Regulation of sporulation in the yeast *Saccharomyces cerevisiae*

Iga Piekarska¹, Joanna Rytka¹ and Bozenna Rempola²*

¹Department of Genetics, ²Department of Biophysics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warszawa, Poland

Sporulation of the budding yeast *Saccharomyces cerevisiae* — equivalent to gametogenesis in higher organisms, is a complex differentiation program induced by starvation of cells for nitrogen and carbon. Such environmental conditions activate coordinated, sequential changes in gene expression leading to production of haploid, stress-resistant spores. Sporulation comprises two rounds of meiosis coupled with spore morphogenesis and is tightly controlled to ensure viable progeny. This review concerns the regulation of differentiation process by nutritional and transcriptional signals.

**Keywords:** sporulation, transcriptional regulation, meiosis, *Saccharomyces cerevisiae*

Received: 04 March, 2010; revised: 11 June, 2010; accepted: 08 September, 2010; available on-line: 15 September, 2010

**INTRODUCTION**

Cells of the yeast *Saccharomyces cerevisiae*, being non-motile, depend on the availability of nutrients in their immediate surroundings. In the presence of adequate nutrient supply, regardless of their ploidy, the cells follow the mitotic cycle and proliferate by budding, giving rise to next generations of cells. In the event of complete depletion of essential nutrients, haploid cells enter a dormant stationary phase whereas diploid cells initiate a differentiation pathway called sporulation (reviewed in Kupiec et al., 1997). Two overlapping processes, meiosis and spore morphogenesis, make up the complex developmental program of sporulation (reviewed in Esposito & Klapholz, 1981; Kupiec et al., 1997). Meiosis, reducing the ploidy of the cells, plays a central role in the sexual cycle. It is preceded by a prolonged meiS phase (pre-meiotic S phase) during which a single round of DNA replication occurs. In the next stage, meiotic prophase I, homologous chromosomes pair and exchange DNA through recombination. Following the recombination, the cells undergo two rounds of nuclear division, meiosis I (MI) and meiosis II (MII), leading to the formation of four haploid nuclei. Unlike higher eukaryotes, yeast cells undergo closed meiosis, meaning that the nucleus is surrounded by an intact nuclear envelope throughout the process. The second major event of sporulation is spore morphogenesis, when the four haploid nuclei are packaged into spores which are able to survive for a long time and which protect the genome from damage induced by chemical or physical stress (Smits et al., 2001). The process of spore morphogenesis requires two events. Firstly, prospore membranes (PSMs) are generated around the haploid nuclei to form prospores. Secondly, the newly formed prospores are surrounded by a protective, stress-resistant spore wall. In favorable conditions, each of the spores can germinate and enter the mitotic cell cycle. They can proliferate as haploids or may fuse with cells of the opposite mating type to regenerate diploid cells.

In this review we present state of the knowledge about regulation of sporulation in the yeast *Saccharomyces cerevisiae*, including mechanisms controlling repression of sporulation during vegetative growth and the nutritional and transcriptional signals determining sporulation events.

**SIGNALS CONTROLLING INITIATION OF SPORULATION IN YEAST**

Induction of the sporulation program is a complex and precisely controlled pathway. The cell’s decision to initiate sporulation is regulated by genetic and nutritional signals which in *S. cerevisiae* are composed of: the type of cell, nutrient availability, and respiration (reviewed in Petronczki et al., 2003; Esposito, 2006). The genetic signal determining cell type comes from the mating type (*MAT*) alleles. The ability to sporulate requires the presence of both *MATa* and *MATα* alleles. Thus, only diploid cells that express *MATa* and *MATα*, whose products form the a1/α2 heterodimer, are able to sporulate. The decision to begin sporulation is also controlled by nutritional conditions. Starvation for nitrogen causes cell cycle arrest in the G1 phase, which is necessary for the initiation of sporulation (Hirschberg & Simchen, 1977). The absence of a fermentable carbon source, such as glucose, is also required. Even low concentrations of glucose in the environment (0.2–0.5%) repress sporulation initiation, also under conditions of nitrogen depletion (Honigberg & Purnapatre, 2003). The third nutritional signal that controls the entry into sporulation is the presence of a non-fermentable carbon source, such as acetate, that can be metabolized by respiration. Respiration is required both as a signal for sporulation initiation and as a source providing energy for the subsequent processes (Jambhehar & Amon, 2008). While a non-fermentable carbon source is needed only during the early stages of meiosis, prior to meiotic nuclear division, respiration is necessary throughout meiosis (Jambhehar & Amon, 2008). Therefore, cells must be respiratorily efficient to execute the developmental sporulation program.

Abbreviations: APC, anaphase-promoting complex; EMG, early meiotic gene; IRE, internal regulatory sequence; MAPK, mitogen-activated protein kinase; MMG, middle meiotic gene; MSE, middle sporulation element; NRE, negative regulatory element; PKA, protein kinase A; PSM, prospore membrane; SC, synaptonemal complex; SPB, spindle pole body; UAS, upstream activation sequence; UCS, upstream controlling sequence; URS, upstream repression sequence

---

*e-mail:* bozenna@ibb.waw.pl
The genetic and environmental signals activate a cascade of regulatory proteins resulting in coordinated, sequential changes in the expression of genes involved in various sporulation-specific processes. Microarray analyses have identified more than 1000 yeast genes that are transcriptionally regulated specifically during sporulation. About half of them are down-regulated and half up-regulated during different stages of sporulation (Chu et al., 1998; Primig et al., 2000). Based on the time of their induction, sporulation-specific genes have been classified into three main sequential groups: early, middle, and late genes (Mitchell, 1994; Fig. 1). The time at which a particular gene is expressed is closely correlated with its function. Immediately after the transfer of cells to sporulation medium not only expression of genes involved in sporulation is induced but also that of genes engaged in stress-response metabolic functions (i.e., response to nitrogen and carbon starvation, amino acid biosynthesis and mitochondrial functioning) (Primig et al., 2000). Early sporulation-specific genes are expressed at the beginning of meiotic prophase I, middle genes are transcribed at later stages of meiosis and during initiation of spore morphogenesis, while the expression of late genes is induced during spore formation. Members of each class of sporulation genes have common regulatory sequences within their promoters and are controlled by the same transcription factors; this determines their co-expression and ensures the correct order of activation of subsequent sets of sporulation-specific genes (Mitchell, 1994; Vershon & Pierce, 2000). The set of early genes is induced by Ime1 (Inducer of Meiosis 1) (Kassir et al., 1988; Smith et al., 1993). The major regulator of middle genes is Ndt80p which also autoactivates its own expression (Chu et al., 1998; Chu & Herskowitz, 1998; Pak & Segall, 2002; Pierce et al., 2003). The factors required for the activation of the late genes remain unknown. Each group of genes involved in the sporulation process is repressed in vegetative cells as well as during the remaining parts of the sporulation process.

REPRESSION OF SPORULATION PROGRAM DURING VEGETATIVE GROWTH

The sporulation program is efficiently repressed in the presence of essential nutrients, when cells can divide mitotically. Furthermore, cells undergoing early stages of meiosis may return to vegetative growth if nutrients are provided. These cells complete early meiotic processes, such as replication and recombination, but then they switch to mitotic chromosome segregation and produce two diploid cells (Zenwirth et al., 1997; Friedlander et al., 2006). However, return to growth (RTG) is possible only before a specific stage of sporulation has been reached. This stage of irreversibility, called “commitment to meiosis” (Simchen et al., 1972, Friedlander et al., 2006), occurs after the events of prophase I but before the beginning of the first nuclear division. After this point, cells continue the sporulation process even if nutrients appear in the environment (reviewed in Friedlander et al., 2006; Simchen, 2009). Abandoning the sporulation program at later stages, when serious changes in chromatin structure, cell morphology and cell wall composition have occurred, could be lethal for the cell (Friedlander et al., 2006).

The expression of sporulation-specific genes during vegetative growth is prevented by several repressive mechanisms. These mechanisms are different for early, middle, and late genes. The central component controlling the repression of genes expressed early in the meiotic transcriptional program is a DNA binding protein, Ume6p (Unscheduled Meiotic Gene Expression 6) (Strich et al., 1994; Anderson et al., 1995; Steber et al., 1996). It associates with the Upstream Repression Sequence 1 (URS1) present in the promoters of most early genes and also in genes involved in metabolic responses to nutritional conditions (Strich et al., 1994; Anderson et al., 1995, Williams et al., 2002; Fig. 2A). Ume6p ensures that the cells respond to environmental changes by turning catabolic and anabolic genes on and off, respectively, and it also switches on the genes engaged in initiation and progression of meiosis (Williams et al., 2002). Ume6p activity involves the recruitment of two independent co-repressor complexes. One contains Sin3p and Rpd3p (Kadosh & Struhl, 1998) and the other contains a subunit of a chromatin remodeling complex, Isw2p (Goldmark et al., 2000; Fazzio et al., 2001). The interaction between Ume6p and Sin3p — which forms a complex with the histone deacetylase Rpd3p — leads to local deacetylation of chromatin and to repression of transcription by blocking the functioning of activator proteins (Vershon & Pierce, 2000). Independently of the
Sporulation. Ime1p, the master inducer of sporulation

The integration of the genetic and nutritional signals indicating that the cell is diploid and starved for both a fermentable carbon source and nitrogen causes expression of the transcription factor Ime1p. Ime1p, the key regulator of sporulation, switches on a transcriptional cascade of sporulation-specific genes. The promoter of IME1 is extremely large compared to other yeast genes (2.1 kb) and contains both positive and negative regulatory elements (Sagee et al., 1998). It is divided into four Upstream Controlling Sequences, named UCS1–4, which directly influence IME1 transcription (Fig. 3). UCS2 consists of seven elements: three Upstream Activation Sequences (UASv, UASrm, UASru), two Internal Regulatory Sequences (IREu and IRED), and two Upstream Repression Sequences (URSu and URSd). UCS1 and UCS2 respond to nutritional conditions while UCS3 and UCS4 are required for cell-type control.
The cell-type signal is transmitted to UCS4 via Rme1p (Covitz & Mitchell, 1993), whereas the proteins that mediate the MAT signal via UCS3 are unknown. In haploid cells, Rme1p binds to an Rme1 Repressor Element (RRE) within UCS4 to repress IME1 transcription. In diploid MATa/MATα cells, the cell-type specific α1 and α2 proteins form a heterodimer which binds to regulatory sites in the promoter of RME1 to repress its transcription (Covitz et al., 1991; Herskovitz et al., 1992). The absence of Rme1p derepresses IME1 expression, allowing induction of sporulation. The Rme1p-dependent pathway is not the only mechanism whereby the α1/α2 heterodimer stimulates IME1 and sporulation. The α1/α2 proteins also regulate expression of the IME4 gene which encodes for putative RNA methyltransferase (Clancy et al., 2002) required for the induction of IME1 (Shah & Clancy, 1992). Depending on the cell type, yeast express different IME4 transcripts (Hongay et al., 2006). Haploid cells produce non-coding, antisense IME4 RNA, because its promoter is stronger than the sense one (Govin & Berger, 2009). In diploid cells, the antisense transcription is blocked by the α1/α2 heterodimer which binds downstream of the IME4 ORF to allow sense transcription. The sense transcript codes for the Ime4 protein which activates the expression of IME1. However, the region in the IME1 promoter which responds to Ime4p and the mechanism of Ime1p activation by Ime4p are unknown.

The nutritional signals that regulate IME1 expression are substantially more complex than the regulation by cell type. Several of the IME1 promoter elements function as activator and represser sites sensitive to the availability of nitrogen and to the type of carbon source, such as acetate or glucose. Nitrogen abundance has a repressive effect on IME1 transcription; this signal is sensed by UCS1 (Matsumoto et al., 1983; Fig. 3). Glucose represses IME1 expression via three distinct elements of UCS2: UASru, UASv and IREu, whereas acetate activates IME1 transcription via UASru, UASrm and IREu (reviewed in Kassir et al., 2003).

**Figure 4. Signaling pathways that regulate sporulation**

Nutritional signals are transmitted by nutrient signaling pathways such as glucose repression, alkaline-sensing, Tor2p, RAS and cAMP-PKA, which regulate expression of the master activator of sporulation Ime1p. Glucose represses IME1 transcription by activation of Sok2p repressor and by inhibition of Msn2p-Msn4p and Snf1p activators as well as by inhibition of respiration — a process indispensable for media alkalization, which inhibits Swm1p repressor. Moreover, glucose and nitrogen repress Rim11p and Rim15p kinases needed for full activation of Ime1p. Nutrient starvation decreases Cln/Cdk activity, allowing IME1 expression and Ime1p transport to the nucleus and, in consequence, activation of IME2 and other early sporulation genes (After Honigberg & Purnapatre, 2003; Santangelo, 2006; Zaman et al., 2008).
The cell’s response to glucose and nitrogen depends strongly on RAS and cAMP-dependent protein kinase A (PKA) (Honigberg & Purnapatra, 2003; McDonald et al., 2009). The nitrogen signal is transmitted to the UGS1 element within the IME1 promoter through Cdc25p — a regulator of the RAS adenylate cyclase pathway — causing repression of UGS1 activity (Matsumoto et al., 1983). Glucose regulates sporulation at three different steps: it controls expression of IME1 and IME2 and manages the entry into later sporulation events (Honigberg & Lee, 1998). Extracellular-glucose sensing depends on the transmembrane proteins Rgt2, Snf3, and Gpr1, and on the Hxt hexose transporters, whereas intracellular sensing occurs through the Gpa2p pathway which works in parallel with the RAS pathway to activate PKA (Santangelo, 2006). Activation of PKA, which depends on glucose and other signals, results in repression of IME1 transcription. PKA phosphorylates the stress response factors Msn2p and Msn4p, thus repressing their function as transcriptional activators of stress-response genes (Garreau et al., 2000). Some of these genes are required for IME1 induction (Honigberg & Purnapatra, 2003). Moreover, Msn2p-Msn4p induces transcription of IME1 directly (Sagee et al., 1998). PKA also regulates the activity of Sok2p which, when phosphorylated, represses the transcription of IME1 (Shenhar & Kassir, 2001). Furthermore, glucose and nitrogen signals negatively regulate the kinases Rim15p and Rim11p which phosphorylate Ime1p and promote the induction of sporulation (Rubin-Bejerano et al., 2004; Zaman et al., 2008). Glucose represses the expression of RIM15 whereas the PKA pathway inhibits the activity of Rim15p and Rim11p through inhibitory phosphorylation. Glucose is also sensed in a PKA-independent manner by the Snf1p kinase. Its activity is required for IME1 and IME2 expression (Honigberg & Lee, 1998) and is inhibited by high levels of glucose. Thus, glucose and nitrogen control IME1 expression both by inactivating an activator and by activating a repressor of this gene. In addition, glucose and nitrogen control Imel1p activity also at a post-transcriptional level. Progression through the sporulation program requires that the sporation-initiating nutritional signals remain present.

The presence of a non-fermentable carbon source, which is metabolized respiratorily, leads to the production of CO2 which is partially dissolved in the medium in the form of bicarbonate ion (HCO3-). Hence, the acetate ions in the medium are partially replaced by HCO3-, which results in accumulation of bicarbonate and in consequence alkalization of the medium (Hay-ashi et al., 1998). The high external pH induces the alkaline-sensing pathway that includes cell surface factors and the transcriptional regulator Rim101p. Activation of Rim101p depends on its proteolytic cleavage by the Rim13p protease (Lamb & Mitchell, 2003). The active form of Rim101p represses Smp1p which inhibits IME1 expression probably by binding to the UASrm site in the IME1 promoter (Zaman et al., 2008). Additionally, medium alkalization causes degradation of the Ume3p-Ume5p cyclin-kinase complex. This degradation is required for efficient induction of IME2 expression (Cooper & Strich, 2002). Thus, respiratory metabolism of a non-fermentable carbon source, leading to medium alkalization, promotes sporulation through expression of both IME1 and IME2.

In summary, the proteins that regulate the action of factors required for the initiation of sporulation are controlled by more than one pathway or factor (Honigberg & Purnapatra, 2003). Furthermore, one pathway can regulate the transcription of a gene whose product can then be controlled on a post-translational level by another pathway.

GENES EXPRESSED EARLY IN SPORULATION PROGRAM

After transfer of the cells to sporulation conditions, more than 200 sporulation-specific genes are expressed (Chu et al., 1998; Vershon & Pierce, 2000). This set of genes is termed early meiotic/sporulation genes (EMGs). The proteins they encode are involved in the earliest stages of sporulation, such as premeiotic DNA replication and prophase I events. Prophase I is composed of four substages. During leptotene, telomeres migrate to the spindle pole bodies (SPBs) — a yeast equivalent of the microtubule organization center — and form the telomere bouquet. Next, homologous chromosomes pair and become synapsed along their entire length by proteinaceous synaptonemal complexes (SCs) assembled during zygotene. At pachytene, recombination is completed, and at diplotene, the SCs are dismantled, however, homologous chromosomes remain joined by chiasmata which form as a result of recombination events. Exit from pachytene is also connected with separation of SPBs and modification of kinetochore. The SPBs, which duplicate before meiosis I, at a time close to the onset of premeiotic DNA replication, separate and migrate to opposite sites of the nucleus at diplotene. The modification of kinetochores causes sister kinetochores to orient toward the same SPB in meiosis I. After these

---

**Figure 5. Transcriptional regulation of early sporulation genes**

(A) Imel1p, a transcriptional activator of early genes, binds to specific elements in promoters of early genes and induces their expression. Efficient activation of EMGs requires chromatin-remodeling complex RSC, histone acetyltransferase Gcn5p, and kinases Rim11p, Rim15p and Mck1p (After Inai et al., 2007 Govin & Berger, 2009). (B) Imel1p activates EMG expression by two different pathways: Imel1p-dependent and Imel2p-dependent (After Mitchell, 1994; Guttmann-Raviv et al., 2002).
processes have been completed, the cell enters the first meiotic division.

Based on the time of their expression, three classes of early genes have been distinguished: early I (62 genes), early II (47 genes), and early-middle (95 genes) (Chu et al., 1998; Vershon & Pierce, 2000). Expression of the early II and early-middle gene sets is slightly delayed compared with the early I set. Additionally, the early-middle genes differ from the early I and II classes in that they are re-induced parallel to the induction of middle genes (Chu et al., 1998). Many of the early I and II genes are implicated in meiotic homologous chromosome centromere pairing, synopsis, recombination, and pachytenic checkpoint functions, whereas early middle genes play roles in spindle pole body dynamics or chromatid behavior (Chu et al., 1998).

Expression of early meiotic genes (EMGs) is initiated by Ime1p (Kassir et al., 1988). Most EMG promoters have a common conserved Upstream Repression Sequence (URS1) element which serves as a repressor site during vegetative growth and as an activator site under sporulation conditions. Ime1p activates the expression of early genes by associating with URS1 via Ume6p, a DNA binding repressor (Strich et al., 1994; Fig. 5A). Efficient induction of EMG transcription additionally requires the chromatin-remodeling machinery such as the chromatin-remodeling complex RSC or Gen5p, a histone acetyltransferase which regulates chromatin organization around the URS1 element (Inai et al., 2007). The chromatin-remodeling complex untangles the repressive chromatin structure maintained by Sin3p–Rpd3p and the Isw2p complex during vegetative growth. Gen5p changes the local chromatin structure and alters nucleosome positioning to allow gene expression (Burgess et al., 1999). Gen5p may also cause modification of the higher chromatin structure, thus facilitating the binding of Ime1p independently of nucleosome positioning (Inai et al., 2007).

URS1 is a relatively weak activator sequence, and therefore other activator elements augment the level of early gene expression (Vershon & Pierce, 2000). Many EMGs have activator segments such as TεC or UAS H, which lie upstream of URS1. Also an Abf1p-dependent regulatory element which binds the general transcriptional activator has been identified in several EMG promoters (Schlecht et al., 2008).

The expression of most early genes requires two different pathways, one Ime1p- and the other Ime2p-dependent (Fig. 5B). However, Ime1p is indirectly necessary for both pathways since IME2 expression is positively regulated by Ime1p (Yoshida et al., 1990). Ime1p and Ime2p switch on many of the same genes. Despite the common targets, stimulation of EMG transcription by the Ime1p and Ime2p pathways occurs through different regulatory sites (Mitchell, 1994). Amplification of Ime1p activity by Ime2p action likely ensures a balanced expression of early meiotic genes engaged in the earliest stages of sporulation (Mitchell, 1994). The Ime1-dependent pathway is related to Ume6p, the kinases Rim11p and Rim15p, and to potentially other kinases such as Mek1p (Xiao & Mitchell, 2000). Rim11p and Rim15p phosphorylate Ime1p and Ume6p and stabilize their association (Vidan & Mitchell, 1997; Xiao & Mitchell, 2000). Several previous reports have indicated that Ume6p can act both as a repressor and an activator of genes expressed early in the transcriptional sporulation program, and its conversion from transcriptional repressor to activator is related to its association with the Ime1p inducer (Bowdish et al., 1995; Rubin-Bejerano et al., 1996; Washburn & Esposito, 2001). However, recent literature data support an alternative mechanism suggesting that Ume6p is degraded in cells transferred from growth conditions to sporulation conditions. Its elimination requires entry into sporulation and is connected with a transient interaction with Ime1p (Mallory et al., 2007).

In the Ime2p-dependent pathway, Ime1p plays only an indirect role by stimulating IME2 expression. Ime2p, a CDK-like protein kinase which has autophosphorylation activity, controls the G1–S transition by decreasing the level of Sic1p, an inhibitor of the Cdc–Cdc28 kinase (a B-type cyclin and the catalytic subunit of the main cell cycle cyclin-dependent kinase, respectively) (Benjamin et al., 2003). Degradation of Sic1p results in the activation of the Cdc28–Cdk5,6 complex which promotes the meiotic G1–S phase transition and premeiotic DNA replication (Mitchell, 1994; Vershon & Pierce, 2000; Kassir et al., 2003; Neiman, 2005). Ime2p not only induces early gene expression but also promotes the S–M phase transition by phosphorylation and activation of Ndt80p, a transcriptional activator of middle sporulation genes (Benjamin et al., 2003), and controls chromosome segregation by regulating the activity of the anaphase-promoting complex/cyclosome (APC/C) (Bolte et al., 2002). Later in the sporulation program, Ime2p terminates EMG expression by phosphorylation of Ime1p which targets it for degradation (Guttmann-Raviv et al., 2002). Thus, IME1 expression occurs in a narrow window relative to Ime2p, and Ime2p plays multiple roles in promoting progression through sporulation.

**GENES EXPRESSED IN THE MIDDLE MEIOTIC PHASE**

When cells exit meiotic prophase I, the early meiotic genes are switched off and transcription of about 160 middle meiotic/sporulation genes (MMGs) is induced (Chu et al., 1998; Vershon & Pierce, 2000). Proteins encoded by MMGs are required for the prophase I to metaphase I transition, for meiotic division and for the initiation of spore morphogenesis. Exit from prophase I is dependent on the kinase activity of the cell cycle regulator Cdc28p, which is promoted by the cyclins Cln1p, Cln3p, Cln4p, Cln5p, and Cln6p (Chu & Herskowitz, 1998). Then, two successive rounds of meiotic division occur. During the first, “reductive” division, named meiosis I, homologous chromosomes are pulled to the opposite spindle pole bodies by microtubules, which are attached to the kinetochores. Sister chromatid centro-meres remain linked by a multiprotein cohesion complex and their kinetochores are attached to microtubules emanating from the same SPB. This type of attachment is termed mono-orientation or syntelic attachment. The second, “equational” division, named meiosis II, resembles a mitotic division although, unlike mitosis and meiosis I, it is not preceded by a round of DNA replication. The cohesion complex, which holds sister centromeres together, is destructed, and sister chromatids segregate to opposite SPBs because their kinetochores are attached to microtubules formed by two different SPBs. This is termed bi-orientation of sister chromatids or amphitelic attachment.

The meiotic division is coupled to spore morphogenesis, resulting in the encapsulation of each of the four haploid nuclei into stress-resistant ascospores. Spore morphogenesis comprises prospose membrane formation
and spore wall maturation, and it is initiated during the second meiotic division. In early meiosis II, the newly duplicated SPBs undergo modifications and their cytoplasmic surfaces — the meiotic plaques — become sites of prospore membrane formation (reviewed in Moreno-Borchart & Knop, 2003; Neiman, 2005). The meiotic plaques recruit vesicles carrying cargo for building of the prospore membranes (PSMs). The vesicles fuse to form the initial prospore membrane caps. In later stages of sporulation, PSMs encapsulate the haploid nuclei and four prospores are formed.

Expression of the middle sporulation-specific genes is activated by the global activator Ndt80p that also positively regulates its own expression (Chu et al., 1998; Chu & Herskowitz, 1998; Pak & Segall, 2002; Pierce et al., 2003). This transcription factor, which is expressed earlier than other MMGs, recognizes and binds to the conserved middle sporulation elements (MSEs) that are present upstream of 70% of the middle genes (Chu et al., 1998; Vershon & Pierce, 2000). The MSE sites in different middle genes function differently. Some MSEs play a double role: during sporulation they function as activator sites whereas during vegetative growth, and also in early stages of sporulation, as repressor sites. In other genes of the middle class, the MSEs are strong activator elements but weak repressor sites (Chu et al., 1998; Vershon & Pierce, 2000). The MSE sites in different middle genes function differently. Some MSEs function differently. Some MSEs positively regulate their own expression (Chu & Herskowitz, 1998). However, their induction is probably still dependent on Ndt80p: either Ndt80p binds to non-canonical elements in MMG promoters or it activates other factors that promote expression of these genes (Chu et al., 1998; Vershon & Pierce, 2000). Although the Ndt80p factor plays an essential role in the activation of middle sporulation genes, the expression of some MMGs may be regulated independently of Ndt80p (Chu et al., 1998). Some genes in the MMG class also contain other positive regulatory elements such as URS1 or an Abf1p-binding site (ABS), which increase the expression level (Vershon & Pierce, 2000; Kassir et al., 2003).

Expression of the transcriptional activator of middle genes Ndt80p is induced by Ime1p and Ime2p (Chu & Herskowitz, 1998; Hepworth et al., 1998; Pak & Segall, 2002). The promoter of NDT80 contains two URS sites, termed URS1 and URS2, and also two MSE sequences called MSE1 and MSE2 (Pak & Segall, 2002). URS1 functions either as a repressor site (under growth conditions) or as an activator site (under sporulation conditions), whereas URS2 functions only as an activator-binding site. The MSEs, similarly to URSs, contribute either to both repression and activation of NDT80, depending on the environmental conditions (MSE1) or only to its activation (MSE2) (Pak & Segall, 2002). Thus, in vegetative cells, transcription of NDT80 is repressed by both Sum1p, that binds to MSE1 sites, and by Ume6p, which binds to URS1 sites (Fig. 6A). In sporulation conditions, when Ime1p promotes transcription of early meiotic genes by transient association with Ume6p, activation of NDT80 by Ime1p-Ume6p is initially prevented by the presence of Sum1p at the MSE1 site (Fig. 6B). Then, the repressor activity of Sum1p is inhibited by newly formed Ime2p allowing Ime1p to promote low levels of NDT80 expression. The newly synthesized Ndt80p competes with Sum1p and activates its own expression by binding to the MSE1 site, leading to high NDT80 transcription (Fig. 6C). Full activity of Ndt80p promotes expression of genes coding for B-type cyclins (Clb1, Clb3-Clb6) and proteins required for the meiotic division and for the initiation of spore formation (Chu & Herskowitz, 1998; Hepworth et al., 1998; Pak & Segall, 2002; Fig. 6D).

GENES EXPRESSED LATE IN MEIOSIS

Late meiotic/sporulation genes (LMGs), have been divided into two classes, based on the timing of their expression (Chu et al., 1998; Vershon & Pierce, 2000). The first group, called middle-late genes, consists of about 61 genes that are required for spore formation. Some genes involved in mitochondrial function or vacuolar morphogenesis and inheritance are also initiated during the middle-late stages of sporulation (Chu et al., 1998). The sec-

![Figure 6. Transcriptional regulation of middle sporulation genes](image-url)
Spore formation comprises building of the prosopore membrane and the spore wall. The prosopore membrane (PSM) is initially formed on the outer plaque of the SPB during the middle stages of sporulation, and at the time of nuclear division it extends to engulf the nuclear lobe by vesicle addition. Vesicular transport plays a critical role in this process (Neiman, 1998; Whitaere et al., 2001; Morisihita & Engebrecht, 2005; Morisihita et al., 2007). When the prosopore membrane surrounds the nucleus, changes in organelle structure occur (Chu et al., 1998). At the termination of meiosis II, each PSM closes off so that each haploid nucleus becomes encapsulated, together with the cytoplasmic material and organelles, within a double prosopore membrane. Between the two layers of the PSMs, the spore wall is assembled de novo (reviewed Kupiec et al., 1997; Neiman, 2005). The mature spore wall consists of four layers in the order from inside to outside: mannan, β-1,3-glucan, chitosan, and dityrosine. The two outer layers ensure the resistance of spores to environmental factors. The four spores are connected by interspore chitosan bridges and are enclosed inside the ascus, which derives its plasma membrane and cell wall from the respective mother cell's components.

While the regulation of the early and middle sporulation-specific genes has been thoroughly examined, that of the late genes is still poorly understood. Many genes of the middle-late subclass contain a middle sporulation-like element (MSE) and a negative regulatory element (NRE), which contribute to repression in growing cells and to activation in sporulation conditions (Friesen et al., 1997; Vershon & Pierce, 2000). Despite the presence of the MSE site, these genes are expressed later than the middle sporulation genes, which is due to the NRE element that likely prevents expression during the middle stages of sporulation (Vershon & Pierce, 2000; Fig. 7A). For the high levels of transcription observed for the middle-late genes in sporulating cells, two additional positive sites are required. One is located between the NRE site and the TATA box, the other is located near the transcription start site (Friesen et al., 1997). The factors that bind to the regulatory sites within the promoters of the middle-late genes and the signals that promote their transcription are unknown. However, several factors required for full expression of these genes have been identified (Vershon & Pierce, 2000; Fig. 7B). One of them is the sporulation-specific mitogen-activated protein kinase (MAPK) Smk1p. Unlike other MAP kinases, transcription of SMK1 is activated by the CDK activating kinase Cak1p (Wagner et al., 1997; Schaber et al., 2002) and is regulated via the meiosis-specific activator of the APC complex Ama1p (McDonald et al., 2009). Regulation through the APC complex plays a role in coupling the completion of meiotic division to sporulation (Oelschlaegel et al., 2005; Penkner et al., 2005). Expression of Smk1p is also controlled by the RAS/cAMP pathway (McDonald et al., 2009), suggesting that nutritional signals regulate both early stages of meiosis and spor formation. Another factor that is required for the expression of middle-late genes is Ssp1p, also a component of the sporulation specific MAPK cascade. However, Ssp1p and Smk1p do not function in a linear cascade (Engebrecht, 2003). Ssp1p regulates trafficking of enzymes that are required for the synthesis of the spore wall (Iwamoto et al., 2005). Activation of middle-late sporulation genes also depends on one of the subunits of the APC complex, Swm1p, but this protein acts independently of the Smk1p-Ssp1p MAP kinase cascade (Ufano et al., 1999).

The transcription of the second subclass of late sporulation-specific genes, similarly to the transcription of many middle-late genes, is up-regulated by the Smk1p-Sps1p kinase cascade and by Swm1p (Vershon & Pierce, 2000; Engebrecht, 2003). However, none of these genes contain an MSE site (Vershon & Pierce, 2000). The delay in their induction, compared to that of the middle-late genes, may result from the action of some additional transcriptional regulators or regulatory elements specific to this small group of genes.

CONCLUDING REMARKS

Sporulation — yeast gametogenesis — is in many respects similar to gametogenesis in mammalian cells. Therefore, it has been subject to comprehensive research for many years. It has proven a suitable model for uncovering the genetic architecture of the meiotic process, for genome-wide studies of the control of gene expression during cell differentiation, as well as for investigating the action of signaling pathways. Data derived from both traditional and genomic-scale genetic screens aiming at the identification of cellular processes that are important for sporulation indicates that the majority of genes critical for meiosis and spore formation encode proteins involved in general cellular processes. These genes are engaged in carbon utilization, autophagy, vacuole targeting, general transcription, and vesicle transport. Significant progress has been made in
understanding how the signaling networks coordinate the differentiation program in yeast but our knowledge is still incomplete.

REFERENCES

Anderson SF, Steber CM, Esposito RE, Coleman JE (1995) Ume6, a negative regulator of meiosis in Saccharomyces cerevisiae, contains a C-terminal Zn2Cys6 binuclear cluster that binds the URS1 DNA sequence in a zine-dependent manner. Protein Sci 4: 1832–1843.


I. Piekarska and others

2010


Steger CM, Esposito RE, Coleman JE (1995) UME6, a negative regulator of meiosis in Saccharomyces cerevisiae, contains a C-terminal zinc-finger cluster that binds the URS1 DNA sequence in a zinc-dependent manner. Protein Sci 4: 1832–1843.


