Control of Meiotic Gene Expression in 
Saccharomyces cerevisiae

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INTRODUCTION

Cells of the budding yeast Saccharomyces cerevisiae produce mitotic daughters whenever nutrients are plentiful. However, starvation causes cell growth and mitotic division to cease. One type of cell, the a/a diploid cell, then initiates a sporulation program that leads through meiosis to spore formation. The other two types of cells, a and α haploid cells, may arrest in G1 phase of the mitotic cell cycle. The focus of this article is the regulatory system that permits a/a cells to sporulate. Other reviews have discussed regulation of meiosis in S. cerevisiae (38, 57, 59, 90) and Schizosaccharomyces pombe (64, 116) and meiotic recombination (1a, 48, 81, 82).

Two nutritional conditions are required for sporulation. One is limitation for an essential nutrient. Nitrogen limitation causes efficient sporulation and is generally used in the laboratory to induce sporulation. However, limitation for carbon, phosphate, sulfate, guanine, methionine, and other compounds can also cause sporulation (26, 108). The other condition is absence of a fermentable carbon source, such as glucose. Sporulation medium typically contains acetate, although pyruvate and ethanol are also suitable (26). The carbon source apparently governs the decision between pseudohyphal growth and sporulation: nitrogen limitation in the presence of glucose leads to pseudohyphal growth (29); nitrogen limitation in the absence of glucose leads to sporulation.

The signal that specifies cell type comes from alleles of the mating type locus, or MAT (34). Haploid a and α cells have MATa or MATα alleles, respectively, while diploid a/a cells have both MATa and MATα alleles. The ability to sporulate

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requires the expression of both *MATa* and *MATα*. Thus, diploid a/a and α/α cells, which have two *MATa* or two *MATα* alleles, are unable to sporulate (83). *MATa* and *MATα* specify a1 and a2, respectively, which are subunits of the transcriptional repressor a1-a2 (19, 31). a1-a2 is ultimately responsible for all known a/a cell properties. In this article, I will refer to cells that lack a1 or a2 as non-a/a cells. Because a1-a2 expression is generally restricted to diploid cells, it indicates that cells have the necessary number of chromosomes for successful meiotic divisions.

The landmark events of sporulation have been established by comparison of starved a/a and non-a/a cells (25, 71) (Fig. 1). Cells enter meiosis through the meiotic prophase, which includes a round of DNA synthesis and events associated with recombination: chromosomes condense, transient double-stranded chromosome breaks occur, and gene convertants and recombinants appear. In addition, the synaptonemal complex forms. (See reference 71 for a careful kinetic study.) Cells then go through the meiosis I (reductive) and meiosis II (equational) divisions. Finally, spore walls form through deposition of spore coat materials within a membrane outgrowth near the spindle poles (6, 10).

A set of genes referred to as meiotic genes or sporulation-specific genes display much higher RNA levels in sporulating cells than in either vegetative cells or starved, non-a/α cells (Table 1 and references therein). These genes have been identified through two general approaches. One is based on gene function: mutants with specific meiotic defects (such as recombination, spore packaging, or reductional division) were identified, and studies of the corresponding genes revealed that their transcripts accumulated only in sporulating cells. Not all mutations that cause meiotic defects lie in meiotic genes, however. For example, spo7 mutations block sporulation, but *SPO7* is expressed in vegetative cells (115). The second approach is based on gene expression: genes expressed preferentially in starved a/a cells were identified by differential hybridization (2, 12, 30, 73) or lacZ fusion protein expression screens (13). Surprisingly, the bulk of the genes identified by this second approach turned out to be dispensable for the sporulation program.

Meiotic genes have been divided into three classes—early, middle, and late—based on their time of expression (12, 57, 73, 114). Early genes are expressed at the beginning of meiotic prophase; middle genes are expressed later in prophase; and late genes are expressed around the time of meiotic divisions and spore packaging (Fig. 1). The restructuring of the cell during sporulation leads to differential compartmentation of later transcripts, in the ascus versus spore cytoplasm (49) and even in one spore but not another (6). These temporal groupings are only approximate for two reasons. First, some genes are expressed at unique times. *DI71* and *DI72*, for example, are expressed after middle genes but before late genes (6). Second, the kinetics and synchrony of sporulation vary from strain to strain. Thus, the groupings in Table 1, particularly for middle and late genes, should be considered provisional.

Sporulating cells express many genes that are expressed at similar levels under other circumstances. For example, several heat shock genes are expressed at elevated levels after starvation of both a/a and non-a/a cells (50). Transcripts of many DNA synthesis and repair genes accumulate to elevated levels during meiotic prophase, as they do during mitotic S phase or after DNA damage (40–42, 56, 78, 84). It is noteworthy that increased RNA levels may not cause a corresponding increase in protein product levels (78). Some of these genes are clearly required for sporulation, while others are not (9, 25, 75, 89).

**FIG. 1.** Time course of meiotic events. The main phases of the sporulation program are indicated in relation to the times at which early, middle, and late genes are expressed. The time after starvation at which each phase occurs is indicated for SK-1-derived strains (71) and BR2495-derived strains (65). The relative times of early, middle, and late meiotic gene expression are from references 114, 57, and 65. SC, synaptonemal complex.

**STRUCTURE OF MEIOTIC PROMOTERS**

The regulatory sequences of three early meiotic genes and two later genes have been analyzed in some detail. These studies, combined with studies on the sequences of other meiotic genes, indicate that many early genes have common regulatory sequences. In addition, later genes may share a distinct regulatory sequence. Thus, the temporal sequence of meiotic gene expression may reflect the order in which classes of promoters are activated.

**Early Meiotic Genes**

Functional analysis of 5' regions of the early genes *SPO13*, *HOP1*, and *IME2* suggests four broad conclusions (3, 8, 109). First, these genes contain a site, URS1, that is a repression site in promoters of nonmeiotic genes (102). Second, URS1 represses early meiotic promoters in nonmeiotic cells but stimulates these promoters in meiotic cells. Third, a nearby site often participates along with URS1 in stimulating meiotic gene expression. Finally, many early meiotic promoters have regulatory sequences that are very close to minimal promoter sequences (that is, the TATA and RNA start sites).

Analysis of the *SPO13* regulatory region first implicated a URS1 site in meiosis-specific expression (8). A *spo13-lacZ* fusion that included only *SPO13* sequences between −140 and +45 displayed meiosis-specific expression; the fusion was silent in growing a/a cells and expressed in starved a/a cells. No expression was detected in growing or starved non-a/a cells. A point mutation in the URS1 site (at −92) caused a sixfold decrease in *spo13-lacZ* expression in meiotic cells. Decreased expression was also observed with a *spo13-lacZ* fusion that extended only to −80 and therefore lacked the URS1 site. These findings, together with the observation that many early meiotic genes have URS1 sites (8), indicated that URS1 may have a positive role in early meiotic gene expression.

Both the URS1 point mutation and the deletion to −80 also caused slightly elevated *spo13-lacZ* expression in nonmeiotic cells. Buckingham et al. (8) pointed out that expression may have resulted from adventitious vector upstream activation sequences (UASs) or promoters. However, this observation suggested that the URS1 site in the *SPO13* promoter is a negative site in nonmeiotic cells (8).
### TABLE 1. Sporulation-specific genes

| Time of expression | Gene | Isolation | Function | Regulatory site(s) | Reference |
|--------------------|------|-----------|----------|--------------------|-----------|
| Early              | DMC1 | Expression| Recombination | URS1: -137, TCCGGCGCT; UASH: -168, TGTGAGAaG; Tc: -195, TTTTCTTCG | 2         |
|                    | HOP1 | Function  | Recombination | URS1: -173, TCCGGCGCT; UASH: -198, TGTGAACTG | 35        |
|                    | IME1| Function  | Recombination | URS1: -552, aCGGCAGCT; UASH: -457, TCGGCGGCT | 45        |
|                    | IME2, SME1 | Function | Recombination | URS1: -581, TTTTCTTCG; UASH: -478, TTTTCTTCG | 118       |
|                    | IME4 | Function  | Recombination | -98, TCCGGCGCT | 65        |
|                    | ME14 | Function  | Recombination | -136, TCCGGCGCT; -150, aGGGCAGCT | 53, 80    |
|                    | MER1 | Function  | Splicing | -111, TCCGGCGCT | 23        |
|                    | REC102 | Function | Recombination | -397, TCCGGCGCT | 14, 60    |
|                    | REC104 | Function | Recombination | -93, TCCGGCGCT | 14, 60    |
|                    | REC114 | Function | Recombination | -90, TCCGGCGCT | 14, 60    |
|                    | RED1 | Function  | Recombination | -166, TCCGGCGCT | 105       |
|                    | RIM4 | Function  | IME2 expression | -394, TCCGGCGCT; -336, TCGGCGGCT | 101       |
|                    | SPO11 | Function | Recombination | +163, TCCGGCGCT | 114       |
|                    | SPO13 | Function | Meiosis I division | -96, TCCGGCGCT | 114       |
|                    | SPO16 | Function | Sporulation efficiency | -90, TCCGGCGCT | 114       |
|                    | ZIP1 | Function  | Synaptonemal complex formation | -22, TCCGGCGCT | 104       |

**Middle**

| Gene | Isolation | Function | Regulatory site(s) | Reference |
|------|-----------|----------|--------------------|-----------|
| SIT2 | Expression | Nonessential; unknown | - | 30 |
| SIT3 | Expression | Nonessential; unknown | - | 30 |
| SIT4 | Expression | Nonessential; unknown | - | 30 |
| SPO12 | Function | Meiosis I division | -220, aGGCATC; -345, TGTGAGAaa | 58 |
| SPS1 | Expression | Postmeiotic events | - | 74 |
| SPS2 | Expression | Nonessential; unknown | - | 74 |
| SPS3 | Expression | Unknown | - | 28 |

**Mid-late**

| Gene | Isolation | Function | Regulatory site(s) | Reference |
|------|-----------|----------|--------------------|-----------|
| DIT1 | Function | Spore wall formation | - | 6 |
| DIT2 | Function | Spore wall formation | - | 6 |

**Late**

| Gene | Isolation | Function | Regulatory site(s) | Reference |
|------|-----------|----------|--------------------|-----------|
| SGA1 | Homology | Nonessential; glucoamylase | - | 117 |
| SPR1 | Expression | Nonessential; β-glucanase | - | 12, 69 |
| SPR2 | Expression | Nonessential; unknown | - | 12, 77 |
| SPR3 | Expression | Nonessential; unknown | - | 12 |
| SP5100 | Expression | Spore wall maturation | - | 52 |
| SP5101 | Expression | Unknown | - | 52 |

*a* Conclusions about expression class are generally those drawn in primary references. There is some ambiguity in comparing middle and late genes reported from different laboratories.

*b* Isolation refers to the basis for initial identification of the gene. Function, identified through mutant phenotype; expression, identified by expression pattern of transcript; homology, identified by cross-hybridization with a functionally related gene.

*c* Genes in which insertions or deletions cause no sporulation defect are designated nonessential.

*d* Locations of URS1, UASH, and Tc sites are from references 3, 8, and 109 and from sequences reported for individual genes. Nucleotide coordinates refer to the 5'-most residue of each sequence. Some sites are reported in inverse orientation to simplify comparison. Consensus sequences: URS1, TCGGCGCCT (8); UASH, TGTGAACTG (109); Tc site, TTTTCTTCG (3). Bases that deviate from each consensus are shown in lowercase letters. —, no sequence has been reported.

*e* IME1 is not strictly a meiosis-specific gene (47, 87), as described in the text.
Studies of \textit{HOP1} have also pointed to both positive and negative roles for URS1 (109). \textit{HOP1} sequences between −207 and +18 direct meiosis-specific expression of an \textit{hop1-lacZ} fusion. Destruction of a URS1 site at −173 through a multisite mutation causes midlevel constitutivity: expression is elevated in nonmeiotic cells (growing a/o cells) and reduced in meiotic cells. Although the URS1, mutation reduced meiotic cell-growth (Fig. 3A), this activity may have accumulated during vegetative growth before transfer to sporulation medium. Nonmeiotic expression of a \textit{hop1-lacZ} fusion lacking URS1 was clearly not due to vector sequences, because expression was abolisheing by a second multisite mutation within \textit{HOP1} 5' sequences. This second mutation defined an element called UAS$_{HOP}$, which has UAS activity in nonmeiotic cells when separated from the URS1 site. Therefore, at \textit{HOP1}, URS1 blocks UAS$_{HOP}$ activity in nonmeiotic cells. In addition, URS1 stimulates \textit{HOP1} expression in meiotic cells.

Both \textit{SPO13} and \textit{HOP1} have regulatory regions that are very close to their initiation codons (Table 1). For \textit{SPO13}, RNA start sites have been mapped in the −49 to −10 interval, only 40 to 80 bp from the URS1 site (114). Yeast genes generally have regulatory sequences that lie upstream of, and are separable from, TATA and RNA start sites (98). Many genes have UASs that can confer regulated expression when fused to a heterologous minimal promoter, consisting only of TATA and RNA start sites. However, no meiosis-specific regulatory region has been separated from the minimal promoter elements of \textit{SPO13} and \textit{HOP1}. One explanation is that these promoters simply pose some technical difficulty (e.g., meiotic regulatory sequences and minimal promoter sequences may overlap). A second possibility is that expression of these genes is achieved by an unusual mechanism (e.g., a single site may serve as both an activation sequence and a TATA sequence). The \textit{IME2} gene has a structure more typical of yeast genes. Deletions that abolish expression affect sequences between −584 and −442 (3). Although \textit{IME2} has a long (ca. 300-base) untranslated leader (20), these deletions lie quite far from the RNA start site. This interval has the properties of a meiosis-specific UAS, because placing it upstream of a minimal promoter causes meiosis-specific expression of the reporter gene. In these studies, meiosis-specific expression was assessed by dependence of expression on an activator of meiosis, \textit{IME1} (see below). Further subcloning revealed that this region contains two separable \textit{IME1}-dependent UAS regions, a stronger upstream UAS and a weaker downstream UAS. Thus, \textit{IME2} has a meiosis-specific upstream regulatory region that is separable from other promoter elements.

Mutational analysis of the strong \textit{IME2} UAS indicates that here, as in the \textit{SPO13} and \textit{HOP1} promoters, a URS1 site plays both positive and negative roles (3). A second site, called a T$_{C}$ site, also contributes to UAS activity, much like the \textit{HOP1} promoter. URS1 mutations caused midlevel constitutive (\textit{IME1}-independent) expression, whereas T$_{C}$ site mutations simply reduced UAS activity without relieving \textit{IME1} dependence. These observations led to the suggestion that the URS1 and T$_{C}$ sites have different roles in UAS activity: URS1 is required to confer \textit{IME1} dependence, whereas the T$_{C}$ site adjusts the overall expression level (3).

URS1, UAS$_{HOP}$, and T$_{C}$ sites are found in the regulatory regions of many early meiotic genes (Table 1). The URS1 site generally lies within 200 bp of the initiation codon; \textit{IME2}, \textit{REC102}, and \textit{RIM4} are the exceptions. A subset of genes (\textit{HOP1}, \textit{MER1}, \textit{REC102}, \textit{SPO11}, and \textit{SPO16}) also have a nearby UAS$_{HOP}$ site; a different subset (\textit{IME2}, \textit{IME1}, \textit{MEK1}, \textit{MEK4}, \textit{RED1}, and \textit{RIM4}) have a nearby T$_{C}$ site. The UAS$_{HOP}$ or T$_{C}$ site generally lies upstream of URS1. Some unusual cases include \textit{DMC1}, which has URS1, UAS$_{HOP}$, and T$_{C}$ sites; \textit{SPO13} and \textit{MEI4}, which have URS1 sites without discernible UAS$_{HOP}$ or T$_{C}$ sites; and \textit{SPO11}, which has a URS1 site within the coding region. \textit{MEK1/MRE4} has been cloned and sequenced by two groups, which found one and two URS1 sites, respectively. \textit{IME2} has two pairs of URS1 and T$_{C}$ sites; one pair lies within each of the \textit{IME2} UASs. The weaker \textit{IME2} UAS has a poorer match to the T$_{C}$ site consensus. The middle gene \textit{SPO12} was reported to have both URS1 and UAS$_{HOP}$ homology (109), but both sequences are very poor matches to each consensus. Thus, URS1 and an accompanying UAS$_{HOP}$ or T$_{C}$ site are found near many early meiotic genes.

Two simple models can account for the roles of each site at early meiotic regulatory regions. One possibility is that meiosis-specific expression results only from a unique interaction between URS1 and an accompanying site. Each site in isolation would have properties unrelated to meiotic expression. Meiotic genes without UAS$_{HOP}$ or T$_{C}$ site homology presumably have a similar type of site that has yet to be identified. The second possibility is that the URS1 site is a meiotic on/off switch that specifies which genes may be activated early in meiosis. The accompanying UAS$_{HOP}$ or T$_{C}$ site serves as a gain control that determines the overall level of expression. Independent on/off and gain controls may be useful for a large family of genes with a wide range of expression levels. Studies of the individual sites and the relevant regulatory proteins will be necessary to distinguish these and other more complicated models.

The \textit{IME1} gene is expressed at high levels early in meiosis, yet it has no recognizable URS1 site. \textit{IME1} may be expressed a little earlier than most early meiotic genes (47, 67) and therefore may belong to a distinct expression class. Arguments based on function suggest that \textit{IME1} expression should precede that of other early genes (see below). However, \textit{IME1} is expressed at high levels after heat shock (87) and at the end of exponential growth (47). These conditions do not lead to meiosis, so \textit{IME1} is not strictly a meiosis-specific gene. \textit{IME1} may have an unusual regulatory region because its expression pattern is different from that of most early meiotic genes. However, these observations leave open the possibility that URS1-dependent expression is only one of the ways that early meiosis-specific expression is achieved.

Middle and Late Meiotic Genes

Expression of the late sporulation-specific gene \textit{SGA1} also depends on two sequence elements (48). One element functions in a heterologous promoter as a UAS. Expression of the heterologous gene containing the \textit{SGA1} UAS is blocked by the presence of either glucose or ammonia. The second element, called the negative regulatory element (NRE), functions in a heterologous promoter as a negative site. Expression of the NRE-containing hybrid promoter is restricted to a/o cells and depends on starvation and on the positive meiotic regulators \textit{IME1} and \textit{IME2}. A 17-bp segment of the NRE (AGGGTC CTTTTTGGT) includes 14 identities to a 5' segment of a middle sporulation-specific gene, \textit{SPS4}. Note that expression of \textit{SPS4} and \textit{SGA1} has not been monitored in the same experiment, so the genes may belong to the same temporal class. These observations indicate that expression of some middle and late meiotic genes may depend on relief of repression through the NRE (48). Analysis of the late gene \textit{SPB2} supports the importance of an NRE-like sequence but suggests a positive role for the site
MEIOTIC REGULATORY GENES

Many known genes influence meiotic gene expression. Understanding the function of each relies on understanding its relationship with upstream and downstream regulators. For that reason, this section begins with a summary of the identification and properties of many key regulators. Several groups of genes have been identified through similar strategies. Increased dosage of IME (inducer of meiosis) genes stimulates meiosis in non-a/a cells. Mutations in UME (unscheduled meiotic gene expression) genes permit SPO13 promoter activity in vegetative, non-a/a cells. Mutations in RIM (regulator of inducer of meiosis) genes prevent expression of an ime2-lacZ fusion gene. Mutations affecting cyclic AMP (cAMP-dependent) protein kinase activity affect meiotic gene expression, but these genes are not listed individually and will be discussed only briefly (see reference 7 for a more thorough review of this topic).

IME1. IME1 was identified as a multicopy genomic clone that permits non-a/a diploids to sporulate (45). An ime1 disruption prevents expression of almost all meiotic genes and all tested meiotic events (23, 45, 48, 67, 92, 109, 118). IME1 has no informative homologs (94).

IME2. IME2 (also called SME1, for start of meiosis) was identified as a multicopy genomic clone that permits a/a cells expressing an inhibitor of meiosis, RME1, to undergo recombination (92). IME2 was also isolated as a multicopy clone that permits sporulation in the presence of a nitrogen source (118). An ime2 disruption mutant shows reduced or delayed recombination and DNA synthesis and reduced expression of middle meiotic genes (92, 118). Ime2 is a protein kinase homolog (118).

IME4. IME4 was identified as a clone that enhances RES1-dependent spr3-lacZ expression in non-a/a cells (86). An ime4 disruption reduces or abolishes IME1 and IME2 expression and sporulation.

MCK1. MCK1 (meiosis and centromere regulatory kinase) was identified as a multicopy genomic clone that permits a/a cells expressing RME1 to undergo meiotic recombination (70). MCK1 was independently identified as a multicopy suppressor of mitotic chromosome missegregation arising from centromere mutations (88). An mck1 disruption reduces the rate and efficiency of meiotic gene expression and meiosis, causes accumulation of immature asci, and causes defects in mitotic centromere behavior (70, 88). Mck1 (initially called Ypk1, for yeast protein kinase) is a protein kinase homolog that cofractionates with serine, threonine, and tyrosine kinase activity (18).

MER1. MER1 (meiotic recombination) was identified through a mutation causing production of inviable spores (22), as do many recombination-defective mutations. A mer1 null mutation blocks meiotic recombination. Mer1 is required for splicing of MER2 RNA (24). Mer1 has a motif found in several ribonucleoprotein-associated proteins (63).

RES1. RES1 (Rme1 escape) was discovered through a partially dominant mutation, RES1-1, that permits expression of spr3-lacZ and sporulation of a/a cells expressing Rme1 (44).

RIM1. RIM1 was identified through mutations that prevent expression of an ime2-lacZ fusion (99) and through mutations that permit survival of haploid cells genetically programmed to sporulate (i.e., expressing IME1 constitutively) (66). Recessive rim1 mutations prevent sporulation and meiotic gene expression. Rim1 is a protein kinase homolog (4).

RME1. RME1 (regulator of meiosis) was discovered through an allelic difference among laboratory strains; some have a recessive rme1 mutation that permits non-a/a diploids to sporulate (46). RME1 was also initially called CSP1 (control of sporulation) (39). An rme1 disruption permits IME1 expression and sporulation in non-a/a cells; rme1 mutations do not alter the nutritional requirements for sporulation (68). Overexpression of RME1 blocks IME1 expression and sporulation (15, 68). Rme1 is a zinc finger protein homolog (15).

RPD3. rpd3 (reduced potassium dependency) mutations permit expression of spo13-lacZ in vegetative, non-a/a cells (110), permit ime2-HIS3 expression in Δime1 strains (3), and alter the regulation of many nonmeiotic genes (110). Rpd3 has no informative homologs (110).

SIN3. sin3 (switch-independent) mutations permit expression of spo13-lacZ and several early meiotic genes in vegetative, non-a/a cells (97), permit ime2-HIS3 expression in Δime1 strains (3), and alter the regulation of many nonmeiotic genes (5, 96, 111). SIN3 has also been called RPD1 (111), SDH1 (5), UME4 (97), and GAM2 (119). A sin3 null mutation reduces sporulation efficiency (111). Sin3 is a nuclear protein with four putative paired amphipathic helices (112).

SME2. A multicopy SME2 (start of meiosis) plasmid permits sporulation in the presence of ammonia or glucose (47). A sme2 disruption mutation does not affect sporulation. Increased SME2 dosage may stimulate the expression of one or more late meiotic genes specifically (47).

SME3. A multicopy SME3 (start of meiosis) plasmid permits sporulation in the presence of ammonia or glucose and in non-a/a cells (47). An sme3 disruption mutation does not affect sporulation.

SPS1. SPS1 was identified as a middle sporulation-specific transcript (73). Sps1 mutations block sporulation after the meiotic divisions (74) and cause reduced expression of late meiotic genes (85). Sps1 is a protein kinase homolog (85).

UME1, UME2, UME3. Mutations in these genes permit the expression of several early meiotic genes in vegetative, non-a/a cells (97).

UME6. ume6 loss-of-function mutations permit spo13-lacZ expression in vegetative, non-a/a cells (97) and permit ime2-HIS3 expression in Δime1 strains (3). UME6 was independently identified as CAR80 from its role in expression of the nonmeiotic gene CAR1 (72). Ume6 null mutations reduce sporulation efficiency and spore viability and cause slow growth (3). A different type of allele, originally called rim16-12, was identified as a mutation that permits survival of haploid cells genetically programmed to sporulate (4, 66). rim16-12 causes reduced sporulation and ime2-lacZ expression but does not affect spore viability or growth.

A number of mutations that may affect meiotic RNA levels have not been characterized in great detail. spo17 and spo18
mutations were identified through defects in sporulation-specific glucoamylase activity (95). There is also a large collection of spo7 mutations (107), which block sporulation at various steps.

**REGULATORY PATHWAYS**

The regulation of meiotic genes may be summarized in broad outline very simply. Starvation of α/α cells causes increased expression of IME1; Ime1 then stimulates the expression of many meiotic genes. With this view in mind, I will first examine how these signals govern IME1 expression and then how Ime1 activates meiotic genes. Subsequent sections expand and qualify this simplified view.

**Control of IME1 by the Mating Type Locus**

Meiotic gene expression and sporulation depend on the repressor a1-α2, which determines the a/α cell type. How does a repressor permit cells to enter meiosis? One mechanism involves an inhibitor of meiosis specified by RME1 (Fig. 2). In non-α/a cells, Rme1 blocks meiosis, as indicated by the observation that Rme1 loss-of-function mutations permit non-α/a cells to sporulate (46, 68, 79). In α/a cells, RME1 is transcriptionally repressed through an upstream a1-α2 binding site (15, 68). Thus, RME1 RNA is present at 10- to 20-fold-higher levels in vegetative non-α/a cells than in vegetative α/a cells (68). RME1 RNA levels increase a further 10-fold in non-α/a cells after starvation, so a 100-fold difference in RME1 RNA levels determines the ability or inability to sporulate (17). Expression of RME1 in α/a cells, through increased gene dosage or by fusion to a constitutive promoter, reduces sporulation efficiency and meiotic gene expression (15, 32, 68). Therefore, Ρme1 can inhibit sporulation, regardless of cell type, if it is expressed.

Two observations had suggested that repression of RME1 was not the only mechanism by which a1-α2 stimulates meiosis. First, in comparisons of strains that lack RME1 function, a/α diploids sporulate more efficiently than non-α/a diploids (68). Second, expression of RME1 blocks sporulation more efficiently in non-α/a cells than in α/a cells (93). More direct evidence for an RME1-independent pathway that influences meiosis came from identification of the RES1 and IME4 genes. RES1 was identified through a partially dominant mutation, RES1-1, that permits expression of a sporulation-specific spr3-lacZ fusion in a/a cells carrying a multicopy RME1 plasmid (44). RES1-1 also permits sporulation of non-α/a diploids. Two findings suggest that RES1-1 acts through a different pathway from RME1. First, RES1-1 permits higher levels of sporulation than an rme1 null mutation in non-α/a diploids. Second, RME1 and rme1 mutations have additive effects on the sporulation of non-α/a diploids (44). These independence arguments should be considered provisional, however, because the nature of the RES1-1 alteration (loss or gain of function) is unclear.

An attempt to clone RES1 led to identification of a suppressor, IME4, that specifies a positive regulator of meiosis (86). Increased IME4 dosage permits non-α/a diploids to sporulate; an ime4 insertion mutation blocks sporulation. (In some strains, ime4 mutations have little effect on sporulation, as discussed below.) IME4 expression is meiosis specific: RNA levels are low in vegetative cells and increase in response to nitrogen starvation only in α/a cells. Because a1-α2 is known to act only as a repressor, it was proposed that a1-α2 stimulates IME4 expression indirectly, for example, by repressing a repressor of IME4 (86). (The recent observation that insertions lying 3' to IME4 lead to cell type-independent IME4 expression suggests that a more unusual regulatory mechanism may be involved [11].) These expression and dosage studies indicate that IME4 transmits an a/α cell type signal.

What is the relationship between Rme1 and Ime4? An rme1 mutation does not alter regulation of IME4 expression by a1-α2, so Rme1 is not the hypothetical repressor of IME4 (86). An rme1 mutation can suppress an ime4 insertion mutation to permit expression of the meiotic genes IME1 and IME2. Thus, in principle, Rme1 may act either in parallel to or downstream of Ime4. Given that a1-α2 represses RME1 expression directly (15), the simplest explanation is that Rme1 and Ime4 act in parallel pathways (86).

The ultimate target of regulation by Rme1 and Ime4 is expression of the IME1 gene. IME1 is expressed at low levels in vegetative cells and at 5- to 30-fold-higher levels in starved a/α cells (45, 92). Ime1 is formally a positive regulator of other meiotic genes, because deletion of IME1 prevents the expression of other early (Spo11, Spo13, Mei1, IME2, and Hop1), middle (Sp51 and Sp52), and late (Sga1) meiotic genes (23, 48, 67, 109). The a/α cell type regulatory signal is transmitted by IME1 RNA levels, because expression of IME1 from a cell type-independent promoter permits expression of meiotic genes regardless of cell type (94). Therefore, Ime1 plays a pivotal role in the activation of early meiotic genes.

Ime4 is required to stimulate IME1 expression, because an ime4 mutation blocks IME1 RNA accumulation in starved a/α cells (86). However, suppression studies indicate that Ime4 may have an additional role in stimulating meiosis, although two experiments gave apparently conflicting results. In one experiment, an RES1-1 mutation permits high levels of IME1 RNA accumulation in an ime4 mutant but permits only inefficient sporulation. On the other hand, the presence of an IME1 multicopy plasmid in an ime4 mutant permits efficient sporulation. One idea that reconciles these observations is that Ime1 and Ime4 have partially overlapping functions: overexpression of Ime1 from a multicopy plasmid would alleviate the
need for Ime4, but expression of Ime1 at more normal levels (in the RES1-ime4 mutant) would not (86). In addition, differences in the translation of IME1 RNA in these two situations (87) might account for these results.

IME4 RNA levels respond to both cell type and nutritional signals, as described above (86). Thus, increased IME4 expression may lead directly to increased IME1 expression in nitrogen-starved a/α cells. However, a second nitrogen regulatory pathway must exist, because IME1 is regulated by nitrogen in an ime4 RES1-1 double mutant (86).

Three lines of evidence indicate that IME1 is the target of repression by Rme1. First, Rme1 loss-of-function mutations permit IME1 expression in starved non-a/α cells (45, 92). Second, expression of RME1 (from a constitutive promoter) in a/α cells prevents IME1 expression (15). Third, expression of IME1 from a constitutive promoter overrides the inhibition of meiotic gene expression and sporulation by Rme1 (15). These observations argue that Rme1 blocks IME1 expression in non-a/α cells. In addition, IME1 may be the only gene required for meiosis that is repressed by Rme1.

Rme1 acts over a considerable distance to repress IME1. Early studies suggested that Rme1 might act through a site 3 kb upstream of IME1, because a multicopy plasmid carrying this region could apparently titrate Rme1 activity (32). More recently, deletion analysis has indicated that a 500-bp interval that lies 2 kb upstream of IME1 is required for repression by Rme1 (16). Mobility shift experiments indicate that Rme1, a zinc finger protein, binds to a site in this interval. Oddly, a region containing the Rme1 binding site has the properties of an Rme1-dependent activation sequence when separated from flanking DNA (16). Repression of either IME1 or a heterologous promoter (in artificial constructs) requires the Rme1 binding site together with the adjacent 300-bp interval. Deletion of the Rme1 binding site from the chromosome does not fully relieve Rme1-dependent repression, so there may be other functional Rme1 binding sites (such as the putative site 3 kb upstream). These aspects of Rme1-dependent repression—action over a large distance and dependence on multiple sequence elements—are similar to the properties of the silencers that repress silent mating type information at HML and HMR (51). Whether these analogies reflect more fundamental mechanistic similarities between Rme1-dependent repression and silencing remains to be determined.

Other Regulators of IME1 RNA Levels

The regulation of IME1 RNA levels is complex but can be considered as three phenomena. First, there is a low, basal IME1 RNA level in growing cells. This level is similar in both a/α and non-a/α cells and is reduced by glucose (45). Second, there are elevated IME1 RNA levels under some circumstances not associated with sporulation; these include heat shock (87) and the end of exponential growth (47). Neither of these responses has been compared in a/α and non-a/α cells. Third, there is the high IME1 RNA level observed after nitrogen starvation of a/α cells, which is associated with meiosis (45). This level is 5- to 30-fold higher than the basal level (45, 92); it is possible that differences in the synchrony of the sporulating population account for the differences in maximal RNA levels detected. CAMP depletion can bypass the need for nitrogen starvation to stimulate IME1 expression (and sporulation), but this effect may be an indirect consequence of growth arrest or cell cycle arrest (62, 92). One study argues that mitochondrial function is required for maximal IME1 RNA accumulation (106), but the possibility that energy depletion simply prevented all RNA synthesis was not ruled out. The genes described in this section influence the decision to enter meiosis through effects on IME1 RNA levels, but which of the many possible signals they transmit is unclear.

MCK1, which specifies a putative Ser-Thr-Tyr protein kinase, is expressed at a constant level independent of cell type, glucose, or nitrogen (70). mck1 null mutants reduce sporulation efficiency, cause accumulation of immature asc, and also cause an array of phenotypes that reflect defective mitotic centromere behavior (70, 88). The pleiotropic effects of mck1 mutations raised the question of whether its partial sporulation defect simply reflected general ill health or whether Mck1 was required more directly for IME1 expression. Three observations suggest a more direct role for Mck1 (70). First, overexpression of MCK1 increases IME1 expression in starved a/α cells and accelerates sporulation. This finding suggests that Mck1 activity is normally limiting for IME1 expression. Second, mck1 mutations cause defects in the basal level of IME1 promoter activity in vegetative cells, as assayed with an ime1-HIS3 fusion gene in which the IME1 promoter is fused to the HIS3 coding region. Under these growth conditions, the mck1 mutant displayed no obvious growth or mitotic chromosome segregation defects (88). Third, the slow and inefficient sporulation of mck1 null mutants is suppressed by expression of IME1 from the GAL1 promoter (causing fivefold IME1 overexpression) or ACT1 promoter (causing IME1 expression at roughly the wild-type level) (70, 99). This finding indicates that reduced IME1 expression may be the sole cause of inefficient sporulation in the mutant. However, the ascus maturation defect of mck1 mutants is not suppressed by artificially elevated IME1 expression. Together, these observations argue that Mck1 functions independently to stimulate IME1 expression, ascus maturation, and mitotic centromere behavior.

The RIM1, RIM8, RIM9, and RIM13 genes are also required for IME1 RNA accumulation (99). Mutations in any of these genes lead to reduced IME1 expression in meiotic cells, reduced meiotic gene expression, and slow sporulation. Because the rim mutations are recessive, they are inferred to result in loss of gene function, but this inference is only known to be true for rim1 mutations (100). Like mck1 mutations, rim1/8/9/13 mutations are suppressed by expression of IME1 from the ACT1 promoter and cause reduced ime1-HIS3 expression in vegetative cells. However, these RIM gene products appear to act independently of Mck1, because all rim mck1 double mutants display more severe meiotic gene expression and sporulation defects than the single mutants (99). In contrast, double mutants with two rim mutations are no more defective than rim single mutants. Support for a close functional relationship among the RIM1/8/9/13 gene products comes from their shared pleiotropic mutant phenotypes, including smooth colony morphology (in the otherwise rough SK-1 genetic background) and cold-sensitive growth.

mck1 and rim1/8/9/13 mutations do not affect IME1 expression indirectly through effects on RME1 expression. mck1 rim1 double mutants are defective in sporulation in rme1 deletion strains, and MCK1 and RIM1 are required for the activity of an IME1 promoter fragment that is not repressed by Rme1 (54, 70, 99). Therefore, RME1, MCK1, and RIM1/8/9/13 govern IME1 expression independently.

mck1 and rim1/8/9/13 mutations do not act through effects on IME4 expression, either. The evidence comes from a strain in which ime4 mutations cause a complete sporulation defect in S288C-derived yeast strains (86) but cause only a marginal sporulation defect in SK-1-derived yeast strains (100). In SK-1 strains, mck1 and rim1/8/9/13 mutations cause more severe
sporulation defects than ime4 mutations. Therefore, MCK1 and RIM1/8/9/13 cannot simply be required for IME4 expression. In addition, ime4 mck1 and ime4 rim1 double mutants have more severe sporulation defects than the single mutants (100). These observations suggest that MCK1, RIM1/8/9/13, and IME4 all define independent pathways that stimulate IME1 expression.

The cAMP synthesis and response pathway also influences sporulation, in part, through effects on IME1 expression. Mutations that diminish cAMP-dependent protein kinase activity lead to IME1 expression and sporulation in the absence of nitrogen starvation (7, 62, 92). Mutations that cause elevated, constitutive protein kinase activity lead to failure to express IME1 or to sporulate (62). Although these genetic experiments suggest that the cAMP pathway may respond or govern response to nitrogen levels, the bulk of the evidence favors a role for this pathway in glucose sensing (7, 33). An increased dosage of either of two cAMP phosphodiesterase structural genes permits ime1-his3 expression in mck1 or rim1 null mutants (69a). Therefore, regulation of IME1 by cAMP levels does not require Mck1 or Rim1.

The SME3 gene has the properties of a positive regulator of IME1, because increased SME3 dosage causes elevated IME1 RNA accumulation, particularly in the presence of ammonia or glucose (47). SME3 RNA levels are very low during exponential growth and increase dramatically as cultures reach stationary phase; the response is comparable in a/α and non-a/α cells. Thus, SME3 might relay a signal related to glucose, nitrogen, or growth. However, an sme3 disruption mutation has no effect on sporulation efficiency or, by inference, on IME1 expression. These results may indicate either that SME3 acts in one of several functionally redundant pathways or that SME3 acquires a novel function (stimulation of IME1 expression) only when overexpressed. The relationships between SME3 and other regulators of IME1 expression are unclear at present.

Why should there be such a bewildering array of regulators and pathways that govern IME1 expression? One might argue that the sensitive genetic isolation strategies tend to magnify the effects of minor metabolic perturbations. However, it may be useful for the cell to couple IME1 expression to the sum of several metabolic signals. Thus, the decision to sporulate—which presumably reflects a threshold Ime1 concentration (see below)—would be based on a general picture of nutrient availability. That general picture may ensure that sporulation can be initiated before the nutrient supply is completely exhausted. The large size of the IME1 5′ regulatory region (16, 32) could certainly provide the opportunity for many regulatory proteins to act.

**Functional Roles of Ime1 and Ime2**

Ime1 is ultimately required for the expression of most or all of the early meiotic genes, as judged from the finding that Δime1/[Δime1] diploids fail to express these genes (see above). Experiments in which IME1 is expressed constitutively (cited above) argue that IME1 transmits the a/α cell type signal. Similarly, expression of IME1 in growing cells leads to elevated accumulation of transcripts of the early meiotic genes SP011, SP013, HOP1, and IME2 but not of the middle genes SPS1 and SPS2 (94). These results suggest that early genes are more direct targets of Ime1 than later genes and that IME1 RNA levels are partly responsible for transmitting the starvation signal. Thus, an understanding of Ime1 function is a good starting point for understanding how meiotic genes are regulated.

Ime1 activates meiotic genes through two genetically distinct pathways: one is independent of the IME2 gene product, and the other is dependent upon IME2 (67). I will refer to these pathways as the Ime1 pathway and the Ime2 pathway, respectively (Fig. 3). In wild-type cells, Ime1 is required for both pathways because Ime1 is required for IME2 expression (92, 118). Either pathway can stimulate RNA accumulation from many of the same early meiotic genes, including SP011, SP013, HOP1, and IME2 itself. Each pathway has unique properties as well, so that coordination of the pathways is critical for efficient sporulation.

Evidence for the Ime2 pathway comes from experiments in which IME2 was expressed from a hybrid GAL1-IME2 S′ region (94). Expression of the GAL1-IME2 hybrid gene depends on the galactose regulatory system rather than on Ime1, so that the consequences of Ime2 activity in the absence of Ime1 can be examined directly. A control Δime1/[Δime1] IME2/IME2 diploid failed to express the early genes SP011, SP013, and HOP1 as well as the middle genes SPS1 and SPS2. However, the Δime1/[Δime1] GAL1-IME2/GAL1-IME2 diploid expressed all of those genes. For reasons that are unclear, nitrogen starvation was required for expression of the GAL1-IME2 gene and, consequently, for expression of the early and middle genes. However, these findings clearly indicate that Ime1 can stimulate the expression of several meiotic genes in the absence of Ime1.

Functional expression of meiotic genes through the Ime2 pathway was verified by the ability of Δime1/[Δime1] GAL1-IME2/GAL1-IME2 diploids to undergo meiotic levels of gene conversion at the HIS4 locus and to sporulate (94). However, sporulation of these diploids is aberrant: sporulation is asynchronous and inefficient, spore viability is low, and the frequency of chromosome III disomy among spores is high. These phenotypes are not simply a consequence of Ime2 overexpression, because IME1/[IME1] GAL1-IME2/GAL1-IME2 diploids sporulate with fidelity. These observations argue that Ime1 has some unique role in sporulation that Ime2 cannot carry out. In fact, the early meiotic gene REC114, which is required for recombination, is activated through the Ime1 pathway but not through the Ime2 pathway (76). (It has been suggested that the Ime2 pathway also cannot activate HOP1 expression [109], but HOP1 expression in an ime1 mutant that expresses IME2 was not examined. HOP1 is expressed in a Δime1 GAL1-IME2 strain [93].) Because recombination defects lead to aneuploidy

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**FIG. 3.** Relationship between Ime1, Ime2, and early meiotic genes. Ime1 activates meiotic genes through two pathways. In the Ime1 pathway, Ime1 activates genes independently of Ime2. In the Ime2 pathway, Ime1 acts only indirectly by stimulating expression of IME2; Ime2 then activates genes independently of Ime1. The Ime1 pathway depends on Rim11, Rim15, and Ume6 for activation of IME2 and, possibly, other early meiotic genes. The Ime2 pathway is independent of Rim11 and permits Ime2 to activate its own expression. The symbols in this diagram are the same as for Fig. 2.
and spore inviability, it is possible that failure to express REC114 is responsible for sporulation defects when only the Ime2 pathway is active.

It is evident that the Ime1 pathway stimulates early meiotic genes independently of Ime2 from examination of IME1/IME1 ime2/Δime2 strains (94). These strains express early genes efficiently in response to starvation. Thus, Ime1 can stimulate the expression of several meiotic genes in the absence of Ime2.

Functional expression of meiotic genes through the Ime1 pathway is supported by the finding that ime2 null mutations do not block meiotic gene conversion (94), although the rate of conversion is slowed. However, ime2 null mutants fail to sporulate (92, 118). Thus, Ime2 must have a unique role in sporulation that Ime1 cannot carry out. Some evidence suggests that ime2 null mutants express middle and late meiotic genes poorly (48, 94), so one unique role for Ime2 may be to stimulate later meiotic gene expression. Ime2 is also required to downregulate IME1 RNA levels (92). In wild-type strains, IME1 is expressed only for a brief period of time: IME1 RNA levels are maximal 4 h after starvation (in SK1 strains) and decline at 6 to 8 h. In ime2 null mutants, IME1 RNA levels do not decline until 20 to 30 h. Therefore, Ime2 is formally a negative regulator of IME1 expression. Prolonged expression of IME1 in ime2 mutants probably accounts for their prolonged expression of early meiotic genes (94). The extended expression of IME1 and early meiotic genes may interfere with the progress of ime2 mutants through sporulation.

Ime1 and Ime2 are not homologous and thus may activate meiotic genes through different mechanisms. Ime2 is a serine/threonine protein kinase homolog (118); it has autophosphorylation activity in immune complexes (93). It has been suggested that Ime2 might stimulate meiotic genes by inactivating one of the negative regulators Ume1/2/3/5 or Sin3 (97). Thus far, direct phosphorylation of a regulatory protein by Ime2 has not been demonstrated.

Genetic evidence indicates that Ime1 may activate some meiotic genes by providing a transcriptional activation domain (91). The argument is based on studies of transcriptional activation by a lexA-IME1 fusion-encoded protein, in which the LexA DNA-binding domain is fused to Ime1. Transcriptional activation by LexA-Ime1 was assayed through expression of a gal1-lacZ reporter gene with upstream lexA operators in place of the GAL1 UAS. There are three correlations between the requirements for activation by LexA-Ime1 and for natural Ime1 activity, as assayed through expression of an ime2-lacZ reporter gene. First, four ime1 missense mutations reduce both LexA-Ime1 and Ime1 activities, and intragenic suppressors of two mutations restore both activities. These mutations do not simply reduce accumulation of LexA-Ime1, so they seem to affect intrinsic activity. Second, rim11 mutations block both LexA-Ime1 and Ime1 activities. Third, the central tyrosine-rich region of Ime1, which has the functional properties of an activation domain, can be replaced by the acidic herpesvirus VP16 activation domain to restore Ime1 function. Consistent with the idea that Ime1 functions directly in transcriptional activation is the finding that an Ime1–β-galactosidase fusion protein is concentrated in the nucleus. Although there is no evidence that Ime1 binds directly to DNA, the studies described below (3) suggest that a protein may act as an adaptor to permit Ime1 to bind to DNA.

The Ime1 pathway and Ime2 pathway stimulate early meiotic genes through different sites or combinations of sites. A minimal UAS from IME2 (Tc site and URS1) is activated by the Ime1 pathway and not by the Ime2 pathway (3). The REC114 promoter, which includes UAS1 and URS1 sites, is also activated only through the Ime1 pathway (76). Thus, the URS1-TcC and URS1-UAS1 site combinations may be activated by the Ime1 pathway, whereas an unidentified site (or combination of sites) may be activated by the Ime2 pathway.

Why does a cell need both Ime1 and Ime2 to turn on many of the same genes? Ime2 is a positive regulator of its own expression (3). Therefore, if a cell makes enough Ime1 to activate IME2 expression, Ime2 can amplify Ime1 activity. This arrangement is ideal for converting a graded signal that responds to multiple inputs (IME1 RNA levels) into a qualitative decision to activate meiotic genes and enter meiosis. In addition, amplification of Ime1 activity by Ime2 may ensure balanced expression of the many early genes required for successful recombination and segregation.

Other Positive Regulators of Early Meiotic Genes

The Rim11 and Rim15 gene products are required in addition to Ime1 for IME2 expression (66, 99). Rim11 is clearly a positive regulator of IME2, as determined by studies of bona fide null mutants (4). Rim15 has been identified by a single recessive mutation, so its assignment as a positive regulator of IME2 is tentative. rim11 and rim15 mutations block the activity of an IME2 UAS that responds only to the Ime1 pathway (3), indicating that Rim11 and Rim15 act in the Ime1 pathway. These gene products act in parallel or downstream of Ime1, because they are required for IME2 UAS activity even when IME1 is expressed from the GAL1 promoter (3). Rim11 is required only for the Ime1 pathway, because expression of the GAL1-IME2 hybrid gene activates the IME2 promoter and permits sporulation in a rim11 mutant (66). Whether Rim15 is also specific for the Ime1 pathway is unclear.

Rim11 appears to be more directly required for Ime1 activity than for some other aspect of IME2 UAS activity. This idea comes from the observation that a rim11 mutation blocks transcriptional activation by the LexA-Ime1 fusion protein (91). The rim15 mutation has little effect on LexA-Ime1 activity, so it seems unlikely that Rim11 is simply required for Rim15 expression or activity. Rim11 specifies a serine/threonine protein kinase, as determined by sequence analysis and immune complex phosphorylation assays (4). There is no evidence at present that Ime1 is phosphorylated, so details of the molecular interactions between Ime1 and Rim11 are unknown.

The rim16-12 mutation also blocks IME2 UAS activity without affecting Ime1 polypeptide levels (3, 66). Recent studies indicate that rim16-12 is an unusual allele of UME6 (4), which is discussed below.

Negative Regulators of Early Genes

Two negative regulators, Sin3 and Rpd3, contribute to the proper expression of many early meiotic genes. Null sin3 and rpd3 alleles permit elevated expression of a sop13-lacZ fusion in vegetative, non-a/a cells, so Sin3 and Rpd3 are negative regulators of SPO13 (97, 110). sin3 mutations, which have been characterized more extensively, permit the expression of other early meiotic genes (SPO11, SPO16, and IME2) but not later genes (SPO12 and SP52) in vegetative, non-a/a cells (97). sin3 and rpd3 mutations cause increased expression of a number of nonmeiotic genes, such as HO, TRK2, STE6, and RME1, under inappropriate conditions. More detailed studies indicate that Sin3 and Rpd3 are required for the full range of expression of many regulated genes; that is, sin3 and rpd3 mutations cause elevated expression under repressing or noninducing condi-
tions and cause reduced expression under derepressing or inducing conditions (110, 111). Thus, Sin3 and Rpd3 are referred to as transcriptional modulators. Existing evidence indicates only a negative role for Sin3 and Rpd3 in early meiotic gene expression.

What is the relationship between Sin3, Rpd3, and the positive regulators of early meiotic genes? Sin3 and Rpd3 seem to act in the same pathway, because sin3 rpd3 double mutants express spo13-lacZ during vegetative growth at the same level as either single mutant (110). Relationships with Ime1 and Ime2 have been studied in greater detail with Sin3 than Rpd3. spo13-lacZ is expressed in sin3 mutants, sin3 ime1 double mutants, and sin3 ime2 double mutants (97). Therefore, Sin3 may act downstream or independently of Ime1 and Ime2. Expression of Ime1 in vegetative, non-a/a cells causes expression of early meiotic genes (94), just as a sin3 mutation does, supporting a close functional relationship between Ime1 and Sin3. The sites of action of Ime1 and Sin3 are close or the same, because a sin3 null allele permits activity of a 48-bp IME2 UAS that responds only to the Ime1 pathway (3). However, IME2 UAS activity increases in response to IME1 expression in a sin3 null mutant. Therefore, Ime1 does not stimulate a UAS simply by inactivating Sin3. The observation that an IME2 UAS fragment can be activated by a sin3 null mutation but not by the Ime2 pathway suggests that Ime2 does not simply inactivate Sin3. Thus, it seems likely that Sin3 acts independently of Ime1 and Ime2.

Two observations suggest that Sin3 may act directly as a negative transcriptional regulator. First, Sin3 is concentrated in the nucleus (112). Second, a LexA-Sin3 hybrid protein can block the activation of a reporter gene that contains LexA binding sites (113). The region of Sin3 required for repression by LexA-Sin3 is also required for negative regulation of natural Sin3 target genes. There is no evidence thus far for direct binding of Sin3 to DNA, so Sin3 may exert repression by interacting with a DNA-protein complex.

Recessive mutations in UME1, UME2, UME3, and UME5 cause low-level accumulation of early meiotic RNAs in nonmeiotic cells (97). Accordingly, these genes specify putative negative regulators of early meiotic genes. Detailed characterization of these genes has not been reported.

UME6, a Positive and Negative Regulator of Early Genes

The UME6 gene product has both positive and negative effects on early meiotic gene expression. The gene was first identified as a negative regulator: ume6 mutations permit spo13-lacZ expression in vegetative non-a/a cells (97) and permit IME2 promoter activity, assayed by a fusion to the HIS3 coding region (ime2-HIS3), in ime1 null mutants (3). UME6 is the same gene as CAR80, which was identified as a negative regulator of the arginine catabolic gene CAR1 (72). The connection between CAR1 and early meiotic genes is the URS1 site: URS1 was first discovered as a negative regulatory site in the CAR1 upstream region (102). In fact, a ume6 insertion mutation abolishes repression through URS1 in nonmeiotic cells (72). These findings indicate that UME6 is a negative regulator of meiotic and nonmeiotic genes that acts through URS1.

One might imagine that a Ume6-dependent repression system would compete with an Ime1-dependent activation system at the URS1 sites of early meiotic promoters. This model predicts that ume6 loss-of-function mutations should not interfere with Ime1-dependent activation. In fact, ume6 mutants that express Ime1 might even overexpress meiotic genes. These predictions were not upheld in studies of the IME2 UAS (3). Instead, ume6 mutations that abolish repression also abolish Ime1-dependent activation. These experiments were conducted with a UAS that responds only to the Ime1 pathway and in ime2 mutants, indicating that Ume6 is required for activation through the Ime1 pathway. As determined by immunoblots, Ume6 is not required for Ime1 polypeptide accumulation. Therefore, Ume6 acts in conjunction with or downstream of Ime1 to activate the IME2 UAS. There is no evidence thus far that Ume6 is required for the activation of any other early meiotic genes.

UME6 was also identified through a mutation that may specifically impair its positive role in IME2 expression. The rim16-12 mutation was identified as a mutation that prevents IME2 expression (66). Linkage and complementation analysis indicates that rim16-12 is a ume6 mutation (4). A rim16-12 mutant complements a ume6 insertion mutant for pleiotropic growth defects and spore inviability, indicating that rim16-12 is not a null allele. rim16-12 causes a lower level of ime2-HIS3 expression in vegetative cells than ume6 null mutations, suggesting that repression of the IME2 promoter by Ume6 is intact. Therefore, rim16-12 may cause a specific defect in activation of IME2 and other meiotic genes. Alternatively, rim16-12 may cause the formation of a superrepressor that reduces the expression of all Ume6-repressible genes.

Model for Ime1-Dependent Activation of Early Meiotic Genes

The observations recounted above are consistent with a simple model for the roles of Ume6 and Ime1 in regulation of the IME2 UAS and, possibly, other early meiotic promoters as well (3). In cells that lack Ime1, Ume6 is required for the activity of a repressor that acts through the URS1 site. Ime1 then modifies the repressor to convert it into a positive regulator (Fig. 4).
What is the repressor? The genetic studies described above implicate Ume6 and Sin3 (and probably Rpd3) in repression (3, 97, 113). However, a sin3 mutation does not block repression of the nonmeiotic CYC1 promoter by URS1 (72). Thus, Sin3 may be required for the repression of only a subset of URS1-containing promoters. This subset may be defined by a nearby sequence or by the nature of the activator protein. Given that Ume6 is required for both repression and activation through URS1, the simplest explanation is that Ume6 binds to URS1. However, no observations support that idea at present. The major URS1-binding protein, a heterodimer called BUF (55), is present in ume6 mutant extracts (72). Thus, BUF and Ume6 may associate or modify one another to generate the repressor.

How might Ime1 modify the repressor? The properties of LexA-Ime1 fusion proteins suggest that the role of Ime1 may be to provide a transcriptional activation domain (91). One simple possibility is that Ime1 binds directly to the URS1-repressor complex and, through the presence of an activation domain, converts the negative regulator to a positive regulator. Binding by Ime1 may be facilitated by proteins at a nearby UAS_H or Tac site.

It is tempting to use this model to explain the regulation of all early meiotic genes. However, recall that some early meiotic regulatory regions have separable UAS regions (such as IME2) and others do not (such as HO/P1 and SPO13). This distinction may reflect more fundamental differences in mechanisms of regulation.

**EFFECTS OF STARVATION ON MEIOTIC GENE EXPRESSION**

The studies described above suggest that a cell with high levels of IME1 RNA should express high levels of other early meiotic gene RNAs. However, as mentioned above, heat shock and growth limitation stimulate IME1 RNA accumulation, yet IME2 expression and sporulation do not occur (47, 87). Sporulation might be dismissed as an indirect assay of Ime1 activity, but certainly IME2 RNA should be present in cells that express IME1. The lack of correspondence between IME1 and IME2 RNA levels in growing cells may result from the effects of starvation on IME1 translation (87). Ime1 activity (91), SIN3 expression (112), and meiotic RNA stability (see next section).

The idea that IME1 RNA translation is regulated derives from a comparison of IME1 RNA levels and accumulation of an ime1-lacZ fusion protein (87). Starvation of a/a cells caused a 9-fold increase in IME1 RNA levels but a >3,000-fold increase in β-galactosidase activity. The observation was slightly complicated because the amounts of native IME1 RNA and plasmid-encoded ime1-lacZ RNAs were not distinguished. However, it was observed that IME1 has a long (220- to 280-base) untranslated leader with the potential to form a stem-loop structure. These findings have led to the suggestion that, in growing cells, the stem-loop structure blocks IME1 translation; in starved cells, inhibition of IME1 translation is bypassed (87).

Other effects of starvation are independent of IME1 translation. Expression of IME1 from the GAL1 promoter permits a comparison of growing and starved cells with essentially the same levels of GAL1-IME1 RNA (94) and protein (93). Although the growing cells expressed higher levels of early meiotic RNAs than wild-type cells, starvation caused a further 3- to 10-fold increase in early meiotic gene RNA levels. Two observations may explain this increase. First, Sin3-dependent repression may be lifted in starved cells. This idea derives from the observation that SIN3 RNA is present in growing cells but not in stationary-phase cells (112). Therefore, growth limitation may lead to decreased Sin3 levels. Second, Ime1 may be a more potent transcriptional activator in starved cells. This idea derives from the finding that the LexA-Ime1 fusion protein is a 10-fold-better activator in starved cells than in growing cells (91). The C terminus of Ime1 is required for both the starvation response and Rim11 dependence of LexA-Ime1 (91). Therefore, Rim11 may relay a starvation signal.

**INSTABILITY OF EARLY MEIOTIC TRANSCRIPTS**

Progress through the meiotic prophase can be interrupted by providing nutrients to starved cells (25). These circumstances cause cells to resume mitotic growth (see reference 37 for a more detailed discussion). The ability of cells to rapidly exit the meiotic pathway suggested that meiotic gene transcripts and gene products might be quite unstable. Indeed, providing nutrients to sporulating cells causes the transcripts of three early meiotic genes (SPO11, SPO13, and SPO16) to decay with half-lives of about 3 min (103). The transcripts of two later genes (SPO12 and SPS2) are considerably more stable, with half-lives of 10 to 12 min. Thus, nutrient addition prevents the continued expression of meiotic genes.

The stability of SPO13 RNA is twofold greater in acetate sporation medium than in a similar medium containing glucose (103). This determination was made by interrupting transcription with a temperature-sensitive RNA polymerase mutant. Stability differences were observed with both the native SPO13 gene and an ACT1-SPO13 fusion, in which the ACT1 promoter was fused to the SPO13 coding region. Two control transcripts, those of the native ACT1 gene and a SPO13 promoter-HIS3 fusion, had the same half-lives regardless of the carbon source. Thus, conditions that favor meiosis (presence of acetate and absence of glucose) also increase SPO13 RNA stability.

One major determinant of SPO13 RNA instability lies within the +3 to +262 interval, as determined by deletion and substitution analysis (103). Nonsense or frameshift mutations early in SPO13 stabilize SPO13 RNA, as does inhibition of protein synthesis with verrucarin A. Therefore, the translation of SPO13 RNA leads to its rapid degradation. It is noteworthy that sporulation is associated with decreased translation rates and ribosome numbers (25). The coupling of translation and RNA degradation may ensure that meiotic RNAs are translated under these adverse circumstances before they are degraded.

**MEIOSIS-SPECIFIC SPLICING**

Studies of the genes MER1 and MER2 indicate that a group of genes may be expressed only in meiotic cells through Mer1-dependent, meiosis-specific splicing. mer1 mutants display reduced meiotic recombination rates and, as a consequence, produce inviable spores (22). MERR1 is expressed as an early meiotic gene (23). MER2 was identified as a multicopy genomic clone that improves meiotic gene conversion in a mer1 null mutant (21). (MER2 is the same gene as REC107 [14], in which mutations were identified by their resulting recombination defect [61].) MER2 RNA accumulates in both meiotic and nonmeiotic cells. However, a splicing reaction that removes an 80-base MER2 intron occurs much more efficiently in meiotic cells than in nonmeiotic cells (24). In mer1 mutants, splicing of the MER2 intron is inefficient in both meiotic and nonmeiotic cells. Mer1 is the only meiosis-specific product required for MER2 splicing, because expression of MER1 from
a heterologous promoter in nonmeiotic cells permits efficient MER2 splicing.

Mer1 is not a general splicing factor, because it is expressed only in meiotic cells and is not required for cell viability (23). Mer1 is not even required for all splicing in meiotic cells, because the meiosis-specific MEI4 transcript is spliced efficiently in a mer1 mutant (65). The MER2 5′ splice junction deviates from the strict yeast consensus sequence and may require Mer1 for recognition by the splicing machinery (24). MEI4 and other known intron-containing meiotic genes have consensus 5′ junctions (DMC1 [2], MEI4 [65], and REC114 [76]), so none of these RNAs may require Mer1 for splicing. However, expression of a MER2 cDNA does not completely suppress the spore inviability of a mer1 mutant (24). Therefore, Mer1 may be required for splicing of an unidentified transcript as well as of MER2.

COORDINATION OF EARLY AND LATER TEMPORAL CLASSES

What signals establish the temporal sequence of early and later (that is, middle and late) gene expression? One factor is that later gene expression may be dependent upon early meiotic events (43). Expression of a late spr3-lacZ fusion increases 100-fold after starvation in a wild-type diploid. Expression increases only 2- to 10-fold in a cdc8/cdc8 diploid, which is defective in thymidylate kinase activity and thus in DNA synthesis. DNA synthesis is required for meiotic recombination (9, 25), so the cdc8 mutation may have many indirect effects. However, recombination-defective mutants undergo meiotic divisions and spore formation, suggesting that recombination per se is not required for the expression of later genes (25). Meiotic DNA synthesis may generate a signal that is required for the expression of later sporulation-specific genes. The idea that DNA synthesis dependence is a timing mechanism rests on the (untested) assumption that early gene expression is independent of DNA synthesis.

A second factor that distinguishes some early and later genes is IME2 dependence. Most early genes are expressed in an ime2 mutant, but the later genes SPS1, SPS2, and SGA1 are not (48, 67). Given that IME2 is an early meiotic gene, this dependence may ensure that early genes are expressed before later gene activation.

Recent results indicate that the SPS1 gene product has a positive role in later gene expression. An sps1 mutation leads to a reduction in late gene RNA levels (85). sps1 mutants arrest quite late in sporulation, after the meiotic divisions (74). Thus, the defect in late gene expression cannot be an indirect consequence of a defect in meiotic DNA synthesis. Sps1 is a protein kinase homolog (85), so there must be other members of this transduction pathway. The SPS1 gene itself is a middle gene (74), so SPS1-dependent genes would be silent until the sporulation program is well under way.

CONCLUDING REMARKS

It has been 10 years since the first sporulation-specific transcripts were reported. We now have a wealth of information on the general mechanisms of meiotic gene regulation, promoter structure, meiotic regulatory genes, and the formal pathways in which these regulators act. The challenges over the next few years will be to establish the biochemical mechanisms through which these regulators act and to understand how their activities are coordinated to ensure an orderly developmental program. The mechanisms that maintain the mitotic cell cycle and meiosis as alternatives also remain to be determined. Finally, it will be exciting to see whether meiotic regulatory mechanisms in budding S. cerevisiae are conserved in meiotic cells of other organisms.

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