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# Genome reprogramming during sporulation

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**ABSTRACT When environmental conditions compromise survival, single celled organisms, such as the budding yeast *S. cerevisiae*, induce and complete a differentiation program called sporulation. The first step consists of meiosis, which generates genetic diversity within the eventual haploid cells. The post-meiotic maturation stage reinforces protective barriers, such as the spore wall, against deleterious external conditions. In later stages of sporulation, the spore nucleus becomes highly compacted, likely sharing certain characteristics with the metazoan male gamete, the spermatozoon. The sporulation differentiation program involves many chromatin-related events, including execution of a precise transcription program involving more than one thousand genes. Here, we review how chromatin structure and genome reprogramming regulate the sporulation transcription program, and how post-meiotic events reorganize spore chromatin.**

KEY WORDS: *sporulation, chromatin, compaction, reproduction, development*

Unicellular vegetal and fungal organisms have a restricted choice of growth adaptations to respond to dramatic changes in their environment. Upon nutrient limitation, the budding yeast *Saccharomyces cerevisiae* can switch to a pseudohyphal-type growth, which is characterized by several morphological changes, including invasion of a solid growth media and filamentous growth (Gancedo, 2001). Pseudohyphal growth can be induced by nitrogen limitation, by poor carbon sources such as lactate, or by some organic compounds (Gimeno *et al.*, 1992; Zaragoza and Gancedo, 2000). Pseudohyphal invasion probably reflects the search for nutrients, and escape from a potentially harmful environment (Gimeno *et al.*, 1992; Zaragoza and Gancedo, 2000).

Complete nutrient depletion induces another response, which varies in haploid and diploid yeast. Haploid yeast enter a non-metabolic and quiescent phase, called G0. Diploid yeast undergo a differentiation pathway called sporulation. During sporulation, meiosis generates haploid daughter cells, which then differentiate into stress-resistant spores. In diploid yeast, the choice between pseudohyphal-type growth and sporulation is regulated by the relative abundance of two RNA polymerase II subunits, Rpb4 and Rpb7 (Singh *et al.*, 2007). Rpb4 enhances sporulation efficiency and represses pseudohyphae formation, while Rpb7 acts in reverse (Singh *et al.*, 2007).

Shortly following initiation of sporulation, diploid yeast go through meiosis (Fig. 1). Meiosis has a central role in the sexual reproduction in nearly all eukaryotes, and is critical for generating genetic diversity while conserving a functional genome (Marston and Amon, 2004). The first division deviates from mitotic cell division, in having a prolonged DNA replication phase to allow homologous pairing and recombination during an extended prophase, and disjunction of homologues. In contrast, the second division is similar to mitosis, in that it is short, and it uses the same machinery to disjoin the previously replicated sister chromatids.

The second major event of sporulation is the post-meiotic maturation of spores, when the haploid genome is compacted and protected by the spore wall. This wall is able to ensure spore survival over long times, and protects genome integrity from severe damage induced by chemical or physical stress (Neiman, 2005).

Two main events effect genome programming during sporulation. The first event is execution of a precise transcription program. Upon nitrogen and glucose starvation, the master regulator Ime1 is expressed and induces the expression of early genes encoding the first proteins needed for meiotic S phase (Fig. 1). Secondly, the genome is reorganized during the post meiotic portion of sporulation. During this time haploid spores compact their nucleus, implying a dramatic reorganization of the chromatin structure, including chromatin compaction and germination pre-programming.

*Abbreviations used in this paper:* HAT, histone acetyltransferase; HDAC, histone deacetylase; MSE, middle sporulation element; UAS, upstream activation sequence; UCS, upstream control sequence; URS, upstream repression sequence.

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Several detailed reviews describe transcriptional regulation during sporulation (for more details please refer to Kassir *et al.*, 2003; Kupiec *et al.*, 1997), therefore, we will only briefly review transcription, and instead, focus on genome reprogramming.

## The transcription cascade during sporulation

Approximately 1000 genes are specifically expressed during sporulation (Chu *et al.*, 1998; Primig *et al.*, 2000). Three main groups of genes can be distinguished, called early, middle and late sporulation genes (Chu *et al.*, 1998). However, a more precise evaluation of transcript abundance and timing of expression defines up to 10 clusters of genes (Primig *et al.*, 2000). Each cluster of genes is repressed during vegetative growth and sporulation, except during a limited period of activation.

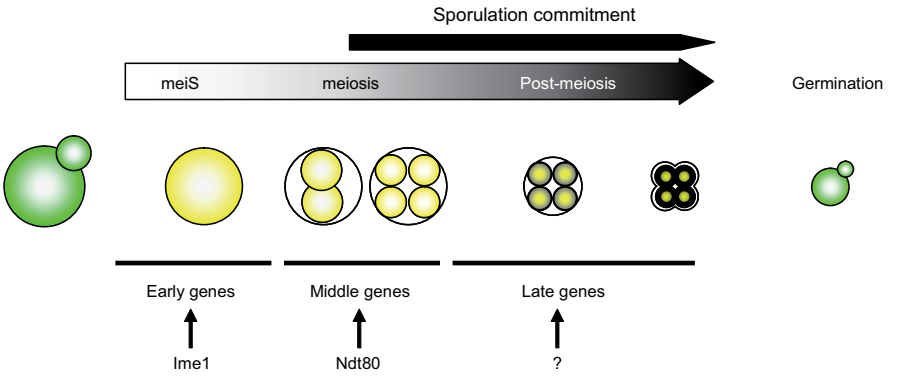


**Fig. 1. Sporulation overview.** *Upon nutrient starvation, diploid yeast ceases vegetative growth (green cells) and induces sporulation (yellow cells). During the meiotic S phase (meiS), the genome is duplicated and prepared for meiosis. Meiosis generates four haploid cells, which differentiate into spores via compaction of the nucleus and the construction of the spore wall. When nutrients become available, spores germinate and begin a new vegetative cycle. Commitment describes a time frame when spores cannot start a new vegetative cycle without finishing the entire sporulation program, even if new nutrients become available. During sporulation, three main classes of genes, called early, middle and late genes, are induced in succession by several key transcription factors (Ime1 and Ntd80). It remains unclear how late genes are induced.*

The early genes are involved in the earliest meiotic events, including meiotic replication, recombination, synaptonemal complex formation, sister chromatid and centromere cohesion during the first prophase. Their induction is controlled by the master inducer Ime1 (Fig. 1).

Middle genes are expressed when cells initiate the first meiotic division. They function mainly in the pachytene checkpoint, including several B-type cyclins, anaphase promoting complex proteins and others factors involved in cell division during meiotic M phase (Primig *et al.*, 2000). The principal regulator of mid-sporulation is Ndt80, which autoactivates its own expression and induces middle gene expression (see Fig 2., Chu and Herskowitz, 1998; Pierce *et al.*, 2003). Late genes are involved in post-meiotic differentiation, and encode proteins required for spore wall maturation.

The timing of gene expression correlates strongly with sporulation functions. Thus, clusters designating unknown genes have been used to provide clues to function, e.g. SPO22 and SPO19 are assigned to meiosis based on their expression pattern (Primig *et al.*, 2000).

## Repression of sporulation during vegetative growth

Diploid yeast must constantly repress sporulation, unless justified by poor environmental conditions. Indeed, initiation of the sporulation program precludes vegetative growth for 24 hours, i.e. for the equivalent of twelve divisions. In other words, a single cell creates four new spores, while several thousand daughter cells might have been generated during the same period. Therefore, sporulation firing is efficiently repressed during vegetative growth and finely controlled under nutrient deprivation. Moreover yeast undergoing early stages of meiosis may return to vegetative growth if nutrients are provided. However, if yeast reach a certain stage of sporulation, they become "committed to sporulate" (Simchen *et al.*, 1972). Commitment occurs after premeiotic DNA replication and recombination, but before meiosis I (Fig. 1). The sporulation process involves critical changes in cell morphology, chromatin structure and cell-wall composition, which may be lethal if abandoned mid-course. Indeed, in the presence of new nutrients, committed cells repress any activation of mitogenic signals to ensure completion of the sporulation program (Friedlander *et al.*, 2006).

### *Sporulation repression in haploid cells*

Repression of sporulation is fundamental to haploid growth, in order to maintain a functional genome. Indeed, ectopic induction of sporulation leads to inappropriate meiosis and production of non-viable cells.

The protein Rme1 represses sporulation in haploid yeast, but also in artificially created diploids that are not appropriate mating types, i.e. MATa/MATa diploids (see Fig. 2B, Kassir and Simchen, 1976). Rme1 inhibits the expression of the master regulator of sporulation, Ime1 (Covitz and Mitchell, 1993). Rme1 binds to two regulatory elements of the *IME1* promoter, named Upstream Control Regions 3 and 4 (UCS3, UCS4, see Fig. 2B and 3; Shimizu *et al.*, 1998). As a repressor, Rme1 modifies the chromatin structure of the *IME1* promoter to prevent the binding of activators. Further, Rme1 recruits Sin4 and Rgr1, which likely modify *IME1* promoter chromatin structure in a repressive manner (Shimizu *et al.*, 1997).

### *Repression of sporulation induced genes during vegetative growth*

During vegetative growth, several repressive mechanisms prevent the expression of the sporulation-specific genes. Interestingly, these repression mechanisms differ for early compared to middle genes. Several early genes are repressed by Ume6 (Fig. 2A), which is a C6 zinc cluster DNA binding protein that binds to an Upstream Repression Sequence 1 (URS1) present in the promoter of numerous early genes (Anderson *et al.*, 1995; Strich *et al.*, 1994). Deletion of *UME6* induces the

expression of early genes as well as non-meiotic genes carrying the URS1 elements in vegetative growth conditions (Bowdish and Mitchell, 1993; Strich *et al.*, 1994). Ume6 interacts with Sin3, which in turn recruits the histone deacetylase Rpd3 to early genes promoters (Fig. 2A, Kadosh and Struhl, 1997). Histone deacetylases (HDAC) are well known repressors of transcription, and are present in key repressor complexes.

Ume6 also recruits Isw2, an ATP-dependant chromatin-remodeling factor. Isw2 recruitment is required for repression of early genes during vegetative growth (Goldmark *et al.*, 2000). Isw2 likely establishes and maintains compact chromatin structure at early gene promoters (Goldmark *et al.*, 2000).

In contrast, middle genes are repressed in haploid and diploid cells by Sum1 (Fig. 2A). More than 50 genes are directly repressed by Sum1 during vegetative growth (Pierce *et al.*, 2003). Sum1 specifically recognizes the Middle Sporulation Element (MSE), a DNA region known to regulate the middle gene class (Pierce *et al.*, 2003; Xie *et al.*, 1999). Sum1 action involves the $NAD^+$ dependant histone deacetylase Hst1, which is closely related to Sir2. The Sum1-Hst1 interaction is mediated by Rfm1, indispensable to Sum1 mediated repression (McCord *et al.*, 2003).

Much less is known about late gene repression. The repressor complex Tup1/Ssn6 is involved in the repression of several sporulation genes, including the late genes *DIT1* and *DIT2* (Fig. 2A), but also the early gene *IME1* (Friesen *et al.*, 1997; Mizuno *et al.*, 1998). Tup1/Ssn6 also recruits HDAC activity as a repression mechanism (for review see Malave and Dent, 2006).

## The master sporulation-inducer *Ime1*

As mentioned above, repression vs. induction of the sporulation program in diploid cells is mainly regulated by nutrient availability. Yeast sense several nutrients, including the quality of the nitrogen or carbon source, but also the presence of phosphate, sulfate, nucleotides and amino acids (Freese *et al.*, 1982; Varma *et al.*, 1985).

Ime1 is believed to be the main inducer of sporulation. *IME1* null mutations prevent detectable expression of all meiotic genes that have been examined, with the exception of *IME4* (see below and for review Kassir *et al.*, 2003; Kupiec *et al.*, 1997). The *IME1* promoter is large (2.1 kb) compared to that of most genes (200-300 bp) in *S. cerevisiae*, and consists of both positive and negative elements (See Fig. 3; Sagee *et al.*, 1998). It is divided into four Upstream Controlling regions, named UCS1 to UCS4 (Fig. 3). UCS2 is itself divided into seven elements: three Upstream Activation Sequences (UASv, UASrm and UASru), two Upstream Repression Sequence (URSd and URSu), and two IRE elements (IREd and IREu). Together, these 10 DNA regions control *IME1* transcription, by integrating signals of the cell ploidy state, and abundance of carbon and nitrogen.

### *Ploidy sensor pathway*

The MAT pathway is a sensor of ploidy and mating type. Diploid MATa/MATα yeast express *MATa1* and *MATα2* genes, whose

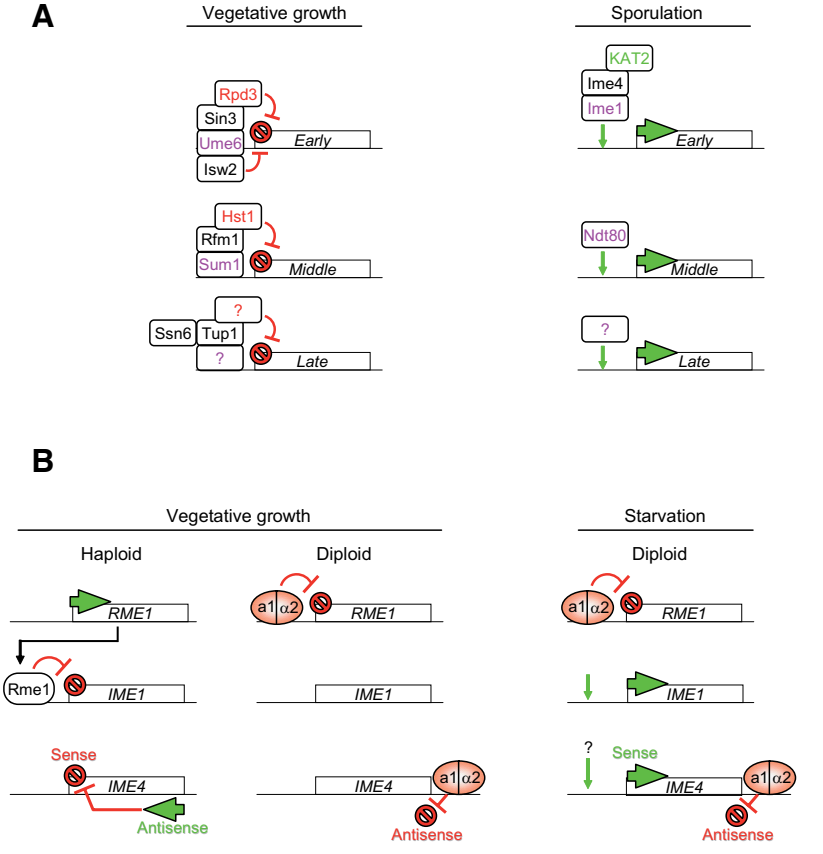


**Fig. 2. Transcription regulation during sporulation. (A)** *Regulation of early, middle and late gene expression. During vegetative growth, the transcriptional repressor Ume6 recognizes a specific sequence in early gene promoters and recruits several complexes essential for their repression. These co-repressors notably include the histone deacetylase Rpd3 and the ATP-dependant remodeling enzyme Isw2. Upon sporulation, Ime1 is responsible for the transcription of early genes. Ime4 and the histone acetyltransferase KAT2/Gcn5 are required for their proper activation. Middle genes are repressed by Sum1, which recruits Rfm1 and the histone deacetylase Hst1. Ntd80 is the main activator of early gene expression during sporulation. Late gene regulation is less clear; repression may involve the co-repressors Ssn6/Tup1. DNA binding proteins are in purple, histone deacetylases are in red, and the histone acetyltransferase is in green. New enzyme nomenclature has been used (Allis* et al., *2007).* **(B)** *Regulation of key transcription factors during sporulation. Rme1 is the main repressor of sporulation in haploid yeast. It recognizes specific elements in the* IME1 *promoter and prevents Ime1 expression. In diploid yeast, the product of the mating type genes MATa1/MATα2 form a heterodimer, which binds to the* RME1 *promoter and prevents Rme1 expression. In diploid cells,* IME1 *is derepressed in vegetative growth conditions and activated upon nutrient starvation. The* IME4 *gene is regulated by competition between a sense and an antisense transcript (Hongay* et al., *2006). The antisense promoter is stronger than the sense one, and antisense transcription inhibits the expression of* IME4 *in* cis *in haploid yeast. In diploid yeast, the a1/α2 heterodimer binds an element downstream of the* IME4 *gene and prevents antisense transcription. During vegetative growth,* IME4 *is derepressed and activated at the beginning of sporulation.*

products form a heterodimer called a1-α2 (Herskowitz *et al.*, 1992). The a1-α2 heterodimer binds to an operator site in the *RME1* promoter and represses transcription (Fig. 2B, Covitz *et al.*, 1991). The absence of Rme1 derepresses *IME1* transcription, allowing sporulation induction upon starvation. Therefore, the presence of this heterodimer is absolutely required for sporulation induction, preventing sporulation in haploid cells, in which meiosis would lead to severe chromosomal abnormalities.

### *Nutrient sensor pathway*

A second pathway regulates sporulation induction, and is based on the nutritional environment. The *IME1* promoter contains several elements sensitive to nitrogen levels and to the type of carbon source, such as glucose or acetate (Fig. 3).

One repressive element is UCS1 in the *IME1* promoter, which responds to nitrogen levels; depletion of UCS1 leads to inappropriate derepression of *IME1* during vegetative growth. In addition, mutants that lack cAMP or AMP dependant-protein kinase activity (PKA) also sporulate in the absence of nitrogen limitation (Matsumoto *et al.*, 1983). The kinase Cdc25, a known PKA and MAP-kinase activator, may transmit the nitrogen signal to UCS1, as repression activity of UCS1 is reduced if *CDC25* is mutated (Matsumoto *et al.*, 1983).

The type of carbon source also regulates sporulation. Glucose represses sporulation, and non-fermentable carbon sources, such as acetate, induce sporulation. The *IME1* promoter senses glucose through UCS2. Three main elements, UASru, UASrm and IREu, repress in the presence of glucose, but activate in the absence of glucose and/or the presence of acetate (Kassir *et al.*, 2003). The positive and negative signaling pathways also likely use the cAMP/PKA pathway (for more details, please refer to Kassir *et al.*, 2003).

To conclude, 10 distinct elements in combinatorial fashion provide the appropriate *IME1* expression pattern (Fig. 3). The elements UCS1, IREu, UASru and UASv integrate several signaling pathways to repress *IME1* transcription in the presence of glucose. In the presence of acetate media, *IME1* is expressed at low levels, resulting from the competition of the repressive action of UCS1, URSu, URSd, IREd and the positive action of UASru, IREu, UASrm and UASv. Upon nitrogen depletion, relief of UCS1 repression promotes the increase of transcription (Kassir *et al.*, 2003).

## *IME1* promoter chromatin structure

Despite the abundance of information on the distinct DNA elements of the *IME1* promoter, and their binding proteins, it is striking that its chromatin structure in repressed or activated states remains uncharacterized. Indeed, chromatin packaging regulates gene access and function during transcription (Li *et al.*, 2007). The chromatin building module, the nucleosome, masks or reveals regulatory DNA sequences to binding proteins. The organization of the chromatin at higher levels also regulates genome accessibility. Thus it is likely that chromatin dynamics regulate the sporulation transcription program.

Histone H2A and H2B abundance regulates *IME1* expression. Sporulation defects occur in strains where expression of H2A/H2B-encoding genes is reduced (Norris and Osley, 1987; Tsui *et al.*, 1997). The limited amount of H2A/H2B lowers *IME1* expression, preventing proper regulation of the transcription cascade and the sporulation program, likely because of perturbation of the fine organization of the *IME1* promoter, rather than by a dramatic change in nucleosome distribution (Hanlon *et al.*, 2003).

We note that most of these data have been generated using a reporter gene under the control of different parts of *IME1* promoter (Kassir *et al.*, 2003). Despite the technical convenience of this approach, it remains questionable whether these DNA constructs reproduce the chromatin state of the endogenous *IME1* promoter. Study of the chromatin structure of the normal *IME1* promoter during sporulation repression and induction may reveal new aspects of its regulation, and explain its sensitivity to H2A/H2B abundance.

Histone variants also modulate transcription (Li *et al.*, 2007). Some sporulation specific genes, such as *DIT1* and *DIT2*, are enriched in H2A.Z during vegetative growth (Raisner *et al.*, 2005). This H2A variant is incorporated into the 5' region of active or inactive genes (Raisner *et al.*, 2005), poising them for rapid induction of transcription (Zhang *et al.*, 2005). Moreover, *DIT1*

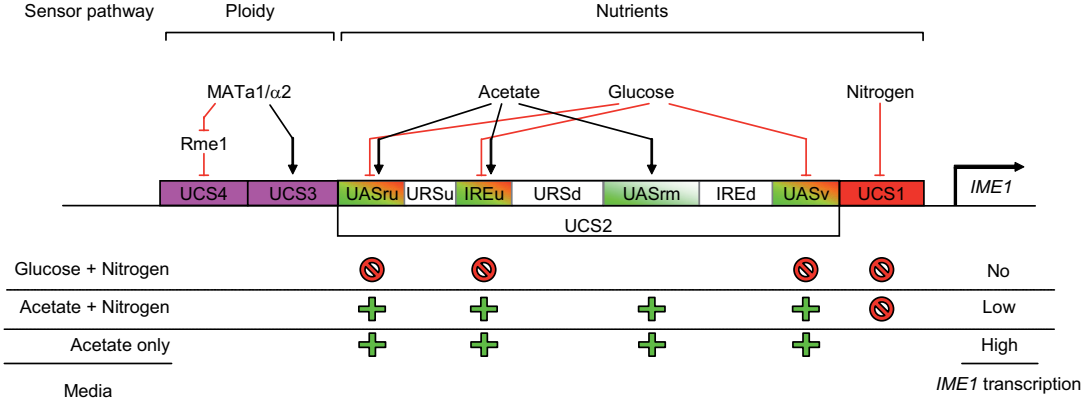


**Fig. 3. *Inducer of meiosis1 (IME1)* promoter regulation.** *The* IME1 *promoter is divided in four Upstream Control Regions, UCS1-4. UCS3 and UCS4 respond to a ploidy control pathway, which prevents sporulation in haploid yeasts. UCS1 responds to nitrogen abundance, and has a repressive effect on* IME1 *transcription. UCS2 responds to the type of carbon source. Glucose represses* IME1 *transcription via the DNA elements UASv, IREu and UASru, whereas acetate activates* IME1 *transcription via UASrm, IREu and UASru. In media containing nitrogen and glucose,* IME1 *is repressed. The balance between UCS2 and UCS1 regions allows a minimal expression of* IME1 *in media containing acetate and nitrogen. In these conditions, the depletion of nitrogen suppresses UCS1 repressive action: sporulation is induced by the overexpression of* IME1. *Red or green elements have respectively repressive or positive effects on* IME1 *transcription. (For more details, please refer to text, Kassir* et al.*, 2003; Kupiec* et al.*, 1997).*

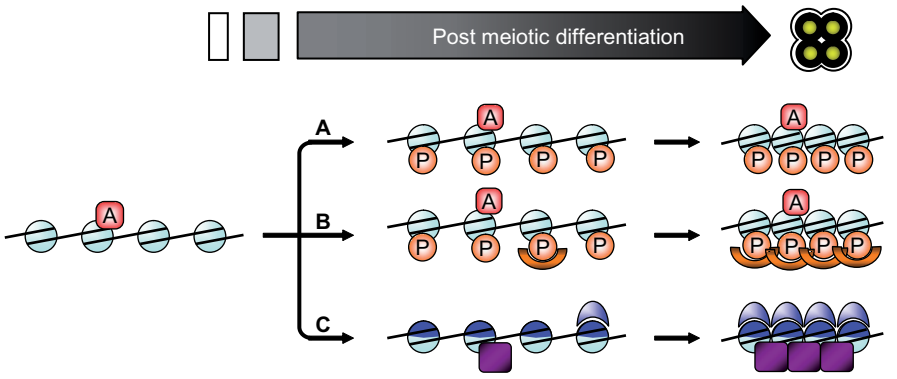


**Fig. 4. Chromatin compaction in spores.** *A model is presented to describe potential mechanisms leading to compaction of chromatin in mature spores. The phosphorylation of serine 1 of histone H4 has been described as a key factor of chromatin compaction (Krishnamoorthy* et al.*, 2006). This modification may directly promote chromatin compaction* **(A)**, *possibly counteracting other 'decompaction' marks (which may persist as epigenetic modifications to specify genes in the next generation of vegetative cells). Certain proteins may recognize H4S1ph to promote chromatin compaction* **(B)**. *Finally, chromatin composition itself may be modified by incorporation of chromatin proteins, such as histone variants (dark blue). These new elements may directly promote chromatin compaction, or may recruit other factors which would achieve the final compaction* **(C)**.

and *DIT2* are repressed by Tup1/Ssn6, which promotes incorporation of H2A.Z during repression of the *GAL1* gene (Gligoris *et al*., 2007). Tup1/Ssn6 is also involved in the repression of *IME1* (Mizuno *et al*., 1998). Two regions present in UCS2 of *the IME1* promoter mediate Tup1/Ssn6 action, but the specific targeting DNA-binding protein is not known (Mizuno *et al*., 1998). Therefore, Tup1/Ssn6 repression of *IME1* could lead to incorporation of H2A.Z, to facilitate rapid transcription activation upon nutrient starvation.

## *IME4* antisense transcription controls sporulation induction

*IME4* is the only sporulation gene whose expression does not depend on Ime1. The mechanism of Ime4 transcription regulation has been revealed recently (Hongay *et al*., 2006), and occurs through a fine balance of sense and antisense transcription (Fig. 2B), expression of which are mutually exclusive. The default transcript is the antisense, as its promoter is stronger than the sense promoter. Therefore haploid yeasts generously express the antisense transcript and do not express Ime4 protein. In diploid cells, the a1/$\alpha$2 heterodimer binds a consensus motif located downstream of *IME4* ORF, and blocks antisense transcription (Fig. 2B). Thus, under nitrogen starvation, *IME4* sense transcription can be induced (Hongay *et al*., 2006). This mechanism uncovers a new way of controlling cell fate in *S. cerevisiae*, since numerous antisense RNAs have been identified but not associated with function (David *et al*., 2006; Samanta *et al*., 2006).

## Activation of sporulation-induced genes

### *Early gene activation*

Early genes are mainly repressed by the HDAC complex Sin3/Rpd3 and the remodeling complex Isw2 (see previous section). Isw2 modifies nucleosome positions to establish a nuclease-inaccessible chromatin structure, masking the TATA box for binding (Goldmark *et al*., 2000). Upon activation, Ime1 recognizes an Upstream Regulation Sequence, URS1, and recruits the remodeling complex RSC, which alters the repressive chromatin structure (Inai *et al*., 2007). RSC specifically counteracts the Isw2-induced structure, since RSC activity is dispensable for *IME2* activation in the absence of Isw2.

Efficient activation of early and middle genes transcription requires Gcn5/KAT2, a histone acetyltransferase (HAT) (Fig. 2A, Burgess *et al*., 1999). However, the kinetics of histone acetylation on the *IME2* promoter is not related to its transcription activation (Inai *et al*., 2007). Acetylation of histones at *IME2* promoter accumulates when a non-fermentable carbon source such as acetate is available, but before the gene is actually transcribed (Inai *et al*., 2007). Therefore acetylation might change higher chromatin structure to facilitate future binding of Ime1, without altering nucleosome positioning and *IME2* repression.

### *Destruction of Ume6 repressor promotes meiotic gene induction*

Ume6 function during induction of sporulation has remained mysterious, as this strong repressor of sporulation during vegetative growth (via HDAC recruitment) is also required for sporulation induction (Bowdish *et al*., 1995). It has been recently shown that Ume6 is rapidly degraded during sporulation induction, and that this degradation is required for the normal expression of early and middle genes, and destruction requires brief interaction with Ime1 (Mallory *et al*., 2007). Thus, Ume6 destruction disrupts HDAC-mediated repression (Mallory *et al*., 2007) to enhance Gcn5-dependent acetylation and promote early and middle gene transcription.

### *Middle and late genes*

Middle and late gene activation are not well understood. Ndt80 is responsible for middle gene activation, by recognition of a specific DNA sequence, the Middle Sporulation Element (MSE). The transcription factor responsible for late gene induction is unknown. However, similar to early genes, the RSC chromatin remodeling complex is required for the full expression expression of late genes, and formation of fully mature spores (Bungard *et al*., 2004).

Currently nothing is known about a role of histone variants during sporulation. It will be be interesting to determine whether H2A.Z is involved in the activation of sporulation-induced genes.

## Post meiotic genome reorganization and compaction

During post-meiosis, newly haploid spores differentiate into fully mature spores, which will protect them from adverse physical and chemical environmental conditions. This process involves

the expression of the late genes, which are mainly involved in spore wall maturation (Neiman, 2005).

The nuclear volume decreases and chromatin is compacted during post meiotic differentiation, but the mechanisms involved remain to be fully elucidated. Phosphorylation of histone H4 serine 1 (H4S1ph) appears to be a key mark of this process. H4S1ph is absent during meiosis, but accumulates dramatically as spores mature (Krishnamoorthy *et al.*, 2006). Moreover, substitution of serine 1 to alanine, which prevents its phosphorylation, leads to increase of nuclear volume, suggesting that H4S1ph is important in chromatin compaction (Krishnamoorthy *et al.*, 2006). It remains to be determined whether H4S1ph acts directly to alter structure. The amino terminal tail extension of H4 is involved in chromatin structure, and interacts with neighboring nucleosomes (Dorigo *et al.*, 2003; Schalch *et al.*, 2005). Moreover, acetylation of H4 Lysine-16 inhibits the formation of higher chromatin structures (Shogren-Knaak *et al.*, 2006). Thus, in an opposite way, H4S1ph may promote chromatin compaction, and counteract 'decompaction' marks (Fig. 4A).

Methylation and acetylation of histones recruits a large diversity of specific proteins, depending on the modified residue (for review see Ruthenburg *et al.*, 2007). Phosphorylation of histones also recruits specific proteins, such as 14-3-3 proteins (Macdonald *et al.*, 2005). In a similar manner, H4S1ph may recruit specific proteins that would promote chromatin compaction (Fig. 4B).

Finally, new proteins may be incorporated into chromatin to promote compaction (Fig. 4C). During spermatogenesis, specific basic proteins, called protamines, replace nearly all histones, and lead to the final packaging of DNA into the sperm head (Balhorn, 2007). This process has long been thought to be restricted to vertebrates, and protamines have been identified in birds, fish, rodents and primates (for review, Balhorn, 2007). However, recent studies demonstrate their existence in insects, where chromatin reorganization during the final stages of spermatogenesis is very similar to mammals (Jayaramaiah Raja and Renkawitz-Pohl, 2005; Rathke *et al.*, 2007). Therefore, chromatin organization and compaction in spores may involve the function of uncharacterized protamine-like proteins, or other proteins.

## Chromatin dynamics during germination

When new nutrients become available, spores resume vegetative growth during germination. However, spore germination can be induced in conditions that do not allow vegetative growth (Granot and Snyder, 1991; Herman and Rine, 1997). Glucose induces the degradation of the spore wall, but is not sufficient to promote bud emergence and mitosis, and, under these conditions spores stop germinating and die rapidly (Granot and Snyder, 1991). This sequence is seen in the induction of a new gene expression program during germination (Joseph-Strauss *et al.*, 2007). Within 5 min of germination a dramatic change in expression of 1000 genes occurs, and half are up- and half are down-regulated. During the first two hours of germination, spores respond only to glucose addition, whereas, during the second phase, cells sense other components, such as nitrogen availability. It remains to be determined why initial sensing is only to carbon source availability.

Interestingly, the gene expression pattern is very similar to the general transcription response of yeast cells to glucose and also closely resembles changes in gene expression observed upon exit from stationary phase (Martinez *et al.*, 2004; Radonjic *et al.*, 2005). During stationary phase, RNA polymerase II has been detected at promoters of genes that will be expressed following exit from starvation (Radonjic *et al.*, 2005). In a similar way, we predict that the first genes to be expressed during germination may be in a similar state, that is, loaded with RNA polymerase II, but in a poised state that prevents transcription elongation.

## Concluding remarks

It is now clear that simple organisms, such as yeast, provide genetic models to unravel complex molecular mechanisms involved in each step of the cell cycle. However, despite wide usage of the yeast model, its only defined differentiation program, sporulation, remains poorly understood.

Nevertheless, sporulation induction is a model of choice to study transcriptional regulation during gametogenesis, as more than one thousand genes are synchronously altered in expression. It is also interesting that yeast sporulation follows a sequence of events similar to higher eukaryotic spermatogenesis. In both cases, genetic information is recombined during meiosis, and then compacted and stored in a unique chromatin structure (that is, compared to vegetative or somatic cells) in haploid, highly differentiated cells. Remarkably, spores germinate to restore a fully functional vegetative cell, just as gametes generate an entire new somatic organism. Yeast genetics and biochemistry has been crucial to our understanding of chromatin dynamics during the main steps of the cell cycle – such as transcription, replication, mitosis and double strand break repair – and has helped to reveal conserved mechanisms in higher eukaryotes, in both normal and pathologic contexts. Thus, continued investigation of the yeast differentiation program during sporulation, will unravel new mechanisms underlying higher eukaryotic development, gametogenesis, germinal and stem cell biology.

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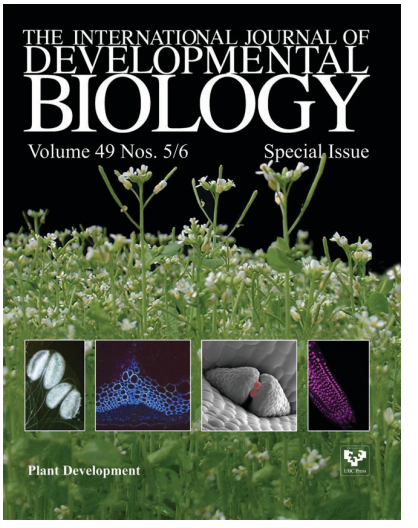


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