Signal pathway integration in the switch from the mitotic cell cycle to meiosis in yeast

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Summary

Diploid yeast, like most eukaryotes, can undergo meiotic differentiation to form haploid gametes. Meiotic differentiation and cell growth (proliferation) are mutually exclusive programs, and in yeast the switch between growth and meiosis is controlled by nutritional signals. The signaling pathways that mediate nutritional controls on meiotic initiation fall into three broad classes: those that respond to nutrient starvation, those that respond to non-fermentable carbon sources, and those that respond to glucose. At the onset of meiosis, nutritional signaling pathways converge on transcriptional regulation of two genes: *IME1*, which encodes a transcription factor; and *IME2*, which encodes a protein kinase. Transcription of *IME1* and *IME2* trigger initiation of meiosis, and the expression of these two genes is linked with one other, with expression of later meiotic genes and with early meiotic events such as DNA replication. In addition, the signaling pathways that control *IME1* and *IME2* expression are themselves integrated through a variety of mechanisms. Thus the signal network that controls the switch from growth to meiotic differentiation provides a signaling code that translates different combinations of extracellular signals into appropriate cellular responses.

Key words: *IME1*, *IME2*, Mitosis, Meiosis, Cell cycle, Sporulation, Signal code

Introduction

Cellular responses are often triggered by combinations of extracellular signals. To combine these different signals into a concerted cellular response, the biochemical pathways that transduce each signal are integrated into signaling networks (Sears and Nevins, 2002; Travnickova-Bendova et al., 2002; Wendland, 2001). Such networks can be considered to provide a 'signal code' to the cell, which translates each possible combination of signals into a positive, negative or neutral effect on a particular cellular response. Signaling networks can be studied effectively in the budding yeast *Saccharomyces cerevisiae*. This genetically tractable eukaryotic microorganism responds precisely to diverse combinations of extracellular nutritional signals.

Here we review recent progress in understanding the nutritional control of meiotic initiation in *S. cerevisiae* with particular focus on how distinct nutritional signals are integrated together into a signaling network. Nutrients also control pseudohyphae formation in this yeast (Gancedo, 2001; Pan et al., 2000; Rua et al., 2001; Wendland, 2001), but this alternative form of cell differentiation is beyond the scope of this Commentary. Similarly, because our focus is on nutritional controls, other controls of meiosis, such as checkpoint controls on the meiosis I division (Murakami and Nurse, 2000; Roeder and Bailis, 2000), will be discussed only briefly. Although we concentrate on *S. cerevisiae*, we also discuss some differences between the signaling network controlling meiotic initiation in this yeast and the analogous signaling network in another yeast, *S. pombe*.

Nutritional signals controlling initiation of meiosis in diploid yeast

Meiosis is the cellular program that transforms a diploid cell into haploid progeny. These haploids then develop into the gametes necessary for sexual reproduction. Meiosis may be considered a specialized form of the mitotic cell cycle: in mitosis each round of DNA replication is followed by a round of chromosome segregation; in meiosis, replication is followed by two sequential rounds of chromosome segregation. In *Saccharomyces cerevisiae*, meiosis occurs in three stages (reviewed in Kupiec et al., 1997). In the first stage, meiotic initiation, key meiotic regulatory genes are expressed as cells transit from the G1 phase of the mitotic cell cycle into the meiotic program. In the second stage, early meiosis, cells pass through DNA replication, pairing of homologous chromosomes and recombination. In the final stage, late meiosis, the chromosomes go through the two sequential rounds of chromosome segregation to form haploid products. In yeast, gamete development immediately follows meiosis and consists of the formation of a spore wall around each haploid genome. The combination of meiosis and spore formation in yeast is collectively called sporulation.

For *S. cerevisiae* to enter meiosis, its nutritional environment must meet three criteria. First, the environment must lack at least one essential growth nutrient (nitrogen limitation is commonly used in the laboratory), which causes the cells to arrest in G1 phase. Second, the environment must contain a non-fermentable carbon source, which can be metabolized through respiration. Third, glucose must be absent from the environment; glucose inhibits meiotic initiation even when the other two criteria are met.

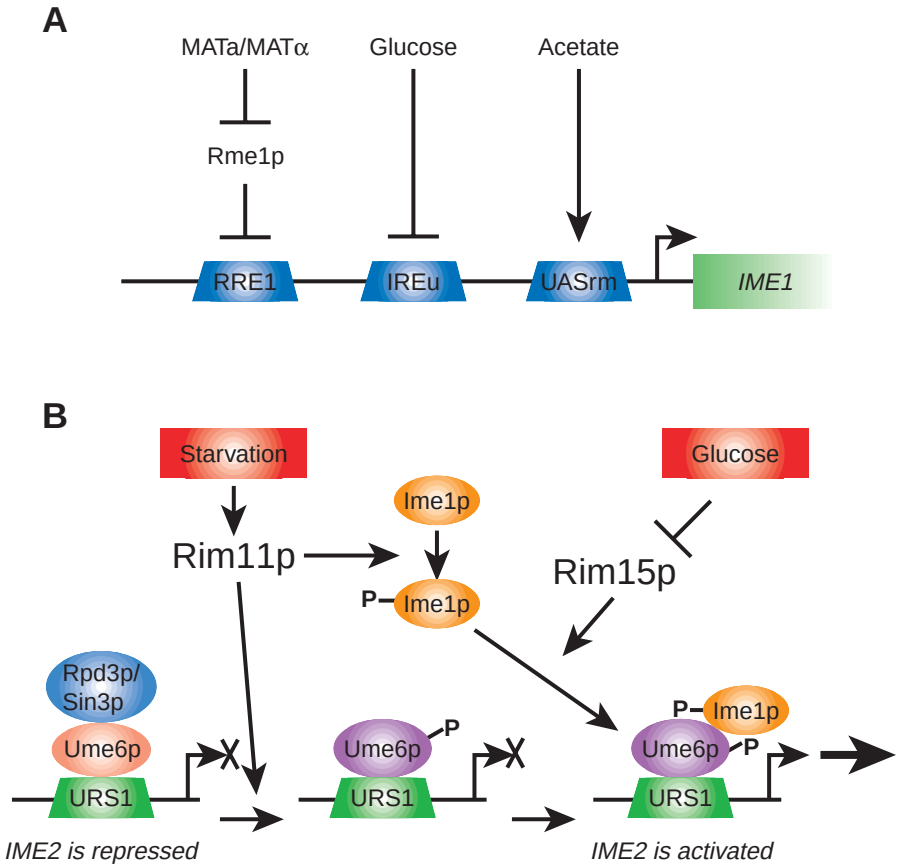

Fig. 1. Mechanisms of signal integration. (A) Convergence of nutritional and cell type control signals on regulation of the *IME1* gene. The different signals shown at the top of the figure act through different regulatory elements of the *IME1* gene. (B) Convergence of nutritional signal on regulation of the *IME2* gene through the URS1 regulatory element.

In other yeast species, initiation of meiosis is regulated by nutritional signals different from those that regulate *S. cerevisiae*. For example, in *Candida lusitaniae*, meiosis and sporulation occur in the presence, but not in the absence, of glucose (Francois et al., 2001). In many yeasts, such as *Candida albicans*, laboratory conditions that promote meiosis have not been identified (Odds et al., 2000). In contrast, the nutritional criteria for initiation of meiosis in *Schizosaccharomyces pombe* are well understood, and these criteria differ in several ways from those controlling meiotic initiation in *S. cerevisiae* (reviewed in Yamamoto, 1996). First, *S. cerevisiae* haploids conjugate under growth conditions and then fuse to form stable diploids, whereas *S. pombe* haploids conjugate under starvation conditions to form diploids that immediately undergo meiosis and sporulation. Second, in *S. pombe*, the environmental signals that trigger the sexual cycle are nitrogen starvation and cellular stress. As discussed below, differences between nutritional controls on meiotic initiation in *S. cerevisiae* and *S. pombe* are attributable to differences in the signaling networks in these two yeast species.

The targets of nutritional regulation of meiotic initiation

Signal integration at early meiotic genes

There are two principal regulators of meiotic initiation: *IME1* (*initiator of meiosis*), which encodes a transcription factor; and *IME2*, which encodes a Ser/Thr protein kinase. The Ime1p transcription factor activates expression of a number of genes expressed early in meiosis, including *IME2*. Both *IME1* and *IME2* are required for normal meiotic initiation (reviewed in Mitchell, 1994), and transcription of both genes is regulated by a combination of different nutritional signals. Interestingly, the mechanisms by which nutritional signals are integrated at these two genes are very different.

Regulation of *IME1* – signal integration at a complex promoter

Different signaling pathways can be integrated by jointly controlling transcription of the same gene (Ptashne and Green, 2002). *IME1*, like other yeast genes with key regulatory roles, has a larger than average regulatory region (approximately 2 kb) (Granot et al., 1989; Rupp et al., 1999). For yeast genes, the entire regulatory region is termed the promoter. The *IME1* promoter can be subdivided into four contiguous regions, upstream control regions 1-4 (UCS1-4), and contains a number of separate regulatory elements within these regions (Sagee et al., 1998). Several of these elements respond to particular nutritional signals (Fig. 1A). For example, glucose represses *IME1* through the 32 bp IREu site, which is approximately 1100 bp upstream from the ORF, whereas acetate activates *IME1* through the UASrm site, which is 800 bp upstream of the ORF (Sagee et al., 1998).

IME1 is fully repressed in growing cells, but, once cells cease growth, it is expressed at a moderate level. Further induction of *IME1* can be prevented by either the presence of glucose or the absence of a non-fermentable carbon source in the nutrient environment (Purnapatre et al., 2002). Because glucose is converted into ethanol at late stages of growth (this is termed the diauxic shift), cells at late stages are primed to initiate meiosis. The diauxic shift may explain why meiosis occurs only at a specific time as yeast colonies mature and preferentially at the edge of these colonies rather than at their centers (Purnapatre and Honigberg, 2002). Most of the growth in a maturing yeast colony occurs at its edge (Meunier and Choder, 1999); so conversion of glucose to ethanol as cells at the periphery of mature colonies become limited for nutrients may provide all the signals necessary for full *IME1* induction.

Cell type and nutritional signals regulate *IME1* transcription through distinct regions of the *IME1* promoter. Cell-type control ensures that *IME1* is not induced in haploid cells under any nutritional conditions. The primary region of the *IME1* promoter required for cell-type control (UCS3 and UCS4) does not overlap with the regions required for nutritional regulation (UCS1 and UCS2) (Covitz and Mitchell, 1993; Sagee et al.,

1998). Rme1p, a transcription factor expressed to much higher levels in haploids than diploids, represses *IME1* through at least two different mechanisms. First, Rme1p binds to the RRE1 site within the UCS4 of *IME1* to directly repress *IME1* transcription. Second, Rme1p binds to a similar site in the promoter of *CLN2*, which encodes a G1 cyclin, to activate its transcription (Blumental-Perry et al., 2002; Frenz et al., 2001). As discussed below, expression of Cln2p inhibits *IME1* expression.

There is no *IME1* ortholog in *S. pombe*; instead nutritional signals regulate *ste11*$^+$ transcription to control entry into the sexual cycle. Like *IME1, ste11*$^+$ encodes a transcription factor, but because conjugation and meiosis are linked processes in *S. pombe*, Ste11 activates genes required for conjugation before it activates genes required for meiosis. Transcription of *ste11*$^+$ is activated by nitrogen starvation and cellular stress through two transcription factors, Atf1-Pcr1 and Rts2, which bind to the *ste11*$^+$ promoter (Higuchi et al., 2002; Kunitomo et al., 2000). Once genes required for conjugation are induced by Ste11 and diploids are formed, the resulting expression of both mating-type alleles inactivates Pat1 (Ran1) kinase, causing hypophosphorylation and further activation of Ste11 (Kitamura et al., 2001; Matsuyama et al., 2000; McLeod et al., 2000). Hypophosphorylated Ste11 is not required for expression of pheromone-response genes, but it is required for expression of early meiotic genes such as *mei2*$^+$ and may further activate transcription of its own gene (Yamamoto, 1996). Thus, *ste11*$^+$has an additional layer of regulation not necessary for *IME1;* it sequentially activates first conjugation genes and then meiotic genes.

Regulation of *IME2* – signal integration at the URS1 site

IME2 is also regulated by several distinct signals, but these signals are integrated at a single regulatory element, the upstream repression site 1 (URS1) (Fig. 1B). URS1 is bound by the Ume6p transcription factor under all conditions tested. When *IME2* is repressed, Ume6p is bound to the Sin3p-Rpd3p complex. The transition from *IME2* in this state to actively expressed *IME2* involves two steps (Washburn and Esposito, 2001). First, the Sin3p-Rpd3p complex is inactivated and may dissociate from Ume6p. Second, Ume6p associates with Ime1p; Ime1p contains a transcriptional activation domain and causes the transcription of *IME2* (Bowdish et al., 1995; Rubin-Bejerano et al., 1996). The stability of this Ume6p-Ime1p complex determines whether *IME2* is transcribed and is regulated by both starvation (G1 arrest) and glucose. Starvation (by nitrogen limitation) activates Rim11p, a Gsk3 family kinase (Xiao and Mitchell, 2000). Rim11p phosphorylates both Ime1p and Ume6p, and this phosphorylation stabilizes the Ume6p-Ime1p association (Malathi et al., 1997; Malathi et al., 1999). In contrast, glucose destabilizes the Ume6p-Ime1p complex by repressing expression of Rim15p, a third kinase required (through an unknown mechanism) for Ume6p-Ime1p association (Vidan and Mitchell, 1997). Thus both nutritional signals converge to regulate *IME2* transcription by modulating the stability of the same transcription factor complex.

The cohort of genes expressed during meiotic initiation

After *IME1* induction, a tightly coordinated transcriptional program ensues. Microarray analysis reveals that approximately 300 genes are induced at least three-fold during meiosis, and this entire program of meiosis can be conveniently grouped into seven sequential waves of expression (Chu et al., 1998; Primig et al., 2000). Members of a group of coexpressed meiotic genes often share common regulatory sites and are regulated by common transcription factors (reviewed in Vershon and Pierce, 2000). For example, in addition to *IME2*, many other early meiotic genes contain a URS1, and several of these genes require *IME1* for activation – for example *HOP1* and *SPO13*. Hop1p is required for pairing of homologous chromosomes, and Spo13p prevents the separation of sister chromatids in the first meiotic division (Lee et al., 2002; Shonn et al., 2002; Woltering et al., 2000).

One way that genes are co-regulated is by a concerted change in the chromatin structure at their promoters. For example, the Ume6p-Sin3p-Rpd3p complex described above (Fig. 1B) represses transcription because Rpd3p deacetylates histones at these promoters (Kadosh and Struhl, 1997; Kadosh and Struhl, 1998; Rundlett et al., 1998). Rpd3p can be recruited to promoters that do not contain URS1 by other transcription factors (Kurdistani et al., 2002). Deacetylation of promoter nucleosomes by Rpd3p prevents association of the SAGA complex with these promoters (Deckert and Struhl, 2002). SAGA contains a histone acetylase, Gcn5p, and indeed as cells initiate meiosis the *IME2* promoter becomes associated with this enzyme (Burgess et al., 1999).

Ume6p also recruits the Isw2p repressor complex to the promoter of *IME2* and many other genes (Fazzio et al., 2001; Goldmark et al., 2000). This complex, which is related to the Swi/Snf family of ATP-dependent chromatin-remodeling enzymes, represses genes by repositioning the promoter nucleosomes into transcriptionally inactive chromatin (Kent et al., 2001; Langst and Becker, 2001). The Isw2p complex contributes to repression of *IME2* and other early meiotic genes during growth and also to their induction under sporulation conditions and hence is required for meiotic initiation (Trachtulcova et al., 2000). Thus, the Isw2p complex, Rpd3p complex, and Gcn5p complex may coordinately regulate many early meiotic genes through a concerted switch in the chromatin structure of these promoters. Two other complexes that remodel chromatin, the RSC (Nps1p-Sth1p) and the Set3 complex, also affect regulation of *IME2* and other early meiotic genes (Pijnappel et al., 2001; Yukawa et al., 1999).

Interlocked regulation of genes expressed at different times in meiosis

Even when *IME1* is overexpressed from a plasmid, *IME2* transcription still requires G1 arrest and is still repressed by glucose. This result is consistent with *IME2* transcription depending on both Ime1p expression and additional nutritional signals that regulate the Ume6p-Ime1p complex. As described below, there are at least two other types of regulation of *IME1* and *IME2*.

IME2 repression of IME1

Expression of Ime2p leads to the eventual repression of *IME1* transcription during late stages of meiosis (Shefer-Vaida et al., 1995; Smith and Mitchell, 1989). More directly, Ime2p kinase

may phosphorylate Ime1p and target it for degradation (Guttmann-Raviv et al., 2002). These two negative-feedback loops on *IME1* expression ensure that Ime1p is expressed in a narrow window relative to Ime2p. The more extended expression of Ime2p is consistent with other results indicating that Ime2p has a continued role during the progression of meiosis (Foiani et al., 1996; Sia and Mitchell, 1995). Taken together, these results suggest that after *IME2* is initially induced by Ime1p, further *IME2* expression does not require Ime1p during later stages of meiosis.

Feedback repression of *IME1* by replication blocks

Meiotic DNA replication is among the first cellular events to occur after *IME1* and *IME2* are induced, and efficient replication during meiosis is dependent on expression of both genes (Foiani et al., 1996). Interestingly, the reverse may also be true. That is, continued expression of *IME2* may depend on efficient progression through meiotic DNA replication, because when replication is blocked by hydroxyurea, the association of Ume6p and Ime1p is inhibited, and *IME2* transcription is repressed (Lamb and Mitchell, 2001). This result suggests that a checkpoint function is induced when replication is blocked that prevents continued initiation of meiosis.

Targets of IME2

Like Ime1p, Ime2p has multiple targets. One such target is Sic1p, an inhibitor of the Clb-Cdc28 kinases (Dirick et al., 1998; Stuart and Wittenberg, 1998). Phosphorylation of Sic1p by Ime2p kinase targets Sic1p for degradation, thus activating Clb-Cdc28p. Clb-Cdc28p kinase induces meiotic DNA replication; thus activation of Ime2p kinase directly induces meiotic DNA replication. During the mitotic cell cycle, Sic1p is phosphorylated by Cln-Cdc28p kinase rather than Ime2p, and phosphorylation at multiple sites on Sic1p is required for Sic1p degradation (Nash et al., 2001a). Possibly, the fact that Ime2p rather than Cln-Cdc28p targets Sic1p for degradation in meiosis explains why initiation of replication is delayed in meiosis relative to its timing in the cell cycle.

A second target for Ime2p is *NDT80*, which encodes a transcription factor that induces genes expressed during middle stages of meiosis (Chu and Herskowitz, 1998; Hepworth et al., 1998). The *NDT80* promoter, in addition to containing two URS1s, also contains two middle sporulation elements (MSEs). Ime1p bound to the *NDT80* URS1 only partially activates *NDT80* transcription when Sum1p, a repressor protein, is bound to MSE (Xie et al., 1999). Ime2p kinase eliminates Sum1p repression at the MSE through an unknown mechanism (Pak and Segall, 2002), and at the same time phosphorylates Ndt80p (Sopko et al., 2002). Once phosphorylated, Ndt80p can bind to MSE to fully induce expression of its own gene and other genes that bear a MSE. For example, Ndt80p is required for transcription of *CLB2* in meiosis (Sopko et al., 2002). *CLB2* contains an MSE and encodes the B-type cyclin that triggers meiotic chromosome segregation. Thus Ime2p, which is expressed early in meiosis, causes expression of the Ndt80p transcription factor, and Ndt80p then induces genes expressed in middle stages of meiosis. Indeed, *IME2* contains an MSE site, which may allow its expression even after *IME1* is repressed.

Possible targets of nutritional controls after meiosis has initiated

After meiosis initiates, progression of the meiotic program depends, in part, on expression of *IME1* and *IME2* and, in part, on continued nutritional signals. Indeed, when *IME1* is overexpressed from a plasmid, the early meiotic events, such as meiotic replication and recombination, occur efficiently even in the absence of a non-fermentable carbon source (Lee and Honigberg, 1996). In contrast, the late meiotic events, such as the meiotic divisions and spore formation, do not occur. These results imply that there are nutritional controls on later meiotic processes that are independent of the nutritional controls on *IME1* and *IME2*. Independent nutritional controls on late meiotic genes may explain why cells in early stages of meiosis are able to abort the meiosis and 'return to growth' when transferred from sporulation conditions to growth conditions. It will be interesting to determine whether critical regulators of middle meiotic gene expression such as Ndt80p and Sum1p are regulated by nutrients separately from the nutritional controls on *IME1* and *IME2*.

In summary, two general features of nutritional regulation of early meiotic genes have emerged over the past few years. First, nutritional signals independently control transcription of several different target genes, for example *IME1*, *IME2* and possibly *NDT80*. Second, expression of each target gene is tightly coordinated with expression of other target genes and with cellular events. The presence of both independent and interlocking controls on key meiotic regulators such as *IME1*, *IME2* and *NDT80* may provide fail-safe mechanisms to ensure that meiotic initiation only occurs under appropriate conditions. In addition, these controls allow cells to continually respond to a changing environment.

Signal transduction pathways that mediate nutritional controls on meiotic initiation

Below we describe the current understanding of the signaling pathways that mediate nutritional control of meiosis in *S. cerevisiae*. We have grouped these pathways with respect to the primary nutritional signal that they respond to (starvation, respiration and glucose). A working model for how these nutritional signaling pathways control meiotic initiation is shown in Fig. 2. This model is currently incomplete but is meant to describe a framework on which a more complete signaling network can be built.

Activation of meiosis by starvation/G1 arrest

S. cerevisiae arrest in G1 in response to starvation (Werner-Washburne et al., 1993), but it is not understood how the absence of any single essential nutrient causes the starvation response. Whatever the mechanism of G1 arrest, cells must arrest before they can initiate meiosis (Hirschberg and Simchen, 1977). As a result, many mutants that affect the timing of G1 arrest will also affect the timing of meiotic initiation. In fact, even auxotrophic markers, such as *ura3* and *leu2*, can affect the timing of meiotic initiation. For this reason,

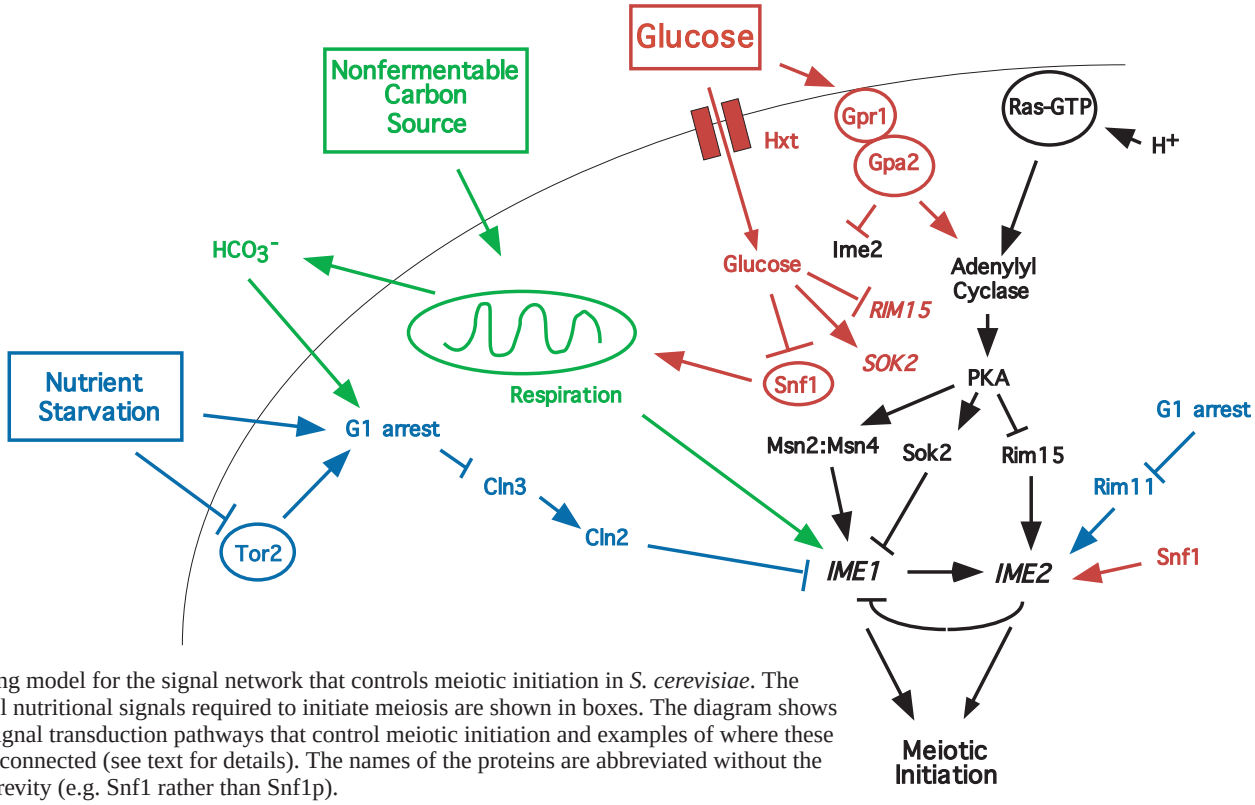

Fig. 2. Working model for the signal network that controls meiotic initiation in *S. cerevisiae*. The three principal nutritional signals required to initiate meiosis are shown in boxes. The diagram shows some of the signal transduction pathways that control meiotic initiation and examples of where these pathways are connected (see text for details). The names of the proteins are abbreviated without the final 'p' for brevity (e.g. Snf1 rather than Snf1p).

the timing of meiotic initiation should only be compared in strains containing exactly the same auxotrophies (Purnapatre et al., 2002).

CLN expression

Many of the signaling pathways that regulate entry into G1 arrest converge to regulate expression of *CLN3*, which encodes a G1 cyclin (Belli et al., 2001; Cherkasova et al., 1999; Jenkins and Hannun, 2001; Nash et al., 2001b). Cln3p is present at constant levels during the cell cycle, but functions primarily to promote the transition from G1 to S phase. When G1 arrest occurs, Cln3p levels decline rapidly (Gallego et al., 1997; Parviz and Heideman, 1998). This decline is also required for meiotic initiation because *CLN3* inhibits *IME1* expression and Ime1p localization to the nucleus (Colomina et al., 1999). During G1 phase, Cln3p activates the Swi4p-Swi6p complex (also called SBF), which is required for expression of two other G1 cyclins, *CLN1* and *CLN2* (Levine et al., 1996). *SWI6* and *CLN2* (but not *CLN1)* are required for repression of *IME1*, although the mechanism for this repression is not known (Purnapatre et al., 2002).

Nitrogen starvation

It is often stated that initiation of meiosis in *S. cerevisiae* requires starvation for nitrogen, but it is not certain if nitrogen directly represses meiotic initiation or if nitrogen starvation promotes meiosis indirectly by causing G1 arrest. Some evidence favors the latter hypothesis, because meiosis is not blocked by the presence of nitrogen when other essential nutrients are limiting (Freese et al., 1982; Freese et al., 1984). On the other hand, several nitrogen-sensing pathways affect the timing of entry into meiosis. Both of the pathways described below, the *TOR2* signaling pathway and the *UPR* pathway, illustrate the difficulty in distinguishing between direct and indirect roles of nitrogen in regulating entry into meiosis.

The *TOR2* pathway modulates several nutritional signaling pathways including the nitrogen discrimination pathway (reviewed in Raught et al., 2001). This pathway is activated when the nutritional environment of the cell contains only poor nitrogen sources. The Tor2p pathway can be activated by the drug rapamycin, and this activation allows meiosis and sporulation under growth conditions. However, microarray analysis indicates that activation of the Tor2p pathway by rapamycin does not directly induce sporulation genes; instead the Tor2p pathway directly controls a number of metabolic genes required for growth arrest (Hardwick et al., 1999). Thus it is likely that the Tor2p pathway stimulates meiosis by causing changes in metabolism that result in G1 arrest.

Another pathway that senses nitrogen concentration and may be involved in the switch from the cell division cycle to meiosis involves *HAC1*. When nitrogen is present at high concentrations, *HAC1* RNA is spliced, and only this form can be translated (Schroder et al., 2000). *HAC1* is required to induce the unfolded protein response (UPR) (Patil and Walter, 2001) and may also regulate entry into meiosis. A *hac1Δ* mutant induces *IME2* more rapidly than the wildtype, and overexpression of spliced *HAC1* (*HAC1*^{i}) delays expression of this gene. The mechanism of *IME2* repression by Hac1p is not known, but *HAC1* is spliced in non-fermentable carbon sources at higher levels than in glucose (Kuhn et al., 2001). Thus nitrogen could repress initiation of meiosis during growth in

non-fermentable carbon sources in part by promoting *HAC1* splicing (Schroder et al., 2000).

Activation of meiosis by non-fermentable carbon sources

IME1 transcription requires respiratory metabolism of a non-fermentable carbon source. When *IME1* is overexpressed from a plasmid, the requirement for respiration to initiate meiosis is bypassed. Respiration leads to the production of CO_2 and hence causes alkalization of the medium. This alkalization may contribute to meiotic initiation (Ohkuni and Yamashita, 2000). For example Rim101p is required both for adaptation to extracellular alkalization (Lamb et al., 2001) and for *IME1* transcription (Su and Mitchell, 1993). In addition, alkalization of the medium may also activate the Srb10p-Srb11p cyclin-kinase complex (also called Ume3p-Ume5p and Ssn3p-Ssn8p), and this activation is required for efficient induction of *IME2* transcription and initiation of meiosis (Cooper and Strich, 2002; Ohkuni and Yamashita, 2000). Thus respiration may promote both *IME1* and *IME2* expression by causing alkalization of the medium.

Repression of meiosis by glucose

Even a relatively low concentration of glucose (0.2-0.5%) inhibits both *IME1* and *IME2* transcription. As described below, glucose probably inhibits meiotic initiation through several different signaling pathways.

A number of cellular processes that respond to glucose are regulated by the glucose repression pathway (reviewed in Johnston and Carlson, 1992). The central component of this pathway is Snf1p kinase, whose activity is inhibited by intracellular glucose. In particular, Snf1p kinase is required for expression of *IME1* and *IME2* (Honigberg and Lee, 1998), and the glucose sensors Rgt2p and Snf3p, which act upstream of *SNF1* (Ozcan and Johnston, 1999), are required to maintain repression of *IME1* during later stages of growth in glucose medium (K.P., Sarah Piccirillo, Rita Lee and S.M.H., unpublished).

Extracellular glucose is also sensed by the G-coupled receptor, Gpr1p (Lorenz et al., 2000; Rolland et al., 2000), which in turn activates Gpa2p, the alpha subunit of a trimeric G-protein complex (Harashima and Heitman, 2002). This complex, once activated, causes activation of protein kinase A (PKA). Although the role of Gpa2p in regulating meiotic initiation has not been established, PKA inhibits both *IME1* and *IME2* transcription as well as promoting growth. In addition, Gpa2p binds directly to Ime2p to repress its kinase activity (Donzeau and Bandlow, 1999).

The ortholog of *GPA2* in *S. pombe* is $gpa2^+$, which also activates PKA. Glucose represses gluconeogenic genes through this pathway, and nitrogen represses $ste11^+$ and the sexual cycle through the same pathway (Higuchi et al., 2002). Nitrogen starvation specifically activates Stm1, which is homologous to G-protein-coupled receptors (Chung et al., 2001). Once activated, Stm1 binds and inhibits Gpa2, allowing $ste11^+$ transcription. Thus, although Gpa2 represses meiotic initiation in both *S. cerevisiae* and *S. pombe*, it is regulated by different signals in the two yeast species.

Glucose transiently activates the Ras pathway under some conditions, and a more sustained activation of Ras is caused by intracellular acidification (Thevelein and de Winde, 1999). *S. cerevisiae* have two *RAS* genes, and expression of one of them, *RAS1*, is induced by glucose. Ras proteins activate both the Cdc42p/Ste20p MAP kinase pathway and the PKA pathway. Although both of these pathways control pseudohyphal differentiation (Mosch et al., 1999; Pan and Heitman, 1999), only the PKA pathway controls meiotic initiation. In *S. pombe*, the single *RAS* homolog, Ras1, activates rather than represses meiotic initiation. *S. pombe* Ras1 does not regulate the PKA pathway, instead it regulates localization of Byr1 and activation of the Byr1/Byr2/Spk1 MAPK pathway (Bauman and Albright, 1998; Ozoe et al., 2002). Thus *S. cerevisiae* Ras proteins activate the PKA pathway to repress meiotic initiation, whereas *S. pombe* Ras1 activates the Byr1 MAPK pathway to stimulate meiotic initiation.

The idea that glucose represses meiotic initiation in *S. cerevisiae* through multiple pathways is consistent with the finding that glucose is still able to repress meiosis when either the PKA pathway or the glucose repression pathway is inactive. For example, glucose represses meiotic initiation in a *cyr1-1* (adenylyl cyclase) mutant even though PKA is inactive in this mutant (Matsumoto et al., 1983), and glucose can also represses meiosis effectively in a *gpa2Δ* mutant (Donzeau and Bandlow, 1999). Conversely, glucose represses meiotic initiation in an *rgt2Δ snf3Δ* double mutant (K.P., S.P., R.L. and S.M.H., unpublished), even though the glucose repression pathway is not fully activated in this mutant.

In summary, each signal that regulates meiotic initiation may be transduced through multiple pathways. For example, nitrogen starvation may stimulate meiotic initiation through both the Tor2p pathway and the Hac1p pathway, and, similarly, glucose may repress meiotic initiation through both the glucose repression pathway and the Gpa2p/PKA pathway. Comparisons between signaling networks controlling meiotic initiation in *S. cerevisiae* and *S. pombe* suggest that each regulatory network has evolved to adapt to a particular ecological niche. Indeed, the same signaling enzymes (e.g. Ras and Gpa2p) are used to different purposes in the two yeast. It seems likely that as each yeast species adapts to particular environments, existing pathways and signaling components are duplicated or modified to provide a specific signal code for the species.

Crosstalk between signaling pathways in control of meiotic initiation

Signaling pathways are integrated into networks when the pathways that transduce these signals converge to regulate the same signal transduction enzyme. Signaling enzymes regulated by more than one signal can be considered to act as nodes in the signaling network. In this section, several examples of this type of integration are discussed.

Integration of glucose and respiratory controls on meiosis

When both glucose and a non-fermentable carbon source are present in the medium, *IME1* expression and initiation of meiosis are completely repressed. This result can be explained because glucose represses Snf1p kinase activity, and Snf1p is

required for expression of respiratory enzymes (Ronne, 1995). In addition to controlling respiration, Snf1p kinase may activate meiotic initiation directly. For example, initiation of meiosis occurs in the absence of respiration when *IME1* is overexpressed from a plasmid, but even *IME1* overexpression is not sufficient to allow efficient meiotic initiation in a *snf1Δ* mutant (Honigberg and Lee, 1998). Thus Snf1p probably promotes meiotic initiation through other targets besides respiratory enzymes.

Signal integration within the PKA pathway

As mentioned above, PKA both promotes growth and represses *IME1* and *IME2* transcription. Because PKA is activated by intracellular cAMP, adenylyl cyclase (Cyr1p/Cdc35p), the enzyme that generates cAMP, is a critical regulator of PKA activity. Adenylyl cyclase activity is controlled by both the Gpa2p pathway (responding to glucose) and the Ras2p pathway (responding to intracellular acidification and possibly other signals); thus adenylyl cyclase can be considered to be a signaling node that integrates these two pathways (Thevelein and de Winde, 1999).

The PKA pathway contains other components that serve as potential signal nodes. For example, *GPR1* transcription is induced when cells are starved for amino acids and nitrogen (Xue et al., 1998). *GPR1* encodes the receptor that activates Gpa2p in response to glucose. A second example is Bcy1p, the regulatory subunit of PKA. Bcy1p is localized primarily in the nucleus during growth in glucose but localized throughout the cell during growth in ethanol, and presumably this localization of PKA controls its activity (Griffioen et al., 2000).

PKA targets as sites for signal integration

Several of the targets of the PKA pathway are also regulated by other signaling pathways, indicating that these targets could serve as signaling nodes. One example is Rim15p kinase, which promotes the interaction of Ume6p and Ime1p (see above). Glucose represses *RIM15* expression, whereas PKA directly inhibits the activity of Rim15p by phosphorylating it. Thus Rim15p represents a signaling node whose expression is controlled by one pathway and whose activity is regulated by another.

IME1 may be repressed by the PKA pathway through the Msn2p-Msn4p transcription complex. PKA hyperphosphorylates Msn2p-Msn4p, which inhibits its function as a transcriptional activator (Garreau et al., 2000). Msn2p-Msn4p activates transcription of many stress-response genes by binding to the stress response element (STRE) present in the promoters of these genes (Smith et al., 1998). Some of these stress-response genes, for example, *TPS1 (*trehalose phosphate synthetase), are also required for induction of *IME1* (De Silva-Udawatta and Cannon, 2001). More directly, an STRE is present in the *IME1* promoter and may be required for *IME1* expression (Sagee et al., 1998).

PKA may also regulate the Msn2p-Msn4p complex by phosphorylating Sok2p (Ward and Garrett, 1994), which is thought to bind Msn2p-Msn4p and convert it to a transcriptional repressor (Shenhar and Kassir, 2001). Independently of the PKA pathway, *SOK2* expression is induced by glucose (Shenhar and Kassir, 2001). Thus, Sok2p regulation is similar to Rim15p regulation. In both cases, PKA regulates the activity of the enzyme, whereas a different pathway regulates its expression.

There are likely to be other PKA targets in meiosis. In fact, cAMP levels drop rapidly as cells initiate meiotic differentiation and then rise again soon after this decrease (Uno et al., 1990). This fluctuation suggests that PKA represses meiotic initiation but stimulates other meiotic events. Indeed although adenylyl cyclase mutants initiate meiosis precociously, they do not progress through sporulation normally, and they form largely two-spored rather than four-spored asci (Uno et al., 1990).

In summary, although the signaling network controlling meiotic initiation is only partially known, several types of node have been revealed. First, the activity of an enzyme can be controlled by more than one pathway or signal. For example, glucose regulates adenylyl cyclase through the Gpa2p pathway whereas intracellular acidification regulates adenylyl cyclase through the Ras2p pathway. Second, one pathway can regulate expression of a gene whereas a second pathway regulates activity of its product. For example, *RIM15* is regulated post-translationally by the PKA pathway, but transcriptionally through an independent pathway. Third, the presence of one signal may regulate the transmission of another signal. For example, when glucose inactivates Snf1p kinase, it blocks expression of genes required for respiration.

Genomic approaches

Genomics provides new tools for investigating signal pathway integration. In particular, microarray analysis has yielded insight into the pattern of genes expressed during meiosis in *S. cerevisiae* (Chu et al., 1998; Primig et al., 2000) and *S. pombe* (Mata et al., 2002). In both yeasts, the first genes to be induced in meiosis encode metabolic and stress-response enzymes. In *S. cerevisiae*, many of these enzymes are required for metabolism of non-fermentable carbon sources, whereas in *S. pombe*, many are required for nitrogen metabolism. In *S. cerevisiae*, approximately one-third of these genes contain a URS1 and require Ume6p for expression (Williams et al., 2002), but induction of many of these genes is transient and probably does not require Ime1p (Primig et al., 2000).

Microarray analysis of genes induced during meiosis has identified a number of genes that were not previously known to function in meiosis, but were subsequently determined to play important roles in the meiotic program (Chu et al., 1998; Gerton and DeRisi, 2002; Rabitsch et al., 2001; Smith et al., 2002; Valencia et al., 2001). Similarly, genomic studies using two-hybrid assays or mass-spectrometric analysis of protein complexes (Gavin et al., 2002; Ho et al., 2002; Ito et al., 2001; Uetz et al., 2000) have identified potential interactions between known meiotic proteins and proteins without a known meiotic function. Identifying which of these interactions are biologically relevant is assisted by comparing data from independent genome-wide interaction studies (von Mering et al., 2002) and correlating protein interaction data with microarray data (Kemmeren et al., 2002).

Genomic analysis has been useful for identifying regulatory elements that are present in meiotic genes. In addition to verifying the presence of URS1 or MSE elements in a large number of meiotic genes, microarray expression data has

identified other putative regulatory elements shared by genes expressed in meiosis (Bussemaker et al., 2001; Pilpel et al., 2001). For example, approximately 75% of the metabolic genes expressed very early in the meiotic program contain at least one of five different short motifs (Bussemaker et al., 2001). Many early meiotic genes also contain an MCB box or both an MCB and an SCB box in their regulatory region (Bussemaker et al., 2001; Pilpel et al., 2001). These regulatory sequences are found in the promoters of genes required for the transition from G1 to S phase during the cell cycle and hence may also be important in induction of genes required for DNA replication in meiosis. In addition to microarray data analysis, an alternative method for identifying regulatory motifs is to compare the regulatory regions of homologous genes in closely related species (Cliften et al., 2001), and the genomes of several of these 'sensu stricto' species have recently been sequenced. As one example, this 'phylogenetic footprinting' analysis has revealed several regulatory elements in addition to the URS1 in the promoter of the early meiotic gene, *REC102* (Jiao et al., 2002).

Completion of the sequencing of the yeast genome (Goffeau, 1996) enabled the creation of a set of mutants that each lack one of approximately 5000 non-essential genes in the genome (Giaever et al., 2002). Homozygous diploid deletion mutants have been used in large-scale screening for sporulation-defective mutants (Briza et al., 2002; Enyenihi and Saunders, 2003). Interestingly, mutants exhibiting defective autophagy, a process of large-scale protein degradation, did not undergo either the meiotic divisions or spore formation. Autophagy may be required in meiosis to salvage nitrogen and other metabolites used for biosynthesis. Another process that may play a role in meiotic initiation is RNA processing. Several proteins that affect *IME1* expression are involved in this process: Rim4p is a putative RNA-binding protein (Soushko and Mitchell, 2000; Deng and Saunders, 2001), Ime4p is an RNA methyltransferease (Clancy et al., 2002), and Ire1p is an RNA endonuclease involved in regulated splicing (Schroder et al., 2000).

Conclusion

The complex and interlocking nutritional controls that regulate initiation and progression of meiosis in yeast could serve at least two biological functions. One function is to ensure that yeast respond appropriately to the variety of complex mixtures of nutrients in its natural environment. A second function is to ensure the fidelity of the regulatory program. Because any single regulatory pathway will fail at some frequency, multiple pathways transmitting the same signal will decrease the chance that meiotic genes are expressed inappropriately.

Our knowledge of the signaling network controlling initiation of meiosis is incomplete, and it is certain that more signaling pathways and more connections between pathways will be discovered. The rapid progress in the field over the past few years can be attributed in part to the synergy created between detailed genetic and biochemical studies on the one hand and genome-wide studies on the other. In the future, as genome data is gathered for more yeast species, comparisons between species may provide an additional way to correlate signal networks with biological function.

The switch between growth and meiosis in yeast provides an opportunity to identify a complete signaling code, a network of signal transduction enzymes that translates different combinations of signals into appropriate cellular responses. One key to identifying such codes will be to determine the involvement (or lack of involvement) of each component of the network in responding to each signal. Identification of such codes in yeast should be help us to decipher the even more complex signaling interactions that occur in higher organisms.

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