow NA.** 2007. Endoplasmic reticulum alpha-glycosidases of *Candida albicans* are required for N glycosylation, cell wall integrity, and normal host-fungus interaction. Eukaryot Cell **6:**2184–2193. http://dx.doi.org/10.1128/EC.00350-07.
558. **Mora-Montes HM, Lopez-Romero E, Zinker S, Ponce-Noyola P, Flores-Carreon A.** 2004. Hydrolysis of $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$ oligosaccharides by a purified alpha-mannosidase from *Candida albicans*. Glycobiology **14:**593–598. http://dx.doi.org/10.1093/glycob/cwh091.
559. **Herscovics A.** 1999. Processing glycosidases of *Saccharomyces cerevisiae*. Biochim Biophys Acta **1426:**275–285. http://dx.doi.org/10.1016/S0304-4165(98)00129-9.
560. **Eades CJ, Hintz WE.** 2000. Characterization of the class I alpha-mannosidase gene family in the filamentous fungus *Aspergillus nidulans*. Gene **255:**25–34. http://dx.doi.org/10.1016/S0378-1119(00)00298-5.
561. **Lobsanov YD, Vallee F, Imberty A, Yoshida T, Yip P, Herscovics A, Howell PL.** 2002. Structure of *Penicillium citrinum* alpha 1,2-mannosidase reveals the basis for differences in specificity of the endoplasmic reticulum and Golgi class I enzymes. J Biol Chem **277:**5620–5630. http://dx.doi.org/10.1074/jbc.M110243200.
562. **Henrissat B, Davies G.** 1997. Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol **7:**637–644. http://dx.doi.org/10.1016/S0959-440X(97)80072-3.
563. **Herscovics A.** 2001. Structure and function of class I alpha 1,2-mannosidases involved in glycoprotein synthesis and endoplasmic reticulum quality control. Biochimie **83:**757–762. http://dx.doi.org/10.1016/S0300-9084(01)01319-0.
564. **Romero PA, Vallee F, Howell PL, Herscovics A.** 2000. Mutation of Arg(273) to Leu alters the specificity of the yeast N-glycan processing class I alpha1,2-mannosidase. J Biol Chem **275:**11071–11074. http://dx.doi.org/10.1074/jbc.275.15.11071.
565. **Kruszewska JS, Perlinska-Lenart U, Gorka-Niec W, Orlowski J, Zembek P, Palamarczyk G.** 2008. Alterations in protein secretion caused by metabolic engineering of glycosylation pathways in fungi. Acta Biochim Pol **55:**447–456.
566. **Sean M.** 2001. What can yeast tell us about N-linked glycosylation in the Golgi apparatus? FEBS Lett **498:**223–227. http://dx.doi.org/10.1016/S0014-5793(01)02488-7.
567. **Lambou K, Perkhofer S, Fontaine T, Latge J-P.** 2010. Comparative functional analysis of the OCH1 mannosyltransferase families in *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Yeast **27:**625–636. http://dx.doi.org/10.1002/yea.1798.
568. **Lussier M, Sdicu AM, Bussey H.** 1999. The *KTR* and *MNN1* mannosyltransferase families of *Saccharomyces cerevisiae*. Biochim Biophys Acta **1426:**323–334. http://dx.doi.org/10.1016/S0304-4165(98)00133-0.
569. **Herscovics A.** 1999. Importance of glycosidases in mammalian glycoprotein biosynthesis. Biochim Biophys Acta **1473:**96–107. http://dx.doi.org/10.1016/S0304-4165(99)00171-3.
570. **Lee J, Park S-H, Stanley P.** 2002. Antibodies that recognize bisected complex N-glycans on cell surface glycoproteins can be made in mice lacking N-acetylglucosaminyltransferase III. Glycoconj J **19:**211–219. http://dx.doi.org/10.1023/A:1024205925263.
571. **El-Ganiny AM, Sanders DAR, Kaminskyj SGW.** 2008. *Aspergillus nidulans* UDP-galactopyranose mutase, encoded by ugmA plays key roles in colony growth, hyphal morphogensis, and conidiation. Fungal Genet Biol **45:**1533–1542. http://dx.doi.org/10.1016/j.fgb.2008.09.008.
572. **Aebi M, Bernasconi R, Clerc S, Molinari M.** 2010. *N*-Glycan structures: recognition and processing in the ER. Trends Biochem Sci **35:**74–82. http://dx.doi.org/10.1016/j.tibs.2009.10.001.
573. **Werner ED, Brodsky JL, McCracken AA.** 1996. Proteasome-dependent endoplasmic reticulum-associated protein degradation: an unconventional route to a familiar fate. Proc Natl Acad Sci U S A **93:**13797–13801. http://dx.doi.org/10.1073/pnas.93.24.13797.
574. **Gentzsch M, Tanner W.** 1997. Protein-*O*-glycosylation in yeast: protein-specific mannosyltransferases. Glycobiology **7:**481–486. http://dx.doi.org/10.1093/glycob/7.4.481.
575. **Kriangkripipat T, Momany M.** 2009. *Aspergillus nidulans* protein O-mannosyltransferases play roles in cell wall integrity and developmental patterning. Eukaryot Cell **8:**1475–1485. http://dx.doi.org/10.1128/EC.00040-09.
576. **Mouyna I, Kniemeyer O, Jank T, Loussert C, Mellado E, Aimanianda V, Beauvais A, Wartenberg D, Sarfati J, Bayry J, Prévost M-C, Brakhage AA, Strahl S, Huerre M, Latgé J-P.** 2010. Members of protein *O*-mannosyltransferase family in *Aspergillus fumigatus* differentially affect growth, morphogenesis and viability. Mol Microbiol **76:**1205–1221. http://dx.doi.org/10.1111/j.1365-2958.2010.07164.x.
577. **Zakrzewska A, Migdalski A, Saloheimo M, Penttila ME, Palamarczyk G, Kruszewska JS.** 2003. cDNA encoding protein O-mannosyltransferase from the filamentous fungus *Trichoderma reesei*; functional equivalence to *Saccharomyces cerevisiae* PMT2. Curr Genet **43:**11–16.
578. **Goto M.** 2007. Protein O-glycosylation in fungi: diverse structures and multiple functions. Biosci Biotechnol Biochem **71:**1415–1427. http://dx.doi.org/10.1271/bbb.70080.
579. **Mora-Montes HM, Bates S, Netea MG, Castillo L, Brand A, Buurman ET, Díaz-Jiménez DF, Jan Kullberg B, Brown AJP, Odds FC, Gow NAR.** 2010. A multifunctional mannosyltransferase family in *Candida albicans* determines cell wall mannan structure and host-fungus interactions. J Biol Chem **285:**12087–12095. http://dx.doi.org/10.1074/jbc.M109.081513.

580. **Munro CA, Bates S, Buurman ET, Hughes HB, Maccallum DM, Bertram G, Atrih A, Ferguson MA, Bain JM, Brand A, Hamilton S, Westwater C, Thomson LM, Brown AJ, Odds FC, Gow NA.** 2005. Mnt1p and Mnt2p of *Candida albicans* are partially redundant alpha-1,2-mannosyltransferases that participate in *O*-linked mannosylation and are required for adhesion and virulence. J Biol Chem **280:**1051–1060. http://dx.doi.org/10.1074/jbc.M411413200.
581. **Kruszewska JS, Saloheimo M, Migdalski A, Orlean P, Penttila M, Palamarczyk G.** 2000. Dolichol phosphate mannose synthase from the filamentous fungus *Trichoderma reesei* belongs to the human and *Schizosaccharomyces pombe* class of the enzyme. Glycobiology **10:**983–991. http://dx.doi.org/10.1093/glycob/10.10.983.
582. **Zembek P, Perlinska-Lenart U, Rawa K, Gorka-Niec W, Palamarczyk G, Kruszewska JS.** 2011. Cloning and functional analysis of the *dpm2* and *dpm3* genes from *Trichoderma reesei* expressed in a *Saccharomyces cerevisiae dpm1*Δ mutant strain. Biol Chem **392:**517–527.
583. **Orlean P, Albright C, Robbins PW.** 1988. Cloning and sequencing of the yeast gene for dolichol phosphate mannose synthase, an essential protein. J Biol Chem **263:**17499–17507.
584. **Haselbeck A, Tanner W.** 1983. *O*-Glycosylation in *Saccharomyces cerevisiae* is initiated at the endoplasmic reticulum. FEBS Lett **158:**335–338. http://dx.doi.org/10.1016/0014-5793(83)80608-5.
585. **Colussi PA, Taron CH, Mack JC, Orlean P.** 1997. Human and *Saccharomyces cerevisiae* dolichol phosphate mannose synthases represent two classes of the enzyme, but both function in *Schizosaccharomyces pombe*. Proc Natl Acad Sci U S A **94:**7873–7878. http://dx.doi.org/10.1073/pnas.94.15.7873.
586. **Kruszewska JS, Butterweck AH, Kurzatkowski W, Migdalski A, Kubicek CP, Palamarczyk G.** 1999. Overexpression of the *Saccharomyces cerevisiae* mannosylphosphodolichol synthase-encoding gene in *Trichoderma reesei* results in an increased level of protein secretion and abnormal cell ultrastructure. Appl Environ Microbiol **65:**2382–2387.
587. **Orlean P, Menon AK.** 2007. Thematic review series: lipid posttranslational modifications. GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycophospholipids. J Lipid Res **48:**993–1011.
588. **Watanabe R, Inoue N, Westfall B, Taron CH, Orlean P, Takeda J, Kinoshita T.** 1998. The first step of glycosylphosphatidylinositol biosynthesis is mediated by a complex of PIG-A, PIG-H, PIG-C and GPI1. EMBO J **17:**877–885. http://dx.doi.org/10.1093/emboj/17.4.877.
589. **Watanabe R, Ohishi K, Maeda Y, Nakamura N, Kinoshita T.** 1999. Mammalian PIG-L and its yeast homologue Gpi12p are N-acetylglucosaminylphosphatidylinositol de-N-acetylases essential in glycosylphosphatidylinositol biosynthesis. Biochem J **339:**185–192.
590. **Maeda Y, Watanabe R, Harris CL, Hong Y, Ohishi K, Kinoshita K, Kinoshita T.** 2001. PIG-M transfers the first mannose to glycosylphosphatidylinositol on the lumenal side of the ER. EMBO J **20:**250–261. http://dx.doi.org/10.1093/emboj/20.1.250.
591. **Del Sorbo G, Schoonbeek H, De Waard MA.** 2000. Fungal transporters involved in efflux of natural toxic compounds and fungicides. Fungal Genet Biol **30:**1–15. http://dx.doi.org/10.1006/fgbi.2000.1206.
592. **Coleman JJ, Mylonakis E.** 2009. Efflux in fungi: la piece de resistance. PLoS Pathog **5:**e1000486. http://dx.doi.org/10.1371/journal.ppat.1000486.
593. **Saier MH, Jr, Beatty JT, Goffeau A, Harley KT, Heijne WH, Huang SC, Jack DL, Jahn PS, Lew K, Liu J, Pao SS, Paulsen IT, Tseng TT, Virk PS.** 1999. The major facilitator superfamily. J Mol Microbiol Biotechnol **1:**257–279.
594. **Paulsen IT, Chen J, Nelson KE, Saier MH, Jr.** 2001. Comparative genomics of microbial drug efflux systems. J Mol Microbiol Biotechnol **3:**145–150.
595. **Paulsen IT, Lewis K.** 2001. Microbial multidrug efflux: introduction. J Mol Microbiol Biotechnol **3:**143–144.
596. **Kovalchuk A, Driessen AJ.** 2010. Phylogenetic analysis of fungal ABC transporters. BMC Genomics **11:**177. http://dx.doi.org/10.1186/1471-2164-11-177.
597. **Ren Q, Chen K, Paulsen IT.** 2007. TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. Nucleic Acids Res **35:**D274–D279. http://dx.doi.org/10.1093/nar/gkl925.
598. **Ren Q, Kang KH, Paulsen IT.** 2004. TransportDB: a relational database of cellular membrane transport systems. Nucleic Acids Res **32:**D284—D288. http://dx.doi.org/10.1093/nar/gkh016.
599. **Ruocco M, Lanzuise S, Vinale F, Marra R, Turra D, Woo SL, Lorito M.** 2009. Identification of a new biocontrol gene in *Trichoderma atroviride*: the role of an ABC transporter membrane pump in the interaction with different plant-pathogenic fungi. Mol Plant Microbe Interact **22:**291–301. http://dx.doi.org/10.1094/MPMI-22-3-0291.
600. **Zhang Y, Zhang Z, Zhang X, Zhang H, Sun X, Hu C, Li S.** 2012. CDR4 is the major contributor to azole resistance among four Pdr5p-like ABC transporters in *Neurospora crassa*. Fungal Biol **116:**848–854. http://dx.doi.org/10.1016/j.funbio.2012.05.002.
601. **Chouaki T, Lavarde V, Lachaud L, Raccurt CP, Hennequin C.** 2002. Invasive infections due to *Trichoderma* species: report of 2 cases, findings of in vitro susceptibility testing, and review of the literature. Clin Infect Dis **35:**1360–1367. http://dx.doi.org/10.1086/344270.
602. **Kratzer C, Tobudic S, Schmoll M, Graninger W, Georgopoulos A.** 2006. *In vitro* activity and synergism of amphotericin B, azoles and cationic antimicrobials against the emerging pathogen *Trichoderma* spp. J Antimicrob Chemother **58:**1058–1061. http://dx.doi.org/10.1093/jac/dkl384.
603. **Ketchum CJ, Schmidt WK, Rajendrakumar GV, Michaelis S, Maloney PC.** 2001. The yeast **a**-factor transporter Ste6p, a member of the ABC superfamily, couples ATP hydrolysis to pheromone export. J Biol Chem **276:**29007–29011. http://dx.doi.org/10.1074/jbc.M100810200.
604. **Ivanova C, Baath JA, Seiboth B, Kubicek CP.** 2013. Systems analysis of lactose metabolism in *Trichoderma reesei* identifies a lactose permease that is essential for cellulase induction. PLoS One **8:**e62631. http://dx.doi.org/10.1371/journal.pone.0062631.
605. **Porciuncula Jde O, Furukawa T, Shida Y, Mori K, Kuhara S, Morikawa Y, Ogasawara W.** 2013. Identification of major facilitator transporters involved in cellulase production during lactose culture of *Trichoderma reesei* PC-3-7. Biosci Biotechnol Biochem **77:**1014–1022. http://dx.doi.org/10.1271/bbb.120992.
606. **Bahn YS, Xue C, Idnurm A, Rutherford JC, Heitman J, Cardenas ME.** 2007. Sensing the environment: lessons from fungi. Nat Rev Microbiol **5:**57–69. http://dx.doi.org/10.1038/nrmicro1578.
607. **Schmoll M.** 2008. The information highways of a biotechnological workhorse: signal transduction in *Hypocrea jecorina*. BMC Genomics **9:**430. http://dx.doi.org/10.1186/1471-2164-9-430.
608. **Neer EJ.** 1995. Heterotrimeric G proteins: organizers of transmembrane signals. Cell **80:**249–257. http://dx.doi.org/10.1016/0092-8674(95)90407-7.
609. **Kroeze WK, Sheffler DJ, Roth BL.** 2003. G-protein-coupled receptors at a glance. J Cell Sci **116:**4867–4869. http://dx.doi.org/10.1242/jcs.00902.
610. **Li L, Wright SJ, Krystofova S, Park G, Borkovich KA.** 2007. Heterotrimeric G protein signaling in filamentous fungi. Annu Rev Microbiol **61:**423–452. http://dx.doi.org/10.1146/annurev.micro.61.080706.093432.
611. **Magalhaes AC, Dunn H, Ferguson SS.** 2012. Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. Br J Pharmacol **165:**1717–1736. http://dx.doi.org/10.1111/j.1476-5381.2011.01552.x.
612. **Omann M, Zeilinger S.** 2010. How a mycoparasite employs G-protein signaling: using the example of *Trichoderma*. J Signal Transduct **2010:**123126.
613. **Reithner B, Brunner K, Schuhmacher R, Peissl I, Seidl V, Krska R, Zeilinger S.** 2005. The G protein alpha subunit Tga1 of *Trichoderma atroviride* is involved in chitinase formation and differential production of antifungal metabolites. Fungal Genet Biol **42:**749–760. http://dx.doi.org/10.1016/j.fgb.2005.04.009.
614. **Zeilinger S, Reithner B, Scala V, Peissl I, Lorito M, Mach RL.** 2005. Signal transduction by Tga3, a novel G protein alpha subunit of *Trichoderma atroviride*. Appl Environ Microbiol **71:**1591–1597. http://dx.doi.org/10.1128/AEM.71.3.1591-1597.2005.
615. **do Nascimento Silva R, Steindorff AS, Ulhoa CJ, Felix CR.** 2009. Involvement of G-alpha protein GNA3 in production of cell wall-degrading enzymes by *Trichoderma reesei* (*Hypocrea jecorina*) during mycoparasitism against *Pythium ultimum*. Biotechnol Lett **31:**531–536. http://dx.doi.org/10.1007/s10529-008-9900-5.
616. **Mukherjee PK, Latha J, Hadar R, Horwitz BA.** 2004. Role of two G-protein alpha subunits, TgaA and TgaB, in the antagonism of plant pathogens by *Trichoderma virens*. Appl Environ Microbiol **70:**542–549. http://dx.doi.org/10.1128/AEM.70.1.542-549.2004.
617. **Seibel C, Gremel G, Silva RD, Schuster A, Kubicek CP, Schmoll M.** 2009. Light-dependent roles of the G-protein subunit GNA1 of *Hypocrea jecorina* (anamorph *Trichoderma reesei*). BMC Biol **7:**58. http://dx.doi.org/10.1186/1741-7007-7-58.

618. **Schmoll M, Schuster A, do Nascimento Silva R, Kubicek CP.** 2009. The G-alpha protein GNA3 of *Hypocrea jecorina* (anamorph *Trichoderma reesei*) regulates cellulase gene expression in the presence of light. Eukaryot Cell **8:**410–420. http://dx.doi.org/10.1128/EC.00256-08.
619. **Tisch D, Kubicek CP, Schmoll M.** 2011. New insights into the mechanism of light modulated signaling by heterotrimeric G-proteins: ENVOY acts on *gna1* and *gna3* and adjusts cAMP levels in *Trichoderma reesei* (*Hypocrea jecorina*). Fungal Genet Biol **48:**631–640. http://dx.doi.org/10.1016/j.fgb.2010.12.009.
620. **Tisch D, Schuster A, Schmoll M.** 2014. Crossroads between light response and nutrient signalling: ENV1 and PhLP1 act as mutual regulatory pair in *Trichoderma reesei*. BMC Genomics **15:**425. http://dx.doi.org/10.1186/1471-2164-15-425.
621. **Krueger D, Koch J, Barthelmess IB.** 1990. cpc-2, a new locus involved in general control of amino acid synthetic enzymes in *Neurospora crassa*. Curr Genet **18:**211–215. http://dx.doi.org/10.1007/BF00318383.
622. **Gruber S, Omann M, Zeilinger S.** 2013. Comparative analysis of the repertoire of G protein-coupled receptors of three species of the fungal genus *Trichoderma*. BMC Microbiol **13:**108. http://dx.doi.org/10.1186/1471-2180-13-108.
623. **Kulkarni RD, Thon MR, Pan H, Dean RA.** 2005. Novel G-protein-coupled receptor-like proteins in the plant-pathogenic fungus *Magnaporthe grisea*. Genome Biol **6:**R24. http://dx.doi.org/10.1186/gb-2005-6-3-r24.
624. **Lafon A, Han KH, Seo JA, Yu JH, d'Enfert C.** 2006. G-protein and cAMP-mediated signaling in aspergilli: a genomic perspective. Fungal Genet Biol **43:**490–502. http://dx.doi.org/10.1016/j.fgb.2006.02.001.
625. **Omann MR, Lehner S, Escobar Rodriguez C, Brunner K, Zeilinger S.** 2012. The seven-transmembrane receptor Gpr1 governs processes relevant for the antagonistic interaction of *Trichoderma atroviride* with its host. Microbiology **158:**107–118. http://dx.doi.org/10.1099/mic.0.052035-0.
626. **Karpichev IV, Cornivelli L, Small GM.** 2002. Multiple regulatory roles of a novel *Saccharomyces cerevisiae* protein, encoded by YOL002c, in lipid and phosphate metabolism. J Biol Chem **277:**19609–19617. http://dx.doi.org/10.1074/jbc.M202045200.
627. **Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, Funk WD.** 2005. PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. J Mol Evol **61:**372–380. http://dx.doi.org/10.1007/s00239-004-0375-2.
628. **DeZwaan TM, Carroll AM, Valent B, Sweigard JA.** 1999. *Magnaporthe grisea* Pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues. Plant Cell **11:**2013–2030.
629. **Affeldt KJ, Brodhagen M, Keller NP.** 2012. *Aspergillus oxylipin* signaling and quorum sensing pathways depend on g protein-coupled receptors. Toxins (Basel) **4:**695–717. http://dx.doi.org/10.3390/toxins4090695.
630. **Chung KS, Won M, Lee SB, Jang YJ, Hoe KL, Kim DU, Lee JW, Kim KW, Yoo HS.** 2001. Isolation of a novel gene from *Schizosaccharomyces pombe*: stm1+ encoding a seven-transmembrane loop protein that may couple with the heterotrimeric Gα 2 protein, Gpa2. J Biol Chem **276:**40190–40201. http://dx.doi.org/10.1074/jbc.M100341200.
631. **Ross EM, Wilkie TM.** 2000. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem **69:**795–827. http://dx.doi.org/10.1146/annurev.biochem.69.1.795.
632. **Lee BN, Adams TH.** 1994. Overexpression of flbA, an early regulator of *Aspergillus* asexual sporulation, leads to activation of brlA and premature initiation of development. Mol Microbiol **14:**323–334. http://dx.doi.org/10.1111/j.1365-2958.1994.tb01293.x.
633. **Han KH, Seo JA, Yu JH.** 2004. Regulators of G-protein signalling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulates asexual sporulation through attenuation of GanB (Gα) signalling. Mol Microbiol **53:**529–540. http://dx.doi.org/10.1111/j.1365-2958.2004.04163.x.
634. **Zhang H, Tang W, Liu K, Huang Q, Zhang X, Yan X, Chen Y, Wang J, Qi Z, Wang Z, Zheng X, Wang P, Zhang Z.** 2011. Eight RGS and RGS-like proteins orchestrate growth, differentiation, and pathogenicity of *Magnaporthe oryzae*. PLoS Pathog **7:**e1002450. http://dx.doi.org/10.1371/journal.ppat.1002450.
635. **Wright SJ, Inchausti R, Eaton CJ, Krystofova S, Borkovich KA.** 2011. RIC8 is a guanine-nucleotide exchange factor for G alpha subunits that regulates growth and development in *Neurospora crassa*. Genetics **189:**165–176. http://dx.doi.org/10.1534/genetics.111.129270.
636. **Li Y, Yan X, Wang H, Liang S, Ma WB, Fang MY, Talbot NJ, Wang ZY.** 2010. MoRic8 is a novel component of G-protein signaling during plant infection by the rice blast fungus *Magnaporthe oryzae*. Mol Plant Microbe Interact **23:**317–331. http://dx.doi.org/10.1094/MPMI-23-3-0317.
637. **Lee MJ, Dohlman HG.** 2008. Coactivation of G protein signaling by cell-surface receptors and an intracellular exchange factor. Curr Biol **18:**211–215. http://dx.doi.org/10.1016/j.cub.2008.01.007.
638. **D'Souza CA, Heitman J.** 2001. Conserved cAMP signaling cascades regulate fungal development and virulence. FEMS Microbiol Rev **25:**349–364. http://dx.doi.org/10.1111/j.1574-6976.2001.tb00582.x.
639. **Friedl MA, Kubicek CP, Druzhinina IS.** 2008. Carbon source dependence and photostimulation of conidiation in *Hypocrea atroviridis*. Appl Environ Microbiol **74:**245–250. http://dx.doi.org/10.1128/AEM.02068-07.
640. **Gresik M, Kolarova N, Farkas V.** 1989. Light-stimulated phosphorylation of proteins in cell-free extracts from *Trichoderma viride*. FEBS Lett **248:**185–187. http://dx.doi.org/10.1016/0014-5793(89)80458-2.
641. **Sestak S, Farkas V.** 1993. Metabolic regulation of endoglucanase synthesis in *Trichoderma reesei:* participation of cyclic AMP and glucose-6-phosphate. Can J Microbiol **39:**342–347. http://dx.doi.org/10.1139/m93-048.
642. **Schuster A, Tisch D, Seidl-Seiboth V, Kubicek CP, Schmoll M.** 2012. Roles of protein kinase A and adenylate cyclase in light-modulated cellulase regulation in *Trichoderma reesei*. Appl Environ Microbiol **78:**2168–2178. http://dx.doi.org/10.1128/AEM.06959-11.
643. **Yang Z.** 2002. Small GTPases: versatile signaling switches in plants. Plant Cell **14**(Suppl):S375–S388.
644. **Downward J.** 2003. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer **3:**11–22. http://dx.doi.org/10.1038/nrc969.
645. **Vasara T, Saloheimo M, Keranen S, Penttila M.** 2001. *Trichoderma reesei rho3* a homologue of yeast RH03 suppresses the growth defect of yeast *sec15-1* mutation. Curr Genet **40:**119–127. http://dx.doi.org/10.1007/s002940100245.
646. **Wennerberg K, Rossman KL, Der CJ.** 2005. The Ras superfamily at a glance. J Cell Sci **118:**843–846. http://dx.doi.org/10.1242/jcs.01660.
647. **van Dam TJ, Bos JL, Snel B.** 2011. Evolution of the Ras-like small GTPases and their regulators. Small GTPases **2:**4–16. http://dx.doi.org/10.4161/sgtp.2.1.15113.
648. **Francis SM, Gas ME, Daugeron MC, Bravo J, Seraphin B.** 2012. Rbg1-Tma46 dimer structure reveals new functional domains and their role in polysome recruitment. Nucleic Acids Res **40:**11100–11114. http://dx.doi.org/10.1093/nar/gks867.
649. **Rojas AM, Fuentes G, Rausell A, Valencia A.** 2012. The Ras protein superfamily: evolutionary tree and role of conserved amino acids. J Cell Biol **196:**189–201. http://dx.doi.org/10.1083/jcb.201103008.
650. **Mirisola MG, Longo VD.** 2011. Conserved role of Ras-GEFs in promoting aging: from yeast to mice. Aging (Albany, NY) **3:**340–343.
651. **Kwon MJ, Arentshorst M, Roos ED, van den Hondel CA, Meyer V, Ram AF.** 2011. Functional characterization of Rho GTPases in *Aspergillus niger* uncovers conserved and diverged roles of Rho proteins within filamentous fungi. Mol Microbiol **79:**1151–1167. http://dx.doi.org/10.1111/j.1365-2958.2010.07524.x.
652. **Boureux A, Vignal E, Faure S, Fort P.** 2007. Evolution of the Rho family of ras-like GTPases in eukaryotes. Mol Biol Evol **24:**203–216.
653. **Etienne-Manneville S, Hall A.** 2002. Rho GTPases in cell biology. Nature **420:**629–635. http://dx.doi.org/10.1038/nature01148.
654. **Harris SD.** 2011. Cdc42/Rho GTPases in fungi: variations on a common theme. Mol Microbiol **79:**1123–1127. http://dx.doi.org/10.1111/j.1365-2958.2010.07525.x.
655. **Dichtl K, Helmschrott C, Dirr F, Wagener J.** 2012. Deciphering cell wall integrity signalling in *Aspergillus fumigatus*: identification and functional characterization of cell wall stress sensors and relevant Rho GTPases. Mol Microbiol **83:**506–519. http://dx.doi.org/10.1111/j.1365-2958.2011.07946.x.
656. **Wendland J, Philippsen P.** 2001. Cell polarity and hyphal morphogenesis are controlled by multiple rho-protein modules in the filamentous ascomycete *Ashbya gossypii*. Genetics **157:**601–610.
657. **Zheng W, Chen J, Liu W, Zheng S, Zhou J, Lu G, Wang Z.** 2007. A Rho3 homolog is essential for appressorium development and patho-

genicity of *Magnaporthe grisea*. Eukaryot Cell **6:**2240–2250. http://dx.doi.org/10.1128/EC.00104-07.
658. **Vasara T, Salusjarvi L, Raudaskoski M, Keranen S, Penttila M, Saloheimo M.** 2001. Interactions of the *Trichoderma reesei rho3* with the secretory pathway in yeast and *T. reesei*. Mol Microbiol **42:**1349–1361.
659. **Estravis M, Rincon S, Perez P.** 2012. Cdc42 regulation of polarized traffic in fission yeast. Commun Integr Biol **5:**370–373. http://dx.doi.org/10.4161/cib.19977.
660. **Scheffer J, Chen C, Heidrich P, Dickman MB, Tudzynski P.** 2005. A CDC42 homologue in *Claviceps purpurea* is involved in vegetative differentiation and is essential for pathogenicity. Eukaryot Cell **4:**1228–1238. http://dx.doi.org/10.1128/EC.4.7.1228-1238.2005.
661. **Mahlert M, Leveleki L, Hlubek A, Sandrock B, Bolker M.** 2006. Rac1 and Cdc42 regulate hyphal growth and cytokinesis in the dimorphic fungus *Ustilago maydis*. Mol Microbiol **59:**567–578. http://dx.doi.org/10.1111/j.1365-2958.2005.04952.x.
662. **Zheng W, Zhao Z, Chen J, Liu W, Ke H, Zhou J, Lu G, Darvill AG, Albersheim P, Wu S, Wang Z.** 2009. A Cdc42 ortholog is required for penetration and virulence of *Magnaporthe grisea*. Fungal Genet Biol **46:**450–460. http://dx.doi.org/10.1016/j.fgb.2009.03.005.
663. **Chen C, Ha YS, Min JY, Memmott SD, Dickman MB.** 2006. Cdc42 is required for proper growth and development in the fungal pathogen Colletotrichum trifolii. Eukaryot Cell **5:**155–166. http://dx.doi.org/10.1128/EC.5.1.155-166.2006.
664. **Freitag J, Lanver D, Bohmer C, Schink KO, Bolker M, Sandrock B.** 2011. Septation of infectious hyphae is critical for appressoria formation and virulence in the smut fungus *Ustilago maydis*. PLoS Pathog **7:**e1002044. http://dx.doi.org/10.1371/journal.ppat.1002044.
665. **Jimenez B, Arends M, Esteve P, Perona R, Sanchez R, Ramon y Cajal S, Wyllie A, Lacal JC.** 1995. Induction of apoptosis in NIH 3T3 cells after serum deprivation by overexpression of rho-p21, a GTPase protein of the Ras superfamily. Oncogene **10:**811–816.
666. **Hlubek A, Schink KO, Mahlert M, Sandrock B, Bolker M.** 2008. Selective activation by the guanine nucleotide exchange factor Don1 is a main determinant of Cdc42 signalling specificity in *Ustilago maydis*. Mol Microbiol **68:**615–623. http://dx.doi.org/10.1111/j.1365-2958.2008.06177.x.
667. **Kang PJ, Beven L, Hariharan S, Park HO.** 2010. The Rsr1/Bud1 GTPase interacts with itself and the Cdc42 GTPase during bud-site selection and polarity establishment in budding yeast. Mol Biol Cell **21:**3007–3016. http://dx.doi.org/10.1091/mbc.E10-03-0232.
668. **Park HO, Chant J, Herskowitz I.** 1993. BUD2 encodes a GTPase-activating protein for Bud1/Rsr1 necessary for proper bud-site selection in yeast. Nature **365:**269–274. http://dx.doi.org/10.1038/365269a0.
669. **Takemoto D, Kamakura S, Saikia S, Becker Y, Wrenn R, Tanaka A, Sumimoto H, Scott B.** 2011. Polarity proteins Bem1 and Cdc24 are components of the filamentous fungal NADPH oxidase complex. Proc Natl Acad Sci U S A **108:**2861–2866. http://dx.doi.org/10.1073/pnas.1017309108.
670. **Li H, Barker BM, Grahl N, Puttikamonkul S, Bell JD, Craven KD, Cramer RA, Jr.** 2011. The small GTPase RacA mediates intracellular reactive oxygen species production, polarized growth, and virulence in the human fungal pathogen *Aspergillus fumigatus*. Eukaryot Cell **10:**174–186. http://dx.doi.org/10.1128/EC.00288-10.
671. **Semighini CP, Harris SD.** 2008. Regulation of apical dominance in *Aspergillus nidulans* hyphae by reactive oxygen species. Genetics **179:**1919–1932. http://dx.doi.org/10.1534/genetics.108.089318.
672. **Yang Z, Dickman MB.** 1999. *Colletotrichum trifolii* mutants disrupted in the catalytic subunit of cAMP-dependent protein kinase are nonpathogenic. Mol Plant Microbe Interact **12:**430–439. http://dx.doi.org/10.1094/MPMI.1999.12.5.430.
673. **Aguirre J, Lambeth JD.** 2010. Nox enzymes from fungus to fly to fish and what they tell us about Nox function in mammals. Free Radic Biol Med **49:**1342–1353. http://dx.doi.org/10.1016/j.freeradbiomed.2010.07.027.
674. **Aguirre J, Rios-Momberg M, Hewitt D, Hansberg W.** 2005. Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol **13:**111–118. http://dx.doi.org/10.1016/j.tim.2005.01.007.
675. **Chant J, Stowers L.** 1995. GTPase cascades choreographing cellular behavior: movement, morphogenesis, and more. Cell **81:**1–4. http://dx.doi.org/10.1016/0092-8674(95)90363-1.
676. **Norton TS, Fortwendel JR.** 2014. Control of Ras-mediated signaling in *Aspergillus fumigatus*. Mycopathologia **178:**325–330. http://dx.doi.org/10.1007/s11046-014-9765-1.
677. **Zhu Y, Fang HM, Wang YM, Zeng GS, Zheng XD, Wang Y.** 2009. Ras1 and Ras2 play antagonistic roles in regulating cellular cAMP level, stationary-phase entry and stress response in *Candida albicans*. Mol Microbiol **74:**862–875. http://dx.doi.org/10.1111/j.1365-2958.2009.06898.x.
678. **Fortwendel JR, Fuller KK, Stephens TJ, Bacon WC, Askew DS, Rhodes JC.** 2008. *Aspergillus fumigatus* RasA regulates asexual development and cell wall integrity. Eukaryot Cell **7:**1530–1539. http://dx.doi.org/10.1128/EC.00080-08.
679. **Fortwendel JR, Zhao W, Bhabhra R, Park S, Perlin DS, Askew DS, Rhodes JC.** 2005. A fungus-specific ras homolog contributes to the hyphal growth and virulence of *Aspergillus fumigatus*. Eukaryot Cell **4:**1982–1989. http://dx.doi.org/10.1128/EC.4.12.1982-1989.2005.
680. **Harispe L, Portela C, Scazzocchio C, Penalva MA, Gorfinkiel L.** 2008. Ras GTPase-activating protein regulation of actin cytoskeleton and hyphal polarity in *Aspergillus nidulans*. Eukaryot Cell **7:**141–153. http://dx.doi.org/10.1128/EC.00346-07.
681. **Wang P, Cardenas ME, Cox GM, Perfect JR, Heitman J.** 2001. Two cyclophilin A homologues with shared and distinct functions important for growth and virulence of *Cryptococcus neoformans*. EMBO Rep **2:**511–518. http://dx.doi.org/10.1093/embo-reports/kve109.
682. **Buhr TL, Oved S, Truesdell GM, Huang C, Yarden O, Dickman MB.** 1996. A kinase-encoding gene from *Colletotrichum trifolii* complements a colonial growth mutant of *Neurospora crassa*. Mol Gen Genet **251:**565–572.
683. **Muller P, Katzenberger JD, Loubradou G, Kahmann R.** 2003. Guanyl nucleotide exchange factor Sql2 and Ras2 regulate filamentous growth in Ustilago maydis. Eukaryot Cell **2:**609–617. http://dx.doi.org/10.1128/EC.2.3.609-617.2003.
684. **Warwar V, Dickman MB.** 1996. Effects of calcium and calmodulin on spore germination and appressorium development in *Colletotrichum trifolii*. Appl Environ Microbiol **62:**74–79.
685. **Buhr TL, Dickman MB.** 1997. Gene expression analysis during conidial germ tube and appressorium development in *Colletotrichum trifolii*. Appl Environ Microbiol **63:**2378–2383.
686. **Stricker AR, Grosstessner-Hain K, Wurleitner E, Mach RL.** 2006. Xyr1 (xylanase regulator 1) regulates both the hydrolytic enzyme system and D-xylose metabolism in *Hypocrea jecorina*. Eukaryot Cell **5:**2128–2137. http://dx.doi.org/10.1128/EC.00211-06.
687. **Brefort T, Doehlemann G, Mendoza-Mendoza A, Reissmann S, Djamei A, Kahmann R.** 2009. *Ustilago maydis* as a pathogen. Annu Rev Phytopathol **47:**423–445. http://dx.doi.org/10.1146/annurev-phyto-080508-081923.
688. **Waugh MS, Vallim MA, Heitman J, Alspaugh JA.** 2003. Ras1 controls pheromone expression and response during mating in *Cryptococcus neoformans*. Fungal Genet Biol **38:**110–121. http://dx.doi.org/10.1016/S1087-1845(02)00518-2.
689. **Mukherjee M, Mukherjee PK, Kale SP.** 2007. cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. Microbiology **153:**1734–1742. http://dx.doi.org/10.1099/mic.0.2007/005702-0.
690. **Park HO, Kang PJ, Rachfal AW.** 2002. Localization of the Rsr1/Bud1 GTPase involved in selection of a proper growth site in yeast. J Biol Chem **277:**26721–26724. http://dx.doi.org/10.1074/jbc.C200245200.
691. **Park HO, Bi E, Pringle JR, Herskowitz I.** 1997. Two active states of the Ras-related Bud1/Rsr1 protein bind to different effectors to determine yeast cell polarity. Proc Natl Acad Sci U S A **94:**4463–4468. http://dx.doi.org/10.1073/pnas.94.9.4463.
692. **Bauer Y, Knechtle P, Wendland J, Helfer H, Philippsen P.** 2004. A Ras-like GTPase is involved in hyphal growth guidance in the filamentous fungus *Ashbya gossypii*. Mol Biol Cell **15:**4622–4632. http://dx.doi.org/10.1091/mbc.E04-02-0104.
693. **Pulver R, Heisel T, Gonia S, Robins R, Norton J, Haynes P, Gale CA.** 2013. Rsr1 focuses Cdc42 activity at hyphal tips and promotes maintenance of hyphal development in *Candida albicans*. Eukaryot Cell **12:**482–495. http://dx.doi.org/10.1128/EC.00294-12.
694. **Feng P, Xie Z, Sun J, Zhang J, Li X, Lu C, Xi L.** 2010. Molecular cloning, characterization and expression of PmRsr1, a Ras-related gene from yeast form of *Penicillium marneffei*. Mol Biol Rep **37:**3533–3540. http://dx.doi.org/10.1007/s11033-009-9947-y.
695. **Tsao CC, Chen YT, Lan CY.** 2009. A small G protein Rhb1 and a

GTPase-activating protein Tsc2 involved in nitrogen starvation-induced morphogenesis and cell wall integrity of *Candida albicans*. Fungal Genet Biol **46:**126–136. http://dx.doi.org/10.1016/j.fgb.2008.11.008.

696. **Bazafkan H, Tisch D, Schmoll M.** 2014. Regulation of glycoside hydrolase expression in *Trichoderma*, p 291–307. *In* Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG (ed), Biotechnology and biology of *Trichoderma*. Elsevier, Oxford, United Kingdom.
697. **Dennerlein S, Rozanska A, Wydro M, Chrzanowska-Lightowlers ZM, Lightowlers RN.** 2010. Human ERAL1 is a mitochondrial RNA chaperone involved in the assembly of the 28S small mitochondrial ribosomal subunit. Biochem J **430:**551–558. http://dx.doi.org/10.1042/BJ20100757.
698. **Uchiumi T, Ohgaki K, Yagi M, Aoki Y, Sakai A, Matsumoto S, Kang D.** 2010. ERAL1 is associated with mitochondrial ribosome and elimination of ERAL1 leads to mitochondrial dysfunction and growth retardation. Nucleic Acids Res **38:**5554–5568. http://dx.doi.org/10.1093/nar/gkq305.
699. **Heupel S, Roser B, Kuhn H, Lebrun MH, Villalba F, Requena N.** 2010. Erl1, a novel era-like GTPase from *Magnaporthe oryzae*, is required for full root virulence and is conserved in the mutualistic symbiont Glomus intraradices. Mol Plant Microbe Interact **23:**67–81. http://dx.doi.org/10.1094/MPMI-23-1-0067.
700. **Salus SS, Demeter J, Sazer S.** 2002. The Ran GTPase system in fission yeast affects microtubules and cytokinesis in cells that are competent for nucleocytoplasmic protein transport. Mol Cell Biol **22:**8491–8505. http://dx.doi.org/10.1128/MCB.22.24.8491-8505.2002.
701. **Smirlis D, Boleti H, Gaitanou M, Soto M, Soteriadou K.** 2009. *Leishmania donovani* Ran-GTPase interacts at the nuclear rim with linker histone H1. Biochem J **424:**367–374. http://dx.doi.org/10.1042/BJ20090576.
702. **Hillig RC, Renault L, Vetter IR, Drell T, IV, Wittinghofer A, Becker J.** 1999. The crystal structure of rna1p: a new fold for a GTPase-activating protein. Mol Cell **3:**781–791. http://dx.doi.org/10.1016/S1097-2765(01)80010-1.
703. **Lal K, Field MC, Carlton JM, Warwicker J, Hirt RP.** 2005. Identification of a very large Rab GTPase family in the parasitic protozoan Trichomonas vaginalis. Mol Biochem Parasitol **143:**226–235. http://dx.doi.org/10.1016/j.molbiopara.2005.06.008.
704. **Pereira-Leal JB.** 2008. The Ypt/Rab family and the evolution of trafficking in fungi. Traffic **9:**27–38. http://dx.doi.org/10.1111/j.1600-0854.2007.00667.x.
705. **Fuchs U, Steinberg G.** 2005. Endocytosis in the plant-pathogenic fungus *Ustilago maydis*. Protoplasma **226:**75–80. http://dx.doi.org/10.1007/s00709-005-0109-3.
706. **Le Crom S, Schackwitz W, Pennacchio L, Magnuson JK, Culley DE, Collett JR, Martin J, Druzhinina IS, Mathis H, Monot F, Seiboth B, Cherry B, Rey M, Berka R, Kubicek CP, Baker SE, Margeot A.** 2009. Tracking the roots of cellulase hyperproduction by the fungus *Trichoderma reesei* using massively parallel DNA sequencing. Proc Natl Acad Sci U S A **106:**16151–16156. http://dx.doi.org/10.1073/pnas.0905848106.
707. **Maresova L, Vydareny T, Sychrova H.** 2012. Comparison of the influence of small GTPases Arl1 and Ypt6 on yeast cells' tolerance to various stress factors. FEMS Yeast Res **12:**332–340. http://dx.doi.org/10.1111/j.1567-1364.2011.00780.x.
708. **Bui QT, Golinelli-Cohen MP, Jackson CL.** 2009. Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. Mol Genet Genomics **282:**329–350. http://dx.doi.org/10.1007/s00438-009-0473-3.
709. **van der Vaart A, Griffith J, Reggiori F.** 2010. Exit from the Golgi is required for the expansion of the autophagosomal phagophore in yeast *Saccharomyces cerevisiae*. Mol Biol Cell **21:**2270–2284. http://dx.doi.org/10.1091/mbc.E09-04-0345.
710. **Lettner T, Zeidler U, Gimona M, Hauser M, Breitenbach M, Bito A.** 2010. *Candida albicans* AGE3, the ortholog of the *S. cerevisiae* ARF-GAP-encoding gene GCS1, is required for hyphal growth and drug resistance. PLoS One **5:**e11993. http://dx.doi.org/10.1371/journal.pone.0011993.
711. **Reis K, Fransson A, Aspenstrom P.** 2009. The Miro GTPases: at the heart of the mitochondrial transport machinery. FEBS Lett **583:**1391–1398. http://dx.doi.org/10.1016/j.febslet.2009.04.015.
712. **Michel AH, Kornmann B.** 2012. The ERMES complex and ER-mitochondria connections. Biochem Soc Trans **40:**445–450. http://dx.doi.org/10.1042/BST20110758.
713. **Koshiba T, Holman HA, Kubara K, Yasukawa K, Kawabata S, Okamoto K, MacFarlane J, Shaw JM.** 2011. Structure-function analysis of the yeast mitochondrial Rho GTPase, Gem1p: implications for mitochondrial inheritance. J Biol Chem **286:**354–362. http://dx.doi.org/10.1074/jbc.M110.180034.
714. **Schmidt S, Sohrmann M, Hofmann K, Woollard A, Simanis V.** 1997. The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. Genes Dev **11:**1519–1534. http://dx.doi.org/10.1101/gad.11.12.1519.
715. **Feoktistova A, Morrell-Falvey J, Chen JS, Singh NS, Balasubramanian MK, Gould KL.** 2012. The fission yeast septation initiation network (SIN) kinase, Sid2, is required for SIN asymmetry and regulates the SIN scaffold, Cdc11. Mol Biol Cell **23:**1636–1645. http://dx.doi.org/10.1091/mbc.E11-09-0792.
716. **Hergovich A, Hemmings BA.** 2012. Hippo signalling in the G2/M cell cycle phase: lessons learned from the yeast MEN and SIN pathways. Semin Cell Dev Biol **23:**794–802. http://dx.doi.org/10.1016/j.semcdb.2012.04.001.
717. **Bedhomme M, Jouannic S, Champion A, Simanis V, Henry Y.** 2008. Plants, MEN and SIN. Plant Physiol Biochem **46:**1–10. http://dx.doi.org/10.1016/j.plaphy.2007.10.010.
718. **Cohen P.** 2000. The regulation of protein function by multisite phosphorylation: a 25 year update. Trends Biochem Sci **25:**596–601. http://dx.doi.org/10.1016/S0968-0004(00)01712-6.
719. **Kosti I, Mandel-Gutfreund Y, Glaser F, Horwitz BA.** 2010. Comparative analysis of fungal protein kinases and associated domains. BMC Genomics **11:**133. http://dx.doi.org/10.1186/1471-2164-11-133.
720. **Deshmukh K, Anamika K, Srinivasan N.** 2010. Evolution of domain combinations in protein kinases and its implications for functional diversity. Prog Biophys Mol Biol **102:**1–15. http://dx.doi.org/10.1016/j.pbiomolbio.2009.12.009.
721. **Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S.** 2002. The protein kinase complement of the human genome. Science **298:**1912–1934. http://dx.doi.org/10.1126/science.1075762.
722. **Park G, Servin JA, Turner GE, Altamirano L, Colot HV, Collopy P, Litvinkova L, Li L, Jones CA, Diala FG, Dunlap JC, Borkovich KA.** 2011. Global analysis of serine-threonine protein kinase genes in *Neurospora crassa*. Eukaryot Cell **10:**1553–1564. http://dx.doi.org/10.1128/EC.05140-11.
723. **Chen P, Lee KS, Levin DE.** 1993. A pair of putative protein kinase genes (YPK1 and YPK2) is required for cell growth in *Saccharomyces cerevisiae*. Mol Gen Genet **236:**443–447.
724. **Morawetz R, Mischak H, Goodnight J, Lendenfeld T, Mushinsky JF, Kubicek CP.** 1994. A protein kinase-encoding gene, pkt1, from *Trichoderma reesei*, homologous to the yeast YPK1 and YPK2 (YKR2) genes. Gene **146:**309–310. http://dx.doi.org/10.1016/0378-1119(94)90311-5.
725. **Morawetz R, Lendenfeld T, Mischak H, Muhlbauer M, Gruber F, Goodnight J, de Graaff LH, Visser J, Mushinski JF, Kubicek CP.** 1996. Cloning and characterisation of genes (*pkc1* and *pkcA*) encoding protein kinase C homologues from *Trichoderma reesei* and *Aspergillus niger*. Mol Gen Genet **250:**17–28.
726. **Lendenfeld T, Kubicek CP.** 1998. Characterization and properties of protein kinase C from the filamentous fungus *Trichoderma reesei*. Biochem J **330:**689–694. http://dx.doi.org/10.1042/bj3300689.
727. **Wolanin PM, Thomason PA, Stock JB.** 2002. Histidine protein kinases: key signal transducers outside the animal kingdom. Genome Biol **3:**REVIEWS3013.
728. **Bahn YS.** 2008. Master and commander in fungal pathogens: the two-component system and the HOG signaling pathway. Eukaryot Cell **7:**2017–2036. http://dx.doi.org/10.1128/EC.00323-08.
729. **Dongo A, Bataille-Simoneau N, Campion C, Guillemette T, Hamon B, Iacomi-Vasilescu B, Katz L, Simoneau P.** 2009. The group III two-component histidine kinase of filamentous fungi is involved in the fungicidal activity of the bacterial polyketide ambruticin. Appl Environ Microbiol **75:**127–134. http://dx.doi.org/10.1128/AEM.00993-08.
730. **Dong W, Tang X, Yu Y, Nilsen R, Kim R, Griffith J, Arnold J, Schuttler HB.** 2008. Systems biology of the clock in *Neurospora crassa*. PLoS One **3:**e3105. http://dx.doi.org/10.1371/journal.pone.0003105.
731. **Catlett NL, Yoder OC, Turgeon BG.** 2003. Whole-genome analysis of

two-component signal transduction genes in fungal pathogens. Eukaryot Cell **2:**1151–1161. http://dx.doi.org/10.1128/EC.2.6.1151-1161.2003.

732. **Tian C, Li J, Glass NL.** 2011. Exploring the bZIP transcription factor regulatory network in *Neurospora crassa*. Microbiology **157:**747–759. http://dx.doi.org/10.1099/mic.0.045468-0.
733. **Tian C, Beeson WT, Iavarone AT, Sun J, Marletta MA, Cate JH, Glass NL.** 2009. Systems analysis of plant cell wall degradation by the model filamentous fungus *Neurospora crassa*. Proc Natl Acad Sci U S A **106:**22157–22162. http://dx.doi.org/10.1073/pnas.0906810106.
734. **Lamb TM, Goldsmith CS, Bennett L, Finch KE, Bell-Pedersen D.** 2011. Direct transcriptional control of a p38 MAPK pathway by the circadian clock in *Neurospora crassa*. PLoS One **6:**e27149. http://dx.doi.org/10.1371/journal.pone.0027149.
735. **Oide S, Liu J, Yun SH, Wu D, Michev A, Choi MY, Horwitz BA, Turgeon BG.** 2010. Histidine kinase two-component response regulator proteins regulate reproductive development, virulence, and stress responses of the fungal cereal pathogens *Cochliobolus heterostrophus* and *Gibberella zeae*. Eukaryot Cell **9:**1867–1880. http://dx.doi.org/10.1128/EC.00150-10.
736. **Wu D, Oide S, Zhang N, Choi MY, Turgeon BG.** 2012. ChLae1 and ChVel1 regulate T-toxin production, virulence, oxidative stress response, and development of the maize pathogen *Cochliobolus heterostrophus*. PLoS Pathog **8:**e1002542. http://dx.doi.org/10.1371/journal.ppat.1002542.
737. **Jones CA, Greer-Phillips SE, Borkovich KA.** 2007. The response regulator RRG-1 functions upstream of a mitogen-activated protein kinase pathway impacting asexual development, female fertility, osmotic stress, and fungicide resistance in *Neurospora crassa*. Mol Biol Cell **18:**2123–2136. http://dx.doi.org/10.1091/mbc.E06-03-0226.
738. **Banno S, Noguchi R, Yamashita K, Fukumori F, Kimura M, Yamaguchi I, Fujimura M.** 2007. Roles of putative His-to-Asp signaling modules HPT-1 and RRG-2, on viability and sensitivity to osmotic and oxidative stresses in *Neurospora crassa*. Curr Genet **51:**197–208. http://dx.doi.org/10.1007/s00294-006-0116-8.
739. **Kassir Y, Rubin-Bejerano I, Mandel-Gutfreund Y.** 2006. The *Saccharomyces cerevisiae* GSK-3 beta homologues. Curr Drug Targets **7:**1455–1465. http://dx.doi.org/10.2174/1389450110607011455.
740. **Martinek S, Inonog S, Manoukian AS, Young MW.** 2001. A role for the segment polarity gene shaggy/GSK-3 in the *Drosophila* circadian clock. Cell **105:**769–779. http://dx.doi.org/10.1016/S0092-8674(01)00383-X.
741. **Tataroglu O, Lauinger L, Sancar G, Jakob K, Brunner M, Diernfellner AC.** 2012. Glycogen synthase kinase is a regulator of the circadian clock of *Neurospora crassa*. J Biol Chem **287:**36936–36943. http://dx.doi.org/10.1074/jbc.M112.396622.
742. **Betina V, Zajacova J.** 1978. Regulation of periodicity and intensity of photoinduced conidiation of *Trichoderma viride*. Folia Microbiol (Praha) **23:**453–459. http://dx.doi.org/10.1007/BF02885575.
743. **Deitzer GF, Horwitz BA, Gressel J.** 1988. Rhythms in blue-light-induced conidiation of wild type and a mutant strain of *Trichoderma harzianum*. Photochem Photobiol **47:**425–431. http://dx.doi.org/10.1111/j.1751-1097.1988.tb02747.x.
744. **Bottner CA, Schmidt H, Vogel S, Michele M, Kaufer NF.** 2005. Multiple genetic and biochemical interactions of Brr2, Prp8, Prp31, Prp1 and Prp4 kinase suggest a function in the control of the activation of spliceosomes in *Schizosaccharomyces pombe*. Curr Genet **48:**151–161. http://dx.doi.org/10.1007/s00294-005-0013-6.
745. **Montembault E, Dutertre S, Prigent C, Giet R.** 2007. PRP4 is a spindle assembly checkpoint protein required for MPS1, MAD1, and MAD2 localization to the kinetochores. J Cell Biol **179:**601–609. http://dx.doi.org/10.1083/jcb.200703133.
746. **Benjamin KR, Zhang C, Shokat KM, Herskowitz I.** 2003. Control of landmark events in meiosis by the CDK Cdc28 and the meiosis-specific kinase Ime2. Genes Dev **17:**1524–1539. http://dx.doi.org/10.1101/gad.1101503.
747. **Foiani M, Nadjar-Boger E, Capone R, Sagee S, Hashimshoni T, Kassir Y.** 1996. A meiosis-specific protein kinase, Ime2, is required for the correct timing of DNA replication and for spore formation in yeast meiosis. Mol Gen Genet **253:**278–288. http://dx.doi.org/10.1007/s004380050323.
748. **Garrido E, Perez-Martin J.** 2003. The *crk1* gene encodes an Ime2-related protein that is required for morphogenesis in the plant pathogen *Ustilago maydis*. Mol Microbiol **47:**729–743. http://dx.doi.org/10.1046/j.1365-2958.2003.03323.x.
749. **Chen RE, Thorner J.** 2007. Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. Biochim Biophys Acta **1773:**1311–1340. http://dx.doi.org/10.1016/j.bbamcr.2007.05.003.
750. **Rispail N, Soanes DM, Ant C, Czajkowski R, Grunler A, Huguet R, Perez-Nadales E, Poli A, Sartorel E, Valiante V, Yang M, Beffa R, Brakhage AA, Gow NA, Kahmann R, Lebrun MH, Lenasi H, Perez-Martin J, Talbot NJ, Wendland J, Di Pietro A.** 2009. Comparative genomics of MAP kinase and calcium-calcineurin signalling components in plant and human pathogenic fungi. Fungal Genet Biol **46:**287–298. http://dx.doi.org/10.1016/j.fgb.2009.01.002.
751. **Chang L, Karin M.** 2001. Mammalian MAP kinase signalling cascades. Nature **410:**37–40. http://dx.doi.org/10.1038/35065000.
752. **Bruno KS, Morrell JL, Hamer JE, Staiger CJ.** 2001. SEPH, a Cdc7p orthologue from *Aspergillus nidulans*, functions upstream of actin ring formation during cytokinesis. Mol Microbiol **42:**3–12.
753. **Al Quobaili F, Montenarh M.** 2012. CK2 and the regulation of the carbohydrate metabolism. Metabolism **61:**1512–1517. http://dx.doi.org/10.1016/j.metabol.2012.07.011.
754. **Apostolaki A, Harispe L, Calcagno-Pizarelli AM, Vangelatos I, Sophianopoulou V, Arst HN, Jr, Penalva MA, Amillis S, Scazzocchio C.** 2012. *Aspergillus nidulans* CkiA is an essential casein kinase I required for delivery of amino acid transporters to the plasma membrane. Mol Microbiol **84:**530–549. http://dx.doi.org/10.1111/j.1365-2958.2012.08042.x.
755. **Schmoll M, Tian C, Sun J, Tisch D, Glass NL.** 2012. Unravelling the molecular basis for light modulated cellulase gene expression: the role of photoreceptors in *Neurospora crassa*. BMC Genomics **13:**127. http://dx.doi.org/10.1186/1471-2164-13-127.
756. **Tisch D, Schmoll M.** 2011. Novel approaches to improve cellulase biosynthesis for biofuel production. Adjusting signal transduction pathways in the biotechnological workhorse *Trichoderma reesei*, p 199–224. *In* dos Santos Bernardes MA (ed), Biofuel production: recent developments and prospects. Intech, Rijeka, Croatia.
757. **Seidl V, Gamauf C, Druzhinina IS, Seiboth B, Hartl L, Kubicek CP.** 2008. The *Hypocrea jecorina* (*Trichoderma reesei*) hypercellulolytic mutant RUT C30 lacks a 85 kb (29 gene-encoding) region of the wild-type genome. BMC Genomics **9:**327. http://dx.doi.org/10.1186/1471-2164-9-327.
758. **Diernfellner AC, Schafmeier T.** 2011. Phosphorylations: making the *Neurospora crassa* circadian clock tick. FEBS Lett **585:**1461–1466. http://dx.doi.org/10.1016/j.febslet.2011.03.049.
759. **Mehra A, Shi M, Baker CL, Colot HV, Loros JJ, Dunlap JC.** 2009. A role for casein kinase 2 in the mechanism underlying circadian temperature compensation. Cell **137:**749–760. http://dx.doi.org/10.1016/j.cell.2009.03.019.
760. **Yang Y, Cheng P, Liu Y.** 2002. Regulation of the *Neurospora* circadian clock by casein kinase II. Genes Dev **16:**994–1006. http://dx.doi.org/10.1101/gad.965102.
761. **Lee N, D'Souza CA, Kronstad JW.** 2003. Of smuts, blasts, mildews, and blights: cAMP signaling in phytopathogenic fungi. Annu Rev Phytopathol **41:**399–427. http://dx.doi.org/10.1146/annurev.phyto.41.052002.095728.
762. **Colabardini AC, Brown NA, Savoldi M, Goldman MH, Goldman GH.** 2013. Functional characterization of *Aspergillus nidulans ypkA*, a homologue of the mammalian kinase SGK. PLoS One **8:**e57630. http://dx.doi.org/10.1371/journal.pone.0057630.
763. **Mazzei GJ, Schmid EM, Knowles JK, Payton MA, Maundrell KG.** 1993. A Ca(2+)-independent protein kinase C from fission yeast. J Biol Chem **268:**7401–7406.
764. **Watanabe M, Chen CY, Levin DE.** 1994. *Saccharomyces cerevisiae* PKC1 encodes a protein kinase C (PKC) homolog with a substrate specificity similar to that of mammalian PKC. J Biol Chem **269:**16829–16836.
765. **Paravicini G, Mendoza A, Antonsson B, Cooper M, Losberger C, Payton MA.** 1996. The *Candida albicans* PKC1 gene encodes a protein kinase C homolog necessary for cellular integrity but not dimorphism. Yeast **12:**741–756. http://dx.doi.org/10.1002/(SICI)1097-0061(19960630)12:8<741::AID-YEA967>3.0.CO;2-G.
766. **Schuster A, Bruno KS, Collett JR, Baker SE, Seiboth B, Kubicek CP, Schmoll M.** 2012. A versatile toolkit for high throughput functional

genomics with *Trichoderma reesei*. Biotechnol Biofuels **5:**1. http://dx.doi.org/10.1186/1754-6834-5-1.
767. **Khatun R, Lakin-Thomas P.** 2011. Activation and localization of protein kinase C in *Neurospora crassa*. Fungal Genet Biol **48:**465–473. http://dx.doi.org/10.1016/j.fgb.2010.11.002.
768. **Arpaia G, Cerri F, Baima S, Macino G.** 1999. Involvement of protein kinase C in the response of *Neurospora crassa* to blue light. Mol Gen Genet **262:**314–322. http://dx.doi.org/10.1007/s004380051089.
769. **Herrmann M, Spröte P, Brakhage AA.** 2006. Protein kinase C (PkcA) of *Aspergillus nidulans* is involved in penicillin production. Appl Environ Microbiol **72:**2957–2970. http://dx.doi.org/10.1128/AEM.72.4.2957-2970.2006.
770. **Heung LJ, Luberto C, Plowden A, Hannun YA, Del Poeta M.** 2004. The sphingolipid pathway regulates Pkc1 through the formation of diacylglycerol in *Cryptococcus neoformans*. J Biol Chem **279:**21144–21153. http://dx.doi.org/10.1074/jbc.M312995200.
771. **Maerz S, Ziv C, Vogt N, Helmstaedt K, Cohen N, Gorovits R, Yarden O, Seiler S.** 2008. The nuclear Dbf2-related kinase COT1 and the mitogen-activated protein kinases MAK1 and MAK2 genetically interact to regulate filamentous growth, hyphal fusion and sexual development in *Neurospora crassa*. Genetics **179:**1313–1325. http://dx.doi.org/10.1534/genetics.108.089425.
772. **Klimecka M, Muszynska G.** 2007. Structure and functions of plant calcium-dependent protein kinases. Acta Biochim Pol **54:**219–233.
773. **Yang Y, Cheng P, Zhi G, Liu Y.** 2001. Identification of a calcium/calmodulin-dependent protein kinase that phosphorylates the *Neurospora* circadian clock protein FREQUENCY. J Biol Chem **276:**41064–41072. http://dx.doi.org/10.1074/jbc.M106905200.
774. **Pregueiro AM, Liu Q, Baker CL, Dunlap JC, Loros JJ.** 2006. The *Neurospora* checkpoint kinase 2: a regulatory link between the circadian and cell cycles. Science **313:**644–649. http://dx.doi.org/10.1126/science.1121716.
775. **Wullschleger S, Loewith R, Hall MN.** 2006. TOR signaling in growth and metabolism. Cell **124:**471–484. http://dx.doi.org/10.1016/j.cell.2006.01.016.
776. **Teichert S, Wottawa M, Schonig B, Tudzynski B.** 2006. Role of the *Fusarium fujikuroi* TOR kinase in nitrogen regulation and secondary metabolism. Eukaryot Cell **5:**1807–1819. http://dx.doi.org/10.1128/EC.00039-06.
777. **Ptacek J, Devgan G, Michaud G, Zhu H, Zhu X, Fasolo J, Guo H, Jona G, Breitkreutz A, Sopko R, McCartney RR, Schmidt MC, Rachidi N, Lee SJ, Mah AS, Meng L, Stark MJ, Stern DF, De Virgilio C, Tyers M, Andrews B, Gerstein M, Schweitzer B, Predki PF, Snyder M.** 2005. Global analysis of protein phosphorylation in yeast. Nature **438:**679–684. http://dx.doi.org/10.1038/nature04187.
778. **Téllez de I, ñón MT, Torres HN.** 1973. Regulation of glycogen phosphorylase a phosphatase in *Neurospora crassa*. Biochim Biophys Acta **297:**399–412. http://dx.doi.org/10.1016/0304-4165(73)90087-1.
779. **Higuchi S, Tamura J, Giri PR, Polli JW, Kincaid RL.** 1991. Calmodulin-dependent protein phosphatase from *Neurospora crassa*. Molecular cloning and expression of recombinant catalytic subunit. J Biol Chem **266:**18104–18112.
780. **Kothe GO, Free SJ.** 1998. Calcineurin subunit B is required for normal vegetative growth in *Neurospora crassa*. Fungal Genet Biol **23:**248–258. http://dx.doi.org/10.1006/fgbi.1998.1037.
781. **Prokisch H, Yarden O, Dieminger M, Tropschug M, Barthelmess IB.** 1997. Impairment of calcineurin function in *Neurospora crassa* reveals its essential role in hyphal growth, morphology and maintenance of the apical Ca2+ gradient. Mol Gen Genet **256:**104–114. http://dx.doi.org/10.1007/s004380050551.
782. **Szoor B, Dombradi V, Gergely P, Feher Z.** 1997. Purification and characterization of the catalytic subunit of protein phosphatase 1 from *Neurospora crassa*. Acta Biol Hung **48:**289–302.
783. **Szoor B, Feher Z, Bako E, Erdodi F, Szabo G, Gergely P, Dombradi V.** 1995. Isolation and characterization of the catalytic subunit of protein phosphatase 2A from *Neurospora crassa*. Comp Biochem Physiol B Biochem Mol Biol **112:**515–522. http://dx.doi.org/10.1016/0305-0491(95)00110-7.
784. **Yatzkan E, Dombra Di, Yarden VO.** 1999. Detection of a protein phosphatase 2A holoenzyme in *Neurospora crassa*. Fungal Genet Newslett **46:**32–33.
785. **Yatzkan E, Yarden O.** 1999. The B regulatory subunit of protein phosphatase 2A is required for completion of macroconidiation and other developmental processes in *Neurospora crassa*. Mol Microbiol **31:**197–209. http://dx.doi.org/10.1046/j.1365-2958.1999.01161.x.
786. **Yatzkan E, Szoor B, Feher Z, Dombradi V, Yarden O.** 1998. Protein phosphatase 2A is involved in hyphal growth of *Neurospora crassa*. Mol Gen Genet **259:**523–531. http://dx.doi.org/10.1007/s004380050844.
787. **Yatzkan E, Yarden O.** 1995. Inactivation of a single type-2A phosphoprotein phosphatase is lethal in *Neurospora crassa*. Curr Genet **28:**458–466. http://dx.doi.org/10.1007/BF00310816.
788. **Zapella PD, da-Silva AM, da-Costa-Maia JC, Terenzi HF.** 1996. Serine/threonine protein phosphatases and a protein phosphatase 1 inhibitor from *Neurospora crassa*. Braz J Med Biol Res **29:**599–604.
789. **Zeke T, Kokai E, Szoor B, Yatzkan E, Yarden O, Szirak K, Feher Z, Bagossi P, Gergely P, Dombradi V.** 2003. Expression of protein phosphatase 1 during the asexual development of *Neurospora crassa*. Comp Biochem Physiol B Biochem Mol Biol **134:**161–170. http://dx.doi.org/10.1016/S1096-4959(02)00188-4.
790. **Arino J, Casamayor A, Gonzalez A.** 2011. Type 2C protein phosphatases in fungi. Eukaryot Cell **10:**21–33. http://dx.doi.org/10.1128/EC.00249-10.
791. **Dickman MB, Yarden O.** 1999. Serine/threonine protein kinases and phosphatases in filamentious fungi. Fungal Genet Biol **26:**99–117. http://dx.doi.org/10.1006/fgbi.1999.1118.
792. **Pao LI, Badour K, Siminovitch KA, Neel BG.** 2007. Nonreceptor protein-tyrosine phosphatases in immune cell signaling. Annu Rev Immunol **25:**473–523. http://dx.doi.org/10.1146/annurev.immunol.23.021704.115647.
793. **Shi Y.** 2009. Serine/threonine phosphatases: mechanism through structure. Cell **139:**468–484. http://dx.doi.org/10.1016/j.cell.2009.10.006.
794. **Cohen P.** 1989. The structure and regulation of protein phosphatases. Annu Rev Biochem **58:**453–508. http://dx.doi.org/10.1146/annurev.bi.58.070189.002321.
795. **Barford D.** 1996. Molecular mechanisms of the protein serine/threonine phosphatases. Trends Biochem Sci **21:**407–412. http://dx.doi.org/10.1016/S0968-0004(96)10060-8.
796. **Cohen PTW.** 1997. Novel protein serine/threonine phosphatases: variety is the spice of life. Trends Biochem Sci **22:**245–251. http://dx.doi.org/10.1016/S0968-0004(97)01060-8.
797. **Dombrádi V.** 1997. Comparative analysis of Ser/Thr protein phosphatases. Trends Comp Biochem **3:**23–48.
798. **Das AK, Helps NR, Cohen PT, Barford D.** 1996. Crystal structure of the protein serine/threonine phosphatase 2C at 2.0 Å resolution. EMBO J **15:**6798–6809.
799. **Gohla A, Birkenfeld J, Bokoch GM.** 2005. Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. Nat Cell Biol **7:**21–29. http://dx.doi.org/10.1038/ncb1201.
800. **Seifried A, Schultz J, Gohla A.** 2013. Human HAD phosphatases: structure, mechanism, and roles in health and disease. FEBS J **280:**549–571. http://dx.doi.org/10.1111/j.1742-4658.2012.08633.x.
801. **Kerk D, Bulgrien J, Smith DW, Barsam B, Veretnik S, Gribskov M.** 2002. The complement of protein phosphatase catalytic subunits encoded in the genome of Arabidopsis. Plant Physiol **129:**908–925. http://dx.doi.org/10.1104/pp.004002.
802. **Fauman EB, Saper MA.** 1996. Structure and function of the protein tyrosine phosphatases. Trends Biochem Sci **21:**413–417. http://dx.doi.org/10.1016/S0968-0004(96)10059-1.
803. **Ramponi G, Stefani M.** 1997. Structure and function of the low M_r phosphotyrosine protein phosphatases. Biochim Biophys Acta **1341:**137–156. http://dx.doi.org/10.1016/S0167-4838(97)00087-3.
804. **Ducommun B, Draetta G, Young P, Beach D.** 1990. Fission yeast cdc25 is a cell-cycle regulated protein. Biochem Biophys Res Commun **167:**301–309. http://dx.doi.org/10.1016/0006-291X(90)91765-K.
805. **Galaktionov K, Beach D.** 1991. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. Cell **67:**1181–1194. http://dx.doi.org/10.1016/0092-8674(91)90294-9.
806. **Jinno S, Suto K, Nagata A, Igarashi M, Kanaoka Y, Nojima H, Okayama H.** 1994. Cdc25A is a novel phosphatase functioning early in the cell cycle. EMBO J **13:**1549–1556.
807. **Jimenez J, Alphey L, Nurse P, Glover DM.** 1990. Complementation of fission yeast cdc2ts and cdc25ts mutants identifies two cell cycle genes from Drosophila: a cdc2 homologue and string. EMBO J **9:**3565–3571.
808. **Su XD, Taddei N, Stefani M, Ramponi G, Nordlund P.** 1994. The crystal structure of a low-molecular-weight phosphotyrosine pro-

tein phosphatase. Nature **370:**575–578. http://dx.doi.org/10.1038/370575a0.
809. **Wo YY, McCormack AL, Shabanowitz J, Hunt DF, Davis JP, Mitchell GL, Van Etten RL.** 1992. Sequencing, cloning, and expression of human red cell-type acid phosphatase, a cytoplasmic phosphotyrosyl protein phosphatase. J Biol Chem **267:**10856–10865.
810. **Chen CH, Ringelberg CS, Gross RH, Dunlap JC, Loros JJ.** 2009. Genome-wide analysis of light-inducible responses reveals hierarchical light signalling in *Neurospora*. EMBO J **28:**1029–1042. http://dx.doi.org/10.1038/emboj.2009.54.
811. **Stark MJ.** 1996. Yeast protein serine/threonine phosphatases: multiple roles and diverse regulation. Yeast **12:**1647–1675.
812. **Mayer RE, Hendrix P, Cron P, Matthies R, Stone SR, Goris J, Merlevede W, Hofsteenge J, Hemmings BA.** 1991. Structure of the 55-kDa regulatory subunit of protein phosphatase 2A: evidence for a neuronal-specific isoform. Biochemistry **30:**3589–3597. http://dx.doi.org/10.1021/bi00229a001.
813. **McCright B, Virshup DM.** 1995. Identification of a new family of protein phosphatase 2A regulatory subunits. J Biol Chem **270:**26123–26128. http://dx.doi.org/10.1074/jbc.270.44.26123.
814. **Luke MM, Della Seta F, Di Como CJ, Sugimoto H, Kobayashi R, Arndt KT.** 1996. The SAP, a new family of proteins, associate and function positively with the SIT4 phosphatase. Mol Cell Biol **16:**2744–2755. http://dx.doi.org/10.1128/MCB.16.6.2744.
815. **Jacinto E, Guo B, Arndt KT, Schmelzle T, Hall MN.** 2001. TIP41 interacts with TAP42 and negatively regulates the TOR signaling pathway. Mol Cell **8:**1017–1026. http://dx.doi.org/10.1016/S1097-2765(01)00386-0.
816. **Printen JA, Brady MJ, Saltiel AR.** 1997. PTG, a protein phosphatase 1-binding protein with a role in glycogen metabolism. Science **275:**1475–1478. http://dx.doi.org/10.1126/science.275.5305.1475.
817. **Brown N, de Gouvea P, Krohn N, Savoldi M, Goldman G.** 2013. Functional characterisation of the non-essential protein kinases and phosphatases regulating *Aspergillus nidulans* hydrolytic enzyme production. Biotechnol Biofuels **6:**91. http://dx.doi.org/10.1186/1754-6834-6-91.
818. **Ghosh A, Servin JA, Park G, Borkovich KA.** 2014. Global analysis of serine/threonine and tyrosine protein phosphatase catalytic subunit genes in *Neurospora crassa* reveals interplay between phosphatases and the p38 mitogen-activated protein kinase. G3 (Bethesda) **4:**349–365. http://dx.doi.org/10.1534/g3.113.008813.
819. **Son S, Osmani SA.** 2009. Analysis of all protein phosphatase genes in *Aspergillus nidulans* identifies a new mitotic regulator, *fcp1*. Eukaryot Cell **8:**573–585. http://dx.doi.org/10.1128/EC.00346-08.
820. **Taylor GS, Liu Y, Baskerville C, Charbonneau H.** 1997. The activity of Cdc14p, an oligomeric dual specificity protein phosphatase from *Saccharomyces cerevisiae*, is required for cell cycle progression. J Biol Chem **272:**24054–24063. http://dx.doi.org/10.1074/jbc.272.38.24054.
821. **Fox GC, Shafiq M, Briggs DC, Knowles PP, Collister M, Didmon MJ, Makrantoni V, Dickinson RJ, Hanrahan S, Totty N, Stark MJ, Keyse SM, McDonald NQ.** 2007. Redox-mediated substrate recognition by Sdp1 defines a new group of tyrosine phosphatases. Nature **447:**487–492. http://dx.doi.org/10.1038/nature05804.
822. **Smith SV, Kinsey DW.** 1976. Calcium carbonate production, coral reef growth, and sea level change. Science **194:**937–939. http://dx.doi.org/10.1126/science.194.4268.937.
823. **Berridge MJ.** 1997. Elementary and global aspects of calcium signalling. J Exp Biol **200:**315–319.
824. **Navazio L, Baldan B, Moscatiello R, Zuppini A, Woo SL, Mariani P, Lorito M.** 2007. Calcium-mediated perception and defense responses activated in plant cells by metabolite mixtures secreted by the biocontrol fungus *Trichoderma atroviride*. BMC Plant Biol **7:**41. http://dx.doi.org/10.1186/1471-2229-7-41.
825. **Brand A, Gow NAR.** 2009. Mechanisms of hypha orientation of fungi. Curr Opin Microbiol **12:**350–357. http://dx.doi.org/10.1016/j.mib.2009.05.007.
826. **Caracuel-Rios Z, Talbot NJ.** 2008. Silencing the crowd: high-throughput functional genomics in *Magnaporthe oryzae*. Mol Microbiol **68:**1341–1344. http://dx.doi.org/10.1111/j.1365-2958.2008.06257.x.
827. **Cavinder B, Hamam A, Lew RR, Trail F.** 2011. Mid1, a mechanosensitive calcium ion channel, affects growth, development, and ascospore discharge in the filamentous fungus *Gibberella zeae*. Eukaryot Cell **10:**832–841. http://dx.doi.org/10.1128/EC.00235-10.
828. **Hallen HE, Trail F.** 2008. The L-type calcium ion channel Cch1 affects ascospore discharge and mycelial growth in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). Eukaryot Cell **7:**415–424. http://dx.doi.org/10.1128/EC.00248-07.
829. **Jackson SL, Heath IB.** 1989. Effects of exogenous calcium ions on tip growth, intracellular Ca^{2+} concentration, and actin arrays in hyphae of the fungus *Saprolegnia ferax*. Exp Mycol **13:**1–12. http://dx.doi.org/10.1016/0147-5975(89)90002-9.
830. **Kim S, Hu J, Oh Y, Park J, Choi J, Lee YH, Dean RA, Mitchell TK.** 2010. Combining ChIP-chip and expression profiling to model the MoCRZ1 mediated circuit for Ca/calcineurin signaling in the rice blast fungus. PLoS Pathog **6:**e1000909. http://dx.doi.org/10.1371/journal.ppat.1000909.
831. **Mandels M, Reese ET.** 1957. Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J Bacteriol **73:**269–278.
832. **Zelter A, Bencina M, Bowman BJ, Yarden O, Read ND.** 2004. A comparative genomic analysis of the calcium signaling machinery in *Neurospora crassa*, *Magnaporthe grisea*, and *Saccharomyces cerevisiae*. Fungal Genet Biol **41:**827–841. http://dx.doi.org/10.1016/j.fgb.2004.05.001.
833. **Palmer CP, Zhou XL, Lin J, Loukin SH, Kung C, Saimi Y.** 2001. A TRP homolog in *Saccharomyces cerevisiae* forms an intracellular Ca(2+)-permeable channel in the yeast vacuolar membrane. Proc Natl Acad Sci U S A **98:**7801–7805. http://dx.doi.org/10.1073/pnas.141036198.
834. **Ozeki-Miyawaki C, Moriya Y, Tatsumi H, Iida H, Sokabe M.** 2005. Identification of functional domains of Mid1, a stretch-activated channel component, necessary for localization to the plasma membrane and Ca2+ permeation. Exp Cell Res **311:**84–95. http://dx.doi.org/10.1016/j.yexcr.2005.08.014.
835. **Locke EG, Bonilla M, Liang L, Takita Y, Cunningham KW.** 2000. A homolog of voltage-gated Ca(2+) channels stimulated by depletion of secretory Ca(2+) in yeast. Mol Cell Biol **20:**6686–6694. http://dx.doi.org/10.1128/MCB.20.18.6686-6694.2000.
836. **Peiter E, Fischer M, Sidaway K, Roberts SK, Sanders D.** 2005. The *Saccharomyces cerevisiae* Ca2+ channel Cch1pMid1p is essential for tolerance to cold stress and iron toxicity. FEBS Lett **579:**5697–5703. http://dx.doi.org/10.1016/j.febslet.2005.09.058.
837. **Iida H, Nakamura H, Ono T, Okumura MS, Anraku Y.** 1994. MID1, a novel *Saccharomyces cerevisiae* gene encoding a plasma membrane protein, is required for Ca2+ influx and mating. Mol Cell Biol **14:**8259–8271. http://dx.doi.org/10.1128/MCB.14.12.8259.
838. **Bootman MD, Collins TJ, Peppiatt CM, Prothero LS, MacKenzie L, De Smet P, Travers M, Tovey SC, Seo JT, Berridge MJ, Ciccolini F, Lipp P.** 2001. Calcium signalling: an overview. Semin Cell Dev Biol **12:**3–10. http://dx.doi.org/10.1006/scdb.2000.0211.
839. **Mach RL, Zeilinger S, Kristufek D, Kubicek CP.** 1998. Ca2+-calmodulin antagonists interfere with xylanase formation and secretion in *Trichoderma reesei*. Biochim Biophys Acta **1403:**281–289. http://dx.doi.org/10.1016/S0167-4889(98)00068-8.
840. **Axelsen KB, Palmgren MG.** 1998. Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol **46:**84–101. http://dx.doi.org/10.1007/PL00006286.
841. **Sorin A, Rosas G, Rao R.** 1997. PMR1, a Ca2+-ATPase in yeast Golgi, has properties distinct from sarco/endoplasmic reticulum and plasma membrane calcium pumps. J Biol Chem **272:**9895–9901. http://dx.doi.org/10.1074/jbc.272.15.9895.
842. **Moller JV, Juul B, le Maire M.** 1996. Structural organization, ion transport, and energy transduction of P-type ATPases. Biochim Biophys Acta **1286:**1–51. http://dx.doi.org/10.1016/0304-4157(95)00017-8.
843. **Benito B, Garciadeblas B, Rodriguez-Navarro A.** 2000. Molecular cloning of the calcium and sodium ATPases in *Neurospora crassa*. Mol Microbiol **35:**1079–1088. http://dx.doi.org/10.1046/j.1365-2958.2000.01776.x.
844. **Bowman BJ, Abreu S, Margolles-Clark E, Draskovic M, Bowman EJ.** 2011. Role of four calcium transport proteins, encoded by *nca-1*, *nca-2*, *nca-3*, and *cax*, in maintaining intracellular calcium levels in *Neurospora crassa*. Eukaryot Cell **10:**654–661. http://dx.doi.org/10.1128/EC.00239-10.
845. **Vashist S, Frank CG, Jakob CA, Ng DT.** 2002. Two distinctly localized P-type ATPases collaborate to maintain organelle homeostasis required for glycoprotein processing and quality control. Mol Biol Cell **13:**3955–3966. http://dx.doi.org/10.1091/mbc.02-06-0090.

846. **Nguyen QB, Kadotani N, Kasahara S, Tosa Y, Mayama S, Nakayashiki H.** 2008. Systematic functional analysis of calcium-signalling proteins in the genome of the rice-blast fungus, Magnaporthe oryzae, using a high-throughput RNA-silencing system. Mol Microbiol **68:**1348–1365. http://dx.doi.org/10.1111/j.1365-2958.2008.06242.x.
847. **Wisniewski M, Droby S, Chalutz E, Eilam Y.** 1995. Effects of Ca2+ and Mg2+ on *Botrytis cinerea* and *Penicillium expansum* in vitro and on the biocontrol activity of *Candida oleophila*. Plant Pathol **44:**1016–1024. http://dx.doi.org/10.1111/j.1365-3059.1995.tb02660.x.
848. **Patterson RL, van Rossum DB, Nikolaidis N, Gill DL, Snyder SH.** 2005. Phospholipase C-gamma: diverse roles in receptor-mediated calcium signaling. Trends Biochem Sci **30:**688–697. http://dx.doi.org/10.1016/j.tibs.2005.10.005.
849. **Gavric O, dos Santos DB, Griffiths A.** 2007. Mutation and divergence of the phospholipase C gene in *Neurospora crassa*. Fungal Genet Biol **44:**242–249. http://dx.doi.org/10.1016/j.fgb.2006.09.010.
850. **Rho H-S, Jeon J, Lee Y-H.** 2009. Phospholipase C-mediated calcium signalling is required for fungal development and pathogenicity in *Magnaporthe oryzae*. Mol Plant Pathol **10:**337–346. http://dx.doi.org/10.1111/j.1364-3703.2009.00536.x.
851. **Kraus PR, Heitman J.** 2003. Coping with stress: calmodulin and calcineurin in model and pathogenic fungi. Biochem Biophys Res Commun **311:**1151–1157. http://dx.doi.org/10.1016/S0006-291X(03)01528-6.
852. **Juvvadi PR, Kuroki Y, Arioka M, Nakajima H, Kitamoto K.** 2003. Functional analysis of the calcineurin-encoding gene *cnaA* from *Aspergillus oryzae*: evidence for its putative role in stress adaptation. Arch Microbiol **179:**416–422.
853. **Pardo JM, Reddy MP, Yang S, Maggio A, Huh GH, Matsumoto T, Coca MA, Paino-D'Urzo M, Koiwa H, Yun DJ, Watad AA, Bressan RA, Hasegawa PM.** 1998. Stress signaling through Ca2+/calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants. Proc Natl Acad Sci U S A **95:**9681–9686. http://dx.doi.org/10.1073/pnas.95.16.9681.
854. **Rasmussen C, Garen C, Brining S, Kincaid RL, Means RL, Means AR.** 1994. The calmodulin-dependent protein phosphatase catalytic subunit (calcineurin A) is an essential gene in *Aspergillus nidulans*. EMBO J **13:**3917–3924.
855. **Chung KR.** 2003. Involvement of calcium/calmodulin signaling in cercosporin toxin biosynthesis by *Cercospora nicotianae*. Appl Environ Microbiol **69:**1187–1196. http://dx.doi.org/10.1128/AEM.69.2.1187-1196.2003.
856. **Cyert MS, Kunisawa R, Kaim D, Thorner J.** 1991. Yeast has homologues (CNA1 and CNA2 gene products) of mammalian calcineurin, a calmodulin-regulated phosphoprotein phosphatase. Proc Natl Acad Sci U S A **88:**7376–7380. http://dx.doi.org/10.1073/pnas.88.16.7376.
857. **Fox DS, Heitman J.** 2002. Good fungi gone bad: the corruption of calcineurin. Bioessays **24:**894–903. http://dx.doi.org/10.1002/bies.10157.
858. **Withee JL, Mulholland J, Jeng R, Cyert MS.** 1997. An essential role of the yeast pheromone-induced Ca2+ signal is to activate calcineurin. Mol Biol Cell **8:**263–277. http://dx.doi.org/10.1091/mbc.8.2.263.
859. **Seidl V, Seibel C, Kubicek CP, Schmoll M.** 2009. Sexual development in the industrial workhorse *Trichoderma reesei*. Proc Natl Acad Sci U S A **106:**13909–13914. http://dx.doi.org/10.1073/pnas.0904936106.
860. **Helenius A, Trombetta ES, Hebert DN, Simons JF.** 1997. Calnexin, calreticulin and the folding of glycoproteins. Trends Cell Biol **7:**193–200. http://dx.doi.org/10.1016/S0962-8924(97)01032-5.
861. **Saito Y, Ihara Y, Leach MR, Cohen-Doyle MF, Williams DB.** 1999. Calreticulin functions in vitro as a molecular chaperone for both glycosylated and non-glycosylated proteins. EMBO J **18:**6718–6729. http://dx.doi.org/10.1093/emboj/18.23.6718.
862. **Burgoyne RD, Weiss JL.** 2001. The neuronal calcium sensor family of Ca2+-binding proteins. Biochem J **353:**1–12. http://dx.doi.org/10.1042/bj3530001.
863. **Deka R, Kumar R, Tamuli R.** 2011. *Neurospora crassa* homologue of neuronal calcium sensor-1 has a role in growth, calcium stress tolerance, and ultraviolet survival. Genetica **139:**885–894. http://dx.doi.org/10.1007/s10709-011-9592-y.
864. **Hamasaki-Katagiri N, Molchanova T, Takeda K, Ames JB.** 2004. Fission yeast homolog of neuronal calcium sensor-1 (Ncs1p) regulates sporulation and confers calcium tolerance. J Biol Chem **279:**12744–12754. http://dx.doi.org/10.1074/jbc.M311895200.
865. **Saitoh K, Arie T, Teraoka T, Yamaguchi I, Kamakura T.** 2003. Targeted gene disruption of the neuronal calcium sensor 1 homologue in rice blast fungus, *Magnaporthe grisea*. Biosci Biotechnol Biochem **67:**651–653. http://dx.doi.org/10.1271/bbb.67.651.
866. **Coukell B, Cameron A, Perusini S, Shim K.** 2004. Disruption of the NCS-1/frequenin-related *ncsA* gene in *Dictyostelium discoideum* accelerates development. Dev Growth Differ **46:**449–458. http://dx.doi.org/10.1111/j.1440-169x.2004.00761.x.
867. **Wolenski JS.** 1995. Regulation of calmodulin-binding myosins. Trends Cell Biol **5:**310–316. http://dx.doi.org/10.1016/S0962-8924(00)89053-4.
868. **Cheney RE, Mooseker MS.** 1992. Unconventional myosins. Curr Opin Cell Biol **4:**27–35. http://dx.doi.org/10.1016/0955-0674(92)90055-H.
869. **D'Souza VM, Naqvi NI, Wang H, Balasubramanian MK.** 2001. Interactions of Cdc4p, a myosin light chain, with IQ-domain containing proteins in *Schizosaccharomyces pombe*. Cell Struct Funct **26:**555–565. http://dx.doi.org/10.1247/csf.26.555.
870. **Gerke V, Moss SE.** 2002. Annexins: from structure to function. Physiol Rev **82:**331–371. http://dx.doi.org/10.1152/physrev.00030.2001.
871. **Gerke V, Weber K.** 1984. Identity of p36K phosphorylated upon Rous sarcoma virus transformation with a protein purified from brush borders; calcium-dependent binding to non-erythroid spectrin and F-actin. EMBO J **3:**227–233.
872. **Klee CB.** 1988. Ca2+-dependent phospholipid- (and membrane-) binding proteins. Biochemistry **27:**6645–6653. http://dx.doi.org/10.1021/bi00418a001.
873. **Herrera-Estrella A, Horwitz BA.** 2007. Looking through the eyes of fungi: molecular genetics of photoreception. Mol Microbiol **64:**5–15. http://dx.doi.org/10.1111/j.1365-2958.2007.05632.x.
874. **Lledias F, Hansberg W.** 2000. Catalase modification as a marker for singlet oxygen. Methods Enzymol **319:**110–119. http://dx.doi.org/10.1016/S0076-6879(00)19013-5.
875. **Barnett HL, Lilly VG.** 1951. The inhibitory effect of sorbose on fungi. Science **114:**439–440. http://dx.doi.org/10.1126/science.114.2965.439.
876. **Gressel JB, Hartmann KM.** 1968. Morphogenesis in *Trichoderma*: action spectrum of photoinduced sporulation. Planta **79:**271–274. http://dx.doi.org/10.1007/BF00396034.
877. **Kumagai T, Oda Y.** 1969. Blue and near ultraviolet reversible photoreaction in conidial development of the fungus, *Alternaria tomato*. Dev Growth Differ **11:**130–142. http://dx.doi.org/10.1111/j.1440-169X.1969.00130.x.
878. **Casas-Flores S, Rios-Momberg M, Bibbins M, Ponce-Noyola P, Herrera-Estrella A.** 2004. BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. Microbiology **150:**3561–3569. http://dx.doi.org/10.1099/mic.0.27346-0.
879. **Castellanos F, Schmoll M, Martinez P, Tisch D, Kubicek CP, Herrera-Estrella A, Esquivel-Naranjo EU.** 2010. Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. Fungal Genet Biol **47:**468–476. http://dx.doi.org/10.1016/j.fgb.2010.02.001.
880. **Rosales-Saavedra T, Esquivel-Naranjo EU, Casas-Flores S, Martinez-Hernandez P, Ibarra-Laclette E, Cortes-Penagos C, Herrera-Estrella A.** 2006. Novel light-regulated genes in *Trichoderma atroviride*: a dissection by cDNA microarrays. Microbiology **152:**3305–3317. http://dx.doi.org/10.1099/mic.0.29000-0.
881. **Chen CH, Dunlap JC, Loros JJ.** 2010. *Neurospora* illuminates fungal photoreception. Fungal Genet Biol **47:**922–929. http://dx.doi.org/10.1016/j.fgb.2010.07.005.
882. **Schafmeier T, Diernfellner AC.** 2011. Light input and processing in the circadian clock of *Neurospora*. FEBS Lett **585:**1467–1473. http://dx.doi.org/10.1016/j.febslet.2011.03.050.
883. **Smith KM, Sancar G, Dekhang R, Sullivan CM, Li S, Tag AG, Sancar C, Bredeweg EL, Priest HD, McCormick RF, Thomas TL, Carrington JC, Stajich JE, Bell-Pedersen D, Brunner M, Freitag M.** 2010. Transcription factors in light and circadian clock signaling networks revealed by genome wide mapping of direct targets for *Neurospora* White Collar complex. Eukaryot Cell **9:**1549–1556. http://dx.doi.org/10.1128/EC.00154-10.
884. **Esquivel-Naranjo EU, Herrera-Estrella A.** 2007. Enhanced responsiveness and sensitivity to blue light by *blr-2* overexpression in *Trichoderma atroviride*. Microbiology **153:**3909–3922. http://dx.doi.org/10.1099/mic.0.2007/007302-0.
885. **Seibel C, Tisch D, Kubicek CP, Schmoll M.** 2012. ENVOY is a major determinant in regulation of sexual development in *Hypocrea jecorina*

(*Trichoderma reesei*). Eukaryot Cell **11:**885–890. http://dx.doi.org/10.1128/EC.05321-11.
886. **Schmoll M, Franchi L, Kubicek CP.** 2005. Envoy, a PAS/LOV domain protein of *Hypocrea jecorina* (anamorph *Trichoderma reesei*), modulates cellulase gene transcription in response to light. Eukaryot Cell **4:**1998–2007. http://dx.doi.org/10.1128/EC.4.12.1998-2007.2005.
887. **Schmoll M, Zeilinger S, Mach RL, Kubicek CP.** 2004. Cloning of genes expressed early during cellulase induction in *Hypocrea jecorina* by a rapid subtraction hybridization approach. Fungal Genet Biol **41:**877–887. http://dx.doi.org/10.1016/j.fgb.2004.06.002.
888. **Heintzen C, Loros JJ, Dunlap JC.** 2001. The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. Cell **104:**453–464. http://dx.doi.org/10.1016/S0092-8674(01)00232-X.
889. **Schwerdtfeger C, Linden H.** 2001. Blue light adaptation and desensitization of light signal transduction in *Neurospora crassa*. Mol Microbiol **39:**1080–1087. http://dx.doi.org/10.1046/j.1365-2958.2001.02306.x.
890. **Schwerdtfeger C, Linden H.** 2003. VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. EMBO J **22:**4846–4855. http://dx.doi.org/10.1093/emboj/cdg451.
891. **Chen CL, Kuo HC, Tung SY, Hsu PW, Wang CL, Seibel C, Schmoll M, Chen RS, Wang TF.** 2012. Blue light acts as a double-edged sword in regulating sexual development of *Hypocrea jecorina* (*Trichoderma reesei*). PLoS One **7:**e44969. http://dx.doi.org/10.1371/journal.pone.0044969.
892. **Lokhandwala J, Hopkins HC, Rodriguez-Iglesias A, Dattenbock C, Schmoll M, Zoltowski BD.** 2015. Structural biochemistry of a fungal LOV domain photoreceptor reveals an evolutionarily conserved pathway integrating light and oxidative stress. Structure **23:**116–125. http://dx.doi.org/10.1016/j.str.2014.10.020.
893. **Blumenstein A, Vienken K, Tasler R, Purschwitz J, Veith D, Frankenberg-Dinkel N, Fischer R.** 2005. The *Aspergillus nidulans* phytochrome FphA represses sexual development in red light. Curr Biol **15:**1833–1838. http://dx.doi.org/10.1016/j.cub.2005.08.061.
894. **Liu Y, Bell-Pedersen D.** 2006. Circadian rhythms in *Neurospora crassa* and other filamentous fungi. Eukaryot Cell **5:**1184–1193. http://dx.doi.org/10.1128/EC.00133-06.
895. **Brunner M, Kaldi K.** 2008. Interlocked feedback loops of the circadian clock of *Neurospora crassa*. Mol Microbiol **68:**255–262. http://dx.doi.org/10.1111/j.1365-2958.2008.06148.x.
896. **He Q, Cheng P, He Q, Liu Y.** 2005. The COP9 signalosome regulates the *Neurospora* circadian clock by controlling the stability of the SCFFWD-1 complex. Genes Dev **19:**1518–1531. http://dx.doi.org/10.1101/gad.1322205.
897. **Shi M, Collett M, Loros JJ, Dunlap JC.** 2010. FRQ-interacting RNA helicase mediates negative and positive feedback in the *Neurospora* circadian clock. Genetics **184:**351–361. http://dx.doi.org/10.1534/genetics.109.111393.
898. **Nsa IY, Karunarathna N, Liu X, Huang H, Boetteger B, Bell-Pedersen D.** 2015. A novel cryptochrome-dependent oscillator in *Neurospora crassa*. Genetics **199:**233–245. http://dx.doi.org/10.1534/genetics.114.169441.
899. **Bayram O, Braus GH.** 2012. Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. FEMS Microbiol Rev **36:**1–24. http://dx.doi.org/10.1111/j.1574-6976.2011.00285.x.
900. **Bayram O, Krappmann S, Ni M, Bok JW, Helmstaedt K, Valerius O, Braus-Stromeyer S, Kwon NJ, Keller NP, Yu JH, Braus GH.** 2008. VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science **320:**1504–1506. http://dx.doi.org/10.1126/science.1155888.
901. **Bayram O, Krappmann S, Seiler S, Vogt N, Braus GH.** 2008. *Neurospora crassa ve-1* affects asexual conidiation. Fungal Genet Biol **45:**127–138. http://dx.doi.org/10.1016/j.fgb.2007.06.001.
902. **Feder ME, Hofmann GE.** 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol **61:**243–282. http://dx.doi.org/10.1146/annurev.physiol.61.1.243.
903. **Strudwick N, Schröder M.** 2007. The unfolded protein response, p 69–155. *In* Al-Rubeai M, Fussenegger M (ed), Systems biology, vol 5. Springer, Dordrecht, Netherlands.
904. **Do JH, Yamaguchi R, Miyano S.** 2009. Exploring temporal transcription regulation structure of *Aspergillus fumigatus* in heat shock by state space model. BMC Genomics **10:**306. http://dx.doi.org/10.1186/1471-2164-10-306.
905. **Parsell DA, Lindquist S.** 1993. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu Rev Genet **27:**437–496.
906. **Kiang JG, Tsokos GC.** 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol Ther **80:**183–201. http://dx.doi.org/10.1016/S0163-7258(98)00028-X.
907. **Goloubinoff P, Rios PDL.** 2007. The mechanism of Hsp70 chaperones: (entropic) pulling the models together. Trends Biochem Sci **32:**372–380. http://dx.doi.org/10.1016/j.tibs.2007.06.008.
908. **Morano KA, Liu PC, Thiele DJ.** 1998. Protein chaperones and the heat shock response in *Saccharomyces cerevisiae*. Curr Opin Microbiol **1:**197–203. http://dx.doi.org/10.1016/S1369-5274(98)80011-8.
909. **Squina F, Leal J, Cipriano V, Martinez-Rossi N, Rossi A.** 2010. Transcription of the *Neurospora crassa* 70-kDa class heat shock protein genes is modulated in response to extracellular pH changes. Cell Stress Chaperones **15:**225–231. http://dx.doi.org/10.1007/s12192-009-0131-z.
910. **Hafker T, Techel D, Steier G, Rensing L.** 1998. Differential expression of glucose-regulated (*grp78*) and heat-shock-inducible (*hsp70*) genes during asexual development of *Neurospora crassa*. Microbiology **144:**37–43. http://dx.doi.org/10.1099/00221287-144-1-37.
911. **Rautio J, Smit B, Wiebe M, Penttila M, Saloheimo M.** 2006. Transcriptional monitoring of steady state and effects of anaerobic phases in chemostat cultures of the filamentous fungus *Trichoderma reesei*. BMC Genomics **7:**247. http://dx.doi.org/10.1186/1471-2164-7-247.
912. **Montero-Barrientos M, Hermosa R, Nicolás C, Cardoza RE, Gutiérrez S, Monte E.** 2008. Overexpression of a *Trichoderma* HSP70 gene increases fungal resistance to heat and other abiotic stresses. Fungal Genet Biol **45:**1506–1513. http://dx.doi.org/10.1016/j.fgb.2008.09.003.
913. **Montero-Barrientos M, Hermosa R, Cardoza RE, Gutiérrez S, Nicolás C, Monte E.** 2010. Transgenic expression of the *Trichoderma harzianum hsp70* gene increases *Arabidopsis* resistance to heat and other abiotic stresses. J Plant Physiol **167:**659–665. http://dx.doi.org/10.1016/j.jplph.2009.11.012.
914. **Craig EA, Huang P, Aron R, Andrew A.** 2006. The diverse roles of J-proteins, the obligate Hsp70 co-chaperone. Rev Physiol Biochem Pharmacol **156:**1–21.
915. **Kampinga HH, Craig EA.** 2010. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol **11:**579–592. http://dx.doi.org/10.1038/nrm2941.
916. **Young JC, Agashe VR, Siegers K, Hartl FU.** 2004. Pathways of chaperone-mediated protein folding in the cytosol. Nat Rev Mol Cell Biol **5:**781–791. http://dx.doi.org/10.1038/nrm1492.
917. **Hendrick JP, Langer T, Davis TA, Hartl FU, Wiedmann M.** 1993. Control of folding and membrane translocation by binding of the chaperone DnaJ to nascent polypeptides. Proc Natl Acad Sci U S A **90:**10216–10220. http://dx.doi.org/10.1073/pnas.90.21.10216.
918. **Johnson JL, Craig EA.** 2001. An essential role for the substrate-binding region of Hsp40s in *Saccharomyces cerevisiae*. J Cell Biol **152:**851–856. http://dx.doi.org/10.1083/jcb.152.4.851.
919. **Marra R, Ambrosino P, Carbone V, Vinale F, Woo SL, Ruocco M, Ciliento R, Lanzuise S, Ferraioli S, Soriente I, Gigante S, Turra D, Fogliano V, Scala F, Lorito M.** 2006. Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Curr Genet **50:**307–321. http://dx.doi.org/10.1007/s00294-006-0091-0.
920. **Aron R, Lopez N, Walter W, Craig EA, Johnson J.** 2005. *In vivo* bipartite interaction between the Hsp40 Sis1 and Hsp70 in *Saccharomyces cerevisiae*. Genetics **169:**1873–1882. http://dx.doi.org/10.1534/genetics.104.037242.
921. **Schroder H, Langer T, Hartl FU, Bukau B.** 1993. DnaK, DnaJ and GrpE form a cellular chaperone machinery capable of repairing heat-induced protein damage. EMBO J **12:**4137–4144.
922. **Voos W, Gambill BD, Laloraya S, Ang D, Craig EA, Pfanner N.** 1994. Mitochondrial GrpE is present in a complex with hsp70 and preproteins in transit across membranes. Mol Cell Biol **14:**6627–6634. http://dx.doi.org/10.1128/MCB.14.10.6627.
923. **Bolliger L, Deloche O, Glick BS, Georgopoulos C, Jeno P, Kronidou N, Horst M, Morishima N, Schatz G.** 1994. A mitochondrial homolog of bacterial GrpE interacts with mitochondrial hsp70 and is essential for viability. EMBO J **13:**1998–2006.

924. **Laloraya S, Dekker PJ, Voos W, Craig EA, Pfanner N.** 1995. Mitochondrial GrpE modulates the function of matrix Hsp70 in translocation and maturation of preproteins. Mol Cell Biol **15:**7098–7105. http://dx.doi.org/10.1128/MCB.15.12.7098.
925. **Kabani M, BeckerichJ-M, Brodsky JL.** 2002. Nucleotide exchange factor for the yeast Hsp70 molecular chaperone Ssa1p. Mol Cell Biol **22:**4677–4689. http://dx.doi.org/10.1128/MCB.22.13.4677-4689.2002.
926. **Kabani M, BeckerichM, Gaillardin C.** 2000. Sls1p stimulates Sec63p-mediated activation of Kar2p in a conformation-dependent manner in the yeast endoplasmic reticulum. Mol Cell Biol **20:**6923–6934. http://dx.doi.org/10.1128/MCB.20.18.6923-6934.2000.
927. **Doong H, Vrailas A, Kohn EC.** 2002. What's in the 'BAG'? A functional domain analysis of the BAG-family proteins. Cancer Lett **188:**25–32.
928. **Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, Morimoto RI, Reed JC.** 1997. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. EMBO J **16:**4887–4896. http://dx.doi.org/10.1093/emboj/16.16.4887.
929. **Briknarova K, Takayama S, Brive L, Havert ML, Knee DA, Velasco J, Homma S, Cabezas E, Stuart J, Hoyt DW, Satterthwait AC, Llinas M, Reed JC, Ely KR.** 2001. Structural analysis of BAG1 cochaperone and its interactions with Hsc70 heat shock protein. Nat Struct Mol Biol **8:**349–352. http://dx.doi.org/10.1038/86236.
930. **Sondermann H, Scheufler C, Schneider C, Hohfeld J, Hartl FU, Moarefi I.** 2001. Structure of a Bag/Hsc70 complex: convergent functional evolution of Hsp70 nucleotide exchange factors. Science **291:**1553–1557. http://dx.doi.org/10.1126/science.1057268.
931. **Sondermann H, Ho AK, Listenberger LL, Siegers K, Moarefi I, Wente SR, Hartl FU, Young JC.** 2002. Prediction of novel Bag-1 homologues based on structure/function analysis identifies Snl1p as an Hsp70 co-chaperone in *Saccharomyces cerevisiae*. J Biol Chem **277:**33220–33227. http://dx.doi.org/10.1074/jbc.M204624200.
932. **Dragovic Z, Broadley SA, Shomura Y, Bracher A, Hartl FU.** 2006. Molecular chaperones of the Hsp110 family act as nucleotide exchange factors of Hsp70s. EMBO J **25:**2519–2528. http://dx.doi.org/10.1038/sj.emboj.7601138.
933. **Raviol H, Sadlish H, Rodriguez F, Mayer MP, Bukau B.** 2006. Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. EMBO J **25:**2510–2518. http://dx.doi.org/10.1038/sj.emboj.7601139.
934. **Plesofsky-Vig N, Brambl R.** 1998. Characterization of an 88-kDa heat shock protein of *Neurospora crassa* that interacts with Hsp30. J Biol Chem **273:**11335–11341. http://dx.doi.org/10.1074/jbc.273.18.11335.
935. **Picard D.** 2002. Heat-shock protein 90, a chaperone for folding and regulation. Cell Mol Life Sci **59:**1640–1648. http://dx.doi.org/10.1007/PL00012491.
936. **Wandinger SK, Richter K, Buchner J.** 2008. The Hsp90 chaperone machinery. J Biol Chem **283:**18473–18477. http://dx.doi.org/10.1074/jbc.R800007200.
937. **Scroggins BT, Robzyk K, Wang D, Marcu MG, Tsutsumi S, Beebe K, Cotter RJ, Felts S, Toft D, Karnitz L, Rosen N, Neckers L.** 2007. An acetylation site in the middle domain of Hsp90 regulates chaperone function. Mol Cell **25:**151–159. http://dx.doi.org/10.1016/j.molcel.2006.12.008.
938. **Wandinger SK, Suhre MH, Wegele H, Buchner J.** 2006. The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90. EMBO J **25:**367–376. http://dx.doi.org/10.1038/sj.emboj.7600930.
939. **Shiau AK, Harris SF, Southworth DR, Agard DA.** 2006. Structural analysis of *E. coli hsp90* reveals dramatic nucleotide-dependent conformational rearrangements. Cell **127:**329–340. http://dx.doi.org/10.1016/j.cell.2006.09.027.
940. **Richter K, Buchner J.** 2006. hsp90: Twist and Fold. Cell **127:**251–253. http://dx.doi.org/10.1016/j.cell.2006.10.004.
941. **Chen S, Smith DF.** 1998. Hop as an adaptor in the heat shock protein 70 (Hsp70) and Hsp90 chaperone machinery. J Biol Chem **273:**35194–35200. http://dx.doi.org/10.1074/jbc.273.52.35194.
942. **Richter K, Muschler P, Hainzl O, Reinstein J, Buchner J.** 2003. Sti1 is a non-competitive inhibitor of the Hsp90 ATPase. J Biol Chem **278:**10328–10333. http://dx.doi.org/10.1074/jbc.M213094200.
943. **Prodromou C, Siligardi G, O'Brien R, Woolfson DN, Regan L, Panaretou B, Ladbury JE, Piper PW, Pearl LH.** 1999. Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. EMBO J **18:**754–762. http://dx.doi.org/10.1093/emboj/18.3.754.
944. **Matts R, Caplan A.** 2007. Cdc37 and protein kinase folding, p 331–350. *In* Calderwood SK, Sherman MY, Ciocca DR (ed), Heat shock proteins in cancer, vol 2. Springer Netherlands, Dordrecht, Netherlands.
945. **Liang J, Fantes P.** 2007. The *Schizosaccharomyces pombe* Cdc7 protein kinase required for septum formation is a client protein of Cdc37. Eukaryot Cell **6:**1089–1096. http://dx.doi.org/10.1128/EC.00080-07.
946. **Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, YinL-Y, Patterson C.** 1999. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol Cell Biol **19:**4535–4545. http://dx.doi.org/10.1128/MCB.19.6.4535.
947. **Murata S, Chiba T, Tanaka K.** 2003. CHIP: a quality-control E3 ligase collaborating with molecular chaperones. Int J Biochem Cell Biol **35:**572–578. http://dx.doi.org/10.1016/S1357-2725(02)00394-1.
948. **Murata S, Minami Y, Minami M, Chiba T, Tanaka K.** 2001. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. EMBO Rep **2:**1133–1138. http://dx.doi.org/10.1093/embo-reports/kve246.
949. **Dolinski KJ, Cardenas ME, Heitman J.** 1998. CNS1 encodes an essential p60/Sti1 homolog in *Saccharomyces cerevisiae* that suppresses cyclophilin 40 mutations and interacts with Hsp90. Mol Cell Biol **18:**7344–7352. http://dx.doi.org/10.1128/MCB.18.12.7344.
950. **McLaughlin SH, Sobott F, Yao ZP, Zhang W, Nielsen PR, Grossmann JG, Laue ED, Robinson CV, Jackson SE.** 2006. The co-chaperone p23 arrests the Hsp90 ATPase cycle to trap client proteins. J Mol Biol **356:**746–758. http://dx.doi.org/10.1016/j.jmb.2005.11.085.
951. **Panaretou B, Siligardi G, Meyer P, Maloney A, Sullivan JK, Singh S, Millson SH, Clarke PA, Naaby-Hansen S, Stein R, Cramer R, Mollapour M, Workman P, Piper PW, Pearl LH, Prodromou C.** 2002. Activation of the ATPase activity of hsp90 by the stress-regulated co-chaperone aha1. Mol Cell **10:**1307–1318. http://dx.doi.org/10.1016/S1097-2765(02)00785-2.
952. **Swoboda RK, Bertram G, Budge S, Gooday GW, Gow NA, Brown AJ.** 1995. Structure and regulation of the HSP90 gene from the pathogenic fungus *Candida albicans*. Infect Immun **63:**4506–4514.
953. **Pugliese L, Georg RC, Fietto LG, Gomes SL.** 2008. Expression of genes encoding cytosolic and endoplasmic reticulum HSP90 proteins in the aquatic fungus *Blastocladiella emersonii*. Gene **411:**59–68. http://dx.doi.org/10.1016/j.gene.2008.01.005.
954. **Harrar Y, Bellini C, Faure J-D.** 2001. FKBPs: at the crossroads of folding and transduction. Trends Plant Sci **6:**426–431. http://dx.doi.org/10.1016/S1360-1385(01)02044-1.
955. **Tremmel D, Tropschug M.** 2007. *Neurospora crassa* FKBP22 is a novel ER chaperone and functionally cooperates with BiP. J Mol Biol **369:**55–68. http://dx.doi.org/10.1016/j.jmb.2007.01.092.
956. **Nadeau K, Das A, Walsh CT.** 1993. Hsp90 chaperonins possess ATPase activity and bind heat shock transcription factors and peptidyl prolyl isomerases. J Biol Chem **268:**1479–1487.
957. **Mok D, Allan RK, Carrello A, Wangoo K, Walkinshaw MD, Ratajczak T.** 2006. The chaperone function of cyclophilin 40 maps to a cleft between the prolyl isomerase and tetratricopeptide repeat domains. FEBS Lett **580:**2761–2768. http://dx.doi.org/10.1016/j.febslet.2006.04.039.
958. **Partaledis JA, Berlin V.** 1993. The FKB2 gene of *Saccharomyces cerevisiae*, encoding the immunosuppressant-binding protein FKBP-13, is regulated in response to accumulation of unfolded proteins in the endoplasmic reticulum. Proc Natl Acad Sci U S A **90:**5450–5454. http://dx.doi.org/10.1073/pnas.90.12.5450.
959. **Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW.** 1984. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science **226:**544–547. http://dx.doi.org/10.1126/science.6238408.
960. **Wang P, Heitman J.** 2005. The cyclophilins. Genome Biol **6:**226. http://dx.doi.org/10.1186/gb-2005-6-7-226.
961. **Sykes K, Gething MJ, Sambrook J.** 1993. Proline isomerases function during heat shock. Proc Natl Acad Sci U S A **90:**5853–5857. http://dx.doi.org/10.1073/pnas.90.12.5853.
962. **Duina AA, Kalton HM, Gaber RF.** 1998. Requirement for Hsp90 and a CyP-40-type cyclophilin in negative regulation of the heat shock response. J Biol Chem **273:**18974–18978. http://dx.doi.org/10.1074/jbc.273.30.18974.

963. **Pemberton TJ, Kay JE.** 2005. The cyclophilin repertoire of the fission yeast *Schizosaccharomyces pombe*. Yeast **22:**927–945. http://dx.doi.org/10.1002/yea.1288.
964. **Kusmierczyk AR, Martin J.** 2001. Chaperonins - keeping a lid on folding proteins. FEBS Lett **505:**343–347. http://dx.doi.org/10.1016/S0014-5793(01)02838-1.
965. **Gutsche I, Essen L-O, Baumeister W.** 1999. Group II chaperonins: new TRiC(k)s and turns of a protein folding machine. J Mol Biol **293:**295–312. http://dx.doi.org/10.1006/jmbi.1999.3008.
966. **Martin J, Langer T, Boteva R, Schramel A, Horwich AL, Hartl FU.** 1991. Chaperonin-mediated protein folding at the surface of groEL through a 'molten globule'-like intermediate. Nature **352:**36–42. http://dx.doi.org/10.1038/352036a0.
967. **Nielsen KL, McLennan N, Masters M, Cowan NJ.** 1999. A single-ring mitochondrial chaperonin (Hsp60-Hsp10) can substitute for GroEL-GroES in vivo. J Bacteriol **181:**5871–5875.
968. **Reading DS, Hallberg RL, Myers AM.** 1989. Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. Nature **337:**655–659. http://dx.doi.org/10.1038/337655a0.
969. **Rospert S, Glick BS, Jenö P, Schatz G, Todd MJ, Lorimer GH, Viitanen PV.** 1993. Identification and functional analysis of chaperonin 10, the groES homolog from yeast mitochondria. Proc Natl Acad Sci U S A **90:**10967–10971. http://dx.doi.org/10.1073/pnas.90.23.10967.
970. **Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, Neupert W, Hallberg EM, Hallberg RL, Horwich AL.** 1989. Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature **337:**620–625. http://dx.doi.org/10.1038/337620a0.
971. **Hutchinson EG, Tichelaar W, Hofhaus G, Weiss H, Leonard KR.** 1989. Identification and electron microscopic analysis of a chaperonin oligomer from *Neurospora crassa* mitochondria. EMBO J **8:**1485–1490.
972. **Bukau B, Horwich AL.** 1998. The Hsp70 and Hsp60 chaperone machines. Cell **92:**351–366. http://dx.doi.org/10.1016/S0092-8674(00)80928-9.
973. **Mayhew M, da Silva ACR, Martin J, Erdjument-Bromage H, Tempst P, Hartl FU.** 1996. Protein folding in the central cavity of the GroEL-GroES chaperonin complex. Nature **379:**420–426. http://dx.doi.org/10.1038/379420a0.
974. **Ostermann J, Horwich AL, Neupert W, Hartl FU.** 1989. Protein folding in mitochondria requires complex formation with hsp60 and ATP hydrolysis. Nature **341:**125–130. http://dx.doi.org/10.1038/341125a0.
975. **Martin J, Mayhew M, Langer T, Hartl U.** 1993. The reaction cycle of GroEL and GroES in chaperonin-assisted protein folding. Nature **366:**228–233. http://dx.doi.org/10.1038/366228a0.
976. **Mande SC, Mehra V, Bloom BR, Hol WG.** 1996. Structure of the heat-shock protein chaperonin-10 of *Mycobacterium leprae*. Science **271:**203–207. http://dx.doi.org/10.1126/science.271.5246.203.
977. **Rospert S, Junne T, Glick BS, Schatz G.** 1993. Cloning and disruption of the gene encoding yeast mitochondrial chaperonin 10, the homolog of *E. coli* groES. FEBS Lett **335:**358–360. http://dx.doi.org/10.1016/0014-5793(93)80419-U.
978. **Hallberg EM, Shu Y, Hallberg RL.** 1993. Loss of mitochondrial hsp60 function: nonequivalent effects on matrix-targeted and intermembrane-targeted proteins. Mol Cell Biol **13:**3050–3057. http://dx.doi.org/10.1128/MCB.13.5.3050.
979. **Kim S, Willison KR, Horwich AL.** 1994. Cytosolic chaperonin subunits have a conserved ATPase domain but diverged polypeptide-binding domains. Trends Biochem Sci **19:**543–548. http://dx.doi.org/10.1016/0968-0004(94)90058-2.
980. **Nakamoto H, Vigh L.** 2007. The small heat shock proteins and their clients. Cell Mol Life Sci **64:**294–306. http://dx.doi.org/10.1007/s00018-006-6321-2.
981. **Lee GJ, Roseman AM, Saibil HR, Vierling E.** 1997. A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. EMBO J **16:**659–671. http://dx.doi.org/10.1093/emboj/16.3.659.
982. **Haslbeck M.** 2002. sHsps and their role in the chaperone network. Cell Mol Life Sci **59:**1649–1657. http://dx.doi.org/10.1007/PL00012492.
983. **Carver JA, Lindner RA.** 1998. NMR spectroscopy of α-crystallin. Insights into the structure, interactions and chaperone action of small heat-shock proteins. Int J Biol Macromol **22:**197–209.
984. **Liming Y, Qian Y, Pigang L, Sen L.** 2008. Expression of the HSP24 gene from *Trichoderma harzianum* in *Saccharomyces cerevisiae*. J Thermal Biol **33:**1–6. http://dx.doi.org/10.1016/j.jtherbio.2007.08.004.
985. **Montero-Barrientos M, Cardoza R, Gutiérrez S, Monte E, Hermosa R.** 2007. The heterologous overexpression of hsp23, a small heat-shock protein gene from *Trichoderma virens*, confers thermotolerance to *T. harzianum*. Curr Genet **52:**45–53. http://dx.doi.org/10.1007/s00294-007-0140-3.
986. **Bonaccorsi ED, Ferreira AJ, Chambergo FS, Ramos AS, Mantuani MC, Farah JP, Sorio CS, Gombert AK, Tonso A, El-Dorry H.** 2006. Transcriptional response of the obligatory aerobe *Trichoderma reesei* to hypoxia and transient anoxia: implications for energy production and survival in the absence of oxygen. Biochemistry **45:**3912–3924. http://dx.doi.org/10.1021/bi052045o.
987. **Plesofsky-Vig N, Brambl R.** 1995. Disruption of the gene for hsp30, an alpha-crystallin-related heat shock protein of *Neurospora crassa*, causes defects in thermotolerance. Proc Natl Acad Sci U S A **92:**5032–5036. http://dx.doi.org/10.1073/pnas.92.11.5032.
988. **Panaretou B, Zhai C.** 2009. The heat shock proteins: their roles as multi-component machines for protein folding. Fungal Biol Rev **22:**110–119.
989. **Glover JR, Lindquist S.** 1998. Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. Cell **94:**73–82. http://dx.doi.org/10.1016/S0092-8674(00)81223-4.
990. **Parsell DA, Kowal AS, Singer MA, Lindquist S.** 1994. Protein disaggregation mediated by heat-shock protein Hsp104. Nature **372:**475–478. http://dx.doi.org/10.1038/372475a0.
991. **Lee S, Sowa ME, Choi JM, Tsai FT.** 2004. The ClpB/Hsp104 molecular chaperone: a protein disaggregating machine. J Struct Biol **146:**99–105. http://dx.doi.org/10.1016/j.jsb.2003.11.016.
992. **Leonhardt SA, Fearson K, Danese PN, Mason TL.** 1993. HSP78 encodes a yeast mitochondrial heat shock protein in the Clp family of ATP-dependent proteases. Mol Cell Biol **13:**6304–6313. http://dx.doi.org/10.1128/MCB.13.10.6304.
993. **Salusjärvi L, Poutanen M, Pitkänen J-P, Koivistoinen H, Aristidou A, Kalkkinen N, Ruohonen L, Penttilä M.** 2003. Proteome analysis of recombinant xylose-fermenting *Saccharomyces cerevisiae*. Yeast **20:**295–314. http://dx.doi.org/10.1002/yea.960.
994. **Rodriguez-Romero J, Corrochano LM.** 2004. The gene for the heat-shock protein HSP100 is induced by blue light and heat-shock in the fungus *Phycomyces blakesleeanus*. Curr Genet **46:**295–303. http://dx.doi.org/10.1007/s00294-004-0534-4.
995. **Wu C.** 1995. Heat shock transcription factors: structure and regulation. Annu Rev Cell Dev Biol **11:**441–469. http://dx.doi.org/10.1146/annurev.cb.11.110195.002301.
996. **Morimoto RI, Kroeger PE, Cotto JJ.** 1996. The transcriptional regulation of heat shock genes: a plethora of heat shock factors and regulatory conditions, p 139–163. *In* Feige U, Morimoto RI, Yahara I, Polla BS (ed), Stress-inducible cellular responses. Birkhäuser-Verlag, Basel, Switzerland.
997. **Kroeger PE, Morimoto RI.** 1994. Selection of new HSF1 and HSF2 DNA-binding sites reveals difference in trimer cooperativity. Mol Cell Biol **14:**7592–7603. http://dx.doi.org/10.1128/MCB.14.11.7592.
998. **Sorger PK, Nelson HCM.** 1989. Trimerization of a yeast transcriptional activator via a coiled-coil motif. Cell **59:**807–813. http://dx.doi.org/10.1016/0092-8674(89)90604-1.
999. **Wiederrecht G, Seto D, Parker CS.** 1988. Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. Cell **54:**841–853. http://dx.doi.org/10.1016/S0092-8674(88)91197-X.
1000. **Zimarino V, Tsai C, Wu C.** 1990. Complex modes of heat shock factor activation. Mol Cell Biol **10:**752–759. http://dx.doi.org/10.1128/MCB.10.2.752.
1001. **Park J, Park J, Jang S, Kim S, Kong S, Choi J, Ahn K, Kim J, Lee S, Kim S, Park B, Jung K, Kim S, Kang S, Lee YH.** 2008. FTFD: an informatics pipeline supporting phylogenomic analysis of fungal transcription factors. Bioinformatics **24:**1024–1025. http://dx.doi.org/10.1093/bioinformatics/btn058.
1002. **Shelest E.** 2008. Transcription factors in fungi. FEMS Microbiol Lett **286:**145–151. http://dx.doi.org/10.1111/j.1574-6968.2008.01293.x.
1003. **Todd RB, Zhou M, Ohm RA, Leeggangers HA, Visser L, de Vries RP.** 2014. Prevalence of transcription factors in ascomycete and basidiomycete fungi. BMC Genomics **15:**214. http://dx.doi.org/10.1186/1471-2164-15-214.
1004. **MacPherson S, Larochelle M, Turcotte B.** 2006. A fungal family of

transcriptional regulators: the zinc cluster proteins. Microbiol Mol Biol Rev **70:**583–604. http://dx.doi.org/10.1128/MMBR.00015-06.

1005. **Lu J, Cao H, Zhang L, Huang P, Lin F.** 2014. Systematic analysis of Zn2Cys6 transcription factors required for development and pathogenicity by high-throughput gene knockout in the rice blast fungus. PLoS Pathog **10:**e1004432. http://dx.doi.org/10.1371/journal.ppat.1004432.
1006. **Aro N, Saloheimo A, Ilmen M, Penttilä M.** 2001. ACEII, a novel transcriptional activator involved in regulation of cellulase and xylanase genes of *Trichoderma reesei*. J Biol Chem **276:**24309–24314. http://dx.doi.org/10.1074/jbc.M003624200.
1007. **Häkkinen M, Valkonen MJ, Westerholm-Parvinen A, Aro N, Arvas M, Vitikainen M, Penttila M, Saloheimo M, Pakula TM.** 2014. Screening of candidate regulators for cellulase and hemicellulase production in *Trichoderma reesei* and identification of a factor essential for cellulase production. Biotechnol Biofuels **7:**14. http://dx.doi.org/10.1186/1754-6834-7-14.
1008. **Rauscher R, Wurleitner E, Wacenovsky C, Aro N, Stricker AR, Zeilinger S, Kubicek CP, Penttila M, Mach RL.** 2006. Transcriptional regulation of *xyn1*, encoding xylanase I, in *Hypocrea jecorina*. Eukaryot Cell **5:**447–456. http://dx.doi.org/10.1128/EC.5.3.447-456.2006.
1009. **Portnoy T, Margeot A, Seidl-Seiboth V, Le Crom S, Ben Chaabane F, Linke R, Seiboth B, Kubicek CP.** 2011. Differential regulation of the cellulase transcription factors XYR1, ACE2, and ACE1 in Trichoderma reesei strains producing high and low levels of cellulase. Eukaryot Cell **10:**262–271. http://dx.doi.org/10.1128/EC.00208-10.
1010. **Klaubauf S, Narang HM, Post H, Zhou M, Brunner K, Mach-Aigner AR, Mach RL, Heck AJ, Altelaar AF, de Vries RP.** 2014. Similar is not the same: differences in the function of the (hemi-)cellulolytic regulator XlnR (Xlr1/Xyr1) in filamentous fungi. Fungal Genet Biol **72:**73–81. http://dx.doi.org/10.1016/j.fgb.2014.07.007.
1011. **Derntl C, Gudynaite-Savitch L, Calixte S, White T, Mach RL, Mach-Aigner AR.** 2013. Mutation of the xylanase regulator 1 causes a glucose blind hydrolase expressing phenotype in industrially used *Trichoderma* strains. Biotechnol Biofuels **6:**62. http://dx.doi.org/10.1186/1754-6834-6-62.
1012. **Nitta M, Furukawa T, Shida Y, Mori K, Kuhara S, Morikawa Y, Ogasawara W.** 2012. A new Zn(II)(2)Cys(6)-type transcription factor BglR regulates beta-glucosidase expression in *Trichoderma reesei*. Fungal Genet Biol **49:**388–397. http://dx.doi.org/10.1016/j.fgb.2012.02.009.
1013. **Rubio MB, Hermosa R, Reino JL, Collado IG, Monte E.** 2009. Thctf1 transcription factor of *Trichoderma harzianum* is involved in 6-pentyl-2H-pyran-2-one production and antifungal activity. Fungal Genet Biol **46:**17–27. http://dx.doi.org/10.1016/j.fgb.2008.10.008.
1014. **Hollemann T, Bellefroid E, Stick R, Pieler T.** 1996. Zinc finger proteins in early *Xenopus* development. Int J Dev Biol **40:**291–295.
1015. **Iuchi S.** 2001. Three classes of C2H2 zinc finger proteins. Cell Mol Life Sci **58:**625–635. http://dx.doi.org/10.1007/PL00000885.
1016. **Miller J, McLachlan AD, Klug A.** 1985. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus oocytes*. EMBO J **4:**1609–1614.
1017. **Aro N, Ilmen M, Saloheimo A, Penttila M.** 2003. ACEI of *Trichoderma reesei* is a repressor of cellulase and xylanase expression. Appl Environ Microbiol **69:**56–65. http://dx.doi.org/10.1128/AEM.69.1.56-65.2003.
1018. **Strauss J, Mach RL, Zeilinger S, Hartler G, Stoffler G, Wolschek M, Kubicek CP.** 1995. Cre1, the carbon catabolite repressor protein from *Trichoderma reesei*. FEBS Lett **376:**103–107. http://dx.doi.org/10.1016/0014-5793(95)01255-5.
1019. **Antonieto AC, dos Santos Castro L, Silva-Rocha R, Persinoti GF, Silva RN.** 2014. Defining the genome-wide role of CRE1 during carbon catabolite repression in *Trichoderma reesei* using RNA-Seq analysis. Fungal Genet Biol **73:**93–103. http://dx.doi.org/10.1016/j.fgb.2014.10.009.
1020. **Peterson R, Nevalainen H.** 2012. *Trichoderma reesei* RUT-C30: thirty years of strain improvement. Microbiology **158:**58–68. http://dx.doi.org/10.1099/mic.0.054031-0.
1021. **Mello-de-Sousa TM, Gorsche R, Rassinger A, Pocas-Fonseca MJ, Mach RL, Mach-Aigner AR.** 2014. A truncated form of the carbon catabolite repressor 1 increases cellulase production in *Trichoderma reesei*. Biotechnol Biofuels **7:**129.
1022. **Lorito M, Mach RL, Sposato P, Strauss J, Peterbauer CK, Kubicek CP.** 1996. Mycoparasitic interaction relieves binding of the Cre1 carbon catabolite repressor protein to promoter sequences of the *ech42* (endochitinase-encoding) gene in *Trichoderma harzianum*. Proc Natl Acad Sci U S A **93:**14868–14872. http://dx.doi.org/10.1073/pnas.93.25.14868.
1023. **Peterbauer CK, Litscher D, Kubicek CP.** 2002. The *Trichoderma atroviride seb1* (stress response element binding) gene encodes an AGGGG-binding protein which is involved in the response to high osmolarity stress. Mol Genet Genomics **268:**223–231. http://dx.doi.org/10.1007/s00438-002-0732-z.
1024. **Seidl V, Seiboth B, Karaffa L, Kubicek CP.** 2004. The fungal STRE-element-binding protein Seb1 is involved but not essential for glycerol dehydrogenase (*gld1*) gene expression and glycerol accumulation in *Trichoderma atroviride* during osmotic stress. Fungal Genet Biol **41:**1132–1140. http://dx.doi.org/10.1016/j.fgb.2004.09.002.
1025. **Dinamarco TM, Almeida RS, de Castro PA, Brown NA, dos Reis TF, Ramalho LN, Savoldi M, Goldman MH, Goldman GH.** 2012. Molecular characterization of the putative transcription factor SebA involved in virulence in *Aspergillus fumigatus*. Eukaryot Cell **11:**518–531. http://dx.doi.org/10.1128/EC.00016-12.
1026. **Penalva MA, Tilburn J, Bignell E, Arst HN, Jr.** 2008. Ambient pH gene regulation in fungi: making connections. Trends Microbiol **16:**291–300. http://dx.doi.org/10.1016/j.tim.2008.03.006.
1027. **Selvig K, Alspaugh JA.** 2011. pH response pathways in fungi: adapting to host-derived and environmental signals. Mycobiology **39:**249–256. http://dx.doi.org/10.5941/MYCO.2011.39.4.249.
1028. **Moreno-Mateos MA, Delgado-Jarana J, Codon AC, Benitez T.** 2007. pH and PacI control development and antifungal activity in *Trichoderma harzianum*. Fungal Genet Biol **44:**1355–1367. http://dx.doi.org/10.1016/j.fgb.2007.07.012.
1029. **Trushina N, Levin M, Mukherjee PK, Horwitz BA.** 2013. PacC and pH-dependent transcriptome of the mycotrophic fungus *Trichoderma virens*. BMC Genomics **14:**138. http://dx.doi.org/10.1186/1471-2164-14-138.
1030. **He R, Ma L, Li C, Jia W, Li D, Zhang D, Chen S.** 2014. Trpac1, a pH response transcription regulator, is involved in cellulase gene expression in *Trichoderma reesei*. Enzyme Microb Technol **67:**17–26. http://dx.doi.org/10.1016/j.enzmictec.2014.08.013.
1031. **Lambreghts R, Shi M, Belden WJ, Decaprio D, Park D, Henn MR, Galagan JE, Basturkmen M, Birren BW, Sachs MS, Dunlap JC, Loros JJ.** 2009. A high-density single nucleotide polymorphism map for *Neurospora crassa*. Genetics **181:**767–781.
1032. **Selitrennikoff CP, Nelson RE, Siegel RW.** 1974. Phase-specific genes for macroconidiation in *Neurospora crassa*. Genetics **78:**679–690.
1033. **Pare A, Kim M, Juarez MT, Brody S, McGinnis W.** 2012. The functions of grainy head-like proteins in animals and fungi and the evolution of apical extracellular barriers. PLoS One **7:**e36254. http://dx.doi.org/10.1371/journal.pone.0036254.
1034. **Kappel L, Gaderer R, Flipphi M, Seidl-Seiboth V.** 2 October 2015. The N-acetylglucosamine catabolic gene cluster in *Trichoderma reesei* is controlled by the Ndt80-like transcription factor RON1. Mol Microbiol http://dx.doi.org/10.1111/mmi.13256.
1035. **Ruger-Herreros C, Rodriguez-Romero J, Fernandez-Barranco R, Olmedo M, Fischer R, Corrochano LM, Canovas D.** 2011. Regulation of conidiation by light in *Aspergillus nidulans*. Genetics **188:**809–822. http://dx.doi.org/10.1534/genetics.111.130096.
1036. **Wong Sak Hoi J, Dumas B.** 2010. Ste12 and Ste12-like proteins, fungal transcription factors regulating development and pathogenicity. Eukaryot Cell **9:**480–485. http://dx.doi.org/10.1128/EC.00333-09.
1037. **Li D, Bobrowicz P, Wilkinson HH, Ebbole DJ.** 2005. A mitogen-activated protein kinase pathway essential for mating and contributing to vegetative growth in *Neurospora crassa*. Genetics **170:**1091–1104. http://dx.doi.org/10.1534/genetics.104.036772.
1038. **Vallim MA, Miller KY, Miller BL.** 2000. *Aspergillus* SteA (sterile12-like) is a homeodomain-C2/H2-Zn+2 finger transcription factor required for sexual reproduction. Mol Microbiol **36:**290–301. http://dx.doi.org/10.1046/j.1365-2958.2000.01874.x.
1039. **Gruber S, Zeilinger S.** 2014. The transcription factor Ste12 mediates the regulatory role of the Tmk1 MAP kinase in mycoparasitism and vegetative hyphal fusion in the filamentous fungus *Trichoderma atroviride*. PLoS One **9:**e111636. http://dx.doi.org/10.1371/journal.pone.0111636.
1040. **Mendoza-Mendoza A, Pozo MJ, Grzegorski D, Martinez P, Garcia JM, Olmedo-Monfil V, Cortes C, Kenerley C, Herrera-Estrella A.** 2003. Enhanced biocontrol activity of *Trichoderma* through inactiva-

tion of a mitogen-activated protein kinase. Proc Natl Acad Sci U S A **100:**15965–15970. http://dx.doi.org/10.1073/pnas.2136716100.
1041. **Stathopoulos-Gerontides A, Guo JJ, Cyert MS.** 1999. Yeast calcineurin regulates nuclear localization of the Crz1p transcription factor through dephosphorylation. Genes Dev **13:**798–803. http://dx.doi.org/10.1101/gad.13.7.798.
1042. **Thewes S.** 2014. Calcineurin-Crz1 signaling in lower eukaryotes. Eukaryot Cell **13:**694–705. http://dx.doi.org/10.1128/EC.00038-14.
1043. **Simkovic M, Ditte P, Kurucova A, Lakatos B, Varecka L.** 2008. Ca2+-dependent induction of conidiation in submerged cultures of *Trichoderma viride*. Can J Microbiol **54:**291–298. http://dx.doi.org/10.1139/W08-001.
1044. **Vinson C, Acharya A, Taparowsky EJ.** 2006. Deciphering b-ZIP transcription factor interactions *in vitro* and *in vivo*. Biochim Biophys Acta **1759:**4–12. http://dx.doi.org/10.1016/j.bbaexp.2005.12.005.
1045. **Vinson CR, Sigler PB, McKnight SL.** 1989. Scissors-grip model for DNA recognition by a family of leucine zipper proteins. Science **246:**911–916. http://dx.doi.org/10.1126/science.2683088.
1046. **Etxebeste O, Ni M, Garzia A, Kwon NJ, Fischer R, Yu JH, Espeso EA, Ugalde U.** 2008. Basic-zipper-type transcription factor FlbB controls asexual development in *Aspergillus nidulans*. Eukaryot Cell **7:**38–48. http://dx.doi.org/10.1128/EC.00207-07.
1047. **Kong S, Park SY, Lee YH.** 2015. Systematic characterization of the bZIP transcription factor gene family in the rice blast fungus, *Magnaporthe oryzae*. Environ Microbiol **17:**1425–1443. http://dx.doi.org/10.1111/1462-2920.12633.
1048. **Mulder HJ, Saloheimo M, Penttila M, Madrid SM.** 2004. The transcription factor HACA mediates the unfolded protein response in *Aspergillus niger*, and up-regulates its own transcription. Mol Genet Genomics **271:**130–140. http://dx.doi.org/10.1007/s00438-003-0965-5.
1049. **Richie DL, Hartl L, Aimanianda V, Winters MS, Fuller KK, Miley MD, White S, McCarthy JW, Latge JP, Feldmesser M, Rhodes JC, Askew DS.** 2009. A role for the unfolded protein response (UPR) in virulence and antifungal susceptibility in *Aspergillus fumigatus*. PLoS Pathog **5:**e1000258. http://dx.doi.org/10.1371/journal.ppat.1000258.
1050. **Saloheimo M, Valkonen M, Penttila M.** 2003. Activation mechanisms of the HAC1-mediated unfolded protein response in filamentous fungi. Mol Microbiol **47:**1149–1161. http://dx.doi.org/10.1046/j.1365-2958.2003.03363.x.
1051. **Valkonen M, Penttilä M, Saloheimo M.** 2004. The *ire1* and *ptc2* genes involved in the unfolded protein response pathway in the filamentous fungus *Trichoderma reesei*. Mol Genet Genomics **272:**443–451. http://dx.doi.org/10.1007/s00438-004-1070-0.
1052. **Degnan BM, Vervoort M, Larroux C, Richards GS.** 2009. Early evolution of metazoan transcription factors. Curr Opin Genet Dev **19:**591–599. http://dx.doi.org/10.1016/j.gde.2009.09.008.
1053. **Sailsbery JK, Atchley WR, Dean RA.** 2012. Phylogenetic analysis and classification of the fungal bHLH domain. Mol Biol Evol **29:**1301–1318. http://dx.doi.org/10.1093/molbev/msr288.
1054. **Skinner MK, Rawls A, Wilson-Rawls J, Roalson EH.** 2010. Basic helix-loop-helix transcription factor gene family phylogenetics and nomenclature. Differentiation **80:**1–8. http://dx.doi.org/10.1016/j.diff.2010.02.003.
1055. **Lehman JF, Gleason MK, Ahlgren SK, Metzenberg RL.** 1973. Regulation of phosphate metabolism in *Neurospora crassa*. Characterization of regulatory mutants. Genetics **75:**61–73.
1056. **Wu D, Dou X, Hashmi SB, Osmani SA.** 2004. The Pho80-like cyclin of *Aspergillus nidulans* regulates development independently of its role in phosphate acquisition. J Biol Chem **279:**37693–37703. http://dx.doi.org/10.1074/jbc.M403853200.
1057. **Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, Weiss RL, Borkovich KA, Dunlap JC.** 2006. A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. Proc Natl Acad Sci U S A **103:**10352–10357. http://dx.doi.org/10.1073/pnas.0601456103.
1058. **Caruso ML, Litzka O, Martic G, Lottspeich F, Brakhage AA.** 2002. Novel basic-region helix-loop-helix transcription factor (AnBH1) of *Aspergillus nidulans* counteracts the CCAAT-binding complex AnCF in the promoter of a penicillin biosynthesis gene. J Mol Biol **323:**425–439. http://dx.doi.org/10.1016/S0022-2836(02)00965-8.
1059. **Tüncher A, Reinke H, Martic G, Caruso ML, Brakhage AA.** 2004. A basic-region helix-loop-helix protein-encoding gene (*devR*) involved in the development of *Aspergillus nidulans*. Mol Microbiol **52:**227–241. http://dx.doi.org/10.1111/j.1365-2958.2003.03961.x.
1060. **Minie M, Clark D, Trainor C, Evans T, Reitman M, Hannon R, Gould H, Felsenfeld G.** 1992. Developmental regulation of globin gene expression. J Cell Sci Suppl **16:**15–20.
1061. **Omichinski JG, Clore GM, Schaad O, Felsenfeld G, Trainor C, Appella E, Stahl SJ, Gronenborn AM.** 1993. NMR structure of a specific DNA complex of Zn-containing DNA binding domain of GATA-1. Science **261:**438–446. http://dx.doi.org/10.1126/science.8332909.
1062. **Scazzocchio C.** 2000. The fungal GATA factors. Curr Opin Microbiol **3:**126–131. http://dx.doi.org/10.1016/S1369-5274(00)00063-1.
1063. **Idnurm A, Verma S, Corrochano LM.** 2010. A glimpse into the basis of vision in the kingdom Mycota. Fungal Genet Biol **47:**881–892. http://dx.doi.org/10.1016/j.fgb.2010.04.009.
1064. **Sanchez-Arreguin A, Perez-Martinez AS, Herrera-Estrella A.** 2012. Proteomic analysis of *Trichoderma atroviride* reveals independent roles for transcription factors BLR-1 and BLR-2 in light and darkness. Eukaryot Cell **11:**30–41. http://dx.doi.org/10.1128/EC.05263-11.
1065. **Friedl MA, Schmoll M, Kubicek CP, Druzhinina IS.** 2008. Photostimulation of *Hypocrea atroviridis* growth occurs due to a cross-talk of carbon metabolism, blue light receptors and response to oxidative stress. Microbiology **154:**1229–1241. http://dx.doi.org/10.1099/mic.0.2007/014175-0.
1066. **Fu YH, Marzluf GA.** 1990. *nit-2*, the major nitrogen regulatory gene of *Neurospora crassa*, encodes a protein with a putative zinc finger DNA-binding domain. Mol Cell Biol **10:**1056–1065. http://dx.doi.org/10.1128/MCB.10.3.1056.
1067. **Hynes MJ.** 1975. Studies on the role of the *areA* gene in the regulation of nitrogen catabolism in *Aspergillus nidulans*. Aust J Biol Sci **28:**301–313.
1068. **Haas H, Zadra I, Stoffler G, Angermayr K.** 1999. The *Aspergillus nidulans* GATA factor SREA is involved in regulation of siderophore biosynthesis and control of iron uptake. J Biol Chem **274:**4613–4619. http://dx.doi.org/10.1074/jbc.274.8.4613.
1069. **Zhou LW, Haas H, Marzluf GA.** 1998. Isolation and characterization of a new gene, sre, which encodes a GATA-type regulatory protein that controls iron transport in *Neurospora crassa*. Mol Gen Genet **259:**532–540. http://dx.doi.org/10.1007/s004380050845.
1070. **Altomare C, Norvell WA, Bjorkman T, Harman GE.** 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus Trichoderma harzianum rifai 1295-22. Appl Environ Microbiol **65:**2926–2933.
1071. **Feng B, Haas H, Marzluf GA.** 2000. ASD4, a new GATA factor of *Neurospora crassa*, displays sequence-specific DNA binding and functions in ascus and ascospore development. Biochemistry **39:**11065–11073. http://dx.doi.org/10.1021/bi000886j.
1072. **Aramayo R, Peleg Y, Addison R, Metzenberg R.** 1996. *Asm-1*+, a *Neurospora crassa* gene related to transcriptional regulators of fungal development. Genetics **144:**991–1003.
1073. **Iyer SV, Ramakrishnan M, Kasbekar DP.** 2009. *Neurospora crassa fmf-1* encodes the homologue of the *Schizosaccharomyces pombe* Ste11p regulator of sexual development. J Genet **88:**33–39. http://dx.doi.org/10.1007/s12041-009-0005-2.
1074. **Wieser J, Adams TH.** 1995. *flbD* encodes a Myb-like DNA-binding protein that coordinates initiation of *Aspergillus nidulans* conidiophore development. Genes Dev **9:**491–502. http://dx.doi.org/10.1101/gad.9.4.491.
1075. **Forsburg SL, Guarente L.** 1989. Identification and characterization of HAP4: a third component of the CCAAT-bound HAP2/HAP3 heteromer. Genes Dev **3:**1166–1178. http://dx.doi.org/10.1101/gad.3.8.1166.
1076. **McNabb DS, Xing Y, Guarente L.** 1995. Cloning of yeast HAP5: a novel subunit of a heterotrimeric complex required for CCAAT binding. Genes Dev **9:**47–58. http://dx.doi.org/10.1101/gad.9.1.47.
1077. **Tanaka A, Kato M, Hashimoto H, Kamei K, Naruse F, Papagiannopoulos P, Davis MA, Hynes MJ, Kobayashi T, Tsukagoshi N.** 2000. An *Aspergillus oryzae* CCAAT-binding protein, AoCP, is involved in the high-level expression of the Taka-amylase A gene. Curr Genet **37:**380–387. http://dx.doi.org/10.1007/s002940000125.
1078. **Zeilinger S, Ebner A, Marosits T, Mach R, Kubicek CP.** 2001. The *Hypocrea jecorina* HAP 2/3/5 protein complex binds to the inverted CCAAT-box (ATTGG) within the *cbh2* (cellobiohydrolase II-gene) activating element. Mol Genet Genomics **266:**56–63. http://dx.doi.org/10.1007/s004380100518.

1079. **Seabold RR, Schleif RF.** 1998. Apo-AraC actively seeks to loop. J Mol Biol **278:**529–538. http://dx.doi.org/10.1006/jmbi.1998.1713.
1080. **Iyer LM, Koonin EV, Aravind L.** 2002. Extensive domain shuffling in transcription regulators of DNA viruses and implications for the origin of fungal APSES transcription factors. Genome Biol **3:**RESEARCH0012.
1081. **Kredics L, Hatvani L, Naeimi S, Körmöczi P, Manczinger L, Vagvölgyi C, Druzhinina I.** 2013. Biodiversity of the genus *Hypocrea/Trichoderma* in different habitats, p 3–24. *In* Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG (ed), Biotechnology and biology of *Trichoderma*. Elsevier, Oxford, United Kingdom.
1082. **Samuels GJ.** 1996. *Trichoderma*: a review of biology and systematics of the genus. Mycol Res **100:**923–935. http://dx.doi.org/10.1016/S0953-7562(96)80043-8.
1083. **Jaklitsch WM.** 2009. European species of *Hypocrea*. Part I. The green-spored species. Stud Mycol **63:**1–91.
1084. **Jaklitsch WM.** 2011. European species of *Hypocrea*. Part II. Species with hyaline ascospores. Fungal Divers **48:**1–250.
1085. **Puyesky M, Benhamou N, Noyola PP, Bauw G, Ziv T, Van Montagu M, Herrera-Estrella A, Horwitz BA.** 1999. Developmental regulation of cmp1, a gene encoding a multidomain conidiospore surface protein of *Trichoderma*. Fungal Genet Biol **27:**88–99. http://dx.doi.org/10.1006/fgbi.1999.1134.
1086. **Aguilar-Osorio G, Vankuyk PA, Seiboth B, Blom D, Solomon PS, Vinck A, Kindt F, Wosten HA, de Vries RP.** 2010. Spatial and developmental differentiation of mannitol dehydrogenase and mannitol-1-phosphate dehydrogenase in *Aspergillus niger*. Eukaryot Cell **9:**1398–1402. http://dx.doi.org/10.1128/EC.00363-09.
1087. **Metz B, de Vries RP, Polak S, Seidl V, Seiboth B.** 2009. The *Hypocrea jecorina* (syn. *Trichoderma reesei*) *lxr1* gene encodes a D-mannitol dehydrogenase and is not involved in L-arabinose catabolism. FEBS Lett **583:**1309–1313.
1088. **Mikus M, Hatvani L, Neuhof T, Komon-Zelazowska M, Dieckmann R, Schwecke T, Druzhinina IS, von Dohren H, Kubicek CP.** 2009. Differential regulation and posttranslational processing of the class II hydrophobin genes from the biocontrol fungus *Hypocrea atroviridis*. Appl Environ Microbiol **75:**3222–3229. http://dx.doi.org/10.1128/AEM.01764-08.
1089. **Clutterbuck AJ.** 1969. A mutational analysis of conidial development in *Aspergillus nidulans*. Genetics **63:**317–327.
1090. **Adams TH, Wieser JK, Yu JH.** 1998. Asexual sporulation in *Aspergillus nidulans*. Microbiol Mol Biol Rev **62:**35–54.
1091. **Etxebeste O, Garzia A, Espeso EA, Ugalde U.** 2010. *Aspergillus nidulans* asexual development: making the most of cellular modules. Trends Microbiol **18:**569–576. http://dx.doi.org/10.1016/j.tim.2010.09.007.
1092. **Yu JH, Mah JH, Seo JA.** 2006. Growth and developmental control in the model and pathogenic aspergilli. Eukaryot Cell **5:**1577–1584. http://dx.doi.org/10.1128/EC.00193-06.
1093. **Villalobos-Escobedo JM.** 2014. Estudio del efecto de la luz sobre la respuesta al daño mecánico en *Trichoderma atroviride*. M.Sc. thesis. Cinvestav-Langebio, Irapuato, Mexico.
1094. **Park HS, Yu JH.** 2012. Genetic control of asexual sporulation in filamentous fungi. Curr Opin Microbiol **15:**669–677. http://dx.doi.org/10.1016/j.mib.2012.09.006.
1095. **Matsuyama SS, Nelson RE, Siegel RW.** 1974. Mutations specifically blocking differentiation of macroconidia *Neurospora crassa*. Dev Biol **41:**278–287. http://dx.doi.org/10.1016/0012-1606(74)90306-6.
1096. **Springer ML, Yanofsky C.** 1989. A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. Genes Dev **3:**559–571. http://dx.doi.org/10.1101/gad.3.4.559.
1097. **Berlin V, Yanofsky C.** 1985. Isolation and characterization of genes differentially expressed during conidiation of *Neurospora crassa*. Mol Cell Biol **5:**849–855. http://dx.doi.org/10.1128/MCB.5.4.849.
1098. **Hager KM, Yanofsky C.** 1990. Genes expressed during conidiation in Neurospora crassa: molecular characterization of *con-13*. Gene **96:**153–159. http://dx.doi.org/10.1016/0378-1119(90)90247-O.
1099. **Bell-Pedersen D, Dunlap JC, Loros JJ.** 1996. Distinct cis-acting elements mediate clock, light, and developmental regulation of the *Neurospora crassa eas* (*ccg-2*) gene. Mol Cell Biol **16:**513–521. http://dx.doi.org/10.1128/MCB.16.2.513.
1100. **Schmoll M.** 2013. Sexual development in *Trichoderma*: scrutinizing the aspired phenomenon, p 67–86. *In* Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (ed), Trichoderma: biology and applications. CAB International, Oxfordshire, United Kingdom.
1101. **Schmoll M, Wang TF.** 2015. Sexual development in *Trichoderma*. *In* Wendland J (ed), Mycota I, 3rd ed. Springer, Berlin, Germany.
1102. **Seibel C, Tisch D, Kubicek CP, Schmoll M.** 2012. The role of pheromone receptors for communication and mating in *Hypocrea jecorina* (*Trichoderma reesei*). Fungal Genet Biol **49:**814–824. http://dx.doi.org/10.1016/j.fgb.2012.07.004.
1103. **Freitag M, Williams RL, Kothe GO, Selker EU.** 2002. A cytosine methyltransferase homologue is essential for repeat-induced point mutation in *Neurospora crassa*. Proc Natl Acad Sci U S A **99:**8802–8807. http://dx.doi.org/10.1073/pnas.132212899.
1104. **Ni M, Feretzaki M, Sun S, Wang X, Heitman J.** 2011. Sex in fungi. Annu Rev Genet **45:**405–430. http://dx.doi.org/10.1146/annurev-genet-110410-132536.
1105. **Leeder AC, Palma-Guerrero J, Glass NL.** 2011. The social network: deciphering fungal language. Nat Rev Microbiol **9:**440–451. http://dx.doi.org/10.1038/nrmicro2580.
1106. **Schmoll M, Seibel C, Tisch D, Dorrer M, Kubicek CP.** 2010. A novel class of peptide pheromone precursors in ascomycetous fungi. Mol Microbiol **77:**1483–1501. http://dx.doi.org/10.1111/j.1365-2958.2010.07295.x.
1107. **Zickler D.** 2009. Observing meiosis in filamentous fungi: *Sordaria* and *Neurospora*. Methods Mol Biol **558:**91–114. http://dx.doi.org/10.1007/978-1-60761-103-5_7.
1108. **Chuang YC, Li WC, Chen CL, Hsu PW, Tung SY, Kuo HC, Schmoll M, Wang TF.** 2015. *Trichoderma reesei* meiosis generates segmentally aneuploid progeny with higher xylanase-producing capability. Biotechnol Biofuels **8:**30. http://dx.doi.org/10.1186/s13068-015-0202-6.
1109. **Dodd SL, Lieckfeldt E, Samuels GJ.** 2003. *Hypocrea atroviridis* sp. nov., the teleomorph of *Trichoderma atroviride*. Mycologia **95:**27–40. http://dx.doi.org/10.2307/3761959.
1110. **Chaverri P, Samuels GJ, Stewart EL.** 2001. *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. Mycologia **93:**1113–1124. http://dx.doi.org/10.2307/3761672.
1111. **Mitchell AP.** 1994. Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol Rev **58:**56–70.
1112. **Neiman AM.** 2011. Sporulation in the budding yeast *Saccharomyces cerevisiae*. Genetics **189:**737–765. http://dx.doi.org/10.1534/genetics.111.127126.
1113. **Kassir Y, Granot D, Simchen G.** 1988. IME1, a positive regulator gene of meiosis in S. cerevisiae. Cell **52:**853–862. http://dx.doi.org/10.1016/0092-8674(88)90427-8.
1114. **Mitchell AP, Driscoll SE, Smith HE.** 1990. Positive control of sporulation-specific genes by the IME1 and IME2 products in *Saccharomyces cerevisiae*. Mol Cell Biol **10:**2104–2110. http://dx.doi.org/10.1128/MCB.10.5.2104.
1115. **Su SS, Mitchell AP.** 1993. Identification of functionally related genes that stimulate early meiotic gene expression in yeast. Genetics **133:**67–77.
1116. **Smith HE, Su SS, Neigeborn L, Driscoll SE, Mitchell AP.** 1990. Role of IME1 expression in regulation of meiosis in *Saccharomyces cerevisiae*. Mol Cell Biol **10:**6103–6113. http://dx.doi.org/10.1128/MCB.10.12.6103.
1117. **Li W, Mitchell AP.** 1997. Proteolytic activation of Rim1p, a positive regulator of yeast sporulation and invasive growth. Genetics **145:**63–73.
1118. **Bowdish KS, Mitchell AP.** 1993. Bipartite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae*. Mol Cell Biol **13:**2172–2181. http://dx.doi.org/10.1128/MCB.13.4.2172.
1119. **Bowdish KS, Yuan HE, Mitchell AP.** 1995. Positive control of yeast meiotic genes by the negative regulator UME6. Mol Cell Biol **15:**2955–2961. http://dx.doi.org/10.1128/MCB.15.6.2955.
1120. **Rubin-Bejerano I, Mandel S, Robzyk K, Kassir Y.** 1996. Induction of meiosis in *Saccharomyces cerevisiae* depends on conversion of the transcriptional represssor Ume6 to a positive regulator by its regulated association with the transcriptional activator Ime1. Mol Cell Biol **16:**2518–2526. http://dx.doi.org/10.1128/MCB.16.5.2518.
1121. **Sherwood RK, Scaduto CM, Torres SE, Bennett RJ.** 2014. Convergent evolution of a fused sexual cycle promotes the haploid lifestyle. Nature **506:**387–390. http://dx.doi.org/10.1038/nature12891.
1122. **Hutchison EA, Glass NL.** 2010. Meiotic regulators Ndt80 and Ime2 have different roles in *Saccharomyces* and *Neurospora*. Genetics **185:**1271–1282. http://dx.doi.org/10.1534/genetics.110.117184.

1123. **Vidal M, Gaber RF.** 1991. RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*. Mol Cell Biol **11:**6317–6327. http://dx.doi.org/10.1128/MCB.11.12.6317.

1124. **Vidal M, Strich R, Esposito RE, Gaber RF.** 1991. RPD1 (SIN3/UME4) is required for maximal activation and repression of diverse yeast genes. Mol Cell Biol **11:**6306–6316. http://dx.doi.org/10.1128/MCB.11.12.6306.

1125. **Strich R, Slater MR, Esposito RE.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc Natl Acad Sci U S A **86:**10018–10022. http://dx.doi.org/10.1073/pnas.86.24.10018.

1126. **Davis L, Smith GR.** 2001. Meiotic recombination and chromosome segregation in *Schizosaccharomyces pombe*. Proc Natl Acad Sci U S A **98:**8395–8402. http://dx.doi.org/10.1073/pnas.121005598.

1127. **Watanabe Y, Yamamoto M.** 1994. *S. pombe mei2+* encodes an RNA-binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell **78:**487–498. http://dx.doi.org/10.1016/0092-8674(94)90426-X.

1128. **Ding R, Smith GR.** 1998. Global control of meiotic recombination genes by *Schizosaccharomyces pombe rec16* (*rep1*). Mol Gen Genet **258:**663–670. http://dx.doi.org/10.1007/s004380050780.

1129. **Zhu Y, Takeda T, Whitehall S, Peat N, Jones N.** 1997. Functional characterization of the fission yeast start-specific transcription factor Res2. EMBO J **16:**1023–1034. http://dx.doi.org/10.1093/emboj/16.5.1023.

1130. **Miyamoto M, Tanaka K, Okayama H.** 1994. res2+, a new member of the cdc10+/SWI4 family, controls the 'start' of mitotic and meiotic cycles in fission yeast. EMBO J **13:**1873–1880.

1131. **St-Andre O, Lemieux C, Perreault A, Lackner DH, Bahler J, Bachand F.** 2010. Negative regulation of meiotic gene expression by the nuclear poly(A) -binding protein in fission yeast. J Biol Chem **285:**27859–27868. http://dx.doi.org/10.1074/jbc.M110.150748.

1132. **Harigaya Y, Tanaka H, Yamanaka S, Tanaka K, Watanabe Y, Tsutsumi C, Chikashige Y, Hiraoka Y, Yamashita A, Yamamoto M.** 2006. Selective elimination of messenger RNA prevents an incidence of untimely meiosis. Nature **442:**45–50. http://dx.doi.org/10.1038/nature04881.

1133. **Cole F, Keeney S, Jasin M.** 2010. Evolutionary conservation of meiotic DSB proteins: more than just Spo11. Genes Dev **24:**1201–1207. http://dx.doi.org/10.1101/gad.1944710.

1134. **De Veaux LC, Hoagland NA, Smith GR.** 1992. Seventeen complementation groups of mutations decreasing meiotic recombination in *Schizosaccharomyces pombe*. Genetics **130:**251–262.

1135. **Tesse S, Storlazzi A, Kleckner N, Gargano S, Zickler D.** 2003. Localization and roles of Ski8p protein in *Sordaria* meiosis and delineation of three mechanistically distinct steps of meiotic homolog juxtaposition. Proc Natl Acad Sci U S A **100:**12865–12870. http://dx.doi.org/10.1073/pnas.2034282100.

1136. **Richard GF, Kerrest A, Lafontaine I, Dujon B.** 2005. Comparative genomics of hemiascomycete yeasts: genes involved in DNA replication, repair, and recombination. Mol Biol Evol **22:**1011–1023. http://dx.doi.org/10.1093/molbev/msi083.

1137. **Keeney S.** 2007. Spo11 and the formation of DNA double-strand breaks in meiosis, p 81–123. *In* Egel R, Lankenau DH (ed), Rocombination and meiosis: crossing-over and disjunction, vol 2. Springer-Verlag, Berlin, Germany.

1138. **Milman N, Higuchi E, Smith GR.** 2009. Meiotic DNA double-strand break repair requires two nucleases, MRN and Ctp1, to produce a single size class of Rec12 (Spo11)-oligonucleotide complexes. Mol Cell Biol **29:**5998–6005. http://dx.doi.org/10.1128/MCB.01127-09.

1139. **Krogh BO, Symington LS.** 2004. Recombination proteins in yeast. Annu Rev Genet **38:**233–271. http://dx.doi.org/10.1146/annurev.genet.38.072902.091500.

1140. **Stracker TH, Theunissen JW, Morales M, Petrini JH.** 2004. The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. DNA Repair (Amst) **3:**845–854. http://dx.doi.org/10.1016/j.dnarep.2004.03.014.

1141. **Garcia V, Phelps SE, Gray S, Neale MJ.** 2011. Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. Nature **479:**241–244. http://dx.doi.org/10.1038/nature10515.

1142. **Bishop DK, Park D, Xu L, Kleckner N.** 1992. DMC1: a meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:**439–456. http://dx.doi.org/10.1016/0092-8674(92)90446-J.

1143. **Shinohara A, Ogawa H, Ogawa T.** 1992. Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. Cell **69:**457–470. http://dx.doi.org/10.1016/0092-8674(92)90447-K.

1144. **Shinohara A, Shinohara M.** 2004. Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination. Cytogenet Genome Res **107:**201–207. http://dx.doi.org/10.1159/000080598.

1145. **San Filippo J, Sung P, Klein H.** 2008. Mechanism of eukaryotic homologous recombination. Annu Rev Biochem **77:**229–257. http://dx.doi.org/10.1146/annurev.biochem.77.061306.125255.

1146. **Tsubouchi H, Roeder GS.** 2006. Budding yeast Hed1 down-regulates the mitotic recombination machinery when meiotic recombination is impaired. Genes Dev **20:**1766–1775. http://dx.doi.org/10.1101/gad.1422506.

1147. **Cloud V, Chan YL, Grubb J, Budke B, Bishop DK.** 2012. Rad51 is an accessory factor for Dmc1-mediated joint molecule formation during meiosis. Science **337:**1222–1225. http://dx.doi.org/10.1126/science.1219379.

1148. **Hyppa RW, Smith GR.** 2010. Crossover invariance determined by partner choice for meiotic DNA break repair. Cell **142:**243–255. http://dx.doi.org/10.1016/j.cell.2010.05.041.

1149. **Nowrousian M, Stajich JE, Chu M, Engh I, Espagne E, Halliday K, Kamerewerd J, Kempken F, Knab B, Kuo HC, Osiewacz HD, Poggeler S, Read ND, Seiler S, Smith KM, Zickler D, Kuck U, Freitag M.** 2010. *De novo* assembly of a 40 Mb eukaryotic genome from short sequence reads: *Sordaria macrospora*, a model organism for fungal morphogenesis. PLoS Genet **6:**e1000891. http://dx.doi.org/10.1371/journal.pgen.1000891.

1150. **Lao JP, Hunter N.** 2010. Trying to avoid your sister. PLoS Biol **8:**e1000519. http://dx.doi.org/10.1371/journal.pbio.1000519.

1151. **Schwacha A, Kleckner N.** 1995. Identification of double Holliday junctions as intermediates in meiotic recombination. Cell **83:**783–791. http://dx.doi.org/10.1016/0092-8674(95)90191-4.

1152. **Cromie GA, Hyppa RW, Taylor AF, Zakharyevich K, Hunter N, Smith GR.** 2006. Single Holliday junctions are intermediates of meiotic recombination. Cell **127:**1167–1178. http://dx.doi.org/10.1016/j.cell.2006.09.050.

1153. **Lynn A, Soucek R, Borner GV.** 2007. ZMM proteins during meiosis: crossover artists at work. Chromosome Res **15:**591–605. http://dx.doi.org/10.1007/s10577-007-1150-1.

1154. **Storlazzi A, Gargano S, Ruprich-Robert G, Falque M, David M, Kleckner N, Zickler D.** 2010. Recombination proteins mediate meiotic spatial chromosome organization and pairing. Cell **141:**94–106. http://dx.doi.org/10.1016/j.cell.2010.02.041.

1155. **Sym M, Engebrecht JA, Roeder GS.** 1993. ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell **72:**365–378. http://dx.doi.org/10.1016/0092-8674(93)90114-6.

1156. **Cheng CH, Lo YH, Liang SS, Ti SC, Lin FM, Yeh CH, Huang HY, Wang TF.** 2006. SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of *Saccharomyces cerevisiae*. Genes Dev **20:**2067–2081. http://dx.doi.org/10.1101/gad.1430406.

1157. **Espagne E, Vasnier C, Storlazzi A, Kleckner NE, Silar P, Zickler D, Malagnac F.** 2011. Sme4 coiled-coil protein mediates synaptonemal complex assembly, recombinosome relocalization, and spindle pole body morphogenesis. Proc Natl Acad Sci U S A **108:**10614–10619. http://dx.doi.org/10.1073/pnas.1107272108.

1158. **Lin FM, Lai YJ, Shen HJ, Cheng YH, Wang TF.** 2010. Yeast axial-element protein, Red1, binds SUMO chains to promote meiotic interhomologue recombination and chromosome synapsis. EMBO J **29:**586–596. http://dx.doi.org/10.1038/emboj.2009.362.

1159. **Spirek M, Estreicher A, Csaszar E, Wells J, McFarlane RJ, Watts FZ, Loidl J.** 2010. SUMOylation is required for normal development of linear elements and wild-type meiotic recombination in *Schizosaccharomyces pombe*. Chromosoma **119:**59–72. http://dx.doi.org/10.1007/s00412-009-0241-5.

1160. **Lu BC.** 1993. Spreading the synaptonemal complex of *Neurospora crassa*. Chromosoma **102:**464–472. http://dx.doi.org/10.1007/BF00357101.

1161. **Lu BC.** 2006. Karyotyping of *Neurospora crassa* using synaptonemal complex spreads of translocation quadrivalents. Genome **49:**612–618. http://dx.doi.org/10.1139/G06-008.

1162. **Navadgi-Patil VM, Burgers PM.** 2009. A tale of two tails: activation of DNA damage checkpoint kinase Mec1/ATR by the 9-1-1 clamp and by

Dpb11/TopBP1. DNA Repair (Amst) **8:**996–1003. http://dx.doi.org/10.1016/j.dnarep.2009.03.011.
1163. **Eichinger CS, Jentsch S.** 2011. 9-1-1: PCNA's specialized cousin. Trends Biochem Sci **36:**563–568. http://dx.doi.org/10.1016/j.tibs.2011.08.002.
1164. **Carballo JA, Johnson AL, Sedgwick SG, Cha RS.** 2008. Phosphorylation of the axial element protein Hop1 by Mec1/Tel1 ensures meiotic interhomolog recombination. Cell **132:**758–770. http://dx.doi.org/10.1016/j.cell.2008.01.035.
1165. **Niu H, Wan L, Busygina V, Kwon Y, Allen JA, Li X, Kunz RC, Kubota K, Wang B, Sung P, Shokat KM, Gygi SP, Hollingsworth NM.** 2009. Regulation of meiotic recombination via Mek1-mediated Rad54 phosphorylation. Mol Cell **36:**393–404. http://dx.doi.org/10.1016/j.molcel.2009.09.029.
1166. **Niu H, Wan L, Baumgartner B, Schaefer D, Loidl J, Hollingsworth NM.** 2005. Partner choice during meiosis is regulated by Hop1-promoted dimerization of Mek1. Mol Biol Cell **16:**5804–5818. http://dx.doi.org/10.1091/mbc.E05-05-0465.
1167. **Wan L, de los Santos T, Zhang C, Shokat K, Hollingsworth NM.** 2004. Mek1 kinase activity functions downstream of RED1 in the regulation of meiotic double strand break repair in budding yeast. Mol Biol Cell **15:**11–23.
1168. **Terentyev Y, Johnson R, Neale MJ, Khisroon M, Bishop-Bailey A, Goldman AS.** 2010. Evidence that MEK1 positively promotes interhomologue double-strand break repair. Nucleic Acids Res **38:**4349–4360. http://dx.doi.org/10.1093/nar/gkq137.
1169. **Goldfarb T, Lichten M.** 2010. Frequent and efficient use of the sister chromatid for DNA double-strand break repair during budding yeast meiosis. PLoS Biol **8:**e1000520. http://dx.doi.org/10.1371/journal.pbio.1000520.
1170. **Kim KP, Weiner BM, Zhang L, Jordan A, Dekker J, Kleckner N.** 2010. Sister cohesion and structural axis components mediate homolog bias of meiotic recombination. Cell **143:**924–937. http://dx.doi.org/10.1016/j.cell.2010.11.015.
1171. **Ho HC, Burgess SM.** 2011. Pch2 acts through Xrs2 and Tel1/ATM to modulate interhomolog bias and checkpoint function during meiosis. PLoS Genet **7:**e1002351. http://dx.doi.org/10.1371/journal.pgen.1002351.
1172. **Chen C, Jomaa A, Ortega J, Alani EE.** 2014. Pch2 is a hexameric ring ATPase that remodels the chromosome axis protein Hop1. Proc Natl Acad Sci U S A **111:**E44–E53. http://dx.doi.org/10.1073/pnas.1310755111.
1173. **Lo YH, Chuang CN, Wang TF.** 2014. Pch2 prevents Mec1/Tel1-mediated Hop1 phosphorylation occurring independently of Red1 in budding yeast meiosis. PLoS One **9:**e85687. http://dx.doi.org/10.1371/journal.pone.0085687.
1174. **Wu HY, Burgess SM.** 2006. Two distinct surveillance mechanisms monitor meiotic chromosome metabolism in budding yeast. Curr Biol **16:**2473–2479. http://dx.doi.org/10.1016/j.cub.2006.10.069.
1175. **Matos J, Blanco MG, Maslen S, Skehel JM, West SC.** 2011. Regulatory control of the resolution of DNA recombination intermediates during meiosis and mitosis. Cell **147:**158–172. http://dx.doi.org/10.1016/j.cell.2011.08.032.
1176. **Oh SD, Lao JP, Hwang PY, Taylor AF, Smith GR, Hunter N.** 2007. BLM ortholog, Sgs1, prevents aberrant crossing-over by suppressing formation of multichromatid joint molecules. Cell **130:**259–272. http://dx.doi.org/10.1016/j.cell.2007.05.035.
1177. **Jessop L, Lichten M.** 2008. Mus81/Mms4 endonuclease and Sgs1 helicase collaborate to ensure proper recombination intermediate metabolism during meiosis. Mol Cell **31:**313–323. http://dx.doi.org/10.1016/j.molcel.2008.05.021.
1178. **Rabitsch KP, Toth A, Galova M, Schleiffer A, Schaffner G, Aigner E, Rupp C, Penkner AM, Moreno-Borchart AC, Primig M, Esposito RE, Klein F, Knop M, Nasmyth K.** 2001. A screen for genes required for meiosis and spore formation based on whole-genome expression. Curr Biol **11:**1001–1009. http://dx.doi.org/10.1016/S0960-9822(01)00274-3.
1179. **Denisenko O, Bomsztyk K.** 2002. Yeast hnRNP K-like genes are involved in regulation of the telomeric position effect and telomere length. Mol Cell Biol **22:**286–297. http://dx.doi.org/10.1128/MCB.22.1.286-297.2002.
1180. **Rabitsch KP, Petronczki M, Javerzat JP, Genier S, Chwalla B, Schleiffer A, Tanaka TU, Nasmyth K.** 2003. Kinetochore recruitment of two nucleolar proteins is required for homolog segregation in meiosis I. Dev Cell **4:**535–548. http://dx.doi.org/10.1016/S1534-5807(03)00086-8.
1181. **Bando M, Katou Y, Komata M, Tanaka H, Itoh T, Sutani T, Shirahige K.** 2009. Csm3, Tof1, and Mrc1 form a heterotrimeric mediator complex that associates with DNA replication forks. J Biol Chem **284:**34355–34365. http://dx.doi.org/10.1074/jbc.M109.065730.
1182. **Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, Brown PO, Herskowitz I.** 1998. The transcriptional program of sporulation in budding yeast. Science **282:**699–705. http://dx.doi.org/10.1126/science.282.5389.699.
1183. **Pak J, Segall J.** 2002. Regulation of the premiddle and middle phases of expression of the NDT80 gene during sporulation of *Saccharomyces cerevisiae*. Mol Cell Biol **22:**6417–6429. http://dx.doi.org/10.1128/MCB.22.18.6417-6429.2002.
1184. **Xie J, Pierce M, Gailus-Durner V, Wagner M, Winter E, Vershon AK.** 1999. Sum1 and Hst1 repress middle sporulation-specific gene expression during mitosis in *Saccharomyces cerevisiae*. EMBO J **18:**6448–6454. http://dx.doi.org/10.1093/emboj/18.22.6448.
1185. **Sourirajan A, Lichten M.** 2008. Polo-like kinase Cdc5 drives exit from pachytene during budding yeast meiosis. Genes Dev **22:**2627–2632. http://dx.doi.org/10.1101/gad.1711408.
1186. **Dementhon K, Iyer G, Glass NL.** 2006. VIB-1 is required for expression of genes necessary for programmed cell death in *Neurospora crassa*. Eukaryot Cell **5:**2161–2173. http://dx.doi.org/10.1128/EC.00253-06.
1187. **Xiong Y, Sun J, Glass NL.** 2014. VIB1, a link between glucose signaling and carbon catabolite repression, is essential for plant cell wall degradation by *Neurospora crassa*. PLoS Genet **10:**e1004500. http://dx.doi.org/10.1371/journal.pgen.1004500.
1188. **Katz ME, Gray KA, Cheetham BF.** 2006. The *Aspergillus nidulans xprG* (*phoG*) gene encodes a putative transcriptional activator involved in the response to nutrient limitation. Fungal Genet Biol **43:**190–199. http://dx.doi.org/10.1016/j.fgb.2005.12.001.
1189. **Yang YL, Wang CW, Leaw SN, Chang TP, Wang IC, Chen CG, Fan JC, Tseng KY, Huang SH, Chen CY, Hsiao TY, Hsiung CA, Chen CT, Hsiao CD, Lo HJ.** 2012. R432 is a key residue for the multiple functions of Ndt80p in *Candida albicans*. Cell Mol Life Sci **69:**1011–1023. http://dx.doi.org/10.1007/s00018-011-0849-5.
1190. **Dirick L, Goetsch L, Ammerer G, Byers B.** 1998. Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science **281:**1854–1857. http://dx.doi.org/10.1126/science.281.5384.1854.
1191. **Henderson KA, Kee K, Maleki S, Santini PA, Keeney S.** 2006. Cyclin-dependent kinase directly regulates initiation of meiotic recombination. Cell **125:**1321–1332. http://dx.doi.org/10.1016/j.cell.2006.04.039.
1192. **Dahmann C, Futcher B.** 1995. Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics **140:**957–963.
1193. **Carlile TM, Amon A.** 2008. Meiosis I is established through division-specific translational control of a cyclin. Cell **133:**280–291. http://dx.doi.org/10.1016/j.cell.2008.02.032.
1194. **Castro A, Bernis C, Vigneron S, Labbe JC, Lorca T.** 2005. The anaphase-promoting complex: a key factor in the regulation of cell cycle. Oncogene **24:**314–325. http://dx.doi.org/10.1038/sj.onc.1207973.
1195. **Cooper KF, Mallory MJ, Egeland DB, Jarnik M, Strich R.** 2000. Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. Proc Natl Acad Sci U S A **97:**14548–14553. http://dx.doi.org/10.1073/pnas.250351297.
1196. **Jantti J, Aalto MK, Oyen M, Sundqvist L, Keranen S, Ronne H.** 2002. Characterization of temperature-sensitive mutations in the yeast syntaxin 1 homologues Sso1p and Sso2p, and evidence of a distinct function for Sso1p in sporulation. J Cell Sci **115:**409–420.
1197. **Yang HJ, Nakanishi H, Liu S, McNew JA, Neiman AM.** 2008. Binding interactions control SNARE specificity in vivo. J Cell Biol **183:**1089–1100. http://dx.doi.org/10.1083/jcb.200809178.
1198. **Neiman AM.** 1998. Prospore membrane formation defines a developmentally regulated branch of the secretory pathway in yeast. J Cell Biol **140:**29–37. http://dx.doi.org/10.1083/jcb.140.1.29.
1199. **Nakase Y, Nakamura-Kubo M, Ye Y, Hirata A, Shimoda C, Nakamura T.** 2008. Meiotic spindle pole bodies acquire the ability to assemble the spore plasma membrane by sequential recruitment of sporulation-specific components in fission yeast. Mol Biol Cell **19:**2476–2487. http://dx.doi.org/10.1091/mbc.E08-02-0118.
1200. **Shimoda C.** 2004. Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking. J Cell Sci **117:**389–396.
1201. **Carroll GC.** 1967. The ultrastructure of ascospore delimitation in *Saccobolus kerverni*. J Cell Biol **33:**218–224. http://dx.doi.org/10.1083/jcb.33.1.218.

1202. **Zickler D.** 1970. Division spindle and centrosomal plaques during mitosis and meiosis in some Ascomyctes. Chromosoma **30:**287–304. http://dx.doi.org/10.1007/BF00321062.
1203. **Raju NB.** 1980. Meiosis and ascospore genesis in *Neurospora*. Eur J Cell Biol **23:**208–223.
1204. **Bertin A, McMurray MA, Grob P, Park SS, Garcia G, III, Patanwala I, Ng HL, Alber T, Thorner J, Nogales E.** 2008. *Saccharomyces cerevisiae* septins: supramolecular organization of heterooligomers and the mechanism of filament assembly. Proc Natl Acad Sci U S A **105:**8274–8279. http://dx.doi.org/10.1073/pnas.0803330105.
1205. **Pablo-Hernando ME, Arnaiz-Pita Y, Tachikawa H, del Rey F, Neiman AM, Vazquez de Aldana CR.** 2008. Septins localize to microtubules during nutritional limitation in *Saccharomyces cerevisiae*. BMC Cell Biol **9:**55. http://dx.doi.org/10.1186/1471-2121-9-55.
1206. **Tachikawa H, Bloecher A, Tatchell K, Neiman AM.** 2001. A Gip1p-Glc7p phosphatase complex regulates septin organization and spore wall formation. J Cell Biol **155:**797–808. http://dx.doi.org/10.1083/jcb.200107008.
1207. **Maier P, Rathfelder N, Maeder CI, Colombelli J, Stelzer EH, Knop M.** 2008. The SpoMBe pathway drives membrane bending necessary for cytokinesis and spore formation in yeast meiosis. EMBO J **27:**2363–2374. http://dx.doi.org/10.1038/emboj.2008.168.
1208. **Knop M, Strasser K.** 2000. Role of the spindle pole body of yeast in mediating assembly of the prospore membrane during meiosis. EMBO J **19:**3657–3667. http://dx.doi.org/10.1093/emboj/19.14.3657.
1209. **Moreno-Borchart AC, Strasser K, Finkbeiner MG, Shevchenko A, Shevchenko A, Knop M.** 2001. Prospore membrane formation linked to the leading edge protein (LEP) coat assembly. EMBO J **20:**6946–6957. http://dx.doi.org/10.1093/emboj/20.24.6946.
1210. **Nickas ME, Neiman AM.** 2002. Ady3p links spindle pole body function to spore wall synthesis in *Saccharomyces cerevisiae*. Genetics **160:**1439–1450.
1211. **Suda Y, Nakanishi H, Mathieson EM, Neiman AM.** 2007. Alternative modes of organellar segregation during sporulation in *Saccharomyces cerevisiae*. Eukaryot Cell **6:**2009–2017. http://dx.doi.org/10.1128/EC.00238-07.
1212. **Iwamoto MA, Fairclough SR, Rudge SA, Engebrecht J.** 2005. Saccharomyces cerevisiae Sps1p regulates trafficking of enzymes required for spore wall synthesis. Eukaryot Cell **4:**536–544. http://dx.doi.org/10.1128/EC.4.3.536-544.2005.
1213. **Huang LS, Doherty HK, Herskowitz I.** 2005. The Smk1p MAP kinase negatively regulates Gsc2p, a 1,3-beta-glucan synthase, during spore wall morphogenesis in *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A **102:**12431–12436. http://dx.doi.org/10.1073/pnas.0502324102.
1214. **Coluccio A, Bogengruber E, Conrad MN, Dresser ME, Briza P, Neiman AM.** 2004. Morphogenetic pathway of spore wall assembly in *Saccharomyces cerevisiae*. Eukaryot Cell **3:**1464–1475. http://dx.doi.org/10.1128/EC.3.6.1464-1475.2004.
1215. **Li J, Agarwal S, Roeder GS.** 2007. SSP2 and OSW1, two sporulation-specific genes involved in spore morphogenesis in *Saccharomyces cerevisiae*. Genetics **175:**143–154.
1216. **Sarkar PK, Florczyk MA, McDonough KA, Nag DK.** 2002. SSP2, a sporulation-specific gene necessary for outer spore wall assembly in the yeast *Saccharomyces cerevisiae*. Mol Genet Genomics **267:**348–358. http://dx.doi.org/10.1007/s00438-002-0666-5.
1217. **Briza P, Eckerstorfer M, Breitenbach M.** 1994. The sporulation-specific enzymes encoded by the DIT1 and DIT2 genes catalyze a two-step reaction leading to a soluble LL-dityrosine-containing precursor of the yeast spore wall. Proc Natl Acad Sci U S A **91:**4524–4528. http://dx.doi.org/10.1073/pnas.91.10.4524.
1218. **Felder T, Bogengruber E, Tenreiro S, Ellinger A, Sa-Correia I, Briza P.** 2002. Dtrlp, a multidrug resistance transporter of the major facilitator superfamily, plays an essential role in spore wall maturation in *Saccharomyces cerevisiae*. Eukaryot Cell **1:**799–810. http://dx.doi.org/10.1128/EC.1.5.799-810.2002.
1219. **Benitez T, Villa TG, Acha IG.** 1975. Chemical and structural differences in mycelial and regeneration walls of *Trichoderma viride*. Arch Microbiol **105:**277–282. http://dx.doi.org/10.1007/BF00447147.
1220. **Benitez T, Villa TG, Garcia Acha I.** 1976. Some chemical and structural features of the conidial wall of *Trichoderma viride*. Can J Microbiol **22:**318–321. http://dx.doi.org/10.1139/m76-046.
1221. **Mata J, Lyne R, Burns G, Bahler J.** 2002. The transcriptional program of meiosis and sporulation in fission yeast. Nat Genet **32:**143–147. http://dx.doi.org/10.1038/ng951.
1222. **Rodriguez RJ, White JF, Jr, Arnold AE, Redman RS.** 2009. Fungal endophytes: diversity and functional roles. New Phytol **182:**314–330. http://dx.doi.org/10.1111/j.1469-8137.2009.02773.x.
1223. **Harman GE, Howell CR, Viterbo A, Chet I, Lorito M.** 2004. *Trichoderma* species: opportunistic, avirulent plant symbionts. Nat Rev Microbiol **2:**43–56. http://dx.doi.org/10.1038/nrmicro797.
1224. **Shoresh M, Harman GE, Mastouri F.** 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol **48:**21–43. http://dx.doi.org/10.1146/annurev-phyto-073009-114450.
1225. **Yedidia II, Benhamou N, Chet II.** 1999. Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl Environ Microbiol **65:**1061–1070.
1226. **Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi IY, Holmes KA.** 2006. Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta **224:**1449–1464. http://dx.doi.org/10.1007/s00425-006-0314-0.
1227. **Shoresh M, Harman GE.** 2008. The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. Plant Physiol **147:**2147–2163. http://dx.doi.org/10.1104/pp.108.123810.
1228. **Alfano G, Ivey ML, Cakir C, Bos JI, Miller SA, Madden LV, Kamoun S, Hoitink HA.** 2007. Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. Phytopathology **97:**429–437. http://dx.doi.org/10.1094/PHYTO-97-4-0429.
1229. **Jones JD, Dangl JL.** 2006. The plant immune system. Nature **444:**323–329. http://dx.doi.org/10.1038/nature05286.
1230. **Frischmann A, Neudl S, Gaderer R, Bonazza K, Zach S, Gruber S, Spadiut O, Friedbacher G, Grothe H, Seidl-Seiboth V.** 2013. Self-assembly at air/water interfaces and carbohydrate binding properties of the small secreted protein EPL1 from the fungus *Trichoderma atroviride*. J Biol Chem **288:**4278–4287. http://dx.doi.org/10.1074/jbc.M112.427633.
1231. **Ron M, Avni A.** 2004. The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. Plant Cell **16:**1604–1615. http://dx.doi.org/10.1105/tpc.022475.
1232. **Bar M, Sharfman M, Ron M, Avni A.** 2010. BAK1 is required for the attenuation of ethylene-inducing xylanase (Eix)-induced defense responses by the decoy receptor LeEix1. Plant J **63:**791–800. http://dx.doi.org/10.1111/j.1365-313X.2010.04282.x.
1233. **Hanania U, Furman-Matarasso N, Ron M, Avni A.** 1999. Isolation of a novel SUMO protein from tomato that suppresses EIX-induced cell death. Plant J **19:**533–541. http://dx.doi.org/10.1046/j.1365-313X.1999.00547.x.
1234. **Rotblat B, Enshell-Seijffers D, Gershoni JM, Schuster S, Avni A.** 2002. Identification of an essential component of the elicitation active site of the EIX protein elicitor. Plant J **32:**1049–1055. http://dx.doi.org/10.1046/j.1365-313X.2002.01490.x.
1235. **Ruocco M, Lanzuise S, Lombardi N, Woo SL, Vinale F, Marra R, Varlese R, Manganiello G, Pascale A, Scala V, Turra D, Scala F, Lorito M.** 2015. Multiple roles and effects of a novel *Trichoderma* hydrophobin. Mol Plant Microbe Interact **28:**167–179. http://dx.doi.org/10.1094/MPMI-07-14-0194-R.
1236. **Viterbo A, Chet I.** 2006. TasHyd1, a new hydrophobin gene from the biocontrol agent *Trichoderma asperellum*, is involved in plant root colonization. Mol Plant Pathol **7:**249–258. http://dx.doi.org/10.1111/j.1364-3703.2006.00335.x.
1237. **Van Wees SC, Van der Ent S, Pieterse CM.** 2008. Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol **11:**443–448. http://dx.doi.org/10.1016/j.pbi.2008.05.005.
1238. **Lo Presti L, Lanver D, Schweizer G, Tanaka S, Liang L, Tollot M, Zuccaro A, Reissmann S, Kahmann R.** 2015. Fungal effectors and plant susceptibility. Annu Rev Plant Biol **66:**513–545. http://dx.doi.org/10.1146/annurev-arplant-043014-114623.
1239. **Kamoun S.** 2007. Groovy times: filamentous pathogen effectors revealed. Curr Opin Plant Biol **10:**358–365. http://dx.doi.org/10.1016/j.pbi.2007.04.017.
1240. **Hogenhout SA, Van der Hoorn RA, Terauchi R, Kamoun S.** 2009. Emerging concepts in effector biology of plant-associated organisms.

Mol Plant Microbe Interact **22:**115–122. http://dx.doi.org/10.1094/MPMI-22-2-0115.

1241. **Oliva R, Win J, Raffaele S, Boutemy L, Bozkurt TO, Chaparro-Garcia A, Segretin ME, Stam R, Schornack S, Cano LM, van Damme M, Huitema E, Thines M, Banfield MJ, Kamoun S.** 2010. Recent developments in effector biology of filamentous plant pathogens. Cell Microbiol **12:**705–715. http://dx.doi.org/10.1111/j.1462-5822.2010.01471.x.
1242. **Zuccaro A, Lahrmann U, Guldener U, Langen G, Pfiffi S, Biedenkopf D, Wong P, Samans B, Grimm C, Basiewicz M, Murat C, Martin F, Kogel KH.** 2011. Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*. PLoS Pathog **7:**e1002290. http://dx.doi.org/10.1371/journal.ppat.1002290.
1243. **Martin F, Aerts A, Ahren D, Brun A, Danchin EG, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V, Salamov A, Shapiro HJ, Wuyts J, Blaudez D, Buee M, Brokstein P, Canback B, Cohen D, Courty PE, Coutinho PM, Delaruelle C, Detter JC, Deveau A, DiFazio S, Duplessis S, Fraissinet-Tachet L, Lucic E, Frey-Klett P, Fourrey C, Feussner I, Gay G, Grimwood J, Hoegger PJ, Jain P, Kilaru S, Labbe J, Lin YC, Legue V, Le Tacon F, Marmeisse R, Melayah D, Montanini B, Muratet M, Nehls U, Niculita-Hirzel H, Oudot-Le Secq MP, Peter M, Quesneville H, Rajashekar B, Reich M, Rouhier N, Schmutz J, Yin T, Chalot M, Henrissat B, Kues U, Lucas S, Van de Peer Y, Podila GK, Polle A, Pukkila PJ, Richardson PM, Rouze P, Sanders IR, Stajich JE, Tunlid A, Tuskan G, Grigoriev IV.** 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature **452:**88–92. http://dx.doi.org/10.1038/nature06556.
1244. **Kloppholz S, Kuhn H, Requena N.** 2011. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. Curr Biol **21:**1204–1209. http://dx.doi.org/10.1016/j.cub.2011.06.044.
1245. **Schafer P, Pfiffi S, Voll LM, Zajic D, Chandler PM, Waller F, Scholz U, Pons-Kuhnemann J, Sonnewald S, Sonnewald U, Kogel KH.** 2009. Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with *Piriformospora indica*. Plant J **59:**461–474. http://dx.doi.org/10.1111/j.1365-313X.2009.03887.x.
1246. **Bittel P, Robatzek S.** 2007. Microbe-associated molecular patterns (MAMPs) probe plant immunity. Curr Opin Plant Biol **10:**335–341. http://dx.doi.org/10.1016/j.pbi.2007.04.021.
1247. **Saunders DG, Win J, Cano LM, Szabo LJ, Kamoun S, Raffaele S.** 2012. Using hierarchical clustering of secreted protein families to classify and rank candidate effectors of rust fungi. PLoS One **7:**e29847. http://dx.doi.org/10.1371/journal.pone.0029847.
1248. **Shabab M, Shindo T, Gu C, Kaschani F, Pansuriya T, Chintha R, Harzen A, Colby T, Kamoun S, van der Hoorn RA.** 2008. Fungal effector protein AVR2 targets diversifying defense-related cys proteases of tomato. Plant Cell **20:**1169–1183. http://dx.doi.org/10.1105/tpc.107.056325.
1249. **Doehlemann G, van der Linde K, Assmann D, Schwammbach D, Hof A, Mohanty A, Jackson D, Kahmann R.** 2009. Pep1, a secreted effector protein of *Ustilago maydis*, is required for successful invasion of plant cells. PLoS Pathog **5:**e1000290. http://dx.doi.org/10.1371/journal.ppat.1000290.
1250. **Dong S, Yin W, Kong G, Yang X, Qutob D, Chen Q, Kale SD, Sui Y, Zhang Z, Dou D, Zheng X, Gijzen M, Wang B MTY.** 2011. *Phytophthora sojae* avirulence effector Avr3b is a secreted NADH and ADP-ribose pyrophosphorylase that modulates plant immunity. PLoS Pathog **7:**e1002353. http://dx.doi.org/10.1371/journal.ppat.1002353.
1251. **Leuthner B, Aichinger C, Oehmen E, Koopmann E, Muller O, Muller P, Kahmann R, Bolker M, Schreier PH.** 2005. A H_2O2-producing glyoxal oxidase is required for filamentous growth and pathogenicity in *Ustilago maydis*. Mol Genet Genomics **272:**639–650. http://dx.doi.org/10.1007/s00438-004-1085-6.
1252. **Stergiopoulos I, van den Burg HA, Okmen B, Beenen HG, van Liere S, Kema GH, de Wit PJ.** 2010. Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dicots and monocots. Proc Natl Acad Sci U S A **107:**7610–7615. http://dx.doi.org/10.1073/pnas.1002910107.
1253. **de Jonge R, Thomma BP.** 2009. Fungal LysM effectors: extinguishers of host immunity? Trends Microbiol **17:**151–157. http://dx.doi.org/10.1016/j.tim.2009.01.002.
1254. **de Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, van der Krol S, Shibuya N, Joosten MH, Thomma BP.** 2010. Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science **329:**953–955. http://dx.doi.org/10.1126/science.1190859.
1255. **Hajri A, Brin C, Hunault G, Lardeux F, Lemaire C, Manceau C, Boureau T, Poussier S.** 2009. A "repertoire for repertoire" hypothesis: repertoires of type three effectors are candidate determinants of host specificity in Xanthomonas. PLoS One **4:**e6632. http://dx.doi.org/10.1371/journal.pone.0006632.
1256. **Koeck M, Hardham AR, Dodds PN.** 2011. The role of effectors of biotrophic and hemibiotrophic fungi in infection. Cell Microbiol **13:**1849–1857. http://dx.doi.org/10.1111/j.1462-5822.2011.01665.x.
1257. **Rosso MN, Vieira P, de Almeida-Engler J, Castagnone-Sereno P.** 2011. Proteins secreted by root-knot nematodes accumulate in the extracellular compartment during root infection. Plant Signal Behav **6:**1232–1234. http://dx.doi.org/10.4161/psb.6.8.16290.
1258. **Kamoun S.** 2006. A catalogue of the effector secretome of plant-pathogenic oomycetes. Annu Rev Phytopathol **44:**41–60. http://dx.doi.org/10.1146/annurev.phyto.44.070505.143436.
1259. **Giraldo MC, Dagdas YF, Gupta YK, Mentlak TA, Yi M, Martinez-Rocha AL, Saitoh H, Terauchi R, Talbot NJ, Valent B.** 2013. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus *Magnaporthe oryzae*. Nat Commun **4:**1996.
1260. **Choi J, Park J, Kim D, Jung K, Kang S, Lee YH.** 2010. Fungal secretome database: integrated platform for annotation of fungal secretomes. BMC Genomics **11:**105. http://dx.doi.org/10.1186/1471-2164-11-105.
1261. **Gu B, Kale SD, Wang Q, Wang D, Pan Q, Cao H, Meng Y, Kang Z, Tyler BM, Shan W.** 2011. Rust secreted protein Ps87 is conserved in diverse fungal pathogens and contains a RXLR-like motif sufficient for translocation into plant cells. PLoS One **6:**e27217. http://dx.doi.org/10.1371/journal.pone.0027217.
1262. **Perez A, Pedros B, Murgui A, Casanova M, Lopez-Ribot JL, Martinez JP.** 2006. Biofilm formation by *Candida albicans* mutants for genes coding fungal proteins exhibiting the eight-cysteine-containing CFEM domain. FEMS Yeast Res **6:**1074–1084. http://dx.doi.org/10.1111/j.1567-1364.2006.00131.x.
1263. **Dubey MK, Jensen DF, Karlsson M.** 2014. Hydrophobins are required for conidial hydrophobicity and plant root colonization in the fungal biocontrol agent *Clonostachys rosea*. BMC Microbiol **14:**18. http://dx.doi.org/10.1186/1471-2180-14-18.
1264. **Linder MB, Szilvay GR, Nakari-Setala T, Penttila ME.** 2005. Hydrophobins: the protein-amphiphiles of filamentous fungi. FEMS Microbiol Rev **29:**877–896. http://dx.doi.org/10.1016/j.femsre.2005.01.004.
1265. **Kubicek CP, Baker S, Gamauf C, Kenerley CM, Druzhinina IS.** 2008. Purifying selection and birth-and-death evolution in the class II hydrophobin gene families of the ascomycete *Trichoderma/Hypocrea*. BMC Evol Biol **8:**4. http://dx.doi.org/10.1186/1471-2148-8-4.
1266. **Plett JM, Martin F.** 2011. Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. Trends Genet **27:**14–22. http://dx.doi.org/10.1016/j.tig.2010.10.005.
1267. **Talbot NJ, Ebbole DJ, Hamer JE.** 1993. Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. Plant Cell **5:**1575–1590.
1268. **Degani O, Lev S, Ronen M.** 2013. Hydrophobin gene expression in the maize pathogen *Cochliobolus heterostrophus*. Physiol Mol Plant Pathol **83:**25–34. http://dx.doi.org/10.1016/j.pmpp.2013.03.003.
1269. **Baccelli I.** 2014. Cerato-platanin family proteins: one function for multiple biological roles? Front Plant Sci **5:**769.
1270. **Pazzagli L, Seidl-Seiboth V, Barsottini M, Vargas WA, Scala A, Mukherjee PK.** 2014. Cerato-platanins: elicitors and effectors. Plant Sci **228:**79–87. http://dx.doi.org/10.1016/j.plantsci.2014.02.009.
1271. **Bonazza K, Gaderer R, Neudl S, Przylucka A, Allmaier G, Druzhinina IS, Grothe H, Friedbacher G, Seidl-Seiboth V.** 2015. The fungal cerato-platanin protein EPL1 forms highly ordered layers at hydrophobic/hydrophilic interfaces. Soft Matter **11:**1723–1732. http://dx.doi.org/10.1039/C4SM02389G.
1272. **Gaderer R, Lamdan NL, Frischmann A, Sulyok M, Krska R, Horwitz BA, Seidl-Seiboth V.** 2015. Sm2, a paralog of the *Trichoderma* cerato-platanin elicitor Sm1, is also highly important for plant protection conferred by the fungal-root interaction of *Trichoderma* with maize. BMC Microbiol **15:**2. http://dx.doi.org/10.1186/s12866-014-0333-0.
1273. **Frias M, Gonzalez C, Brito N.** 2011. BcSpl1, a cerato-platanin family protein, contributes to *Botrytis cinerea* virulence and elicits the hyper-

sensitive response in the host. New Phytol **192:**483–495. http://dx.doi.org/10.1111/j.1469-8137.2011.03802.x.
1274. **Scholze H, Boch J.** 2010. TAL effector-DNA specificity. Virulence **1:**428–432. http://dx.doi.org/10.4161/viru.1.5.12863.
1275. **Muller O, Schreier PH, Uhrig JF.** 2008. Identification and characterization of secreted and pathogenesis-related proteins in *Ustilago maydis*. Mol Genet Genomics **279:**27–39. http://dx.doi.org/10.1007/s00438-007-0291-4.
1276. **Lanver D, Mendoza-Mendoza A, Brachmann A, Kahmann R.** 2010. Sho1 and Msb2-related proteins regulate appressorium development in the smut fungus *Ustilago maydis*. Plant Cell **22:**2085–2101. http://dx.doi.org/10.1105/tpc.109.073734.
1277. **Perez-Nadales E, Di Pietro A.** 2011. The membrane mucin Msb2 regulates invasive growth and plant infection in *Fusarium oxysporum*. Plant Cell **23:**1171–1185. http://dx.doi.org/10.1105/tpc.110.075093.
1278. **Dean P.** 2011. Functional domains and motifs of bacterial type III effector proteins and their roles in infection. FEMS Microbiol Rev **35:**1100–1125. http://dx.doi.org/10.1111/j.1574-6976.2011.00271.x.
1279. **Dinkel H, Michael S, Weatheritt RJ, Davey NE, Van Roey K, Altenberg B, Toedt G, Uyar B, Seiler M, Budd A, Jodicke L, Dammert MA, Schroeter C, Hammer M, Schmidt T, Jehl P, McGuigan C, Dymecka M, Chica C, Luck K, Via A, Chatr-Aryamontri A, Haslam N, Grebnev G, Edwards RJ, Steinmetz MO, Meiselbach H, Diella F, Gibson TJ.** 2012. ELM: the database of eukaryotic linear motifs. Nucleic Acids Res **40:**D242–D251. http://dx.doi.org/10.1093/nar/gkr1064.
1280. **Senchou V, Weide R, Carrasco A, Bouyssou H, Pont-Lezica R, Govers F, Canut H.** 2004. High affinity recognition of a *Phytophthora* protein by *Arabidopsis* via an RGD motif. Cell Mol Life Sci **61:**502–509. http://dx.doi.org/10.1007/s00018-003-3394-z.
1281. **Stassen JH, Van den Ackerveken G.** 2011. How do oomycete effectors interfere with plant life? Curr Opin Plant Biol **14:**407–414. http://dx.doi.org/10.1016/j.pbi.2011.05.002.
1282. **Mitchell A, Chang HY, Daugherty L, Fraser M, Hunter S, Lopez R, McAnulla C, McMenamin C, Nuka G, Pesseat S, Sangrador-Vegas A, Scheremetjew M, Rato C, Yong SY, Bateman A, Punta M, Attwood TK, Sigrist CJ, Redaschi N, Rivoire C, Xenarios I, Kahn D, Guyot D, Bork P, Letunic I, Gough J, Oates M, Haft D, Huang H, Natale DA, Wu CH, Orengo C, Sillitoe I, Mi H, Thomas PD, Finn RD.** 2015. The InterPro protein families database: the classification resource after 15 years. Nucleic Acids Res **43:**D213–D221. http://dx.doi.org/10.1093/nar/gku1243.
1283. **Bode W, Greyling HJ, Huber R, Otlewski J, Wilusz T.** 1989. The refined 2.0 Å X-ray crystal structure of the complex formed between bovine beta-trypsin and CMTI-I, a trypsin inhibitor from squash seeds (*Cucurbita maxima*). Topological similarity of the squash seed inhibitors with the carboxypeptidase A inhibitor from potatoes. FEBS Lett **242:**285–292.
1284. **Kufner I, Ottmann C, Oecking C, Nurnberger T.** 2009. Cytolytic toxins as triggers of plant immune response. Plant Signal Behav **4:**977–979. http://dx.doi.org/10.4161/psb.4.10.9669.
1285. **Ottmann C, Luberacki B, Kufner I, Koch W, Brunner F, Weyand M, Mattinen L, Pirhonen M, Anderluh G, Seitz HU, Nurnberger T, Oecking C.** 2009. A common toxin fold mediates microbial attack and plant defense. Proc Natl Acad Sci U S A **106:**10359–10364. http://dx.doi.org/10.1073/pnas.0902362106.
1286. **Bae H, Bowers JH, Tooley PW, Bailey BA.** 2005. NEP1 orthologues encoding necrosis and ethylene inducing proteins exist as a multigene family in *Phytophthora megakarya*, causal agent of black pod disease on cacao. Mycol Res **109:**1373–1385. doi:10.1017/S0953756205003941.
1287. **Bae H, Kim MS, Sicher RC, Bae HJ, Bailey BA.** 2006. Necrosis- and ethylene-inducing peptide from *Fusarium oxysporum* induces a complex cascade of transcripts associated with signal transduction and cell death in *Arabidopsis*. Plant Physiol **141:**1056–1067. http://dx.doi.org/10.1104/pp.106.076869.
1288. **Kleemann J.** 2010. Identification and functional characterization of secreted effector proteins of the hemibiotrophic fungus *Colletotrichum higginsianum*. Ph.D. thesis. University of Cologne, Cologne, Germany.
1289. **Motteram J, Kufner I, Deller S, Brunner F, Hammond-Kosack KE, Nurnberger T, Rudd JJ.** 2009. Molecular characterization and functional analysis of MgNLP, the sole NPP1 domain-containing protein, from the fungal wheat leaf pathogen *Mycosphaerella graminicola*. Mol Plant Microbe Interact **22:**790–799. http://dx.doi.org/10.1094/MPMI-22-7-0790.
1290. **Dong S, Kong G, Qutob D, Yu X, Tang J, Kang J, Dai T, Wang H, Gijzen M, Wang Y.** 2012. The NLP toxin family in *Phytophthora sojae* includes rapidly evolving groups that lack necrosis-inducing activity. Mol Plant Microbe Interact **25:**896–909. http://dx.doi.org/10.1094/MPMI-01-12-0023-R.
1291. **Tzima AK, Paplomatas EJ, Tsitsigiannis DI, Kang S.** 2012. The G protein beta subunit controls virulence and multiple growth- and development-related traits in *Verticillium dahliae*. Fungal Genet Biol **49:**271–283. http://dx.doi.org/10.1016/j.fgb.2012.02.005.
1292. **Mukherjee M, Kim JE, Park YS, Kolomiets MV, Shim WB.** 2011. Regulators of G-protein signalling in *Fusarium verticillioides* mediate differential host-pathogen responses on nonviable versus viable maize kernels. Mol Plant Pathol **12:**479–491. http://dx.doi.org/10.1111/j.1364-3703.2010.00686.x.
1293. **Yu HY, Seo JA, Kim JE, Han KH, Shim WB, Yun SH, Lee YW.** 2008. Functional analyses of heterotrimeric G protein G alpha and G beta subunits in *Gibberella zeae*. Microbiology **154:**392–401. http://dx.doi.org/10.1099/mic.0.2007/012260-0.
1294. **Vandermarliere E, Lammens W, Schoepe J, Rombouts S, Fierens E, Gebruers K, Volckaert G, Rabijns A, Delcour JA, Strelkov SV, Courtin CM.** 2010. Crystal structure of the noncompetitive xylanase inhibitor TLXI, member of the small thaumatin-like protein family. Proteins **78:**2391–2394. http://dx.doi.org/10.1002/prot.22737.
1295. **Grenier J, Potvin C, Trudel J, Asselin A.** 1999. Some thaumatin-like proteins hydrolyse polymeric beta-1,3-glucans. Plant J **19:**473–480. http://dx.doi.org/10.1046/j.1365-313X.1999.00551.x.
1296. **Vasconcelos EA, Santana CG, Godoy CV, Seixas CD, Silva MS, Moreira LR, Oliveira-Neto OB, Price D, Fitches E, Filho EX, Mehta A, Gatehouse JA, Grossi-De-Sa MF.** 2011. A new chitinase-like xylanase inhibitor protein (XIP) from coffee (*Coffea arabica*) affects soybean Asian rust (*Phakopsora pachyrhizi*) spore germination. BMC Biotechnol **11:**14. http://dx.doi.org/10.1186/1472-6750-11-14.
1297. **Gusakov AV.** 2010. Proteinaceous inhibitors of microbial xylanases. Biochemistry (Mosc) **75:**1185–1199. http://dx.doi.org/10.1134/S0006297910100019.
1298. **Takahashi-Ando N, Inaba M, Ohsato S, Igawa T, Usami R, Kimura M.** 2007. Identification of multiple highly similar XIP-type xylanase inhibitor genes in hexaploid wheat. Biochem Biophys Res Commun **360:**880–884. http://dx.doi.org/10.1016/j.bbrc.2007.06.151.
1299. **Poisson G, Chauve C, Chen X, Bergeron A.** 2007. FragAnchor: a large-scale predictor of glycosylphosphatidylinositol anchors in eukaryote protein sequences by qualitative scoring. Genomics Proteomics Bioinformatics **5:**121–130. http://dx.doi.org/10.1016/S1672-0229(07)60022-9.
1300. **Choudhary V, Schneiter R.** 2012. Pathogen-related yeast (PRY) proteins and members of the CAP superfamily are secreted sterol-binding proteins. Proc Natl Acad Sci U S A **109:**16882–16887. http://dx.doi.org/10.1073/pnas.1209086109.
1301. **Gibbs GM, Roelants K, O'Bryan MK.** 2008. The CAP superfamily: cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins: roles in reproduction, cancer, and immune defense. Endocr Rev **29:**865–897. http://dx.doi.org/10.1210/er.2008-0032.
1302. **Dixon DC, Cutt JR, Klessig DF.** 1991. Differential targeting of the tobacco PR-1 pathogenesis-related proteins to the extracellular space and vacuoles of crystal idioblasts. EMBO J **10:**1317–1324.
1303. **Kombrink A, Sanchez-Vallet A, Thomma BP.** 2011. The role of chitin detection in plant–pathogen interactions. Microbes Infect **13:**1168–1176. http://dx.doi.org/10.1016/j.micinf.2011.07.010.
1304. **Marshall R, Kombrink A, Motteram J, Loza-Reyes E, Lucas J, Hammond-Kosack KE, Thomma BP, Rudd JJ.** 2011. Analysis of two in planta expressed LysM effector homologues from the fungus *Mycosphaerella graminicola* reveals novel functional properties and contributions to virulence on wheat. Plant Physiol **156:**756–769. http://dx.doi.org/10.1104/pp.111.176347.
1305. **Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, Terauchi R, Nishizawa Y, Shibuya N, Thomma BP, Talbot NJ.** 2012. Effector-mediated suppression of chitin-triggered immunity by Magnaporthe oryzae is necessary for rice blast disease. Plant Cell **24:**322–335. http://dx.doi.org/10.1105/tpc.111.092957.
1306. **Seidl-Seiboth V, Zach S, Frischmann A, Spadiut O, Dietzsch C, Herwig C, Ruth C, Rodler A, Jungbauer A, Kubicek CP.** 2013. Spore germination of *Trichoderma atroviride* is inhibited by its LysM protein TAL6. FEBS J **280:**1226–1236. http://dx.doi.org/10.1111/febs.12113.
1307. **Angot A, Vergunst A, Genin S, Peeters N.** 2007. Exploitation of

eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III and type IV secretion systems. PLoS Pathog **3:**e3. http://dx.doi.org/10.1371/journal.ppat.0030003.

1308. **Rosebrock TR, Zeng L, Brady JJ, Abramovitch RB, Xiao F, Martin GB.** 2007. A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. Nature **448:**370–374. http://dx.doi.org/10.1038/nature05966.
1309. **Guindon S, Dufayard JF, Hordijk W, Lefort V, Gascuel O.** 2009. PhyML: fast and accurate phylogeny reconstruction by maximum likelihood. Infect Genet Evol **9:**384–385.
1310. **Geyer R, Wee S, Anderson S, Yates J, Wolf DA.** 2003. BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol Cell **12:**783–790. http://dx.doi.org/10.1016/S1097-2765(03)00341-1.
1311. **Kay S, Hahn S, Marois E, Hause G, Bonas U.** 2007. A bacterial effector acts as a plant transcription factor and induces a cell size regulator. Science **318:**648–651. http://dx.doi.org/10.1126/science.1144956.
1312. **Tkach JM, Yimit A, Lee AY, Riffle M, Costanzo M, Jaschob D, Hendry JA, Ou J, Moffat J, Boone C, Davis TN, Nislow C, Brown GW.** 2012. Dissecting DNA damage response pathways by analysing protein localization and abundance changes during DNA replication stress. Nat Cell Biol **14:**966–976. http://dx.doi.org/10.1038/ncb2549.
1313. **García-Esquivel M, Esquivel-Naranjo EU, Hernández-Oñate M, Ibarra-Laclette E, Herrera-Estrella A.** The *Trichoderma atroviride* cryptochrome/photolyase genes regulate the expression of *blr1*-independent genes both in red and blue light. Fungal Biol, in press.

Monika Schmoll received her degree and Ph.D. on the topic of regulation of cellulase expression and signal transduction in the filamentous fungus *Hypocrea jecorina* (*Trichoderma reesei*) at the Vienna University of Technology, Vienna, Austria, in the group of Christian Kubicek. Besides gaining postdoctoral experience and building her own group at the Vienna University of Technology, she has been a visiting scientist in the laboratory of N. Louise Glass (Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA), the University of Rome La Sapienza, and the University of Szeged, Hungary. In 2012, Dr. Schmoll moved to the Austrian Institute of Technology (AIT) in Tulln, Austria, where she is now group leader and senior scientist. In March 2013 she completed her habilitation at the Vienna University of Technology in the field of Molecular Genetics and Genomics. The primary research field of Dr. Schmoll is the interconnection between light response, sexual development, and metabolism, with an emphasis on effects on cellulase gene expression in the filamentous fungus *Trichoderma reesei*. She showed for the first time that cellulase gene expression is modulated by light in *T. reesei* and since then elucidated important mechanistic details on the underlying mechanism. Her group discovered the sexual cycle in the biotechnological workhorse *Trichoderma*, which had previously been considered asexual. Recently, her group also showed that communication upon sexual development is mediated by regulation of secondary metabolism. Her work with *Trichoderma* is complemented by contributions to genome annotation of several fungi (*Trichoderma* spp., *Aspergillus nidulans*, *Postia placenta*, *Ceriporiopsis subvermispora*, *Phlebiopsis gigantea*), especially in the field of signal transduction.

Alfredo Herrera-Estrella was born and grew up in Mexico City. He graduated as a Biochemical Engineer from the National School of Biological Sciences in 1985. He worked on his Ph.D. thesis (1986 to 1990), focusing on the T-DNA transfer process from *Agrobacterium tumefaciens* to plants, under the direction of Marc Van Montagu at the State University of Ghent in Belgium. He described for the first time *Agrobacterium* virulence proteins capable of carrying the T-DNA into the plant cell nucleus, and he began studies of the mycoparasitic process of the biocontrol agent *Trichoderma atroviride*. Professor Herrera-Estrella pioneered the development of molecular tools for the study of a biocontrol agent with the establishment of transformation systems and cloning of the first mycoparasitism related genes. After returning to Mexico, he continued those studies at the Irapuato Unit of the Center for Research and Advanced Studies (1991 to 2004) and began working towards the elucidation of the mechanisms involved in light perception in *Trichoderma*. In 2000, he was awarded the prize of the Mexican Academy of Sciences. By 2004, he became involved in the establishment, in Mexico, of the National Laboratory of Genomics for Biodiversity. Since then, he and his group have been involved in functional genomics projects, both in crop plants and fungi. Professor Herrera-Estrella continues his efforts in the elucidation of signaling cascades triggering asexual development in fungi. In particular, his group has been using transcriptomic approaches for this purpose. Recently, he has been involved in the study of the response to injury in fungi and the role of noncoding RNAs in this phenomenon.