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Review Article

Mitochondria–nucleus network for genome stability

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ABSTRACT

The proper functioning of the cell depends on preserving the cellular genome. In yeast cells, a limited number of genes are located on mitochondrial DNA. Although the mechanisms underlying nuclear genome maintenance are well understood, much less is known about the mechanisms that ensure mitochondrial genome stability. Mitochondria influence the stability of the nuclear genome and vice versa. Little is known about the two-way communication and mutual influence of the nuclear and mitochondrial genomes. Although the mitochondrial genome replicates independent of the nuclear genome and is organized by a distinct set of mitochondrial nucleoid proteins, nearly all genome stability mechanisms responsible for maintaining the nuclear genome, such as mismatch repair, base excision repair, and double-strand break repair via homologous recombination or the nonhomologous end-joining pathway, also act to protect mitochondrial DNA. In addition to mitochondria-specific DNA polymerase γ, the polymerases α, η, ζ, and Rev1 have been found in this organelle. A nuclear genome instability phenotype results from a failure of various mitochondrial functions, such as an electron transport chain activity breakdown leading to a decrease in ATP production, a reduction in the mitochondrial membrane potential ($\Delta\Psi$), and a block in nucleotide and amino acid biosynthesis. The loss of $\Delta\Psi$ inhibits the production of iron–sulfur prosthetic groups, which impairs the assembly of Fe–S proteins, including those that mediate DNA transactions; disturbs iron homeostasis; leads to oxidative stress; and perturbs wobble tRNA modification and ribosome assembly, thereby affecting translation and leading to proteotoxic stress. In this review, we present the current knowledge of the mechanisms that govern mitochondrial genome maintenance and demonstrate ways in which the impairment of mitochondrial function can affect nuclear genome stability.

Contents

Abbreviations: used: AP, apurinic/apyrimidinic, i.e., abasic; BER, base excision repair; DSB, DNA double-strand break; 5′-dRP, 5′-deoxyribose phosphate; CIA, cytosolic iron–sulfur protein assembly; DG, DNA glycosylase; DRM(D), direct repeat-mediated (deletion); ETC, electron transport chain; HR, homologous recombination; ISC, iron–sulfur cluster; LP BER, long-patch BER; MDR, multidrug resistance; MEPS, minimum efficiency processing segment; MMS, methyl methanesulfonate; NHEJ, nonhomologous end-joining pathway; 3′-PUA, 3′-phospho-α,β-unsaturated aldehyde; RCR, rolling-circle replication; RDR, recombination-dependent replication; ROS, reactive oxygen species; SP BER, short-patch BER; SSA, single-strand annealing; SSB, DNA single-strand break; TCA, tricarboxylic acid cycle, citric acid cycle, Krebs cycle; TLS, translesion synthesis; $\Delta\Psi$, mitochondrial membrane potential

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Introduction

Mitochondria are double-membrane-bound organelles that are thought to have evolved from endosymbiotic α-proteobacteria and that are essential for the viability of eukaryotic cells. Mitochondria perform a variety of functions: produce bulk cellular ATP through the energy conversion reactions of oxidative phosphorylation and the tricarboxylic acid cycle; maintain and express the residual mitochondrial genome that is required for oxidative phosphorylation; perform important steps of heme biosynthesis, amino acid biosynthesis, nucleotide biosynthesis, the urea cycle, and fatty acid metabolism; and participate in apoptosis and reactive oxygen species production. Mitoproteomics studies have indicated that there are ca. 1000 mitochondrial proteins in cells of the yeast *Saccharomyces cerevisiae* [1,2] and ca. 1500 proteins in human mitochondria [3]. Only a small fraction of these proteins is encoded by mitochondrial genomes, and this group primarily includes proteins involved in oxidative phosphorylation and ATP synthesis (8 proteins in yeast mitochondria and 13 proteins in human mitochondria, because yeast mitochondria lack subunits of the respiratory chain complex I that are present in human mitochondria), as well as rRNA and tRNA genes that are required for mitochondrial gene expression. Consequently, the vast majority of mitochondrial proteins are encoded by the nuclear genome, synthesized on cytosolic ribosomes and imported into the mitochondria [4,5]. None of the mitochondrial functions listed above are essential for the viability of *S. cerevisiae* cells, yet protein import into mitochondria is essential [4], highlighting the importance of this cellular compartment. One of the recently recognized essential roles played by mitochondria is the generation and maturation of Fe–S prosthetic groups (iron–sulfur clusters, ISCs)[1] for both mitochondrial and extramitochondrial Fe–S proteins [6,7]. ISCs are crucial for facilitating redox enzyme activities and are required for enzymes engaged in DNA replication; they are also components of various protein complexes, including cytosolic ribosomes [8–10]. ISC synthesis is important not only for Fe–S protein assembly, but also for metal ion homeostasis, the proper cellular stress response, and the execution of apoptosis. Not surprisingly, a defect in this particular mitochondrial function was found to be responsible for the mitochondria-dependent nuclear mutator phenotype [11]. Mitochondrial DNA-encoded genetic information, although minuscule compared to that of the nuclear genome, is required for the proper functioning of mitochondria and subsequently for nuclear genome maintenance [12]; thus, eukaryotic cells have retained mitochondrial DNA (mtDNA) throughout evolution, and special mechanisms have evolved to preserve mtDNA.

Maintenance of the mitochondrial genome

How is the ability to maintain stable mitochondrial DNA determined in S. cerevisiae cells?

Before describing the mtDNA maintenance pathways that function in yeast cells, we will introduce a few terms that are specific to *S. cerevisiae* mitochondrial genetics and describe the nonrespiratory reporter gene that was engineered into the yeast mitochondrial genome and shown to be a useful tool for assaying mtDNA stability in this organism. Because *S. cerevisiae* cells remain viable upon the loss of functional mtDNA, known as the *rho*$^+$ mitochondrial genome, the so-called cytoplasmic *petite* mutants, i.e., nonrespiring clones that form minute colonies on standard glucose-limited medium, can be isolated easily (reviewed in [13]). These mutants arise from either extensive deletions of mtDNA followed by reamplification of the remaining fragment (*rho*$^-$ genomes/mutants) or a complete loss of mtDNA (*rho*^{0} mutants). Therefore, at least three *rho* genotypes are possible: *rho*$^+$ (wild-type), *rho*$^-$ (partial deletion of the mitochondrial genome), and *rho*^{0} (complete loss of mtDNA).

Although *rho*$^-$ and *rho*^{0} *petites* are phenotypically identical, they probably originate from fundamentally different mechanisms. *Petites* with no mtDNA are often produced by a failure to transmit mtDNA to the progeny, whereas *rho*$^-$ *petites* most likely result from recombination events that form partially deleted and rearranged mtDNA molecules (reviewed in [13,14]). The *petite* mutations are markedly pleiotropic because the mutants are unable to perform mitochondrial protein synthesis (essential components of the mitochondrial translation apparatus are encoded by mtDNA, see Introduction). This feature is mainly the consequence of the nuclear transcriptional reprogramming that occurs in yeast cells in response to a *rho*$^-$/*rho*^{0} mutation (reviewed in [15]). Spontaneous cytoplasmic *petite* mutants are quite common; in saturated cultures on glucose-containing medium, the fraction of *petite* cells may reach 1–2% [14,16,17]. However, the frequency of spontaneous and induced mitochondrial point mutations is several orders of magnitude lower than the incidence of *petite* mutants [18–21]. This dramatic difference clearly indicates that the mechanisms underlying the generation of these two types of alterations in mtDNA, cytoplasmic *petite* and point mutations, are divergent.

The levels of mitochondrial point mutagenesis can be estimated from the frequency of mutants that are resistant to antibiotics that target various mitochondrial processes. These antibiotics include the following: (1) erythromycin and chloramphenicol, which inhibit mitochondrial protein synthesis; (2) oligomycin, which inhibits mitochondrial ATP synthase; and (3) antimycin, which blocks electron flow through the respiratory chain at complex III [13,22]. Erythromycin

resistance is acquired through mutations at several positions in the mitochondrial 21S rRNA gene [23]. Oligomycin resistance is acquired through specific point mutations in the mitochondrial *OLI*1 and *OLI*2 genes, which encode subunits 9 and 6, respectively, of the mitochondrial ATPase complex [24,25]. Resistance to antimycin A was attributed to point mutations in the mitochondrial gene *COB,* which codes for cytochrome *b* [26]. However, certain mutations conferring resistance to all of these antibiotics are localized to the nuclear genome, but these nuclear mutations are relatively rare in the case of erythromycin resistance. Each case of antibiotic-resistance mutagenesis that can be attributed to mitochondrial genome instability needs to be reevaluated to exclude the presence of nuclear mutations that present identical phenotypes. In questionable cases, a genetic test verifying the non-Mendelian vs Mendelian inheritance of the resistance phenotype should be performed as proposed by Baruffini et al. [17]. This step is particularly important when testing mutants that are deficient in DNA repair factors that have both nuclear and mitochondrial versions. As discussed below, numerous proteins engaged in DNA transactions are shared by both the nucleus and the mitochondria.

To complete the overview of *S. cerevisiae* mtDNA mutants, point mutations in the mtDNA, the so-called *mit*$^-$ *rho*$^+$ mutations, can be isolated, and these mutations cause respiratory deficiency through the impairment of only one gene (or a few genes) within the components of the respiratory chain without disrupting the integrity of the *rho*$^+$ genome [27]. These mutants exhibit proficient mitochondrial gene expression. Spontaneous *mit*$^-$ mutations are usually reversible, in contrast to *rho*$^-$ and *rho*^{0} mutations, and are as rare as other mitochondrial point mutations (see the discussion above). Mitochondrial mutants of this type are mentioned here because a special *mit*$^-$ *rho*$^+$ strain was constructed with a mitochondrial nonrespiratory reporter gene *ARG*8^m [28]. In this reporter gene, the sequence of *ARG*8 was recoded to fit the yeast mitochondrial genetic code for coding the same protein as the wild-type nuclear gene *ARG*8, synthesized, and inserted into yeast mtDNA in place of the *COX*3 gene. The *ARG*8 gene encodes acetylornithine aminotransferase, a mitochondrial matrix enzyme that catalyzes the fourth step in arginine biosynthesis. The mitochondrial *cox*3: *ARG*8^m gene fully complemented the arginine auxotrophy of a strain lacking nuclear *ARG*8. As expected, expression of the gene was dependent on the mitochondrial translational activator complex specific to *COX*3 mitochondrial mRNA. This reporter gene was used in many studies of mitochondrial gene expression at many locations in the mitochondrial genome and with various modifications, helping to decipher the intricacies of the gene-specific posttranscriptional mechanisms that control the expression of mitochondrial genes in *S. cerevisiae* (the limited space and scope of this review do not allow citing all of the literature on the subject, but it is reviewed in Lipinski et al. [29]).

Another significant modification of *cox*3: *ARG*8^m, published in 2000, aimed to measure the stability of repetitive (microsatellite) sequences in mtDNA [30]. The authors inserted a series of out-of-frame poly(AT) or poly(GT) tracts into the *cox*3: *ARG*8^m gene to screen for repeat rearrangement events that restored reporter expression. Using this system, the authors showed that poly(AT) tracts, which often occur as natural mitochondrial microsatellites, are much more stable than are poly(GT) tracts and that frameshift mutations usually involve deletions rather than additions of repeat units. The difference in stability between poly(AT) and poly(GT) tracts may not depend as much on their sequences as on the ability of the former to form hairpin secondary structures if present in single-stranded DNA (ssDNA). In contrast, in the nuclear genome, poly(GT) and poly(AT) tracts had similar stabilities, and changes to these sequences usually involved additions rather than deletions. These results suggest that the mechanisms mediating the stability of microsatellite sequences in mtDNA differ from the corresponding mechanisms that function in the nucleus. Another interesting difference between the stability of mitochondrial and nuclear microsatellites is a significantly increased stability (by 100-fold) of mitochondrial microsatellite tracts in diploid cells compared with the stability of the corresponding tracts in haploid cells [30,31], whereas no difference between diploid and haploid cells was observed in the frequency of frameshift mutations generated within nuclear microsatellite sequences. The researchers showed that the increased stability of mtDNA in diploid cells was due to ploidy alone, rather than mating type-specific gene expression, but they did not find the exact mechanism to account for this ploidy effect. Later, the group of E. Sia improved their reporter strains for assaying frameshift mutations by constructing a respiratory-competent strain with poly(GT) tracts that interrupted the *ARG*8^m inserted into the mtDNA in a manner that allowed gene expression of the whole mitochondrial genome [32]. Moreover, the same group made significant progress in the study of mtDNA repair pathways by constructing a strain carrying mtDNA with the *COX*2 gene interrupted by a functional *ARG*8^m gene flanked by 96-bp direct repeats. Using this reporter strain, the authors measured the rate at which direct repeat-mediated deletions (DRM(D)) arise in the mitochondrial genome of *S. cerevisiae* [33]. The results showed that the DRM(D) reporter can be destabilized by at least two independent pathways. One predominant pathway generates only deletions in noncrossover arrangement (which may be due to polymerase slippage, template switch, or a single-strand annealing event), and the other pathway, which operates at a much lower efficiency, generates reciprocal products in addition to deletions. Consequently, the minor pathway displays the hallmarks of a recombination pathway involving a structure-specific endonuclease that resolves Holliday junctions. However, an important piece of information about the other, predominant DRM(D)-destabilizing pathway came from experiments with the DRM(D) reporter in a strain carrying the *mip*1-D347A mutation, which causes a strong defect in the 3′→5′ exonuclease activity of mitochondrial polymerase [18]. The deletion rate in the exonuclease mutant was 4-fold lower than that in *MIP*1 strains [33], and the authors speculated that most of the polymerase slippage events that lead to direct repeat deletions in mtDNA may occur during proofreading. Interestingly, the set of DRM(D) reporters constructed by E. Sia's lab with direct repeats of varying lengths ranging from 33 to 96bp displayed the inverse relationship between the repeat length and the stability of the repeat. The shortest repeat was the most stable, and the longest was the least. The DRM(D) reporter with even a shorter repeat of 21bp displayed 1000-fold higher stability that the most stable reporter among those with longer repeats. As the authors have pointed out, the results show that the minimal efficient processing segment (MEPS) for the mitochondrial DRM(D) rearrangement pathway falls between 33 and 21bp. Apparently, for repeats of shorter length than the mitochondrial MEPS other mechanisms than those mediating DRM(D) rearrangements start to predominate, thereby promoting repeat stability. To confirm the notion about the different mode of repeat stability regulation for direct repeats shorter than the mitochondrial MEPS, Stumpf and Copeland [34] showed that in the case of the shortest (21-bp direct repeat) and the most stable DRM(D) reporter the expression of an exonuclease-deficient allele of *MIP*1 destabilized the repeat sequence, opposite to the above-mentioned suppression effect of *mip*1-D347A on the stability of the 96-bp reporter. Therefore, studying the DRM(D) reporters will bring a better understanding of the complicated mitochondrial pathways that counteract genome rearrangements during mtDNA replication.

As mentioned above, the minor DRM(D)-destabilizing pathway appears to be associated with crossover-linked homologous recombination (HR). The primary role of HR, in both this system and all others, is to repair two types of harmful lesions, double-strand breaks (DSBs) and single-strand breaks (SSBs), that arise in

DNA after replication fork collapse, either from the processing of spontaneous damage or from exposure to DNA-damaging agents [35]. As in the nucleus, HR in mitochondria may take place to support mtDNA stability or to destabilize the mitochondrial genome depending on the circumstances. A simple distinction between these two effects of HR on genome integrity is associated with the choice of partner sequences selected for homologous pairing: allelic or nonallelic sequences. HR between allelic sequences is likely to result in an error-free repair event and plays a stabilizing role in the mitochondrial genome integrity. In contrast, HR between nonallelic sequences, e.g., between the repeated sequences in the DRM(D) reporter system, is likely to produce genomic rearrangements and, as a result, this recombination event may destabilize the mtDNA. In mitochondria, owing to the known properties of yeast mitochondrial genomes (high copy number, presence of repetitive sequences), there are many occasions for both allelic and nonallelic HR events. Because the DRM(D) reporter system detects nonallelic recombination events, an experimental system was proposed for testing allelic HR in yeast mitochondria based on heteroallelic recombination in crosses between strains carrying different defective alleles of the original *cox*3Δ: *ARG*8^{m} reporter [36]. The results obtained with this experimental system are described in later sections.

Mitochondrial nucleoid proteins in S. cerevisiae

Mitochondrial DNA is packaged in compact, though dynamic, nucleoprotein complexes known as nucleoids [37,38]. Yeast cells contain up to 40 nucleoids, whereas even 10 to 20 times as many nucleoids can be found in human cells. The number of molecules of mtDNA in one yeast nucleoid varies depending on the growth conditions; only 1 or 2 molecules of mtDNA are found in the nucleoids of aerobically growing cells, and 20 mtDNA molecules can be found in the nucleoids of cells grown under anaerobiosis. The protein components of mitochondrial nucleoids from various organisms have been identified. These proteins can be divided into four groups: (1) proteins with known functions in DNA transactions and packaging; (2) proteins engaged in protein quality control, i.e., chaperonins and proteases; (3) various metabolic enzymes (some of them are characterized as bifunctional, see below); and (4) cytoskeletal components (in nucleoids from higher eukaryotes). The nucleoid-associated metabolic enzymes (group 3) share little overlap among species, which is not surprising because bifunctional metabolic enzymes seem to connect mtDNA maintenance to various metabolic or environmental signals that can be quite divergent for organisms from different phylogenetic lineages [38]. However, one basic mitochondrial nucleoid protein is conserved from yeast to human cells. The main packaging protein in nucleoids is a protein from the eukaryotic high-mobility group family: the Abf2 protein in yeast mitochondria and the mitochondrial transcription factor A (TFAM) in human mitochondria.

In contrast to TFAM, no specific role for Abf2 in regulating transcription in yeast mitochondria has been uncovered. Abf2 binds most of the mitochondrial genome, with a minor preference for GC-rich sequences [39]. Many lines of evidence suggest that the abundance of Abf2 determines the degree of compaction and the structure of nucleoids. Nevertheless, Abf2 is not required for the maintenance of *rho*$^{+}$ genomes. Diffley and Stillman [40] showed that *abf*2-null mutants lose their mtDNA when grown on a rich glucose medium, but they can be grown on medium with nonfermentable substrates as the sole carbon sources or with other nonrepressing substrates [41]. Under these conditions, the expression of another mitochondrial nucleoid protein, Aco1, is induced in yeast cells because the Hap2–5 transcription complex, which is required for the expression of many mitochondrial proteins that function in electron transport and the tricarboxylic acid (TCA) cycle, becomes glucose derepressed [42]. The expression of *ACO*1 is also positively regulated by the transcription factors Rtg1p and Rtg3p, which are effectors of the mitochondria-to-nucleus retrograde (RTG) signaling pathway that responds to the level of cellular glutamate [42]. The Aco1 protein is a bifunctional ISC protein in yeast mitochondria that serves as both the mitochondrial enzyme in the TCA cycle and the nucleoid protein required for the maintenance of *rho*$^{+}$ mtDNA; *aco*1-null mutants are invariably *rho*^{0} [41,43]. The two functions of Aco1 are fulfilled by separate domains of the protein; a subset of *aco*1 mutants is deficient in the catalytic activity of aconitase (disrupting the ISC of the enzyme), but maintain *rho*$^{+}$ mtDNA [41]. Thus, the overproduction of Aco1 in the mitochondrial nucleoid can partially replace the Abf2 protein, suggesting that Aco1 may provide some level of mtDNA packaging or protection. However, a recent study provided an alternative explanation for the loss of mtDNA in *aco*1-null strains and showed that mtDNA loss after the deletion of *ACO*1 is merely an indirect consequence of citrate overproduction by the perturbed TCA cycle and the resulting iron-dependent oxidative stress in mitochondria [44]. These results are described in more detail in a later section. Nevertheless, even if Aco1 is not required for the maintenance of mtDNA and mtDNA loss in *aco*1-null cells is dependent on citrate accumulation, these insights do not contradict the earlier findings on the bifunctional role of Aco1 in the yeast mitochondrion. In addition to Aco1, another yeast mitochondrial nucleoid-associated metabolic enzyme, Ilv5 (which is involved in branched-chain amino acid biosynthesis as the acetohydroxy acid reductoisomerase), can replace Abf2 under conditions of general amino acid control pathway induction in cells growing without the amino acids isoleucine, leucine, and valine [45] or in cells in which the *ILV*5 gene is overexpressed [46].

Furthermore, under conditions in which Aco1 replaces Abf2 (i.e., in *abf*2Δ cells growing on nonrepressing carbon sources), mitochondrial nucleoids are less protected, and their DNA is more sensitive to nuclease attack [47] and oxidative damage [19]. Using the *ARG*8^{m} reporters described above, Sia et al. [48] showed that *abf*2Δ strains growing under conditions that maintain *rho*$^{+}$ mtDNA exhibit the following: (1) increased instability of microsatellite sequences in mtDNA, indicating elevated susceptibility to polymerase slippage during the replication of microsatellite repeats, and (2) increased instability of direct repeats (the DRM (D) reporter) in mtDNA, suggesting a defect in the mechanisms that mediate nonallelic recombination surveillance in mitochondria. In fact, the two mutant phenotypes of *abf*2Δ strains are consistent with one another because the critical destabilizing pathway appears to involve polymerase slippage events in both reporter systems [30,33], as discussed above. Surprisingly, *abf*2Δ deletion mutants did not show any change in the number of point mutations in the mtDNA relative to the reference strain [48]. Thus, nucleoid-associated Abf2 may participate in a mechanism that prevents undesirable polymerase slippage events. Certainly, this role of Abf2 is critical for the basic function of the protein in the maintenance of *rho*$^{+}$ mtDNA. The results discussed above also imply that, to a certain extent, both Aco1 and Ilv5 can replace Abf2 in the nucleoid function associated with preventing rearrangements in mtDNA.

Major mtDNA-packaging proteins, such as Abf2, may participate in diverse mtDNA transactions. We have presented evidence of the role of nucleoid proteins in the prevention of mitochondrial genome rearrangements, but strong evidence indicates that Abf2 is also a positive factor in HR. MacAlpine et al. [49] showed by two-dimensional (2D) gel electrophoresis that Abf2 promotes or stabilizes Holliday recombination junction intermediates in *rho*$^{+}$ mtDNA in vivo, but does not influence the high levels of recombination intermediates found in the mtDNA of *rho*$^{-}$ *petite* mutants. Therefore, Abf2 is a positive factor for certain recombination processes in mitochondria but is not involved in other complex recombination pathways in yeast mitochondria. Strangely, support for this function of Abf2 came from unexpected sources: studies on mtDNA

replication in mammalian cells. In transgenic mice overexpressing human TFAM, a dramatic increase in Holliday junctions and complex mtDNA forms was detected [50]. Not surprisingly, human TFAM expressed in yeast cells was shown to rescue the loss-of-mtDNA phenotype of *abf*2Δ cells [51], suggesting evolutionary conservation of the functions mediated by these mtDNA-packaging proteins in maintaining the mitochondrial genome.

Replication of yeast mitochondrial DNA

The mitochondrial genome in budding yeast cells is replicated by a dedicated mitochondrial polymerase, Mip1. Strains lacking the *MIP*1 gene are *rho*^{0} [43,52], indicating that Mip1 is the major, if not unique (see below), replicative DNA polymerase in the mitochondria of this species. The yeast polymerase γ functions as a single-subunit enzyme that forms only the catalytic subunit of Mip1 [53,54]; this polymerase differs from the mitochondrial polymerases of other eukaryotes, which are usually hetero-oligomeric [55]. In addition, an in vitro study showed that Mip1 is a highly processive DNA polymerase with strong strand-displacement activity [54]. As discussed below, this polymerase activity is required in certain DNA repair pathways and in the Okazaki fragment processing pathway in the nucleus. The catalytic subunit of polymerase γ is phylogenetically related to the prokaryotic PolA family, and its amino acid sequence has been well conserved throughout the evolution of eukaryotic cells. The N-terminal part of the protein contains two domains endowed with 5′-dRP (5′-deoxyribose phosphate) lyase and 3′→5′ exonuclease proofreading activities [55]. The former activity is important for elimination of the dRP moiety necessary for single-nucleotide gap-filling during base excision repair (see below), whereas the latter is crucial for ensuring the fidelity of mtDNA replication by polymerase γ. The above-mentioned *mip*1-D347A mutation, which causes a strong defect in the 3′→5′ exonuclease activity of the mitochondrial polymerase, elicits a several hundredfold increase in the frequency of spontaneous mutations, leading to erythromycin resistance and a severalfold increase in the incidence of *rho*$^-$ *petite* mutants [18].

The *MIP*1 gene in budding yeast has been used as a model to study pathogenic mutations in *POLG* (also called *POLG1;* the gene encoding the catalytic subunit of human polymerase γ) associated with several mitochondrial diseases found in patients suffering from aggressive Alpers syndrome to mild progressive external ophthalmoplegia [56,57]. These studies have determined DNA replication defects that are conferred within 30 mutations and strongly support a model in which these mutations contribute to the manifestation of disease. Moreover, Baruffini et al. [58] showed that the increase in *petite* frequency in a subset of these Mip1-deficient mutants can be suppressed by an increase in the mitochondrial dNTP pool or exposure to the antioxidant dihydrolipoic acid, which presumably decreases reactive oxygen species (ROS). Mutator mice, which express an exonuclease-deficient mtDNA polymerase γ, age prematurely owing to the accumulation of point mutations and mtDNA chromosomal breakage [59,60]. Surprisingly, impaired mitochondrial respiratory activity in these mice does not lead to increased oxidative stress but is associated with the activation of apoptosis and the ensuing tissue progenitor dysfunction. This result is consistent with the notion that mitochondrial dysfunction not only correlates with aging but also represents an important causative aging factor.

A very recent report examined disease-associated mutations that affect conserved regions of the Mip1 polymerase [61]. In that study, various *mip*1 mutations were tested in heterozygous strains harboring both a mutant copy of the *MIP*1 gene and the wild-type copy, and the strains were exposed to the exogenous base-alkylating agent, methyl methanesulfonate (MMS). In this experimental setup, mutations that disrupted Mip1 polymerase activity, but not those that inactivated exonuclease activity, caused an increased frequency of point mutations in mtDNA after MMS exposure. First, these results indicate that the exposure to mutagenic compounds may greatly increase mtDNA mutagenesis, indicating the necessity of including environmental factors in the study of the mechanisms responsible for maintaining mtDNA stability. Second, the results imply that polymerase switching events occur during mtDNA replication and that one polymerase can stall replication and be replaced by another polymerase. In this case, stalling by a variant Mip1 polymerase is presumed to produce ssDNA intermediates that tend to accumulate irreparable damage during exposure to the mutagenic agent, leading to misincorporations after the wild-type polymerase resumes replication of the mtDNA region.

With the exception of the major replicative mitochondrial polymerase identity, not much is known with comparable certainty about mtDNA replication in *S. cerevisiae* cells. Maleszka et al. [62] presented convincing evidence that mtDNA is not predominantly circular but rather forms heterogeneous linear tandem (concatameric) arrays of two to seven genomic units, and only a small fraction of the mtDNA assumes a circular form. Furthermore, because the same authors detected circular molecules of mtDNA with either single- or double-stranded tails (lariats) by electron microscopy, they proposed a rolling-circle replication (RCR) mechanism as the model of yeast mtDNA replication. This mode of replication also implies that the synthesis of the leading and lagging strands is uncoupled in mitochondria. Replication of mtDNA in yeast cells appears to be initiated by two alternative, nonexclusive mechanisms: (1) transcription-dependent initiation that is mediated by the mitochondrial RNA polymerase Rpo41 from three or four replication origins [63,64] and (2) recombination-dependent replication (RDR) that is mediated, at least partially, by the mitochondrial recombinase Mhr1 ([65,66], reviewed in [29]). In the first mechanism, the replication origins are transcription sites that are recognized by Rpo41 to initiate the synthesis of transcripts that are then processed to produce primers for replication. Upon inactivation of the *RPO*41 gene, yeast cells are not able to maintain *rho*$^+$ mitochondrial genomes, but *rho*$^-$ genomes, even those containing mostly repeated *ori* sequences, are still replicated [67,68]. Because the deficiency of mitochondrial protein synthesis leads to mitochondrial genome instability and the accumulation of cells with mitochondria carrying *rho*$^-$ genomes [69], whether Rpo41 is required for the replication of *rho*$^+$ mtDNA remains unclear. The second mechanism was proposed to explain the finding that the major species of mtDNA in mother cells is concatamers, whereas those in buds are circular monomers [65]. The Mhr1 protein is required for the transmission of nascent concatameric mtDNA into the buds. The protein was shown in vitro to catalyze a recombinase reaction of ssDNA pairing with homologous dsDNA to form heteroduplex joints, which are intermediates of HR. However, Mhr1 is not structurally related to the RecA family of recombinases, and the pairing reaction mediated by Mhr1 is ATP-independent and topologically different from that catalyzed by RecA [70]. In the model proposed by Ling and Shibata [65], Mhr1-mediated pairing generates a primer for mtDNA RCR that leads to the formation of concatameric DNA. This model was supported by several subsequent studies reviewed by Lipinski et al. [29], the most notable of which was a study indicating that ROS exposure regulates mtDNA copy number by promoting Mhr1-dependent initiation of RCR at the ROS-generated DSB ends [71]. Nevertheless, Mhr1 does not appear to be the only mitochondrial recombinase [72]. A similar conclusion was also proposed in a recent study on the genome-wide recombination mapping of the mitochondrial genome in *S. cerevisiae* using high-throughput sequencing [73]. In addition, the severe unconditional phenotype of the *MHR*1 deletion due to the loss of *rho*$^+$ genomes [43] is inconsistent with the mild phenotype of the *mhr*1-1 mutant (temperature-dependent loss of *rho*$^+$ mtDNA). This allele encodes a mutated Mhr1 protein, which is inactive in the pairing reaction in vitro, in contrast to wild-type Mhr1 [65]. Therefore, Mhr1 may

play another, not-yet-identified but indispensable, role in the maintenance of *rho*$^+$ mtDNA. Significant support for the RDR mechanisms of mtDNA replication came from a recent study utilizing two-dimensional agarose gel electrophoresis to analyze replication intermediates formed by mtDNA isolated from another yeast species, *Candida albicans* [74]. The authors provided clear evidence that HR, not transcription, predominantly initiates mtDNA replication in this species. It is thought possible that budding yeast cells use a mixed mode of replication initiation that is partly transcription driven and partly recombination driven.

We have even less clear physical evidence about how lagging-strand synthesis proceeds in yeast mitochondria. Surprisingly, nearly the entire Okazaki fragment processing apparatus appears to be present in yeast mitochondria, including the largest subunit of polymerase α, Pol1, with its accessory subunit Pol12 [75]. Whether these proteins are involved in lagging-strand synthesis or play other roles in mitochondria (see below) remains unknown. The Pif1 helicase (with the activity of a 5′→3′ DNA helicase) localizes to both the nucleus and the mitochondria [76,77]. The Rad27 flap endonuclease (the human FEN1 ortholog) is also a dual-localization protein [78,79]. Finally, the essential Dna2 nuclease/helicase presumably localizes to yeast mitochondria [80], although its mitochondrial function, which is overshadowed by its essential nuclear function, has not been characterized in yeast cells. The activities of all three of these proteins have been characterized in detail, and all steps of the Okazaki fragment processing pathway in which they participate in the yeast nucleus were reconstituted in vitro using purified proteins and model substrates [81,82]. The dual localization of these three proteins is conserved across a diverse range of eukaryotic cells, from budding yeast to human cells [83–85], suggesting that these proteins play one or more fundamental roles in mtDNA metabolism.

The evidence supporting the role of Dna2 in mtDNA stability is particularly fascinating. Dna2 is an essential multitasking enzyme with dual-polarity exo/endonuclease and 5′→3′ DNA helicase activity that is involved in Okazaki fragment processing [81], as mentioned above, and DSB repair [86]. First, owing to its enzymatic versatility, Dna2 may participate in mtDNA replication as a replicative helicase together with the Pif1 and Hmi1 helicases, which are thought to play this role in yeast mitochondria [54]. Second, Dna2 may also participate in lagging-strand synthesis in a manner similar to the mechanism of reconstituted Okazaki fragment processing. Third, Dna2 may provide the 5′-strand resection activity necessary for homologous replication, including RDR initiation. Finally, the enzyme may play a role in DNA repair pathways (see below). Interestingly, Dna2 contains a conserved ISC domain that spans the nuclease active site in the N-terminal part of the protein. Mutations that alter the cysteine residues within the domain involved in metal coordination reduce the nuclease activity and ATPase activity of Dna2, suggesting that the nuclease and helicase activities are coupled [87]. A metal cluster-defective enzyme can have substantial nuclease activity for ssDNA substrates, but lacks the ability to act on other DNA configurations, indicating that both metal-containing and metal-free enzyme variants play distinct roles in vivo. Accordingly, Dna2 activity may be regulated by its association with ISC, although the prosthetic group is required for the essential function of Dna2.

The Pif1 helicase has been implicated in several mtDNA transactions, from replication through recombination [reviewed in 14] to repair [19,88,89]. The most significant new finding for Pif1 helicase is its involvement in the replication of hard-to-replicate secondary DNA structures, particularly G-quadruplets (G4 structures; [90,91]). G4 DNA structures are stable four-stranded secondary structures held together by non-Watson–Crick G–G base pairs; despite their conservation and functional significance, these structures appear to slow replication and introduce breaks in Pif1-deficient cells [92]. The Pif1 helicase, which exhibits potent G4 structure unwinding activity in vitro, was suggested to be the accessory replicative helicase that resolves G4 structures to prevent fork stalling and DNA breakage during replication. This activity of Pif1 is likely to be very important for the maintenance of mtDNA because there is a 10-fold higher concentration of G4 motifs in AT-rich yeast mitochondrial DNA than in nuclear DNA [90]. This conclusion is consistent with the results of the 2D gel analysis by Cheng et al. [89], who studied mtDNA replication intermediates in *pif*1-null cells, showing that in cells lacking Pif1 mtDNA breaks at specific sites even if unchallenged with genotoxic conditions.

Other mitochondrial DNA polymerases

The set of mitochondrial polymerases in yeast cells is not restricted merely to the replicative polymerases, polymerase γ and polymerase α, but also includes translesion synthesis (TLS) polymerases. TLS polymerases usually replace stalled replicative DNA polymerases at damaged template strands to bypass a variety of lesions [93]. Zhang et al. [94] presented the first report to demonstrate that DNA polymerase ζ (Rev3/Rev7) and Rev1 function in the mitochondria of *S. cerevisiae* in separate pathways. Polymerase ζ is a B-family DNA polymerase that participates in DNA translesion synthesis, which is responsible for both spontaneous and damage-induced mutagenesis in all eukaryotic cells [95]. Its activity tends to generate mutations because it lacks 3′→5′ exonucleolytic proofreading activity and extends mismatched primer-template termini [96]. The *REV*1 gene encodes a deoxycytidyl transferase that preferentially incorporates deoxycytidine opposite an abasic site [97]. Rev1 also interacts with Rev3 in vitro, and this interaction strongly stimulates the activity of polymerase ζ and contributes to its targeting to the replication fork stalled at a DNA lesion [98]. Thus, the expectation was that mutants lacking genes coding for these TLS polymerases would exhibit a mitochondrial anti-mutator phenotype. A study by Kalifa and Sia [99] analyzed mitochondrial genome mutagenesis in strains with individual deletions of *REV*3, *REV*7, and *REV*1 using the mitochondrial reporters described above. Although polymerase ζ and Rev1 are responsible for the majority of spontaneous and UV-induced frameshift mutations associated with mitochondrial microsatellite sequences, mutant strains lacking polymerase ζ, but not those lacking Rev1, showed, contrary to expectations, an increase in spontaneous mitochondrial point mutagenesis as measured by the frequency of erythromycin-resistant mutants. In addition, the loss of all three proteins led to significantly decreased levels of UV-induced *petite* mutants while simultaneously increasing the levels of point mutations in UV-surviving cells. Therefore, in contrast to TLS repair in the nucleus, polymerase ζ and Rev1 appear to function in separate, largely independent pathways in yeast mitochondria. Second, the damage tolerance pathway (or pathways) in yeast mitochondria is more mutagenic than those of polymerase ζ and Rev1 and is responsible for generating mutations in mtDNA in the absence of TLS polymerases. In several reported cases, mitochondrial base excision repair (BER) pathways appear to contribute to mitochondrial mutagenesis. Whether the increased mitochondrial mutagenesis observed to be associated with *rev*3Δ, *rev*7Δ, and *rev*1Δ depends on factors that are known to function in mitochondrial BER remains unclear.

In light of a recent report on the actual four-subunit structure of nuclear TLS-proficient polymerase ζ as an ISC-dependent complex with Pol31 and Pol32 [100], both polymerase ζ and Rev1 may form mitochondria-specific complexes that exhibit distinct properties compared with those of the Rev3/Rev7- and Rev1-containing complexes that function in the nucleus. Whether Pol31 and Pol32 or Pol30/proliferating cell nuclear antigen (PCNA) are present in

mitochondria remains a matter of debate and should be experimentally verified. Mitochondrially localized Pol30 in yeast cells has been reported previously [101], but in classical Western blot analysis of mitochondrial proteins, anti-PCNA antibodies are used as a control for the nucleocytosolic contamination of mitochondrial extracts (e.g., [102]). Consequently, there is no agreement about the presence of Pol30 in mitochondria. Adding another twist to the story about mitochondrial TLS polymerases, Baruffini et al. [103] showed that *REV*3 overexpression reduced the level of *petite* mutants accumulating in cultures of strains that bear a subclass of pathological mutations in the *MIP*1 gene. This effect was dependent on Rev7 but not Rev1. In addition, the overexpression of both *REV*3 and *REV*1, but not of each individual gene, resulted in a significant reduction in the frequency of erythromycin-resistant mutants in several *mip*1 strains, indicating the complexity of the pathways involved in maintaining mtDNA stability. Notably, the current evidence indicates that the human orthologs of Rev1, Rev3, and Rev7 do not localize to mitochondria in human cells [94].

Finally, the first report on the mitochondrial localization and function of another TLS polymerase in yeast cells has been published [104]. Polymerase η can accurately and efficiently bypass UV-induced cyclobutane thymine dimers in both yeast and human cells [105,106]. In the study by Chatterjee et al. [104], polymerase η (Rad30) localized to yeast mitochondria and, together with polymerase ζ, prevented the accumulation of UV-induced point mutations. However, mitochondrial Rad30 also prevents the UV-induced instability of rho^+ genomes responsible for increased *petite* mutant generation upon UV exposure, thereby opposing the activity of polymerase ζ (see above). Thus, Rad30 appears to act in at least two mtDNA repair pathways: one pathway reduces UV-induced base substitutions, presumably with polymerase ζ in two-step TLS, as has been proposed for other TLS polymerases [107], and the other pathway counteracts UV-induced rearrangements of mtDNA (that lead to *petite* formation) by an unknown mechanism. Whether polymerase η functions in the mitochondria of mammalian cells is unknown.

Mitochondrial base excision repair (mtBER)

Short-patch BER is mediated by monofunctional and bifunctional glycosylases

A prevalent form of DNA lesions, in both the nuclear and the mitochondrial DNA of all aerobic organisms, is damage to bases caused by endogenously produced metabolic by-products, including ROS, or by exogenous genotoxic agents. If they do not distort the DNA helix, these lesions are primarily repaired by BER [108,109]. BER is thought to be the simplest and best-characterized repair pathway; however, recent advances in the understanding of the association between BER and single-strand break repair, which processes various termini that cannot be ligated, challenge this view [110]. The mtBER pathway operates as one of the main repair mechanisms in mitochondria of all eukaryotic organisms, although in mammalian cells, the mtBER system seems to be more complex and versatile than that in *S. cerevisiae* cells (Table 1). The repair of mtDNA in mammalian cells was the subject of excellent reviews by Kazak et al. [117] and Sykora et al. [118].

The basic BER pathway comprises four steps: (1) base lesion recognition and excision by a DNA glycosylase followed by cleavage of the resulting abasic site, (2) DNA end-processing, (3) gap-filling, and (4) ligation. Nonbulky base modifications following oxidation, deamination, and alkylation are recognized by specialized DNA glycosylases. The proteins belong to a large family of enzymes that cleave the N-glycosylic bond linking a specific type of modified DNA base to deoxyribose within the sugar–phosphate backbone of DNA. The DNA glycosylases (DGs) are categorized into two groups: monofunctional and bifunctional. The monofunctional DG cleaves the N-glycosylic bond, leaving an intact abasic (apurinic/apyrimidinic; AP) site in DNA and the free base as the product. The bifunctional DG cleaves the N-glycosylic bond and the phosphodiester backbone via AP lyase activity (see below).

In yeast mitochondria, the one monofunctional DG, Ung1, removes uracil generated by cytosine deamination (removal of U:G mispairs) or misincorporated dUMP [125,126]. Similar to almost all mtBER enzymes in yeast, with the exception of mitochondrial polymerase, the Ung1 glycosylase localizes to both the nucleus and the mitochondria. The inactivation of the *UNG*1 gene leads only to a modestly increased frequency of nonrespiring mutants (the authors of the cited study state that the mutants maintain intact mitochondrial genomes) and a virtually unchanged accumulation of point mutations in mtDNA, conferring resistance to erythromycin compared to the wild-type strain. Interestingly, the overexpression of *UNG*1 increased the frequency of mutations in the mtDNA, suggesting that the elevated level of the Ung1 glycosylase is mutagenic [126]. Monofunctional glycosylases cleave only N-glycosylic bonds, at which point Apn1, the AP endonuclease in *S. cerevisiae* mitochondria [127], cuts the phosphodiester backbone to generate a strand break with 3′-OH and 5′-dRP ends [reviewed in [120]]. The repair can then be completed by either short-patch (SP)/single-nucleotide or long-patch (LP) BER. The distinction between these two alternative pathways is the size of the patch being repaired: one nucleotide in the case of SP BER, as described above, and two or more nucleotides in the case of LP BER [reviewed in [110]]. First, we describe the former pathway.

Upon AP endonuclease incision of an AP site, 3′-OH can serve as an appropriate substrate for DNA synthesis or ligation, but the 5′-dRP end blocks ligation and must be processed further to generate a 5′-phosphate. The conserved catalytic subunits of polymerase γ from various organisms have 5′-dRP lyase activity [55]. The 5′-dRP lyase converts 5′-dRP to a ligatable 5′-phosphate. Two bifunctional glycosylases have been identified in *S. cerevisiae* mitochondria: Ogg1 excises an abundant oxidative lesion in DNA, 7,8-dihydro-8-oxo-2′-deoxyguanine (8-oxoG), opposite to cytosine [128], and Ntg1, which is a homolog of *Escherichia coli* endonuclease III, cleaves oxidized pyrimidines [129]. These proteins have both glycosylase and AP lyase activities. Thus, upon removal of the damaged base, Ogg1 and Ntg1 cut the DNA backbone 3′ to the AP site and generate single-nucleotide gapped DNA with 3′-phospho-α,β-unsaturated aldehyde (3′-PUA) and 5′-phosphate groups at the ends through a β-elimination mechanism. In this scenario, 3′-PUA blocks DNA synthesis and must be processed by the 3′-phosphodiesterase activity of Apn1 before DNA polymerase can resume synthesis. In the case of repair initiated by a monofunctional DG, the 5′-dRP lyase successfully converts the 5′-blocked group to 5′-phosphate, or in the case of repair initiated by a bifunctional DG, the 3′-phosphodiesterase activity of the AP endonuclease converts 3′-PUA to 3′-OH. In both subpathways of SP BER, the latter group can be used as a substrate by a DNA polymerase to fill the single-nucleotide gap generated by base excision and cleavage of the phosphate–sugar backbone. In yeast mitochondria, the gap-filling polymerase is probably polymerase γ, Mip1; however, as mentioned above, more polymerases (e.g., Rev1 and polymerases α, η, ζ) are present than just the main mitochondrial replicative polymerase. Whether these polymerases compete with Mip1 to fill gaps during BER remains to be determined. Finally, in yeast mitochondria, the essential nuclear–mitochondrial ATP-dependent ligase Cdc9 links the 5′ and 3′ ends to restore a continuous DNA strand [130].

In *S. cerevisiae,* deletions of the genes encoding the two bifunctional glycosylases, Ogg1 and Ntg1, on their own do not lead to strong spontaneous mitochondrial mutator phenotypes. Inactivation of the *OGG*1 gene was reported several times in the context of the effect on mitochondrial genome stability. The mitochondrial phenotype exhibited by the *ogg*1Δ deletion strains appears to depend on some unknown features of the genetic background. Singh et al. [131] reported that a deletion of *OGG*1 in their laboratory strain led to a

Table 1
Comparison of the proteins responsible for the maintenance and stability of the mitochondrial genome across phyla.

	Saccharomyces cerevisiae	*Candida albicans*	*Homo sapiens*	Kegg ID and description (for human proteins)	Associated diseases (number of associated diseases)
Mitochondrial replicative proteins					
Pol γ	Mip1	Mip1	POLG1	Polymerase (DNA directed), γ (EC 2.7.7.7)	(23) e.g., chronic progressive external ophthalmoplegia (PEO), ataxia, Alpers syndrome, Parkinson disease
Pol γ-associated subunit			POLG2	Polymerase (DNA directed), γ2, accessory subunit (EC 2.7.7.7)	(6) e.g., PEO with mtDNA deletions autosomal dominant, Alpers syndrome
DNA helicase Twinkle			TWINKLE	Chromosome 10 open reading frame 2 (EC 3.6.4.12)	(11) e.g., PEO autosomal dominant, spinocerebellar ataxia, infantile onset spinocerebellar ataxia
ssDNA-binding protein	Rim1	Rim1	SSBP1	Single-stranded DNA binding protein 1, mitochondrial	
Main nucleoid protein	Abf2	Gcf1	TFAM	Transcription factor A, mitochondrial	
BER enzyme	Ogg1	Ogg1	OGG1	8-Oxoguanine DNA glycosylase (EC 4.2.99.18)	(55) e.g., renal cell carcinoma (somatic), Cockayne syndrome, familial adenomatous polyposis, cholangiocarcinoma, lung cancer
BER enzyme	Ntg1	–	NTHL1[a]	nth endonuclease III-like 1 (*Escherichia coli*) (EC 4.2.99.18)	(5) e.g., primary sclerosing cholangitis, sclerosing cholangitis
BER enzyme	Apn1	Apn1	APEX1[a]	APEX nuclease (multifunctional DNA repair enzyme) 1 (EC 4.2.99.18)	(15) e.g., amyotrophic lateral sclerosis, breast cancer, cystic fibrosis, respiratory failure, attenuated familial adenomatous polyposis
BER enzyme	Ung1	Ung1	UNG	Uracil-DNA glycosylase (EC 3.2.2.27)	(12) e.g., immunodeficiency with hyper Igm type 5, colon adenocarcinoma, colorectal cancer, Bloom syndrome, rubella, congenital rubella
BER enzyme	–	–	MUTYH	mutY homolog (*E. coli*) (EC 3.2.2.–)	(63) e.g., multiple colorectal adenomas, MUTYH-associated polyposis, familial adenomatous polyposis, gastric cancer (somatic), familial colorectal cancer
BER enzyme	–	–	NEIL1	nei endonuclease VIII-like 1 (homolog of *E. coli* NEI) (EC 4.2.99.18)	(15) e.g., primary sclerosing cholangitis, sclerosing cholangitis, familial colorectal cancer, steroid-resistant nephrotic syndrome
BER enzyme	–	–	NEIL2	nei endonuclease VIII-like 2 (homolog of *E. coli* NEI) (EC 4.2.99.18)	
Long-patch BER	Rad27	Rad27	FEN1	Flap structure-specific endonuclease 1 (EC 3.1.–.–)	(11) e.g., Werner syndrome, vitelliform macular dystrophy, macular dystrophy
Nuclease	Nuc1	Nuc1	EXOG (LP BER)	Endo/exonuclease (5′–3′), endonuclease G-like (EC 3.1.30.–)	
Nuclease	Nuc1	Nuc1	ENDOG (mtDNA degradation?)	Endonuclease G (EC 3.1.30.-)	(8) e.g., osteosarcoma, mitochondrial disorders
LP BER, Okazaki FP	Dna2	Dna2	DNA2	DNA replication helicase/nuclease 2 homolog (yeast) (EC 3.6.4.12)	(5) e.g., PEO with mtDNA deletions autosomal dominant, Seckel syndrome type 8
Okazaki FP, other role in replication?	Pif1	orf19.6133	PIF1	PIF1 5′–3′ DNA helicase homolog (*S. cerevisiae*) (EC 3.6.4.12)	
SSB repair	Rad28[b]	orf19.268	ERCC8	Excision repair cross-complementing rodent repair deficiency, complementation group 8 (Cockayne syndrome A)	(8) e.g., Cockayne syndrome, UV-sensitive syndrome 2, xeroderma pigmentosum, group C
SSB repair	Rad26[b]	Rad26	ERCC6	Excision repair cross-complementing rodent repair deficiency, complementation group 6 (Cockayne syndrome B)	(21) e.g., Cockayne syndrome type II, cerebro-oculofacioskeletal syndrome, xeroderma pigmentosum, UV-sensitive syndrome 1, age-related macular degeneration, lung cancer
SSB repair	Hnt3[b]	Hnt3	APTX	Aprataxin (EC 3.–.–.–)	(12) e.g., APTX-related coenzyme Q10 deficiency, early-onset ataxia with oculomotor apraxia and hypoalbuminemia, ataxia, apraxia, Friedreich ataxia
SSB repair	Tdp1[b]	–	TDP1	Tyrosyl-DNA phosphodiesterase 1 (EC 3.1.4)	(10) e.g., spinocerebellar ataxia with axonal neuropathy, autosomal recessive, Ollier disease, spinocerebellar degeneration
Mismatch repair	Msh1	Msh1	Not present; MSH5[c]	mutS homolog 5 (*E. coli*)	(5) e.g., premature ovarian failure, common variable immunodeficiency
Mismatch repair: recognition of single-base mismatches and small insertion/deletion loops	Msh1		YBX1[a]	Y box binding protein 1	
Transcription/replication pausing	?	?	mTERF1–3[d]	Mitochondrial transcription termination factors	mTERF1 (1), e.g., Melas syndrome
DSB repair	Mre11	Mre11	MRE11	MRE11 meiotic recombination 11 homolog A (*S. cerevisiae*)	(13) e.g., ataxia telangiectasia-like syndrome
DSB repair	Yku70	Yku70/ Hdf1	XRCC6	X-ray repair complementing defective repair in Chinese hamster cells 6	(4) e.g., systemic lupus erythematosus
DSB repair	Yku80[b]	Yku80/ orf19.2383	XRCC5	X-ray repair complementing defective repair in Chinese hamster cells 5 (DSB rejoining)	(11) e.g., tinea capitis
DSB repair	Rad51[b]	Rad51	RAD51	RAD51 recombinase	(58) e.g., breast cancer, Bloom syndrome, mirror movements

All of the information listed is available in [111–116]. The reader is referred to these sources, as well as the references therein, for further details. More reference information on the human proteins can be found in [117,118]. The names of human proteins follow the HUGO Gene Nomenclature Committee guidelines. There are no data on the mitochondrial localization of the listed *C. albicans* proteins, with the exception of Gcf1 [124].

[a] A functional equivalent rather than a strict homolog of the *S. cerevisiae* protein (NTHL1 reviewed in [119], APEX1 reviewed in [119,120], YBX1 reviewed in [121]).
[b] Mitochondrial localization was not shown or is not obvious.
[c] A homolog of the *S. cerevisiae,* but it is unknown whether it is a functional equivalent [122].
[d] There are no homologs in fungi; present in metazoans and plants [123].

2-fold increase in the generation of spontaneous *rho*$^-$ *petite* mutants compared with the wild-type strain, but increased point mutagenesis of mtDNA measured by the frequency of erythromycin-resistant mutants was not observed. Thus, in that genetic background, the absence of Ogg1 in mitochondria results in an increased incidence of mitochondrial genome rearrangements but no difference in point mutations of the G→T transversion type that are typically induced by unrepaired 8-oxoG lesions [128]. However, Dzierzbicki et al. [20] showed in another strain that the inactivation of *OGG*1 leads to a 10-fold increase in mitochondrial point mutations that confer resistance to oligomycin; however, consistent with the previous report, they did not observe an increased incidence of erythromycin-resistant mutants in the *ogg*1Δ strain cultures. The group also determined that G→T transversions in the mitochondrial target gene *OLI*1 were 10 times more frequent in *ogg*1Δ cells than in wild-type cells. In the third study [21], Pogorzala et al. inactivated the *OGG*1 gene in yet another strain. In that strain, the deletion of *OGG*1 caused a significant severalfold increase in the frequency of mitochondrial erythromycin-resistance point mutations. Interestingly, despite the differences in all three studies, mitochondrial *ogg*1Δ-specific phenotypes were suppressed by the concomitant deletion of the *NTG*1 gene, suggesting that the two glycosylases, which have diverse substrate specificities (see above), compete for the same substrate. Notably, Sandigursky et al. [132] reported that purified yeast Ogg1 efficiently removed sugar–phosphate residues at incised 3′-AP sites during the AP lyase reaction (i.e., the enzyme has deoxyribophosphodiesterase activity that removes the 3′ unsaturated aldehydic group, described as 3′-PUA above, leaving a 3′-OH). Therefore, the Ogg1 enzyme may be endowed with three activities that allow it to efficiently process its cognate lesion and leave a one-nucleotide gap that can readily be filled by a DNA polymerase without the need for any AP endonuclease activity. Consequently, if Ogg1 cleaves the modified base, the repair is accomplished efficiently by the enzyme to replace the damaged base with the correct one. However, when Ntg1 excises the base instead of Ogg1, a mutagenic product, which is probably the blocking end 3′-PUA, is generated after the AP lyase reaction. As for the identity of the modified base recognized by both Ogg1 and Ntg1, yeast Ogg1 is thought to be an 8-oxo-guanine DNA glycosylase, but it also excises 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-G [128]), which, together with Fapy-A (4,6-diamino-5-formamidopyrimidine), is one of the major lesions generated in DNA by hydroxyl radical attack and UV radiation [133]. Ntg1 has also been shown to excise Fapy-G (but not 8-oxoG opposite cytosine) along with Fapy-A and a number of modified pyrimidines [129]. The true biological effects of both Fapy-A and Fapy-G have been recognized only recently [133]. Both lesions lead to misincorporations and do not block DNA chain elongation, as was believed for a long time. Polymerases misincorporate adenine paired with Fapy-G, resulting in G→T transversions. Therefore, this change is the same type of mutation as that caused by 8-oxoG [128]. Thus, the modified lesion recognized by both Ogg1 and Ntg1 is probably Fapy-G, but its excision by Ntg1 produces a promutagenic intermediate that may produce either point mutations or genome rearrangements, depending on the genetic background. Because the most likely promutagenic intermediate produced by Ntg1 is the 3′-blocked end produced in the AP lyase reaction, Ntg1 may share a pathway with Apn1, the downstream component of the SP BER pathway, in this promutagenic processing of Fapy-G. This argument is supported by the observation that the inactivation of either *NTG*1 or *APN*1 suppresses the increase in point mutagenesis in the *ogg*1Δ strain [21]. Nevertheless, these results are difficult to generalize because both the phenotypes and the interactions among deletions of the three genes and other genes engaged in mtDNA repair seem to be largely genetic context-dependent [21,36]. Therefore, in the discussion below, we stress only the consistent evidence.

Several other studies have implicated Ntg1 glycosylase in the production of promutagenic or recombinogenic intermediates. Upon hydrogen peroxide treatment of isolated mitochondria, Ntg1 was reported to be responsible for generating DSBs (in an enzymatically unclear reaction) in the mitochondrial origin of replication *ori5*, which initiates Mhr1-dependent recombination and leads to an increased mtDNA copy number in response to oxidative stress [71,134]. Consistently, Schroeder and Shadel [135] recently showed that *ntg*1Δ strains exhibit a decreased mtDNA copy number in comparison to that of the reference strains, although the *ntg*1Δ strains do not exhibit a respiration defect. Phadnis et al. [136] reported that the loss of Ntg1 suppressed mitochondrial point mutations, frameshift mutations measured by mitochondrial microsatellite instability, and deletions in mtDNA between direct repeats, supporting the conclusion that the products of the broad-specificity Ntg1-mediated AP lyase activity may contribute to mtDNA instability. The overexpression of *APN*1 in the wild-type strain led to decreased point mutagenesis in the mtDNA, suggesting that downstream BER reactions are rate-limiting for the efficient completion of lesion processing by mitochondrial BER pathways under these conditions. In contrast, the overproduction of Apn1 in an *ntg*1Δ strain significantly increased the level of mitochondrial point mutations, indicating that Apn1 also generates mutagenic intermediates in the absence of Ntg1 glycosylase. In *ntg*1Δ cells, the products that are generated in wild-type cells by Ntg1-mediated AP lyase reactions do not accumulate, and the Apn1 endonuclease is diverted to other potential sites of action. The enzyme is endowed with a variety of activities associated with processing strand breaks involving blocked 3′ termini [reviewed in 120]. The enzyme can also directly cut the DNA strand upstream of the primary lesion (independent of *N*-glycosylases) in a process termed nucleotide incision repair that leaves the damaged base on a 5′-flap that may be processed further by LP BER (see below). Consistent with the reports by Phadnis et al. [136] and Pogorzala et al. [21], another study of mitochondrial microsatellite instability in yeast cells showed that Apn1 overproduction causes increased poly (GT) microsatellite instability in mtDNA, probably by converting AP sites and oxidized bases into more toxic strand breaks. This effect was counteracted by Ogg1 overexpression [127]. According to other reports, the loss of the *APN*1 gene leads to only a weak increase, at most, in the level of spontaneous point mutations [36,137], but a significant increase is observed in the number of mitochondrial mutations induced upon exposure to the alkylating agent methyl methanesulfonate [137,138]. These results indicate that Apn1 in mitochondria is involved in the repair of alkylation-induced lesions in the mtDNA, as in the nucleus. Other factors that interact with Apn1 during MMS-induced lesion repair in yeast mitochondria remain unknown.

With regard to short-patch mtBER in yeast cells, it may seem puzzling that yeast strains lacking mtBER enzymes that were conservatively maintained in the mitochondrial compartment during the evolution of eukaryotic cells do not exhibit any strong mitochondrial phenotypes indicating the significant disruption of mtDNA stability. On the contrary, under certain circumstances, the partial absence of BER enzymes seems to stabilize mtDNA. However, the characterization of yeast mtBER was restricted mostly to studying various single haploid deletion mutants under standard laboratory conditions of relative nutrient abundance and comfort, in other words, under conditions that are entirely artificial for yeast cells. For the most part, natural yeast habitats are harsher than laboratory conditions, and yeast cells are frequently exposed to various chemical or physical mutagenic factors that may damage mtDNA. Additionally, *S. cerevisiae* cells are usually diploid, which may be relevant for mtDNA stability [31]. Are the artificial laboratory conditions of our experiments with yeast the reason for the apparent paucity of mitochondrial phenotypes in mtBER mutants? This question cannot be answered until it can be experimentally

tested. The above-cited studies on MMS-induced mtDNA mutagenesis [61,137,138] and oxidative damage in mtDNA [88] show that using an appropriate deleterious agent to increase the level of specific DNA damage can reveal the functions of various mtDNA repair pathways that are usually weakly detectable. There is another reason that can explain why so many deletions of genes encoding mitochondrial proteins are not associated with discernible phenotypes. The redundancy of biochemical pathways contributes substantially to the robustness of cellular systems. Although several reports studied mutations in two separate mtDNA repair pathways, the redundancies of these mitochondrial processes remain largely unexplored. For example, the lack of Ntg1 glycosylase combined with a *pif*1-null allele or *abf*2-null allele led to a significantly increased frequency of *petite* mutations in comparison to either single mutant [19]. The mechanism for this process remains unknown, although it is presumably associated with the role of Ntg1 in the regulation of mtDNA copy number [135], as mentioned above. These results would imply that the Pif1 helicase and Abf2 protein function in one or more pathways independent of an Ntg1-dependent pathway involved in the maintenance of *rho*$^{+}$ mtDNA and the stability of its sequence. This example clearly shows that a mitochondrial DNA repair pathway can be fully understood only in the context of other related pathways.

Long-patch BER

The BER pathway described above falls into the category of short-patch/single-nucleotide BER, but long-patch BER is an alternative pathway. This repair pathway is required if the 5′ end generated in the first steps of BER (i.e., base excision and AP lyase-mediated strand cleavage of the resulting AP site), usually 5′-dRP, cannot be removed by the polymerase, e.g., the end is further oxidized. In this case, the 5′-blocking group could be removed by the 5′-flap endonuclease 1 (Rad27 in yeast cells; FEN-1 in mammalian cells), which is also known to be involved in removing 5′-RNA primers from Okazaki fragments during DNA replication. However, before the flap endonuclease can act, the 5′-blocking group has to be converted into a 5′-flap by strand-displacement synthesis performed by one of the DNA polymerases, mainly yeast polymerase γ [54]. In the nuclei of mammalian cells, the LP BER steps other than flap cleavage are identical to those of DNA replication, involving DNA polymerases δ/ε and nuclear DNA ligase I. These enzymes, including FEN-1, are recruited by the sliding clamp PCNA that enhances their activity. Several recent studies reported the identities of three mammalian mitochondrial LP BER enzymes: flap endonuclease FEN-1 [84], a second flap endonuclease DNA2 [139,140], and 5′-exo/endonuclease EXOG [141]. Nevertheless, the precise contribution of these nucleases to mitochondrial LP BER remains controversial. EXOG, an ssDNA nuclease and a paralog of endonuclease G (ENDOG), was implicated in the removal of the 5′-blocking residue and is required for repairing endogenous SSBs in the mitochondrial genome [141]. Interestingly, EXOG depletion induces persistent SSBs in the mtDNA, enhances ROS levels, and induces apoptosis in normal cells but not in cells devoid of mtDNA. This finding suggests that persistent SSBs in the mitochondrial genome alone could trigger apoptotic signaling in mammalian cells. Establishing the precise roles of these enzymes in maintaining the integrity of the mitochondrial genome needs further investigation. In particular, the yeast orthologs of mammalian mitochondrial LP BER proteins require further study because, somewhat paradoxically, we know less about LP BER pathways in yeast mitochondria than in mammalian cells.

The LP BER pathway in the mitochondria of *S. cerevisiae* cells remains largely unknown. The ortholog of DNA2, yeast nuclease/helicase Dna2, was mentioned above. Its role in yeast mitochondria has not been characterized. In yeast cell nuclei, Rad27 plays a role in the cleavage of 5′-DNA flaps created during Okazaki fragment processing, in the prevention of sequence duplications and repeat sequence expansions, in DSB repair by nonhomologous end-joining (NHEJ), and in the processing of intermediates during nuclear LP BER [142–145]. The first report on Rad27 in yeast mitochondria revealed unexpected findings about the mitochondrial function of the endonuclease [78]. Strains lacking Rad27 displayed increased rates of erythromycin-resistance point mutagenesis but conversely exhibited decreased instability of microsatellite repeats and less frequent deletions mediated by direct repeats in mtDNA. These results suggest that the lack of Rad27 in mitochondria leads to the accumulation of point mutations, which may be attributed to the largely abolished mitochondrial LP BER. However, in the absence of Rad27, small-scale (microsatellite alterations) and large-scale genome rearrangements (direct-repeat mediated deletions), which are thought to be mediated mostly by polymerase slippage or template switching, are strongly inhibited. Rad27 activity may favor polymerase slippage/template switch events in mitochondria by producing nicks or small gaps upon 5′-flap removal during mitochondrial LP BER. We noticed that defects in strains lacking Rad27 reproduce the phenotypes of strains lacking *REV*3 on a larger scale (although data on the stability of the DRM(D) reporter in *rev*3Δ strains are not available), suggesting that Rad27 and Rev3 may function in the same pathway in mitochondria. Another interesting experiment would be to verify whether the mitochondrial mutator phenotype of *RAD*27 inactivation depends on the activity of any mitochondrial glycosylase(s) or the AP endonuclease, because LP BER may be required for the removal of 5′-blocked ends generated by BER pathways. A similar scenario has been described in the nucleus [143].

The Nuc1 nuclease, an *S. cerevisiae* ortholog of mammalian ENDOG and EXOG nucleases [146,147], exhibits both endo- and 5′→3′ exonuclease activities [148]. Nuc1 is located in the mitochondria yet translocates into the nuclei of apoptotic cells [146]. Nuc1 is involved in mtDNA recombination as a 5′→3′ exonuclease [149]. A more recent study showed that the generation of oxidative stress-induced rearrangements in mtDNA is markedly reduced in strains lacking the *NUC*1 gene [150]. This observation suggests that the fate of at least certain types of oxidative lesions in mtDNA, presumably strand breaks, is dependent on the activity of the enzyme. Nuc1 can produce recombinogenic DNA ends by introducing single-strand gaps into dsDNA using its endonuclease and 5′→3′ exonuclease activities. Under conditions of acute oxidative stress accompanied by the shortage of intramitochondrial energy, e.g., in cells treated with antimycin A while growing on a respiratory substrate, Nuc1 activity may lead to extensive degradation of damaged mtDNA. Combined with certain nonallelic recombination events that the Msh1p activity fails to restrain (see below), this Nuc1p-mediated processing is likely to be the first step that eventually culminates in the formation of *rho*$^{-}$ *petite* mutants. Whether Dna2, Rad27, and Nuc1 function in yeast mitochondria in the LP BER pathway(s) remains to be established.

Mitochondrial mismatch repair and homologous recombination in mitochondria

Msh1-dependent pathway

The *MSH*1 gene encodes a protein that is homologous to the *E. coli* MutS protein [151]. Strains lacking the gene exhibit a *petite* phenotype due to large-scale mitochondrial genome rearrangements, suggesting that Msh1 plays an important role in the maintenance of mtDNA stability. The Msh1 protein was characterized in vitro as a DNA-binding ATPase that recognizes mismatches [152]. MutS homologs generally act by initiating the correction of mismatches that arise during either replication or recombination

(reviewed in [153]). For the first time, the increased accumulation of point mutations in the mtDNA of mutants defective in Msh1 function was described for an *msh*1Δ/*MSH*1 heterozygous strain [154]. Therefore, the *MSH*1 gene is haploinsufficient, suggesting that the Msh1 level is fine-tuned for optimal mitochondrial genome maintenance. A functional Msh1p is required in yeast mitochondria to prevent the accumulation of point mutations in mtDNA as a crucial factor in the proposed, but not-yet-characterized, pathway of mitochondrial mismatch repair. Furthermore, Msh1p activity suppresses direct repeat-mediated deletions, thereby inhibiting genomic rearrangements that may result in defective mtDNA [32]. In addition, a heterozygous *msh*1Δ/*MSH*1 strain with poly(GT) tracts inserted into mtDNA exhibited increased rates of microsatellite repeat-dependent frameshift mutations in mtDNA [30]. However, high overexpression of the *MSH*1 gene leads to the instability of *rho*$^+$ genomes by an unknown mechanism, and moderate overproduction of Msh1 increases the stability of poly(GT) tracts [155]; this result is consistent with the opposite destabilization effect in the partially Msh1-depleted heterozygous strain (see above, [154]). Moderate *MSH*1 overexpression also suppresses the mitochondrial mutator phenotype of an *ogg*1Δ mutant [20] and the mitochondrial mutator phenotype of a *sod*2Δ mutant [36]. Moreover, the increased dosage of wild-type *MSH*1, but not of mutant alleles coding for deficient variants of the protein, results in enhanced allelic mitochondrial HR [36]. In contrast, transformation of the same *MSH*1-overexpressing plasmid into a strain with mtDNA containing the DRM(D) reporter decreased the frequency of direct repeat-mediated deletions in mtDNA (A. Kaniak-Golik, unpublished results). Consequently, Msh1 promotes allelic HR in mitochondria but inhibits nonallelic DRM(D) events and GT-repeat frameshift rearrangements. We propose that the Msh1 protein scans the mtDNA duplexes generated during either replication or recombination-associated strand invasion or strand annealing for the presence of mismatches and directs the duplexes into either HR combined with mismatch correction or heteroduplex rejection and inhibition of the incipient recombination, depending on the quality of the duplex.

New findings on mitochondrial recombination pathways in yeast cells

The pathways of mitochondrial recombination in yeast are not well understood. Our knowledge on the subject has increased by only a small amount since the review compendium by Contamine and Picard [14] was published. In this section, we review only the newest and most significant findings. A recent review by Chen [156] provides a more general and detailed overview of the current knowledge on mtDNA recombination in various organisms. We should also acknowledge a recent study, mentioned above, by Fritsch et al. [73] that promises to provide fresh insight into the mechanisms of mitochondrial recombination in the near future. This study presents a new procedure for using high-throughput sequencing to monitor mtDNA recombination in diploid yeast cells.

Mre11–Rad50–Xrs2 complex and Ku proteins function in mitochondria. Two major DSB repair pathways have been studied extensively, albeit only in nuclear genomes thus far: homologous recombination and nonhomologous end-joining. HR is initiated when the DSB end is resected by nucleases and helicases to generate 3′-ssDNA overhangs. In the nucleus, the MRX complex (Mre11–Rad50–Xrs2) initiates 5′-end resection, which is further processed by the Exo1 and Dna2–Sgs1 nucleases [86]. The resulting 3′-ssDNA overhangs are used by recombinases to invade homologous duplex DNA for priming subsequent DNA synthesis. Joint molecule intermediates generated during HR can be resolved by diverse mechanisms to produce crossover or noncrossover products [35]. Alternatively, long homologous 3′-ssDNA overhangs produced by the resection of repeated sequences can be annealed with a concomitant deletion of the intervening sequence between repeats, leading to recombination by a single-strand annealing mechanism (see above reference). This mechanism represents one possible source of genome rearrangements. In NHEJ, DSB ends are blocked from 5′-end resection by bound Ku proteins, Yku70 and Yku80, which form a heterodimer. The Ku heterodimer promotes direct ligation of the DSB ends, but NHEJ is error prone and frequently results in small insertions, deletions, and substitutions at the joint site [157]. Both the HR and the NHEJ pathways may lead to genome rearrangements when they rely on nonallelic sequences.

The most significant new finding indicating the involvement of HR and NHEJ in maintaining the stability of mtDNA was reported by Kalifa et al. [158]. The authors showed that the inactivation of crucial DSB repair pathways mediated by the MRX complex and Ku proteins synergistically decreased the rate of DRM(D) deletions in yeast mtDNA. The results suggest that these deletions originate mostly from the processing of DSB lesions in the repeated sequences by two independent pathways: mitochondrial MRX and mitochondrial Ku proteins. Surprisingly, the decrease in DRM deletions in mtDNA in a strain lacking both the MRX and the Ku complexes was associated with a modest, although significant, increase in spontaneous *petite* mutants. These results indicate that the mechanisms of DRM deletions and rearrangements responsible for *petite* formation are mechanistically different, and under these conditions, both pathways, mitochondrial HR and NHEJ, contribute to a certain extent to the maintenance of the *rho*$^+$ genome. However, a current report by Dzierzbicki et al. [150] showed that mutants deficient in MRX function displayed increased susceptibility to oxidative stress-induced rearrangements in mtDNA in antimycin A-treated cells. Under these conditions, the inactivation of *RAD*50 was epistatic to a *YKU*70 deletion. The increased *petite* generation in cultures of antimycin A-treated *rad*50Δ cells correlated with their decreased potential for sustaining mitochondrial allelic HR. Thus, under certain conditions, the mitochondrial Rad50-dependent pathway plays a significant role in the maintenance of *rho*$^+$ mtDNA. Other factors acting in this mtDNA repair pathway remain to be established. Similarly, the components of mitochondrial NHEJ have not been identified.

Mechanisms that generate deletions in mitochondrial DNA have been intensively studied in mammalian cells subjected to oxidative stress during aging and disease (reviewed in [159]). Deletions are usually flanked by direct repeats, suggesting that these repeats may be involved in the formation of deletions. Two mechanisms are considered for the generation of mtDNA deletions: (1) polymerase slippage on repeated sequences or (2) DSB repair between repeats. However, mtDNA deletions have been suggested as being more likely to occur during DSB repair of damaged DNA rather than during replication [159]. The presence of multiple homology-dependent and homology-independent (NHEJ?) pathways underlying DSB repair was proposed to explain the observed patterns of mtDNA deletions [160,161]. Therefore, yeast cells may provide a good model system for studying mitochondrial pathways of DSB repair and the generation of mtDNA deletions.

Mgm101, a Rad52-like protein, functions not only in mitochondria but also in the nucleus. The mitochondrial nucleoid protein Mgm101 has been known for some time to be essential for the maintenance of *rho*$^+$ mtDNA but not *rho*$^-$ genomes [162]. Mgm101-deficient cells were more sensitive to mtDNA damage induced by UV irradiation and were hypersensitive to mtDNA damage induced by γ rays and hydrogen peroxide treatment. Consequently, Mgm101 was proposed to perform an essential function in the repair of oxidatively damaged mtDNA that is crucial to maintaining the mitochondrial genome [163]. Recently, new evidence was published [164], showing that Mgm101 shares conserved motifs with the N-terminal ssDNA-annealing (SSA) domain

of the yeast Rad52 protein. Rad52 is known mostly as a mediator of Rad51 recombinase activity in the nucleus, but the protein also has SSA activity. Consistently, the authors demonstrated that purified Mgm101 promotes SSA reactions in vitro in the presence of the mitochondrial ssDNA-binding protein Rim1. Moreover, Mgm101-deficient cells exhibited decreased rates of repeat-mediated deletions in mtDNA (the DRM(D) reporter). This observation agrees with previous conclusions indicating the predominantly noncrossover character of deletions found in the mitochondrial DRM(D) reporter [33] and correlates with the notion that a mitochondrial SSA pathway participates in repeat-mediated deletion events. The elucidation of mitochondrial Mgm101 function will shed light on the role of recombination-related processes for the maintenance and repair of mtDNA. Surprisingly, a new function of Mgm101 in an unexpected location has recently been reported. The protein was shown to form a complex with the FANCM ortholog Mph1 helicase and MutSα (Msh2–Msh6 complex) in the nucleus and participate in the recombination-dependent repair of interstrand crosslink lesions in nuclear DNA [165]. Does Mgm101 function in an analogous pathway in yeast mitochondria? If so, does Msh1 replace the nuclear MutSα in this mitochondrial Mgm101-dependent process? Which mitochondrial helicase associates with Mgm101 in mitochondria? These and other questions concerning Mgm101 should be addressed soon by researchers studying mtDNA repair pathways in budding yeast.

Degradation of mtDNA in the network of mtDNA maintenance pathways

Because the generation of antimycin A-induced large-scale deletions in mtDNA has been reported to be dependent on the Nuc1 protein [150], one possibility is that under conditions of oxidative stress, Nuc1 may be involved in the selective degradation of damaged copies of mtDNA. Previously, mitochondria were postulated to be capable of removing damaged mtDNA owing to the presence of multiple copies of mtDNA. More specifically, the mtDNA damage-inducible nuclease Din7p, a paralog of Rad27 and other Rad2 family nucleases, was suggested as a good candidate to accomplish this goal [166]. In addition, degradation processes operate on oxidatively damaged mtDNA in mammalian cells [167]. Presumably, the degradation of oxidatively damaged mtDNA, together with mtDNA repair pathways such as mtBER, may represent a component of complex machinery that is responsible for protecting the mitochondrial genome integrity. At present, evidence supports another mechanism of mitochondrial degradation. This process is an autophagy-dependent, mitochondria-specific degradation pathway mediated by Atg32 and Atg11 that has been shown to be important for preventing the accumulation of cytoplasmic *petite* mutants in nitrogen-starved cultures [168]. The authors suggested that under these conditions, the mitophagy pathway simply degrades excess mitochondria to suppress ROS production and mtDNA damage. Future studies should elucidate the connections between the mitochondrial nucleases mentioned above and the mitophagy pathway, if such connections exist.

Mitochondrial influence on the nuclear genome

Functional mitochondria are crucial to maintaining the stability of cellular genetic information. This requirement is not limited to the mitochondrial genome, which encodes organellar rRNA, tRNA, and a handful of proteins including components of the respiratory chain, proteins needed in large amounts intramitochondrially, or proteins whose expression needs tight feedback control. Functional mitochondria are also important for the stability of the nuclear genome. In yeast cells, mitochondrial dysfunction leads to a nuclear mutator phenotype (measured by the frequency of canavanine-resistant colonies), regardless of whether this dysfunction can be attributed to blocking oxidative phosphorylation by antimycin A at mitochondrial complex III, deletions in mtDNA (*rho*$^-$), or the complete loss of mtDNA (*rho*^{0}) [12]. Superficially, it can be assumed that a dysfunctional mitochondrion generates reactive oxygen species, which will cause a severalfold increase in the nuclear mutation level. However, this simple explanation is not applicable here. Although antimycin A treatment leads to increased intracellular ROS levels, the intracellular levels of ROS are decreased in *rho*$^-$ and *rho*^{0} strains. Moreover, nuclear mutations arising in *rho*^{0} cells depend on the Rev1 protein and DNA polymerase ζ, whereas those resulting from antimycin A treatment do not. In a recent study, Dirick et al. [169] showed that the genomic instability specific to *rho*^{0} diploid cells is due to DNA breaks and mitotic recombination, occurs mostly in nondividing cells, and tends to fluctuate depending on the environmental conditions. Consequently, mitochondrial dysfunction is mutagenic, and multiple pathways are likely to be responsible for the resulting nuclear mutator phenotype. Similar conclusions can be drawn from other studies showing that ROS elevation due to mitochondrial disorder and subsequent oxidative damage causes destabilization of the mitochondrial and/or nuclear genome [170,171]. Other studies have shown that the nuclear DNA instability phenotype is not generated by mitochondrial ROS and instead have implicated such processes as dNTP production [172], iron–sulfur cluster formation [11], metal ion detoxification/homeostasis [173], or apoptosis disturbances [174]. How does the mitochondrion influence nuclear genome stability? The mitochondrion affects stability in a number of ways. After time and according to distinctive kinetics, every malfunction of a mitochondrial process can provoke mitochondrial disorder, which in turn leads to genome instability. In the following sections, this concept will be supported with numerous examples.

When considering mitochondrial dysfunction, it is important to differentiate between a mutation in a single mitochondrial gene encoding a component of the respiratory chain, which is necessary for proper mitochondrial function, and *rho*$^-$ or *rho*^{0} mutations. All of these mutations affect cellular energy production by oxidative phosphorylation, but the former preserves the mitochondrial genome and the production of mitochondrially encoded proteins, whereas the latter disrupt the ability to synthesize those proteins (components of the mitochondrial translation apparatus that are required for the process are also encoded by mtDNA, see Introduction). Respiratory-deficient *rho*$^+$ mutants do not exhibit as robust a nuclear mutator phenotype as that of *rho*^{0} strains [11,169]. Therefore, a mere respiratory deficiency is not sufficient for marked genomic instability. Whether *rho*$^-$ strains exhibit the nuclear mutator phenotype of *rho*^{0} cells or that of respiratory-deficient *rho*$^+$ cells has not been reported. Interestingly, increasing the inner mitochondrial membrane potential ($\Delta\Psi$) stabilizes the nuclear genome of *rho*^{0} cells [11,169], suggesting that a deficiency in an essential cell-viability mitochondrial function is associated with the nuclear instability of *rho*^{0} strains.

Mutations that affect both the mitochondrial and the nuclear genomes

Certain genes are indispensable for maintaining both the mitochondrial and the nuclear genomes. Mutations in these genes may result in mtDNA damage and lead to mitochondrial genome loss and mitochondria dysfunction through a sequence of steps. Because these mutations affect the nuclear genome simultaneously, the extent to which mitochondrial dysfunction contributes to nuclear genome destabilization is difficult to determine. The products of these genes include proteins involved in various pathways of DNA repair, e.g., the catalytic subunit of DNA polymerase ζ Rev3, which is engaged in postreplication repair [94,99,103]; the Mre11–Rad50–

Xrs2 and Yku70/80 complexes, which are responsible for DSB repair via NHEJ [158]; the uracil-DNA glycosylase Ung1, which is responsible for the repair of uracil in DNA formed by spontaneous cytosine deamination [126]; and Ogg1 and Ntg1 glycosylases, as well as the AP-endonuclease Apn1, which are involved in oxidative DNA damage removal via BER [127,136,137].

Mutations that perturb the mitochondrial and nuclear genomes in various ways

The next class of mutations affects genes whose products play distinct roles in mitochondria and the nucleus. For example, a mutation in the *DEF*1 gene (encoding a protein involved in DNA damage-induced degradation of RNA polymerase II) leads to mtDNA loss (*rho*^{0} phenotype), whereas the same mutation influences telomere length in nuclear DNA. The reintroduction of a wild-type copy of the *DEF*1 gene into *def*1Δ mutant cells stimulates telomere elongation but is unable to restore the *rho*$^+$ phenotype. This result shows that the telomere-shortening effect in *def*1Δ cells is not a consequence of mtDNA loss but that both phenotypes are independent [175]. Another example of a dual-function protein is homocitrate synthase (Lys20), a mitochondrial enzyme that catalyzes the first step in lysine biosynthesis. Surprisingly, Lys20 also localizes to the nucleus, where it associates with chromatin and is linked to the key process of DNA damage repair through the histone acetyltransferase Esa1 and through the alternate histone variant H2A.Z to contribute to histone acetylation and phosphorylation of the checkpoint protein Rad53, respectively [176]. The chromatin-linked roles of Lys20 are dependent on its nuclear localization but independent of its catalytic activity. However, Lys20 seems to link cellular metabolism with chromatin function.

Another metabolic link can be found for the fumarase Fum1. This mitochondrial enzyme is involved in the TCA cycle and has an alternative cellular distribution. Upon induction of DNA damage, the cytosolic fumarase fraction is recruited to the nucleus and plays a key role in the protection of cells from DNA damage, especially from DSBs. The DNA damage defense potential of fumarase depends on its enzymatic activity. A lack of Fum1 can be complemented by high concentrations of its product, fumaric acid [177]. The finding that fumaric acid but not malic acid can protect cells from DSBs indicates that the conversion of malic acid to fumaric acid is the function of fumarase in the nucleus and suggests that fumaric acid plays a role in sensing, regulating, and/or stabilizing the cellular response machinery after DNA damage. In agreement with these results, human fumarase was found to be a tumor suppressor gene [178]. Our understanding of how fumarase protect cells against tumors is supported by earlier data showing that fumarase inhibition leads to elevated intracellular fumarate. Fumarate acts as a competitive inhibitor of prolyl hydroxylase (HPH), the enzyme that stabilizes hypoxia-inducible factor (HIF), which is a transcription factor that induces the expression of angiogenesis-regulated genes [179]. In mouse embryo fibroblasts lacking fumarase, fumarate accumulation leads to AMP-activated protein kinase (AMPK) activation, which protects cells against apoptosis [180]. Therefore, these data suggest at least two possible indirect mechanisms of fumarate-dependent tumorigenesis: (1) a mechanism mediated by the regulation of HPH activity and HIF protein levels and (2) a mechanism that relies on the pro-oncogenic role of AMPK.

Mutations that impair mitochondrial functions are important for nuclear genome maintenance

In the following section, a group of mutations is described that affects mitochondria and leads to their functional defect, which in turn destabilizes the nuclear genome. Depending on the primary mutation and mitochondrial process being affected, various mechanisms of nuclear genome instability are possible.

Altered function of the electron transport chain (ETC) can provoke nuclear genome instability

Genomic DNA destabilization may be caused by all ETC mutations in genes that meet the following criteria:

- they encode components of respiratory chain complexes, regardless of whether they are encoded by mitochondrial or nuclear DNA;
- they encode proteins involved in the transport of proteins into mitochondria;
- they are implicated in mitochondrial protein synthesis;
- they are engaged in protein folding, prosthetic group preparation, and mounting or functional complex maturation;
- they are involved in the delivery of necessary substrates;
- they prevent macromolecule damage during cellular stress condition; or
- they influence ETC gene expression.

This destabilization, however, can be caused by various cellular pathways. Mitochondrial respiration is the primary source of ROS, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), or hydroxyl radical (HO^-). Electron leakage from the ETC under physiological conditions is responsible for the conversion of approximately 1–2% of consumed oxygen molecules into superoxide [181–183]. Uncoupling the respiratory chain in response to a deletion in a variety of genes encoding mitochondrial proteins, e.g., *NDE*1 (NADH dehydrogenase) and *FMC*1 (involved in ATP synthase complex assembly) [184], or in response to treating cells with an ETC inhibitor (antimycin A) results in elevated ROS production; these increased ROS are followed by an increase in endogenous premutagenic DNA lesion formation, which in turn causes genome destabilization [12,185]. However, oxidative stress generates DNA lesions, such as modified bases (the primary modification being 7,8-dihydro-8-oxoguanine, which leads to GC→TA transversions), abasic sites, and single-strand DNA breaks. When left unrepaired, these forms of DNA damage will cause mutations or block the replication fork, thereby contributing to a number of degenerative processes, including gross chromosomal rearrangements [186,187]. Importantly, not all parts of the respiratory chain are involved in ROS overproduction. Mutations in *ATP*11 (involved in ATP synthase complex assembly), *COX*11 (delivering copper to cytochrome *c* oxidase), *CYT*1 (cytochrome c_1), *PET*117 (cytochrome *c* oxidase assembly), *RIP*1, *QCR*8 (subunits of ubiquinol cytochrome *c* reductase complex), or *TCM*62 (succinate dehydrogenase complex assembly) do not produce an elevation of the ROS level [184]. However, these mutations substantially shorten the life span of cells, make them more sensitive to oxidative stress, and sometimes (*atp*11, *cyt*1, *pet*117, and *rip*1) even cause them to be unable to survive on nonfermentable carbon sources. Interestingly, no significant changes in the mitochondrial membrane potential are detected in these mutants compared to the wild-type strain.

H_2O_2 treatment can provoke electron leak from the ETC, although not all parts of the respiratory chain are sensitive to this effect. Although H_2O_2 inhibits the activities of complexes II and III by oxidizing their regulatory SH groups, H_2O_2 does not affect complex IV, which is protected by its peroxidase activity. However, the reaction of H_2O_2 with complex IV leads to the formation of sulfur-centered radicals that cause lipoperoxidation, which in turn specifically enhances the damage in complex III, stimulates electron leakage and subsequent O_2^- production, and causes iron release from the mitochondrial pool [188]. Therefore, complex III appears to be the major source of ROS [189] and seems to have an

additional physiological function as a signaling molecule during adaptation to hypoxia or as an effector during apoptosis.

Despite the potentially destructive role of ROS in the cell, accumulating data suggest their beneficial role as molecular signals to trigger an adaptive response known as mitohormesis and induce endogenous defense mechanisms by which the cell acquires increased stress resistance and longevity [190,191]. Accordingly, antioxidant dietary supplements that prevent ROS signaling counteract the health-promoting and life-span-extending capabilities of calorie restriction and physical exercise.

Incidentally, in human cells, an alternative source of ROS is complex I, which is not present in yeast. Instead, yeast cells have two NADH dehydrogenases (Nde1 and Nde2) and one NADH-ubiquinone oxidoreductase, Ndi1 [192,193]. ETC-dependent ROS production is possible only when the ETC is functional. The ROS level increases in response to ETC perturbation but substantially decreases when there is no flow of electrons through the ETC, e.g., in rho^0 mutants [12]. Interestingly, under certain circumstances, rho^0 cells have a better chance of survival than rho^+ cells. rho^0 cells are 100-fold more resistant to the lethal effects of ROS generated by bleomycin and phleomycin D than are respiratory-competent cells. Moreover, when the same exogenous oxidative stress was applied to rho^+ cells, the majority of survivors lost mitochondrial function. Furthermore, the lack of DNA repair genes, such as *RAD*52 or *BLM*5, in diploid strains, which is highly mutagenic and causes hypersensitivity to free radicals in respiratory-competent cells, does not affect the resistance of the surviving cells that lack mitochondrial function. Losses in mitochondrial function were dependent only on the dose of the applied chemical. Thus, the inactivation of mitochondrial function may protect cells against the lethal effects of oxygen free radicals [194].

Nevertheless, in wild-type cells, an efficient ETC is required not only for proficient energy delivery but also for the appropriate stress response and nuclear genome preservation. The latter function is connected with the presence of the membrane potential rather than with ROS production. As previously stated, ROS provoke primarily point mutations and cause problems when the cell has a defective capacity to defend against DNA damage. However, the loss of the electrochemical potential $\Delta\Psi$ destroys nearly all mitochondrial functions, mitochondrial transport, prosthetic group production, and mitochondrial biosynthetic processes, and this condition is frequently linked to an elevated rate of rearrangements in nuclear DNA [11,195,196].

Impaired iron–sulfur cluster production leads to nuclear genome rearrangements

A series of elegant experiments showed that the cellular crisis observed in rho^0 cells manifesting as progressively slower growth, cell cycle arrest, and nuclear genome instability correlates with a defect in iron–sulfur cluster biogenesis due to a reduction in the $\Delta\Psi$ [11]. Several pieces of evidence confirmed this conclusion. First, the crisis observed upon mtDNA loss was not a consequence of respiratory deficiency. The deletions of three independent nuclear genes required for respiration at various steps of the ETC, namely *CAT*5 (monooxygenase required for ubiquinone biosynthesis), *RIP*1 (ubiquinol-cytochrome *c* reductase), and *COX*4 (subunit IV of cytochrome *c* oxidase), had no effect on the growth rate, regardless of the mtDNA content in the analyzed cells. Second, cells carrying the *ATP*1-111 allele, which encodes a hyperactive F1 ATP synthase and generates a higher $\Delta\Psi$ in rho^0 than in wild-type rho^0 cells, did not present any detectable crisis. Third, downregulation of the nonmitochondrial, cytosolic Nar1 protein, which is required for cytosolic and nuclear Fe–S protein maturation, was sufficient to cause increased genomic instability in cells with intact mitochondrial function. These results suggest an essential role of mitochondria in the formation of ISCs, which are catalytic and structural components of many cellular proteins, including those involved in DNA replication and repair. Moreover, the proteins involved in ISC biogenesis or in the assembly of various Fe–S proteins are also required to maintain nuclear genome integrity. Mutations in these genes cause a chromosome instability phenotype that is severe for the *CIA*1 and *DRE*2 genes [197] and moderate for the *RLI*1 gene [198]. The *ISA*2 and *NBP*35 genes were identified in genomic screens as being responsible for the maintenance of the diploid genome. In various assays, homozygous *isa*2/*isa*2 and heterozygous *NBP*35/*nbp*35*: kanMX*4 strains manifest a mutator phenotype [199]. Nbp35 is one of several Fe–S proteins (the others are Cfd1, Nfs1, Isu1, Isu2, Ncs2, and Cia1) that are engaged in wobble tRNA modification [200,201]. Because modified nucleosides near the anticodon are important for the proper decoding of mRNA by the ribosome, using the wrong tRNA during translation leads to proteotoxic stress. This proteotoxic stress may subsequently lead to genome destabilization [169,202,203]. In *Schizosaccharomyces pombe* cells, the loss of activity of the cytosolic thiouridylase complex Ctu1–Ctu2 responsible for the 2-thiolation of cytosolic tRNAs causes misreading and frameshifting during translation and ultimately results in severe genome instability [204].

The results obtained using Zim17-depleted strains shed more light on the manner in which disturbing the Fe–S biosynthesis machinery affects the cellular genome. Zim17 functions as a zinc-finger motif heat shock protein and chaperone to maintain the activity of other chaperones, such as the mtHsp70 homologs Ssc1 and Ssq1 [205]. Zim17 directly assists in the functional interaction of chaperones with substrate proteins in a J-type cochaperone-dependent manner [206]. However, cells lacking Zim17 have limited respiration activity, mimic the metabolic response to iron starvation, and suffer from a dramatic increase in nuclear genome recombination [207]. Neither ROS nor deficient DNA repair accounts for the genome hyperrecombination phenotype observed in a *zim*17Δ strain. The lack of the Zim17 protein leads to the following: (1) ISC protein assembly perturbation and the improper assembly of DNA polymerase complexes and (2) the nuclear accumulation of preribosomal particles followed by G1 cell cycle arrest. Therefore, the hyperrecombination phenotype of the *zim*17Δ strain is probably caused by a shortage of proteins needed for faithful DNA replication. The cellular requirement of Zim17 depends on the growth conditions. A *zim*17 conditional mutant growing on fermentable medium, even at a permissive temperature, displayed a tendency to lose its respiratory competence, showed vast aggregations of the mitochondrial Ssq1 chaperone, and was accompanied by a defect in Fe–S protein biogenesis. Under respiring conditions, the mitochondrial Hsp70's Ssc1 and Ssq1 exhibited only a partial aggregation in the same *zim*17 mutants [206].

Strains carrying a mutation in the *MET*18/*MMS*19 gene display a variety of phenotypes, including methionine auxotrophy, sensitivity to genotoxic stress, and the presence of extended telomeres. The role of Met18 in preventing genome instability became more obvious when this protein was found to function as part of the cytosolic iron–sulfur protein assembly (CIA) targeting complex, in which it serves as an adapter between early-acting CIA components and a subset of cellular Fe–S proteins. As shown previously, Met18/Mms19 specifically coimmunoprecipitated with two DNA helicases, Rad3 and Dna2, and several other Fe–S proteins, including Met10, Rli1, and the DNA glycosylase Ntg2. These results explain the involvement of Met18 in methionine biosynthesis, telomere maintenance, and DNA replication and repair [208]. A similar function is carried out by MMS19, an Met18 ortholog in mice [209].

Although the list of ISC-containing proteins in *S. cerevisiae* is incomplete (Table 2), functional annotations of some of the known proteins directly implicate a role in nuclear genome maintenance. The nuclear proteins that contain ISCs include the following: Pri2, a primase involved in lagging-strand DNA synthesis and important in DNA double-strand break repair [215]; the catalytic subunits of

all three replicative DNA polymerases, α, δ, and ε, and the specialized DNA polymerase ζ that is involved in postreplicative repair in yeast [218]; the glycosylase Ntg2, which is involved in base-excision repair [214]; and various helicases, including Rad3, which is involved in nucleotide-excision repair (NER) [220,221], and Dna2, which is required for Okazaki fragment processing and recombinational DNA repair [87]. For all these proteins, iron–sulfur clusters are critical to their molecular functions.

The family of Fe–S proteins includes also cytosolic and mitochondrial metabolic enzymes that are crucial for proper cell functioning and proteins involved in the ETC (Sdh2, Rip1, Cir2), enzymes engaged in the TCA cycle (Aco1), and proteins in various pathways of amino acid (Lys4, Ilv3, Leu1, Met5, Glt1), heme (Hem15), biotin (Bio2), and lipoic acid (Lip5) biosynthesis (Table 2). Unexpectedly, in addition to their enzymatic function, Ilv3 and Aco1 were found to be involved in the maintenance of the nuclear genome [199,223]. As mentioned in a previous section, Aco1 is also essential for mitochondrial genome maintenance for two reasons: (1) it can replace Abf2 in its mtDNA packaging function and physically protect mtDNA [41] and (2) it protects the cell against citrate accumulation and toxicity [44,224]. Two independent explanations of citrate accumulation toxicity exist in the literature. First, Lin et al. [224] link citrate toxicity with the ionized form of this metabolite based on their observation that the effect of elevated cellular citrate was partially alleviated by the addition of iron or by an increase in pH in the growth medium in an *aco*1Δ strain. Second, Farooq et al. [44] believe that citrate toxicity results from citrate/iron chelate formation resulting in oxidative damage to mtDNA. The authors argue that iron homeostasis is a highly complex process and that Lin et al. actually did not show that exogenous iron addition increased the iron levels in the mitochondria. The authors noted differences in strain backgrounds and growth conditions between the two studies, which may have affected the mtDNA loss associated with the *aco*1Δ mutation. Farooq et al. showed that the RTG pathway is activated in *aco*1Δ mutants, leading to increased expression of the gene encoding citrate synthase. A high level of citrate synthase leads to iron accumulation in the mitochondria, where H_2O_2 is present because of Sod1 superoxide dismutase activity. Subsequently, the accumulated iron reacts with H_2O_2 to generate very active hydroxyl radicals that destabilize the mtDNA. The fact that mutations in *RTG* genes, genes encoding citrate synthase, mitochondrial iron transporters, or superoxide dismutase Sod1, all prevented mtDNA loss in *aco*1Δ cells strongly supports the second explanation [44]. Additional arguments in favor of this hypothesis come from the works of Chen et al. [225]. These authors show that although citrate is an iron chelator, the citrate–iron complexes are toxic in the intracellular environment. The chelation of iron by citrate was found to promote the autoxidation of Fe^{2+} (Fe^{2+} citrate$\rightarrow Fe^{3+}$ citrate e^-). The autoxidation of Fe^{2+} results in the peroxidation of lipids in test solutions and in isolated mitochondria. In an intracellular reducing environment, citrate-dependent iron oxidation increases and leads to iron citrate toxicity. However, the data presented above do not necessarily exclude the previously proposed role of Aco1, independent of its enzymatic activity, in mtDNA maintenance [41], as mentioned under *Mitochondrial nucleoid proteins in S. cerevisiae.*

Abnormal dNTP pool size, altered bias, or changed cellular distribution often leads to mutagenesis and/or chromosomal instability

dNTP biosynthesis proceeds partially in mitochondria. Mitochondria are involved in dNTP biosynthesis at various stages of this biological process. For example, the de novo biosynthesis of pyrimidines requires the mitochondrial Ura2 enzyme, the bifunctional carbamoylphosphate synthetase/aspartate transcarbamylase, which catalyzes the first two steps in this process [226]. There are two adenylate kinases required for purine metabolism: Adk2 is a mitochondrial enzyme, and Adk1 is localized in both the mitochondria and the cytoplasm [227]. In addition, mitochondria provide a supply of precursors for purine and pyrimidine biosynthesis via the activity of multiple enzymes, including Shm1, a serine hydroxymethyltransferase, and Idp1, an isocitrate dehydrogenase. Under respiratory conditions, the mitochondrial ETC is the major source of ADP phosphorylation. Finally, mitochondria contribute to the formation of the ribonucleotide-diphosphate reductase complex (RNR). The RNR is the enzyme responsible for the rate-limiting step in dNTP synthesis and its activity is tightly regulated. RNR activity is strongly dependent on mitochondria, which deliver three components necessary for the assembly of the complex: (1) diferric-tyrosyl radical cofactor [Fe_2^{III}-$Y^{\bullet}$], an atypical iron prosthetic group for the enzyme; (2) a monothiol glutaredoxin heme protein complex, Grx3–Grx4; and (3) the ISC assembly complex Dre2–Tah18 [222,228,229]. The electron donors required for RNR reduction reactions, cytoplasmic thioredoxins (Trx1 and Trx2), are also heme proteins [230]. Therefore, the role of mitochondria in cellular dNTP production is crucial.

The size of the dNTP pools and their need to be tightly regulated ensure optimized DNA metabolism. The dNTP pool concentration oscillates during the cell cycle, reaching a maximum during the G1/S transition to prepare cells for the next round of DNA replication and contribute to cycle progression [231]. The dNTP pools expand in response to genotoxic stress conditions and stalling of the replication fork, facilitating the continuation of replication through the DNA lesions [232–237]. However, an elevated dNTP pool size has consequences. As shown for human cells, elevated dNTP pools inhibit the proofreading activity of DNA polymerases, enhance dNTP misincorporation, and often lead to an increased mutation rate [238,239]. Unfortunately, abnormalities in the dNTP pools can provoke point mutations and DNA breaks, which can lead to gross DNA rearrangements in the cellular genome [232,235,240–242]. Thus, maintaining proper dNTP levels is essential for cellular viability and genome stability, particularly during oxidative stress. Cells lacking the peroxiredoxin Tsa1, an enzyme crucial for antioxidant cellular defense and signaling, exhibit elevated levels of dNTPs, which appear to underlie the genome instability in this mutant [243].

Mitochondria are involved in the dNTP supply to other cellular compartments. The imbalance in cytosolic dNTP pools due to mitochondrial dysfunction leads to chromosomal instability, as was shown in yeast and human cells [172,244]. Sometimes, a particular mutation may lead to an imbalance in the dNTP pools in mitochondria. For example, yeast cells lacking the *POS5* gene, which encodes the mitochondrial NADH kinase, contain abnormal dNTP pools that are doubled in size in mutant mitochondria compared to wild-type mitochondria. This difference is responsible for the mutator phenotype of the *pos5* strain; however, the lack of antioxidant protection also contributes to elevated mitochondrial genome mutagenesis [245].

The results of experiments summarized below provide evidence for an additional connection among mitochondria, nucleotides, and genome stability. Because both dNTP synthesis and the ETC require nucleotides as substrates, nucleotide transport to mitochondria is crucial for the proper functioning of these processes. Moreover, one nucleotide, GTP, is involved in iron homeostasis in the mitochondrial matrix. Absence of the yeast mitochondrial GTP/GDP carrier protein Ggc1, in addition to causing decreased levels of matrix GTP and increased levels of matrix GDP, manifests as high cellular iron uptake and remarkable iron accumulation within the mitochondria. The expression of the human nucleoside diphosphate kinase Nm23-H4 in yeast *ggc*1 mutant cells increases the level of GTP at the expense of ATP via γ-phosphate group translocation from ATP to GDP, restoring normal iron regulation. Thus, the Ggc1-dependent GTP/GDP levels in the mitochondrial matrix mediate iron metabolism [246]. Beyond their role in respiration to exchange cytosolic

Table 2
Fe–S proteins in yeast *Saccharomyces cerevisiae* and their function, localization, bound cluster types, and human orthologs, as well as diseases linked to their malfunction and proteins involved in their assembly.

Fe–S protein	Protein name	Localization	Molecular function	Cluster type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Fe–S proteins involved in electron transport						
Succinate dehydrogenase	Sdh2	Mitochondrial inner membrane	Succinate dehydrogenase (ubiquinone) activity, SDH (complex II)	[2Fe–2S]	SDHB	(94) e.g., cancers, tumors, sarcomas, carcinomas, paraganglioma, neuroblastoma, myeloma, leukemia, hyperparathyroidism, atherosclerosis, retinitis, asthma, pheochromocytoma, Cowden syndrome, Huntington disease, Parkinson disease, Alzheimer disease, dementia, narcolepsy, hypertension, ischemia, obesity, diabetes mellitus, insulinoma, diabetic retinopathy, myopathy, lipomatosis
Complex III	Rip1	Mitochondrial inner membrane	Ubiquinol-cytochrome *c* reductase, a Rieske Fe–S protein (complex III)	[2Fe–2S]	UQCRF-S1	(17) e.g., prostate and breast cancer, leukemia, Huntington disease, Alzheimer disease, renal cell carcinoma, myelodysplastic syndromes, Parkinson disease, liver disease
Ferredoxin[a]	Yah1[b]	Mitochondrial matrix	Maturation of Fe–S proteins, biosynthesis of heme A, steroid biosynthesis[c]	[2Fe–2S]	Adreno-doxin/ FDX	(18) e.g., lung cancer, rickets, muscular atrophy
Electron transfer flavoprotein dehydrogenase	Cir2	Mitochondrion	Catabolism of amino acids and choline, β-oxidation of fatty acids	[4Fe–4S]	ETFDH	(22) e.g., myeloma, cancers, mental retardation, hypoglycemia, myopathy, Reye syndrome, riboflavin deficiency, multiple acyl-CoA dehydrogenase deficiency, lipid storage disease, glutaric acidemia II severe neonatal type
Fe–S proteins involved in metabolism						
Aconitase	Aco1	Mitochondrial matrix	TCA cycle; component of the mitochondrial nucleoid	[4Fe–4S]	ACO1	(33) e.g., melanoma, asthma, Alzheimer disease, ataxia, Parkinson disease, cataract, intellectual disability, hepatoblastoma, thalassemia, hemochromatosis, anemia, restless legs syndrome
Homoaconitase	Lys4	Mitochondrial matrix	Biosynthesis of lysine (aconitase-like)	[4Fe–4S]	—	
Dihydroxy acid dehydratase	Ilv3[b]	Mitochondrial matrix	Biosynthesis of branched-chain amino acids	[4Fe–4S]	—	
Lipoate synthase	Lip5	Mitochondrial matrix	Biosynthesis of lipoic acid	[2Fe–2S], [4Fe–4S]	VTA1	(3) e.g., thyroiditis, tonsillitis
Biotin synthase	Bio2	Mitochondrial matrix	Biosynthesis of biotin	[2Fe–2S], [4Fe–4S]	—	
Ferrochelatase	Hem15[b]	Mitochondrial inner membrane	Heme biosynthesis	[2Fe–2S][c]	FECH	(46) e.g., adenocarcinoma, prostate cancer, neuronitis, myelomas, endotheliitis, leukemia, hepatitis, thyroiditis, myeloproliferative disorder, Alzheimer disease, ataxia, arthritis, tonsillitis, anemia, oligodendroglioma, porphyria, keratoderma, Friedreich ataxia, porokeratosis
Isopropylmalate isomerase	Leu1	Cytoplasm	Biosynthesis of leucine (aconitase-like)	[4Fe–4S]	—	
Sulfite reductase	Met5/ Ecm17	Cytoplasm	Biosynthesis of methionine, contains siroheme	[4Fe–4S]	—	
Glutamate dehydrogenase	Glt1	Cytoplasm	Biosynthesis of glutamate	[4Fe–4S]	—	
Fe–S proteins involved in genome stability						
Primase	Pri2[b]	Nucleus	Subunit of DNA primase, synthesis of RNA primer during replication, involved in DSB repair	[4Fe–4S]	PRIM2	(1) cerebritis
DNA polymerase α	Pol1[b]	Nucleus	Catalytic subunit of the DNA polymerase α-primase complex, initiation of DNA replication	[4Fe–4S]	POLA1	(22) e.g., retinoblastoma, age-related macular degeneration, acquired immunodeficiency syndrome, encephalitis, ophthalmoplegia, N syndrome, xeroderma pigmentosum, Netherton syndrome, Alpers syndrome
DNA polymerase δ	Pol3[b]	Nucleus	DNA replication polymerase with proofreading exonuclease activity, lagging-strand synthesis, DNA repair	[4Fe–4S]	POLD1	(42) e.g., labyrinthitis, melanoma, various cancers, myeloma, leukemia, meningioma, obesity, tonsillitis, adenoma, multiple sclerosis, acquired immunodeficiency syndrome, lipodystrophy, encephalitis, leukodystrophy, ophthalmoplegia, mutism, Werner syndrome, xeroderma

Table 2 (*continued*)

Fe–S protein	Protein name	Localization	Molecular function	Cluster type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
DNA polymerase ε	Pol2[b]	Nucleus	DNA replication polymerase with proofreading exonuclease activity, leading-strand synthesis, DNA repair	[4Fe–4S]	POLE	pigmentosum, Cockayne syndrome, Alpers syndrome, progeroid features (34) e.g., leukemia, various cancers, retinitis, myeloma, blindness, colorectal cancer, neuroblastoma, narcolepsy, adenoma, multiple sclerosis, acquired immunodeficiency syndrome, encephalitis, xeroderma pigmentosum, Alpers syndrome, Fils syndrome
DNA polymerase ζ	Rev3	Nucleus	Catalytic subunit of DNA polymerase ζ, involved in TLS during postreplication repair	[4Fe–4S]	POLZ/ REV3L	(27) e.g., leukemia, adenocarcinoma, various cancers, retinitis, inflammatory bowel disease, osteosarcoma, acquired immunodeficiency syndrome, encephalitis, Fanconi anemia, ophthalmoplegia, xeroderma pigmentosum
DNA helicase	Rad3[b,d]	Nucleus	5′–3′ DNA helicase; involved in NER and transcription	[3Fe–4S], [4Fe–4S]	XPD	(3) xeroderma pigmentosum, Cockayne syndrome, trichothiodystrophy
DNA helicase	Dna2[b]	Nucleus and mitochondrion	ATP-dependent DNA helicase; 5′-flap endonucleases; Okazaki fragment processing; DNA repair	[3Fe–4S], [4Fe–4S]	DNA2	(3) myopathy, Werner syndrome, malaria
DNA glycosylase	Ntg2	Nucleus	DNA *N*-glycosylase and AP lyase; BER	[4Fe–4S]	NTHL1	(22) e.g., leukemia, colorectal cancer, adenoma, multiple sclerosis, tuberous sclerosis, cholangitis, mitochondrial disorders
Fe–S proteins involved in regulation and assembly						
RNase L inhibitor	Rli1[b]	Cytoplasm and nucleus	Biogenesis of ribosomes, rRNA processing, translation initiation and termination, assembly of RNA complexes, ABC family ATPase	2 × [4Fe–4S]	ABCE1	(15) e.g., adenocarcinoma, several cancers, neuropathy
Glutaredoxin 2	Grx2	Mitochondrion	Glutathione-dependent oxidoreductase	[2Fe–2S]	GLRX2	(14) e.g., melanoma, cervicitis, lung cancer, ischemia, Parkinson disease
Histone acetyltransferase	Elp3	Nucleus	Histone acetyltransferase subunit of the elongator complex, cluster binds *S*-adenosylmethionine	[4Fe–4S]	ELP3	(3) melanoma, neuronitis, malaria
ISC assembly	Dre2[b]	Cytoplasm and mitochondrial intermembrane space	Electron carrier activity, ISC assembly; cytokine-induced apoptosis inhibitor 1	[2Fe–2S], [4Fe–4S]	Ciapin 1/ anamor-sin	(15) e.g., carcinoma, leukemia, B cell lymphomas, cancers
NADPH-dependent diflavin reductase	Tah18[b]	Mitochondrion	Component of an early step in the CIA, transfers electrons from NADPH to the ISC of Dre2p; plays a prodeath role under oxidative stress; Tah18p-dependent NO synthesis confers high-temperature stress tolerance		NDOR1	(12) e.g., melanoma, teratoma, leukemia
CIA targeting complex	Met18/ Mms19	Cytoplasm	Component of a late step of Fe–S CIA; complex with Cia1p and Cia2p that directs ISC incorporation into a subset of proteins involved in methionine biosynthesis, DNA replication and repair, transcription, and telomere maintenance		MMS19	(7) e.g., ovarian cancer, pancreatic cancer, Alzheimer disease, multiple sclerosis
CIA targeting complex	Cia1[b]	Cytoplasm and nucleus	Component of a late step of CIA; complex with Cia2 and Met18		CIAO1	(3) e.g., sarcomas, Wilms tumor
CIA targeting complex	Cia2[b]	Cytoplasm and nucleus	Component of a late step of CIA; complex with Cia1 and Met18		FAM96B	(2) e.g., endotheliitis, malaria
Fe–S proteins involved in Fe–S protein biogenesis						
Ferredoxin[a]	Yah1[b]	Mitochondrial matrix	Maturation of mitochondrial Fe–S proteins, heme A biosynthesis, steroid biosynthesis[c]	[2Fe–2S]	Adreno-doxin/ FDX	(18) e.g., lung cancer, rickets, muscular atrophy
P-loop ATPase; cysteine desulfurase	Nbp35[b]	Cytoplasm and nucleus	Maturation of cytosolic/nuclear Fe–S proteins, complex with Cfd1; ATPase	[4Fe–4S]	NFS1/ IscS	(7) e.g., hyperalgesia, liver failure, cerebritis, ataxia, anemia
P-loop ATPase	Cfd1[b]	Cytoplasm	Maturation of cytosolic Fe–S proteins, complex with Nbp35 and Nar1; ATPase; tRNA wobble uridine modification	[4Fe–4S]	NUBP2	(7) e.g., endometriosis, ovarian neoplasms, various cancers, syndactyly
Iron-only hydrogenase	Nar1[b]	Cytoplasm and nucleus	Maturation of cytosolic/nuclear Fe–S proteins, binds to Nbp35 and Cia1	Two clusters of complex type	NARFL	(6) e.g., glaucoma, breast cancer, pertussis; genetic knockout causes embryonic lethality
Components involved in Fe–S-cluster biosynthesis						
Sulfur donor	Nfs1[b]	Mitochondrion and nucleus	Cysteine desulfurase; cellular iron ion homeostasis, ISC assembly, tRNA thio-modification, tRNA wobble uridine modification		NFS1	(7) e.g., ataxia, anemia, cerebritis
	Isu1			[2Fe–2S]	ISCU	

Table 2 (*continued*)

Fe–S protein	Protein name	Localization	Molecular function	Cluster type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Sulfur acceptor and scaffold		Mitochondrial matrix	Binding of ferric iron and intermediate ISC, cellular iron ion homeostasis, ISC assembly, tRNA wobble uridine modification; interacts with frataxin			(18) e.g., myopathy, glioblastoma multiforme, several cancers, sideroblastic anemia, Friedreich ataxia, siderosis
ISC biogenesis	Isd11[b]	Mitochondrial matrix	ISC biogenesis desulfurase-interacting protein of 11kDa, stabilizes Nfs1		LYRM4	(9) e.g., inflammatory bowel disease, Crohn disease, spondylitis, schizophrenia, ataxia
Sulfur acceptor and scaffold	Isu2	Mitochondrial matrix	Binding of ferric iron and intermediate ISC	[2Fe–2S]	ISCU	(18) e.g., myopathy, glioblastoma multiforme, several cancers, sideroblastic anemia, Friedreich ataxia, siderosis
Scaffold	Nfu1	Mitochondrial matrix	ISC assembly, maturation of lipoate synthase, and SDH	[4Fe–4S]	HIRIP5/ NFU1	(14) e.g., multiple mitochondrial dysfunctions syndrome type 1, myeloma
Scaffold	Isa1	Mitochondrial matrix	Required for maturation of mitochondrial [4Fe–4S] proteins, complex with Isa2, Isa1 deletion causes loss of mtDNA and respiratory deficiency		ISCA1	(3) choroiditis, malaria; may represent an autoantigen in Sjogren syndrome
Scaffold	Isa2	Mitochondrial matrix	Required for maturation of mitochondrial [4Fe–4S] proteins, complex with Isa1		ISCA2	(3) hemophilia, malaria, tuberculosis
ISC biogenesis	Iba57	Mitochondrial matrix	Isa-interacting protein, biogenesis of [4Fe–4S] clusters		IBA57	(2) multiple mitochondrial dysfunctions syndrome 3, tooth resorption
Ferredoxin[a]	Yah1[b]	Mitochondrial matrix	Reduction of sulfur and iron or intermediate of ISC formation, electron transport	[2Fe–2S]	Adrenodoxin/ FDX	(18) e.g., lung cancer, rickets, muscular atrophy
Ferredoxin reductase	Yfh1[b,d]	Mitochondrial matrix	NAD(P)H-dependent reduction of Yah1, iron chaperone; oxidizes and stores iron; interacts with Isu1p to promote ISC assembly		Frataxin/ FXN	(41) e.g., Friedreich ataxia, ataxia, anemia, adenocarcinoma, cancers, myeloma, Huntington disease, neuropathy, myocardial infarction, diabetes mellitus, chorea mitochondrial disorders
Ferredoxin reductase	Arh1[b]	Mitochondrial inner membrane	NAD(P)H-dependent reduction of Yah1; required for formation of cellular iron–sulfur proteins and involved in heme A biosynthesis		Adrenodoxin reductase/ FDXR	(17) e.g., colorectal cancer, muscular atrophy, prostate cancer
Chaperone	Ssc1[b]	Mitochondrial matrix	Hsp70/DnaK-type molecular chaperone, required for assembly of ISC transfer		HSPA9/ GRP75	(40) e.g., carcinomas, cancers, leukemia, neuroblastoma, ataxia, Alzheimer disease, type 1 diabetes, Parkinson disease, myotonic dystrophy type 2, Down syndrome
Chaperone	Ssq1	Mitochondrial matrix	Hsp70/DnaK-type molecular chaperone, required for assembly of ISC into proteins at a step after cluster synthesis and for maturation of Yfh1p, ATP hydrolyzing component		HSPA9/ GRP75	(40) e.g., carcinomas, cancers, leukemia, neuroblastoma, ataxia, Alzheimer disease, type 1 diabetes, Parkinson disease, myotonic dystrophy type 2, Down syndrome
Cochaperone	Jac1[b]	Mitochondrial matrix	Accessory chaperone of Hsp40/DnaJ type, ATP hydrolyzing component	[4Fe–4S][c]	HSCB	(12) e.g., chromosome aberrations, adenocarcinoma, anemia, ataxia
Cochaperone	Mge1[b]	Mitochondrial matrix	Mitochondrial GrpE, a nucleotide release factor for Ssc1 in protein translocation and folding; cochaperone for Ssq1 in folding of ISC proteins		GRPEL1	(3) various neoplasms, pneumonia malaria
Chaperone	Zim17[b,d]	Mitochondrial matrix	Zinc-finger HSP required for maintenance of Ssq1 and Ssc1 proteins, essential for protein import into mitochondria			
ISC transporter	Atm1[b]	Mitochondrial inner membrane	ABC transporter, export ISC to the cytoplasm, transport of heme[c]		ABCB7	(82) e.g., cancers, myelomas, carcinomas, ataxia, anemia, thrombocytopenia, hypercholesterolemia, liver disease, blindness, Pearson syndrome, Addison disease, substance abuse
ISC assembly	Grx5	Mitochondrial matrix	Monothiol glutaredoxin 5, hydroperoxide and superoxide-radical responsive glutathione-dependent oxidoreductase	[2Fe–2S]	GLRX5	(18) e.g., sideroblastic pyridoxine-refractory autosomal recessive anemia
	Aim1	?	Glutaredoxin-interacting protein, maturation of lipoate synthase and SDH, null mutant displays elevated frequency of mtDNA loss		BOLA3	(8) e.g., multiple mitochondrial dysfunctions syndrome type 1 and 2, myeloma
Fe^{2+} transporter	Mrs3	Mitochondrial inner membrane	Mitochondrial membrane iron transporter active under low-iron conditions, RNA splicing		SLC25-A37/ mitoferrin	(9) e.g., anemia, neoplasms, arthritis

Table 2 (*continued*)

Fe–S protein	Protein name	Localization	Molecular function	Cluster type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Fe^{2+} transporter	Mrs4	Mitochondrial inner membrane	Mitochondrial membrane iron transporter active under low-iron conditions, overproduced during DNA damage, RNA splicing		SLC25-A37/ mitofer-rin	(9) e.g., anemia, neoplasms, arthritis

All information listed is available in [112–115,210–213], and the reader is referred to these sources and the references therein for further details. Additional data have been published in [7,87,214–222].?, function/localization unclear or not yet analyzed.

[a] Ferredoxin is involved in heme and ISC biosynthesis and in Fe–S protein biogenesis.
[b] Essential for viability.
[c] Involves human cells.
[d] Viability depends on the genetic background.

ADP for mitochondrial ATP, the other two nucleotide carriers, ADP/ATP and ATP-Mg/Pi, have a second function to import cytosolic ATP into mitochondria and generate the $\Delta\Psi$. This alternative to the ETC source of the mitochondrial membrane potential is essential for proper functioning of the mitochondrial protein import and assembly systems [195]. Other evidence suggests that the deletion of *RIM2*, the gene encoding the mitochondrial pyrimidine nucleotide transporter, impairs pyrimidine transport and causes defects in iron homeostasis, heme synthesis, and ISC synthesis. Rupturing the mitochondrial membrane to allow the free influx of iron does not restore efficient heme synthesis in the absence of the Rim2 protein. These results indicate that Rim2 is a pyrimidine exchanger with an additional unique function in promoting mitochondrial iron utilization [247]. Moreover, Rim2 was found to act as a multicopy suppressor of *pif*1. The Pif1 protein is a DNA helicase engaged in the replication, recombination, and repair of mtDNA, but it also acts as a catalytic inhibitor of telomerase during telomere addition and DSB repair in the nucleus. In addition to telomeres, rDNA and Okazaki fragments are also Pif1 substrates. Thus, the lack of Rim2 affects both mitochondrial and nuclear genome maintenance [248,249].

Because all mitochondrial nucleotide carriers mentioned above are able to transfer their deoxy derivatives, an alternative explanation for the importance of maintaining the cellular level of nucleotide pools and their particular bias can be proposed while considering the following: (1) the mitochondrial membrane potential is necessary to produce ISC and heme prosthetic groups; (2) ISCs are the true sensors of the cellular iron level, and their abundance regulates the transcription of iron-responsive genes; (3) ISCs subsequently regulate iron homeostasis; and (4) homeostasis protects cells against oxidative stress and metal toxicity. The current assumptions state that the proper level and bias of dNTP pools determine the start of replication, influence DNA synthesis fidelity, and enable the bypass of DNA lesions to rescue the cell from replication block and cell cycle arrest; therefore, these steps contribute to genome stability. However, the abundance of dNTP pools, their cellular distribution, and their dNTP/dNDP/dNMP ratios contribute to the $\Delta\Psi$, which influences a variety of cellular functions, e.g., cellular transport and ISC synthesis. The ISC level determines iron homeostasis and adequate defense against oxidative stress. Because a subset of DNA polymerases and helicases are also Fe–S proteins, the ISC level governs the activities of these enzymes. Therefore, properly balanced dNTPs counteract genome instability by maintaining $\Delta\Psi$. This additional role of dNTPs in the cell is particularly important in *rho*^{0} cells, in which the ETC is dysfunctional; thus, ISC synthesis in these cells relies on alternative $\Delta\Psi$ sources.

Metal homeostasis disruption impairs prosthetic group creation to influence genome stability

Mitochondria are the major site of cellular iron utilization for the synthesis of essential cofactors, such as iron–sulfur clusters and heme. We previously described how ISC synthesis affects genome stability. Heme synthesis is also relevant to this process because a group of proteins possessing this prosthetic group can affect the maintenance of the intact genome.

The heme-binding proteins (listed in Table 3) include the following: proteins important for ETC functioning, e.g., cytochrome *c* (Cyc1), the Cyt1 subunit of cytochrome bc_1 complex subunit (complex III), the Cox1 and Cox2 subunits of cytochrome *c* oxidase (complex IV), cytochrome b_2 (Cyb2, L-lactate cytochrome *c* oxidoreductase), and the cytochrome *c* isoform 2 (Cyc7). The influence of these proteins on genome maintenance was discussed earlier. However, heme-binding proteins are involved in the response to oxidative stress, e.g., mitochondrial cytochrome *c* peroxidase Ccp1 [250] and nitric oxide reductase Yhb1 [251], which serve as oxidative stress signal transducers. The cellular abundance of the latter protein is also enriched in response to DNA replication stress [252]. The heme-binding proteins play a crucial role in the antioxidant defense system. Catalase A (Cta1) and catalase T (Ctt1), the enzymes that decompose H_2O_2 into O_2 and H_2O molecules, are hemoproteins [253]. The activities of catalases significantly reduce the potential ROS-dependent damage in the cell, including that of oxidative DNA lesions, to protect the genome.

Other known proteins possessing a heme prosthetic group are involved in various biosynthetic pathways, such as lipid biosynthesis (Cyb5), ergosterol biosynthesis (Erg11, Erg5), fatty acid synthesis (Ole1), de novo biosynthesis of NAD (Bna2), synthesis of *N,N*-bisformyl dityrosine (Dit2, which is required for spore wall maturation), and amino acid biosynthesis (Met5). Other heme proteins are involved in various metabolic pathways: Sdh4, which is a membrane anchor subunit of succinate dehydrogenase; its homolog Shh4, which couples the TCA cycle with the ETC; and Scs7, which is a sphingolipid α-hydroxylase that functions in the α-hydroxylation of sphingolipid-associated very long chain fatty acids. For some heme-containing proteins, direct evidence exists for their involvement in genome integrity. Strains lacking the damage response protein Dap1, which is involved in the regulation of cytochrome P450, exhibit sensitivity to MMS, telomere elongation, the loss of mitochondrial function, and partial arrest in sterol synthesis. Moreover, after MMS treatment, *dap*1 mutants are arrested as unbudded cells, suggesting that Dap1 is necessary for cell cycle progression after alkylation damage [254]. Another example is the Irc21 protein, which is involved in the resistance to carboplatin and cisplatin, compounds that cause DNA cross-linking, increase the number of spontaneous Rad52p foci [255], and ultimately trigger apoptosis [256].

Mitochondria are the dedicated location for the maturation of various metalloproteins that bind Mn^{2+} or Cu^{2+} ions, including antioxidant defense enzymes. The superoxide dismutase Sod2, which uses manganese as a cofactor, can misincorporate iron when cells are starved for manganese or when the mitochondrial iron homeostasis is disrupted by *grx*5, *ssq*1, or *mtm*1 mutations. This iron misincorporation leads to Sod2 inactivation and occurs

Table 3
The heme proteins in yeast *Saccharomyces cerevisiae* and their function, localization, bound heme types, and human orthologs, as well as diseases linked to their malfunction and proteins involved in their assembly.

Heme protein	Protein name	Localization	Molecular function	Heme type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Heme proteins involved in ETC						
Succinate dehydrogenase	Sdh4	Mitochondrial respiratory chain complex II	Membrane anchor subunit of succinate dehydrogenase that couples the TCA cycle with ETC	Heme	—	—
Succinate dehydrogenase	Shh4[a]	Mitochondrial inner membrane	Putative alternate subunit of succinate dehydrogenase; Sdh4 homolog	Heme	SDHD	(43) e.g., paraganglioma, pheochromocytoma, neurofibromatosis, mitochondrial complex II deficiency
Electron carrier	Cob[b]	Mitochondrial respiratory chain complex III	Cytochrome *b;* subunit of the ubiquinol-cytochrome *c* reductase	2 × heme B	MTCYB	(5) e.g., Leber hereditary optic neuropathy, encephalomyopathy, mitochondrial complex III deficiency
Electron carrier	Cyt1[a]	Mitochondrial respiratory chain complex III	Cytochrome c_1; component of the mitochondrial respiratory chain; subunit of the mitochondrial ubiquinol-cytochrome *c* reductase	Heme	CYC1	(4) e.g., hyperglycemia, mitochondrial complex III deficiency
Electron carrier	Cyc1[a]	Mitochondrial intermembrane space	Cytochrome *c,* isoform 1 (iso-1-cytochrome *c*)	Heme		Insulin-responsive hyperglycemia
Electron carrier	Cyc7	Mitochondrial intermembrane space	Cytochrome *c* isoform 2, expressed under hypoxic conditions (iso-2-cytochrome *c*)	Heme		—
Cytochrome *c* oxidase	Cox1[b]	Mitochondrial respiratory chain complex IV	Subunit I of cytochrome *c* oxidase (complex IV); complex IV is the terminal member of the mitochondrial inner membrane ETC	Heme A	COX1	(5) e.g., prostaglandin-endoperoxide synthase deficiency, cystic fibrosis
Cytochrome *c* oxidase	Cox2[b]	Mitochondrial respiratory chain complex IV	Subunit II of cytochrome *c* oxidase (complex IV)	Heme	COX2	(34) e.g., breast cancer, colorectal cancer, cystic fibrosis, hypertension, adenoma
Cytochrome *c* oxidase	Cox3[b]	Mitochondrial respiratory chain complex IV	Subunit III of cytochrome *c* oxidase (complex IV)	Heme	COX3	—
Heme proteins involved in stress response						
Catalase A	Cta1[a]	Mitochondrial matrix, peroxisomal matrix	Catalase A; breaks down hydrogen peroxide in the peroxisomal matrix formed by acyl-CoA oxidase (Pox1) during fatty acid β-oxidation	Heme	CAT	(150) e.g., acatalasemia, pityriasis versicolor, meningitis, encephalitis, diabetes, asthma, Alzheimer disease, systemic lupus erythematosus, rheumatoid arthritis, cancers
Catalase T	Ctt1	Cytoplasm	Cytosolic catalase T; participates in protection from oxidative damage by hydrogen peroxide	Heme	—	—
Thioredoxin	Trx1	Cytoplasm, fungal-type vacuole, mitochondrial intermembrane space	Cytoplasmic thioredoxin isoenzyme; part of thioredoxin system, which protects cells against oxidative and reductive stress; disulfide oxidoreductase; Tsa1 cofactor; Trx2 paralog	Heme	TXN	(8) myocarditis, allergic bronchopulmonary aspergillosis, ocular hypertension, trypanosomiasis, skin squamous cell carcinoma
Thioredoxin	Trx2[a]	Cytoplasm, fungal-type vacuole	Cytoplasmic thioredoxin isoenzyme; Trx1 paralog	Heme	TXN	(8) myocarditis, allergic bronchopulmonary aspergillosis, ocular hypertension, trypanosomiasis, skin squamous cell carcinoma
Thioredoxin	Trx3[a]	Mitochondrion	Mitochondrial thioredoxin; oxidoreductase required to maintain the redox homeostasis of the cell; forms the mitochondrial thioredoxin system with Trr2, redox state is maintained by both Trr2 and Glr1	Heme	—	—
Oxidoreductase	Grx1	Cytoplasm, nucleus	Glutathione-dependent disulfide oxidoreductase (glutathione peroxidase, glutathione transferase); Grx2 paralog	Heme	—	—
Oxidoreductase	Grx2[a]	Cytoplasm, mitochondrion	Cytoplasmic glutaredoxin (thioltransferase, glutathione-dependent disulfide oxidoreductase); involved in maintaining redox state of target proteins; Grx1 paralog	Heme	—	—
Oxidoreductase	Grx3	Cytoplasm, nucleus	Glutathione-dependent oxidoreductase; hydroperoxide and superoxide-radical responsive; monothiol glutaredoxin subfamily member along with Grx4 and Grx5; Grx4 paralog	Heme	GLRX3	(1) lymphocytic choriomeningitis

Table 3 (*continued*)

Heme protein	Protein name	Localization	Molecular function	Heme type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Oxidoreductase	Grx4	Nucleus	Glutathione-dependent oxidoreductase; hydroperoxide and superoxide-radical responsive; Grx3 paralog	Heme	GLRX3	(1) lymphocytic choriomeningitis
Oxidoreductase	Grx5	Mitochondrial matrix	Glutathione-dependent oxidoreductase; hydroperoxide and superoxide-radical responsive; involved in the synthesis/assembly of ISC	Heme	GLRX5	(4) e.g., sideroblastic anemia, Pearson syndrome, spasticity–ataxia–gait anomalies syndrome
Monothiol glutaredoxin	Grx6	Integral component of membrane, ER, fungal-type vacuole, Golgi apparatus	Monothiol glutaredoxin, more similar in activity to dithiol than other monothiol glutaredoxins; binds ISC; Grx7 paralog	Heme	—	—
Monothiol glutaredoxin	Grx7[a]	Integral component of membrane, fungal-type vacuole, Golgi apparatus	Monothiol glutaredoxin, more similar in activity to dithiol than other monothiol glutaredoxins; Grx6 paralog	Heme	—	—
Dithiol glutaredoxin	Grx8	Cytoplasm	Glutaredoxin that employs a dithiol mechanism of catalysis	Heme	—	—
Peroxidase	Ccp1	Mitochondrial intermembrane space	Mitochondrial cytochrome *c* peroxidase; involved in response to oxidative stress	Heme	—	—
Nitric oxide reductase	Yhb1	Mitochondrial matrix, cytoplasm, nucleus	Nitric oxide reductase; flavohemoglobin involved in NO detoxification; plays a role in the oxidative and nitrosative stress response	Heme	—	—
Oxidoreductase	Cyb2	Mitochondrial intermembrane space	Cytochrome b_2; L-lactate cytochrome *c* oxidoreductase	Heme	—	—
Stress response protein	Dap1[a]	Endosome, membrane	Heme-binding protein; involved in regulation of cytochrome P450 protein Erg11; damage response protein; mutations lead to defects in telomeres, mitochondria, and sterol synthesis	Heme	PGRMC1	(13) e.g., trigeminal neuralgia, spondylolisthesis, parotitis, tracheal stenosis, cancers, carcinomas
Stress response protein	Ynl234w	Cytoplasm	Heme protein involved in stress response; involved in glucose signaling or metabolism; regulated by Rgt1	Heme	—	—
Stress response protein	Irc21	Cytoplasm	Heme protein involved in drug response (carboplatin and cisplatin); shares similarity to a human cytochrome oxidoreductase; null mutant displays increased levels of spontaneous Rad52 foci	Heme	—	—
Disulfide oxidoreductase	Eug1	ER lumen	Heme lyase disulfide oxidoreductase; involved in protein folding; Pdi1 paralog	Heme	—	—
Disulfide isomerase	Pdi1[a,c]	ER lumen	Protein disulfide isomerase; involved in protein folding; Eug1 paralog	Heme	P4HB	(3) malaria, pseudoachondroplasia, malignant glioma
Disulfide isomerase	Mpd1[a]	Fungal-type vacuole	Protein disulfide isomerase, interacts with and inhibits the chaperone activity of Cne1	Heme	—	—
Disulfide isomerase	Mpd2	ER	Protein disulfide isomerase; involved in protein folding	Heme	—	—
Heme proteins involved in metabolism						
Cytochrome b_5	Cyb5	ER membrane	Cytochrome b_5; involved in the sterol and lipid biosynthesis pathways; acts as an electron donor to support sterol C5-6 desaturation; involved in sterol and lipid biosynthesis	Heme	CYB5B	(1) Non-Hodgkin lymphoma
Sterol 14-demethylase	Erg11[c,d]	ER	Lanosterol 14-α-demethylase from cytochrome P450 family; catalyzes the C-14 demethylation of lanosterol to form 4,4″-dimethyl cholesta-8,14,24-triene-3-β-ol; involved in ergosterol biosynthesis	Heme	CYP51A1	(2) Chagas disease, Antley-Bixler syndrome
C-22 sterol desaturase	Erg5[a]	ER	C-22 sterol desaturase; a cytochrome P450 enzyme that catalyzes the formation of the C-22(23) double bond in the sterol side chain in ergosterol biosynthesis; involved in ergosterol biosynthesis	Heme	—	—
Stearoyl-CoA 9-desaturase	Ole1[a]	Integral component membrane, ER	Δ(9) fatty acid desaturase; required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria	Heme	—	—

Table 3 (*continued*)

Heme protein	Protein name	Localization	Molecular function	Heme type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Fatty acid α-hydroxylase	Scs7	Integral component membrane, ER	Sphingolipid α-hydroxylase, which functions in the α-hydroxylation of sphingolipid-associated very long chain fatty acids; the enzyme with both cytochrome b_5-like and hydroxylase/desaturase domains	Heme	FA2H	(11) e.g., fatty acid hydroxylase-associated neurodegeneration, leukodystrophy, spastic paraparesis, spastic paraplegia 35, alcohol-related neurodevelopmental disorder, breast cancer, aceruloplasminemia
Indoleamine 2,3-dioxygenase	Bna2	Cytoplasm	Putative tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase; required for de novo biosynthesis of NAD from tryptophan via kynurenine	Heme	IDO2	(2) pancreatic cancer, pancreatitis
Sulfite reductase	Met5[a]	Cytoplasm, sulfite reductase complex (NADPH)	Sulfite reductase β subunit; involved in amino acid biosynthesis	Heme	MTHFR	(283) e.g., homocystinuria and homocysteinemia due to Mthfr deficiency, neural tube defects, vascular disease, thromboembolism, schizophrenia, acute leukemia
Heme oxygenase	Hmx1[a]	ER, nuclear outer membrane	Heme oxygenase; involved in heme degradation during iron starvation and in the oxidative stress	Heme	—	—
Ferric-chelate reductase	Fre1	Plasma membrane	Ferric reductase and cupric reductase; reduces siderophore-bound iron and oxidized copper before uptake by transporters	Heme	CYBB/ GP91PHOX	(12) e.g., chronic granulomatous disease, atypical mycobacteriosis, familial X-linked 2, ehrlichiosis, diastolic heart failure, low renin hypertension
N-formyltyrosine oxidase	Dit2[a]	?	*N*-formyltyrosine oxidase; a cytochrome P450 involved in synthesis of *N,N*-bisformyl dityrosine required for ascospore wall maturation	Heme	—	—
Disulfide oxidoreductase	Prm4	Integral component of membrane	Pheromone-regulated protein proposed to be involved in mating	Heme	—	—
Transporters						
ATP:ADP antiporter	Aac1[a]	Mitochondrial inner membrane, cytoplasm	Mitochondrial inner membrane ADP/ ATP translocator; exchanges cytosolic ADP for mitochondrially synthesized ATP; heme transporter	Heme	ANT1	(12) e.g., progressive external ophthalmoplegia, hypertrophic cardiomyopathy, diabetes mellitus, myopathy
ATP:ADP antiporter	Aac3	Mitochondrial inner membrane	Mitochondrial inner membrane ADP/ ATP translocator; exchanges cytosolic ADP for mitochondrially synthesized ATP; heme transporter	Heme	ANT1	(12) e.g., progressive external ophthalmoplegia, hypertrophic cardiomyopathy, diabetes mellitus, myopathy
ATP:ADP antiporter	Pet9[c,d]	Mitochondrial inner membrane	Major ADP/ATP carrier of the mitochondrial inner membrane; exchanges cytosolic ADP for mitochondrially synthesized ATP; also imports heme and ATP; phosphorylated	Heme	ANT1	(12) e.g., progressive external ophthalmoplegia, hypertrophic cardiomyopathy, diabetes mellitus, myopathy
Transporter	Ydl119C/ Hem25[a]	Mitochondrial inner membrane	Putative mitochondrial transport protein; mitochondrial transporter family SLC25 member; induced in response to the DNA-damaging agent MMS	Heme	SLC25A38	(3) e.g., sideroblastic anemia, Pearson syndrome
Heme activator proteins						
Transcription factor	Hap1	Nucleus	Zinc finger transcription factor; involved in the complex regulation of gene expression in response to levels of heme and oxygen	Heme	—	—
Transcription factor	Hap2	Nucleus	Subunit of the heme-activated, glucose-repressed Hap2/3/4/5 CCAAT-binding complex, a transcriptional activator and global regulator of respiratory gene expression	Heme	—	—
Transcription factor	Hap3	Nucleus, CCAAT-binding factor complex	Subunit of the Hap2/3/4/5 CCAAT-binding complex; complex is heme-activated and glucose-repressed and a transcriptional activator and global regulator of respiratory gene expression; contains sequences contributing to both complex assembly and DNA binding	Heme	NFYB/ CBFB	(12) e.g., myeloid leukemia, cleidocranial dysplasia, imperforate anus, lymphoplasmacytic lymphoma
Transcription factor	Hap4[a]	Nucleus, CCAAT-binding factor complex	Subunit of the Hap2/3/4/5 CCAAT-binding complex; complex is heme-activated and glucose-repressed and a transcriptional activator and global regulator of respiratory gene expression; provides the principal	Heme	—	—

Table 3 (*continued*)

Heme protein	Protein name	Localization	Molecular function	Heme type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
			activation function of the complex; involved in diauxic shift			
Transcription factor	Hap5[a]	Nucleus, CCAAT-binding factor complex	Subunit of the Hap2/3/4/5 CCAAT-binding complex; complex is heme-activated and glucose-repressed and a transcriptional activator and global regulator of respiratory gene expression; contains sequences contributing to both complex assembly and DNA binding	Heme	—	—
Components involved in heme assembly						
Cytochrome *c*–heme linkage	Cyc2	Extrinsic component of mitochondrial inner membrane	Mitochondrial peripheral inner membrane oxidoreductase; contains a FAD cofactor in a domain exposed in the intermembrane space; probably participates in ligation of heme to acytochromes *c* and c_1 (Cyc1 and Cyt1)	Heme	—	—
Cytochrome c_1–heme linkage	Cyt2	Mitochondrial intermembrane space	Cytochrome c_1 heme lyase; involved in maturation of cytochrome c_1; links heme covalently to apocytochrome c_1	Heme	HCCS	(33) e.g., microphthalmia, congenital diaphragmatic hernia, hepatocellular carcinoma, hepatitis, adenoma
Cytochrome *c*–heme linkage	Cyc3	Mitochondrial intermembrane space	Cytochrome *c* heme lyase (holocytochrome *c* synthase); attaches heme to apocytochrome *c* (Cyc1 or Cyc7)	Heme	—	—
Cytochrome *c* assembly	Cox11[a]	Mitochondrial inner membrane and intermembrane space, mitochondrial ribosome	Copper ion binding protein, required for delivery of copper to Cox1	Heme	COX11	(2) Leigh disease, breast cancer susceptibility
Cytochrome *b* assembly	Cor1	Mitochondrial respiratory chain complex III	Core subunit of the ubiquinol-cytochrome *c* reductase complex (bc_1 complex), which is a component of the mitochondrial inner membrane ETC	Heme	UQCRC1	—
Components involved in heme biosynthesis						
First step	Hem1[c]	Mitochondrial matrix	5-Aminolevulinate synthase; catalyzes the first step in the heme biosynthetic pathway		ALAS2	(20) e.g., X-linked sideroblastic anemia, erythropoietic protoporphyria, sickle cell disease, microcytic anemia
Second step	Hem2[a,c]	Cytoplasm and nucleus	Aminolevulinate dehydratase; a zinc-dependent homo-octameric enzyme, catalyzes the conversion of 5-aminolevulinate to porphobilinogen		ALAD	(19) e.g., porphyria, lead poisoning, tyrosinemia, uremia, hypochromic anemia
Third step	Hem3[c]	Cytoplasm and nucleus	Porphobilinogen deaminase; catalyzes the conversion of 4-porphobilinogen to hydroxymethylbilane		HMBS	(16) e.g., porphyria, autosomal dominant disease, lichen sclerosus, ocular melanoma
Fourth step	Hem4[c]	?	Uroporphyrinogen III synthase; catalyzes the conversion of hydroxymethylbilane to uroporphyrinogen III		UROS	(32) e.g., porphyria, Dubin-Johnson syndrome, hypertrichosis, arthritis, prostate cancer, sarcoma
Fifth step	Hem12[c,d]	Cytoplasm and nucleus	Uroporphyrinogen decarboxylase		UROD	(16) e.g., porphyria cutanea tarda, siderosis, hepatitis, macrocytic anemia
Sixth step	Hem13[c]	Cytoplasm	Coproporphyrinogen III oxidase; an oxygen-requiring enzyme		CPOX	(16) e.g., porphyria, portal hypertension, Kearns-Sayre syndrome, Melas syndrome, xeroderma pigmentosum group A, ovarian epithelial cancer
Seventh step	Hem14[a]	Mitochondrial inner membrane	Protoporphyrinogen oxidase		PPOX	(8) e.g., porphyria
Ferrochelatase	Hem15[c]	Mitochondrial inner membrane	Ferrochelatase; catalyzes the insertion of ferrous iron into protoporphyrin IX, the eighth and final step in the heme biosynthetic pathway	Heme B	FECH	(17) e.g., porphyria, anemia, hepatitis, porokeratosis
Protoheme IX farnesyltransferase	Cox10[a]	Integral component of mitochondrial membrane	Heme A:farnesyltransferase (heme O synthase); catalyzes the first step in the conversion of protoheme to the heme A prosthetic group required for cytochrome *c* oxidase activity	Heme O	COX10	(8) e.g., cytochrome *c* oxidase deficiency, tubulopathy, myopathyleukodystrophy, Leigh syndrome, or infantile hypertrophic cardiomyopathy
Heme acceptor	Cox15	Mitochondrial inner membrane	Protein required for the hydroxylation of heme O to form heme A, which is an essential prosthetic group for cytochrome *c* oxidase	Heme A	COX15	(15) e.g., Leigh disease, hypertrophic cardiomyopathy, mitochondrial DNA deletion syndrome, cytochrome *c* oxidase deficiency disease
Ferredoxin	Yah1[a,c]	Mitochondrial matrix	Ferredoxin required for formation of cellular iron–sulfur proteins and involved in heme A biosynthesis	Heme A	Adrenodoxin/ FDX	(18) e.g., lung cancer, rickets, muscular atrophy

Table 3 (*continued*)

Heme protein	Protein name	Localization	Molecular function	Heme type	Human ortholog	Human diseases associated with the gene (number of associated diseases)
Ferredoxin reductase	Arh1[c,]	Mitochondrial inner membrane	Ferredoxin reductase, which performs NAD(P)H-dependent reduction of Yah1; required for formation of cellular iron–sulfur proteins and involved in heme A biosynthesis	Heme A	Adrenodoxin reductase/ FDXR	(17) e.g., colorectal cancer, muscular atrophy, prostate cancer
SAM uroporphyrinogen III transmethylase	Met1[c,d]	Intracellular	*S*-adenosyl-L-methionine uroporphyrinogen III transmethylase; involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase; required for sulfate assimilation and methionine biosynthesis; catalyzes also the first step in vitamin B12 biosynthesis		–	–
Ferrochelatase	Met8	?	Bifunctional dehydrogenase and ferrochelatase; involved in the biosynthesis of siroheme, a prosthetic group used by sulfite reductase	Siroheme	–	–

All information listed is available in [112–115,210–213], and the reader is referred to these sources and the references therein for further details. Additional data have been published in [250,251,253].?, function/localization unclear or not yet analyzed.

[a] Haploinsufficient.
[b] Mitochondrially encoded.
[c] Essential for viability.
[d] Viability depends on the genetic background.

when the disruption of iron homeostasis is accompanied by an increase in mitochondrial Isu proteins. Studies indicate that the Isu1 and Isu2 proteins and the iron–sulfur pathway can donate iron to Sod2 to cause enzyme deactivation [257], ROS elevation, genotoxic stress, and an elevated mutation rate [258].

Changes in the abundance of proteins involved in mitochondrial ISC synthesis (e.g., Isu1, Yfh1, or Ssq1) modulate expression of the iron-sensing transcription factor Yap5, which is responsible for the elevated expression of the *CCC*1 gene, encoding a vacuolar iron importer that responds to increased cytosolic iron. ISCs themselves are sensors for both the high and the low iron transcriptional responses. The deletion of cytosolic glutaredoxins or the loss of activity of the cytosolic ISC assembly complex proteins did not reduce the expression of Yap5 target genes [259]. Thus, mitochondrial ISC regulates transcription of the iron regulon and iron uptake [260].

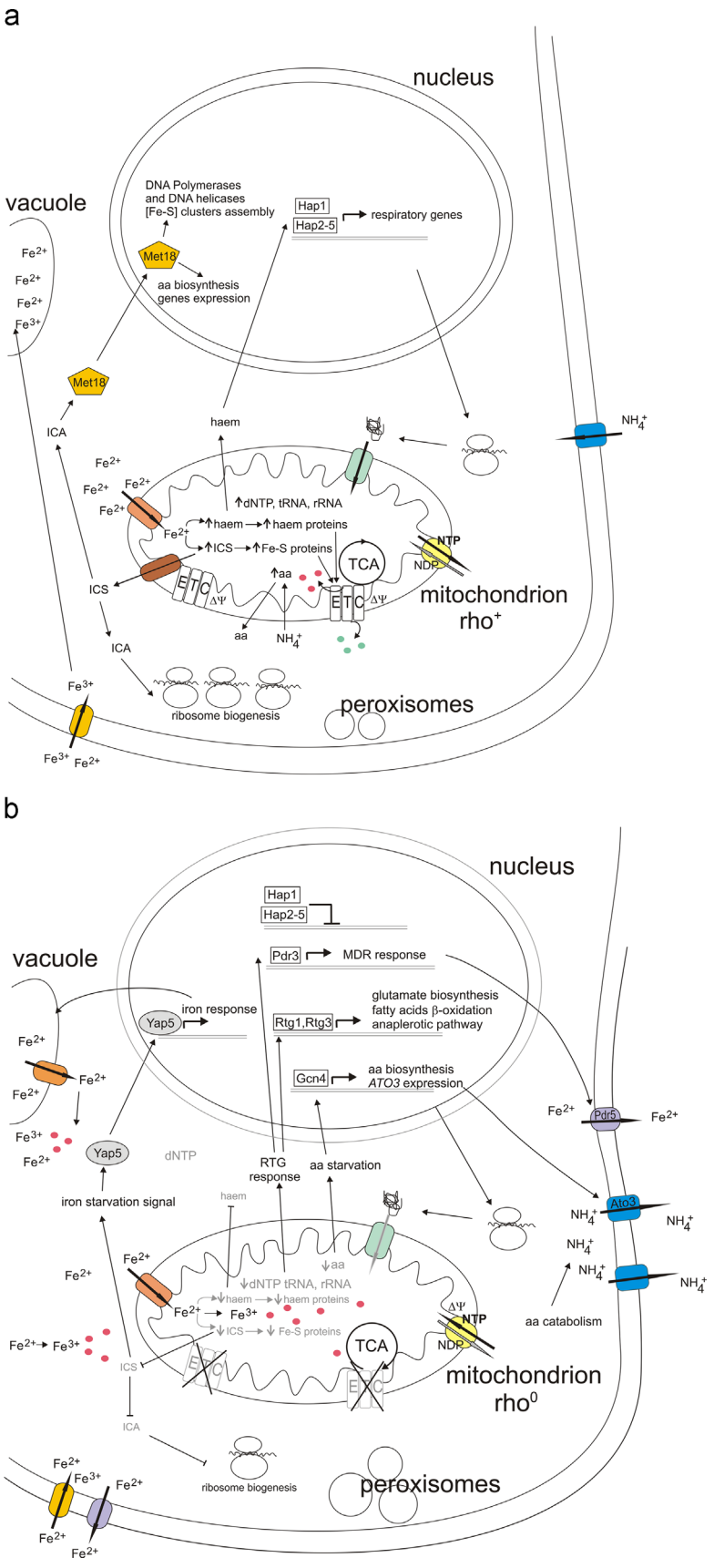
Detoxification and the stress response capacity of mitochondria prevent genotoxic stress and thereby help preserve the genome

Mitochondria have traditionally been recognized as the organelles that produce the energy required to drive the molecular processes of living cells. However, mitochondria have attracted more attention recently as the most important cellular source of free radicals (the primary free radicals O_2^- and nitric oxide (NO), as well as the termination products H_2O_2 and peroxynitrite ($ONOO^-$)), as the main target for free radical regulatory and toxic actions, and finally, as the source of signaling molecules that regulate the cell cycle, proliferation, and apoptosis [261]. To minimize the potentially harmful effects of ROS in mitochondria, a network of antioxidants has evolved that consists of the enzymes superoxide dismutase (Sod2, Sod1), glutathione peroxidase (Gpx1, Gpx2), and peroxiredoxin with thioredoxin peroxidase activity (Prx1), as well as the reductants $NADH_2$, ubiquinol, and reduced glutathione [262–267]. The effective cooperation of antioxidants in this network is crucial to guard mtDNA against ROS-driven damage, to protect mitochondrial function, and, consequently, to maintain the nuclear genome. However, not only mtDNA is threatened when this antioxidant protection fails, but mitochondrion-generated ROS can cause damage in nuclear DNA as well.

The generation of mitochondrial ROS can sometimes be provoked by growth conditions. Ethanol accumulation during fermentation contributes to toxicity in *S. cerevisiae,* impairing viability and fermentative capacity. However, it has recently been shown that ISC mutants show an impaired tolerance to ethanol. These mutants, the *ssq*1Δ, *isa*1Δ, *iba*57Δ, and *grx*5Δ strains, display increased ROS generation, mainly hydrogen peroxide and superoxide, when ethanol accumulates to a toxic concentration during fermentation or upon ethanol treatment. A decreased GSH/GSSG ratio, increased catalase activity, and increased cytochrome *c* release from the mitochondria are observed in the ISC mutants, suggesting that apoptosis is triggered in response to ethanol stress [268].

The mitochondria are exposed to ROS produced locally, and mitochondria are a primary target of cadmium-induced oxidative stress. Prx1 is required to protect against mitochondrial oxidation and

Fig. 1. Mitochondria–nucleus network of *rho*$^+$ and *rho*^{0} cells. Diagrams illustrating the link between mitochondria and the nucleus (A) when the mitochondrial compartment harbors DNA or (B) when it has lost DNA. (A) In *rho*$^+$ cells, the TCA cycle is complete, and the mitochondrial ETC functions properly. The latter process enables amino acid and dNTP biosynthesis, ATP production, and generation of the membrane potential ($\Delta\Psi$), which is necessary for mitochondrial transport and the synthesis of prosthetic groups, such as heme or ISC. Heme stimulates nuclear-encoded respiratory gene expression. The ISC level influences iron homeostasis and is a component of various essential protein complexes, e.g., ribosomes, DNA polymerases, and crucial enzymes from various biosynthetic pathways. The ETC generates ROS, which play two opposite roles as cellular destructors or as signaling molecules to trigger stress defense. (B) In *rho*^{0} cells, the ETC is dysfunctional, and subsequently, the TCA cycle is interrupted and amino acid and dNTP synthesis is impaired. A starvation signal from the mitochondrion induces a retrograde response that launches mechanisms permitting the cell to survive, e.g., peroxisome proliferation (to supplement the interrupted TCA), alternative amino acid synthesis pathways, and anaplerotic pathways. Iron homeostasis disturbances causing oxidative stress via the Fenton reaction and impaired function of DNA polymerases and helicases, which are needed to repair the DNA lesions, are responsible for replication fork blocks and DNA rearrangement events in the nuclear genome. Metallotoxicity and/or oxidative stress trigger the MDR response. A high intracellular level of ammonium, which can induce apoptosis, is lowered by the activity of overproduced cognate carriers. The diagram presents a simplified model for clarity.


a
nucleus
vacuole
DNA Polymerases
and DNA helicases
[Fe-S] clusters assembly
Hap1
Hap2-5
respiratory genes
Met18
aa biosynthesis
genes expression
Fe2+
Fe3+
ICA
haem
NH4+
↑dNTP, tRNA, rRNA
↑haem → ↑haem proteins
↑ICS → ↑Fe-S proteins
TCA
NTP
NDP
ETC
ΔΨ
↑aa
aa
ICS
mitochondrion
rho+
ribosome biogenesis
peroxisomes
b
nucleus
vacuole
Hap1
Hap2-5
Pdr3
MDR response
glutamate biosynthesis
fatty acids β-oxidation
anaplerotic pathway
iron response
Yap5
Rtg1,Rtg3
Gcn4
aa biosynthesis
ATO3 expression
Pdr5
Ato3
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RTG
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aa starvation
iron starvation signal
haem
↓aa
↓dNTP tRNA, rRNA
↓haem → ↓haem proteins
↓ICS → ↓Fe-S proteins
aa catabolism
mitochondrion
rho0
ribosome biogenesis
peroxisomes

plays a specific role in mediating cadmium tolerance [267]. The first step of catalysis performed by all peroxiredoxins results in the oxidation of a conserved peroxidatic cysteine residue to sulfenic acid. However, although most peroxiredoxins can be reactivated via the mitochondrial thioredoxin system Trx3/Trr2, Prx1 is reactivated by the glutathionylation of the catalytic cysteine residue and subsequent reduction by the thioredoxin reductase Trr2 coupled with glutathione.

Because mitochondria play an active role in the biosynthesis of many indispensable compounds and are invaluable in protecting the cell against various stresses (oxidative, heavy metal, acidic, osmotic, or heat shock stress [269]), a system that allows cells to survive mtDNA destruction must exist. In fact, such a system does exist. *rho*^{0} cells respond to the loss of genetic information encoded in mtDNA with the so-called retrograde response. This program of nuclear gene expression comprises the expression of genes involved in various cellular processes: (1) metabolism, e.g., *CIT*1, *CIT*2, *ACO*1, *IDH*1, and *IDH*2 to ensure glutamate biosynthesis, genes encoding peroxisomal proteins (including enzymes of fatty acid β-oxidation that would lead to increased acetyl-CoA production), and genes involved in other anaplerotic pathways [15]; (2) multidrug resistance (MDR); (3) sphingolipid biosynthesis [196,270]; and, finally, (4) ammonium export [271]. The mitochondrial retrograde signaling pathway is mediated by four positive regulatory factors, Rtg1, Rtg2, Rtg3, and Grr1, and four negative regulatory factors, Mks1, Lst8, and two 14-3-3 proteins (Bmh1 and Bmh2). However, few retrograde-responsive genes exhibit RTG-independent expression. Instead, their transcription is regulated by Pdr3 (e.g., *PDR*5 encoding the ABC transporter, which is involved in multidrug and cation resistance and cellular detoxification) or Gcn4 and Ssy1 (e.g., *ATO*3 encoding the ammonium transmembrane transporter). The role of sphingolipids and ammonium is important during cellular exposure to various stresses. Recent work has implicated sphingolipid synthesis and signaling as major mediators of iron toxicity in yeast cells. Myriocin treatment or overexpression of the negative regulator Orm2 blocks sphingolipid synthesis and leads to iron resistance. High-iron conditions that cannot be resolved easily in *rho*^{0} cells, in which ISC synthesis is disturbed owing to ETC failure and subsequent mitochondrial membrane potential loss, result in the upregulation of sphingolipid synthesis. Increasing sphingolipid levels by inactivating Orm2 exacerbates the sensitivity to iron. This toxicity is mediated by sphingolipid signaling. Inactivation of the sphingolipid-activated protein kinases (Pkh1 and Ypk1) and the transcription factor Smp1 enhances the resistance to high-iron conditions [272]. The need for ammonium export arises in *rho*^{0} cells for two reasons: (1) a defect in ammonium assimilation in those cells leads to cellular ammonium excess [271] and (2) excess ammonia stimulates programmed cell death [273]. Thus, to survive, a cell must be able to eliminate ammonia. These results agree with data obtained for mammalian cells, in which ammonia provokes autophagy [274,275].

Apoptosis failure leads to genome instability

Mitochondria play a dual role in cell death. On one hand, various mitochondrial dysfunctions can lead to apoptosis. Several ETC dysfunctions mimic apoptosis via ROS production and cytochrome *c* release. Zdralević et al. stated that programmed cell death and retrograde signaling pathways are the two major intracellular pathways by which yeast cells respond to mitochondrial dysfunction [269]. On the other hand, healthy mitochondria are necessary to execute apoptosis when required by cellular systems. Apoptosis is triggered when the harmful effects of cellular stress fail to permit further healthy existence of the cell. When genomic DNA is subjected to stress, causing severe damage leading to replication block and subsequent mitotic arrest, the death of the affected cell is not a simple repercussion but rather the mandatory event, and this process is called mitotic catastrophe. The cells that escape execution enter endless division problems that result in either senescence culminating in cell death or continued aberrant divisions that result in aneuploidization and often cell death because certain essential genes are missing. Another outcome is mitotic slippage or cytokinesis failure, which results in polyploidization [276]. Both aneuploidy and polyploidy are common phenotypes of various types of cancer cells [277]. Vitale et al. stated that the avoidance of mitotic catastrophe may represent one of the gateways to malignant transformation [276]. Cellular death via mitotic catastrophe, regardless of whether it is enforced by apoptotic, autophagic, or necrotic cell death pathways, has been detected in yeast cells exposed to various endo- and exogenous stresses [278].

Yeast apoptosis shares most of the morphological and biochemical hallmarks of mammalian apoptosis, with phosphatidylserine externalization to the outer layer of the cytoplasmic membrane, DNA fragmentation, chromatin condensation, the involvement of specific proapoptotic proteins (e.g., Aif1 and Bxi1/Ybh3), and the mitochondrion-dependent cell death pathway. This final pathway includes increased ROS production, loss of the $\Delta\Psi$, the appearance of dysfunctional mitochondria, cytochrome *c* release, and the opening of the outer mitochondrial membrane voltage-dependent anion channel reviewed in [261, 279, 280]. We mention a few of the mitochondrial proteins engaged in apoptosis. The referenced papers represent the latest additions to a lengthy apoptosis bibliography.

A recent report showed that Ndi1, a mitochondrial NADH:ubiquinone oxidoreductase that transfers electrons from NADH to ubiquinone in the respiratory chain and has a function similar (though not identical) to that of complex I from higher eukaryotes, is transformed into an apoptosis inducer after various apoptotic stimuli, including H_2O_2, Mn, and acetic acid stresses. The proapoptotic function of Ndi1 relies on its proteolytic cleavage. Under stress conditions, the N-terminal part of the protein that sequesters its toxicity is cleaved. This cleavage activates a new function of this enzyme and permits its escape from the mitochondria to execute its apoptotic function. Therefore, cytochrome *c* is not the only protein that is released from the mitochondria to promote apoptosis [281].

In addition, the Fe–S protein Tah18 is involved in apoptosis. The *TAH*18 gene was previously identified as a synthetic lethal with a conditional mutation in the catalytic subunit of DNA polymerase δ [282]. Later, Tah18 was shown to be involved in the oxidative stress response and to translocate to mitochondria after exposure to stress [102]. Tah18 exhibits NADH and NADPH oxidoreductase activity. The protein has a single molecular function and operates on different substrates depending on changes in the environmental conditions. Therefore, under normal, nonstressful conditions, Tah18 contributes to the early step in cytosolic Fe–S protein assembly by transferring electrons from NADPH to the ISC of Dre2 [228]. Although the Dre2–Tah18 complex is involved in iron reduction, the release of bound iron from Dre2 might be affected by its interaction with Tah18, resulting in an increased production of hydroxyl radicals via the Fenton reaction. Under oxidative stress conditions, Tah18 does not operate on Dre2 (the antiapoptotic protein) but has deleterious effects on mitochondria and promotes cell death [102]. Under high-temperature stress, Tah18 mediates NO synthesis to confer heat shock tolerance to yeast cells [283]. Notably, Dre2, the intramitochondrial partner of Tah18, was directly implicated in genome maintenance because a strain carrying a conditional mutation in the *DRE*2 gene displays a mutator phenotype [197]. However, this mutator phenotype might be linked to another Dre2–Tah18 complex function. The Dre2–Tah18 complex plays a critical role in the biosynthesis of the radical cofactor diferric-tyrosyl, an iron-containing group that is necessary for RNR activity. The depletion of Dre2 affects the transcription of *RNR* genes and the turnover of their mRNAs via the following factors: (1) DNA damage checkpoint activation depending on Mec1, Rad53, and Dun1 and (2) the Aft1/

Aft2-controlled iron regulon. Therefore, cells with diminished Dre2 levels show significantly reduced dNTP levels [284].

Mitochondrial genome maintenance ensures functional mitochondria and contributes to nuclear genome stability

The finding that preserving functional mitochondria influences nuclear genome integrity has been thoroughly documented in previous parts of this paper, and the mechanisms ensuring mitochondrial genome stability were also extensively discussed in a separate section (Fig. 1).

In the concluding remarks, we highlight one more example of an extraordinary and unexpected connection between the mitochondrial and the nuclear genomes. DNA fragments that originate from noncontiguous regions of the mitochondrial genome were transferred during DSB repair to yeast nuclear chromosomes of haploid mitotic cells. The subsequent detailed mitochondrial genome analysis indicated that the yeast nuclear genome contains several short sequences of mitochondrial origin with a size and composition similar to those of the strands used to repair DSBs. These sequences were found primarily in the noncoding regions of the chromosomes. These data suggest that the mitochondrial genome can act as a source of "curative" DNA in the nuclear genome, and from an evolutionary perspective, this process could explain the slow movement of mitochondrial genome fragments into the nuclear DNA [285].

Conclusions

In our review, we summarized convincing data demonstrating that mitochondria are an indispensable cellular compartment for preserving the intact genome of the cell. Experimental data suggest a variety of mitochondrial functions: (1) respiration and ATP delivery, (2) biosynthesis of amino acids, (3) biosynthesis of nucleotides, (4) prosthetic group production (ISC and heme), (5) metal homeostasis, (6) stress signaling, (7) stress defense, and (8) apoptosis execution. These functions are involved in genome maintenance processes, and their proper execution is crucial to prevent genome instability (Fig. 1). However, the mitochondrion, with its ability to produce ROS, is uniquely exposed to oxidative damage. We described how many cellular resources are engaged in maintaining the proper functioning of mitochondria, including their own genome preservation. This exciting entangled two-way network maintains cellular homeostasis, links metabolism with genome stability, and ultimately maintains the balance between life and death. We expect new data will appear in the near future to identify other interesting connections between mitochondria and the nuclear genome that link metabolism with cell cycle progression or arrest and consequent cell proliferation or apoptosis. These connections may eventually help elucidate the association of mitochondrially generated ROS with neurodegenerative diseases, such as Parkinson and Alzheimer diseases, as well as the association of mitochondrial disorders with various cancers and other pathologies. Finally, knowledge of mitochondrion–nucleus interconnections will provide new insights into the aging process and assist in determining how increasing environmental metal pollution interferes with mitochondrial function and cellular homeostasis.s

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