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IME4, a Gene That Mediates *MAT* and Nutritional Control of Meiosis in *Saccharomyces cerevisiae*

JYOTI C. SHAH AND MARY J. CLANCY*

Department of Biological Sciences, University of New Orleans, New Orleans, Louisiana 70148

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In the yeast *Saccharomyces cerevisiae*, sporulation occurs in response to nutritional and genetic signals. The process is initiated when nutrient availability limits mitotic growth, but only in *MAT*a/*MAT*α diploid cells. Under these conditions, the cells express an activator of meiosis (*IME1*), which is required for the expression of early sporulation-specific genes. We describe a new gene, *IME4*, whose activity is essential for *IME1* transcript accumulation and sporulation. The *IME4* transcript was induced in starved *MAT*a/*MAT*α diploids but not in other cell types. In addition, excess *IME4* promoted sporulation in *mat*-insufficient cells. Thus, *IME4* appears to activate *IME1* in response to cell type and nutritional signals. We have also explored the interactions between *IME4* and two genes that are known to regulate *IME1* expression. Normally, cells that lack complete *MAT* information cannot sporulate; when such strains lack *RME1* activity or contain the semidominant *RES1-1* mutation, however, they can express *IME1* and sporulate to low levels. Our results show that *mat*-insufficient strains containing *rme1*::*LEU2* or *RES1-1* bypass mutations still retain *MAT* control of *IME4* expression. Even though *IME4* levels remained low, the *rme1*::*LEU2* and *RES1-1* mutations allowed *IME1* accumulation, implying that these mutations do not require *IME4* to exert their effects. In accord with this interpretation, the *RES1-1* mutation allowed *IME1* accumulation in *MAT*a/*MAT*α strains that contain *ime4*::*LEU2* alleles. These strains still sporulated poorly, suggesting that *IME4* plays a role in sporulation in addition to promoting *IME1* transcript accumulation. *IME4* is located between *ADE5* and *LYS5* on chromosome VII.

Each of the *Saccharomyces cerevisiae* cell types is capable of a unique set of responses to environmental conditions (reviewed in references 6, 12, 13, 15, and 25.) These responses are governed, ultimately, by the combinations of *MAT* (mating type) products that are expressed in particular cells. In wild-type diploids, the combination of the *MAT*a and *MAT*α alleles leads to the formation of a specific repressor, the a1-α2 heterodimer, that is unique to these cells (8–10, 28, 46). As a result of the interaction of this molecule with its many targets, *MAT*a/*MAT*α cells do not mate. Instead, diploid *MAT*a/*MAT*α cells are able to undergo meiosis and spore formation when nitrogen limitation restricts mitotic growth.

Genetic studies have led to the identification of several key molecules that regulate the initiation of sporulation in response to the *MAT* genotype and the nutritional status of the cells (22, 27, 31, 42, 52). Of central importance is an activator of meiosis, the product of the *IME1* gene, which is present at high levels only in starved *MAT*a/*MAT*α diploids (11, 22, 29). *IME1* expression leads to the accumulation of a second activator, *IME2*, and to the activation of additional sporulation-specific genes (29, 42, 43, 52). *mat*-insufficient cells, which do not normally sporulate, can do so when provided with ectopically expressed *IME1*.

IME1 expression is coupled to the *MAT* genotype by the activity of the *RME1* product and at least one additional intermediate (18, 23, 30, 35). *RME1* is a negative regulator of *IME1*; *IME1* accumulation and sporulation can occur when *RME1* activity is low. Levels of *RME1* are normally low in *MAT*a/*MAT*α diploids as a result of the activity of the a1-α2 heterodimer; other cell types, which lack a1/α2, express *RME1* at high levels and do not sporulate (5, 30). Accordingly, *mat*-insufficient strains in which *RME1* has been inactivated by mutation (e.g., *rme1*::*LEU2*) can sporulate to low levels, partially bypassing the requirement for both *MAT* alleles.

An additional gene, *RES1*, was defined by a semidominant mutation (*RES1-1*) that allowed *MAT*a/*MAT*α diploid cells to sporulate efficiently in the presence of excess copies of *RME1* (21). This mutation also allows *mat*-insufficient cells to express *IME1* and to sporulate to low levels. As this gene has not been cloned, the function of the wild-type *RES1* product and the role of *MAT* in regulating its expression or activity are not yet clear. Genetic analysis suggests, however, that *RES1* and *RME1* act independently, since the two mutations together support higher sporulation than either does alone.

Available genetic evidence has strongly suggested that additional *MAT*-dependent functions also participate in the activation of the sporulation process. First, it has not been possible to mimick the *MAT*a/*MAT*α genotype fully by introducing the known bypass mutations into *mat*-insufficient cells; *rme1*::*LEU2* mutations do not support wild-type levels of sporulation, even in combination with *RES1-1* mutations (21, 30). Conversely, overexpression of *RME1* does not fully repress sporulation in *MAT*a/*MAT*α diploids (30). These results would not be expected if, indeed, *RME1* were the sole route through which *MAT* signals are communicated to *IME1*.

We describe below the isolation of a new gene, *IME4*, that appears to define a second pathway through which the mating-type locus controls the initiation of sporulation. As for *IME1*, *IME4* expression was induced in starved *MAT*a/*MAT*α diploids and not in other cell types. Unlike *IME1*, however, *IME4* does not respond to the wild-type *RME1* or *RES1* products; *mat*-insufficient cells that lack *RME1* activity or that contain *RES1-1* mutations still did not express

* Corresponding author.

IME4 at high levels, whereas such cells did express *IME1*. Moreover, excess *IME4* promoted sporulation in *mat*-insufficient cells, whether or not *rme1*::*LEU2* or *RES1-1* bypass mutations were also present.

Under normal genetic conditions, *IME4* is essential for high levels of *IME1* accumulation and for sporulation. That is, *MAT***a**/*MAT*α diploids that carry *ime4*::*LEU2* or *ime4*::*TRP1* null mutations do not sporulate, because they do not accumulate sufficient *IME1*. Accordingly, excess *IME1* suppressed the sporulation defect that resulted from the absence of *IME4*. The defects of *MAT***a**/*MAT*α *ime4*::*TRP1*/*ime4*::*TRP1* strains were also partially suppressed by *RES1-1* or *rme1*::*LEU2* mutations: double-mutant strains expressed wild-type levels of *IME1* and sporulated to low levels, suggesting that *IME4* activity is dispensable for *IME1* activation under these conditions but that the wild-type product is needed for an additional function as well.

MATERIALS AND METHODS

Preparation and analysis of nucleic acids. Routine cloning manipulations were accomplished as described by Sambrook et al. (38) or Davis et al. (7). Restriction enzyme digestions and ligation reactions were performed as directed by the suppliers of the relevant enzymes. Probes were prepared by primer extension using hexanucleotide random primers (Pharmacia) or by in vitro transcription (Stratagene) as directed by the suppliers of the reagents. Yeast genomic DNA for Southern blots was prepared as described by Sherman et al. (40) and hybridized as described previously (4).

RNA was prepared essentially as described by MacDonald et al. (24). Cells were broken with glass beads in the presence of guanidinium salts, and the nucleic acids were precipitated with ethanol. The RNA was selectively precipitated with lithium chloride and ethanol following extraction with phenol and chloroform. RNAs were fractionated and blotted as described previously (21, 49).

Hybridization probes. The probe used to detect *IME1* mRNA and genomic DNA was a 350-bp *Eco*RI fragment from pAM506 (42). For *IME2*, we used a 2.2-kb *Bgl*II-*Hin*dIII fragment equivalent to those in p5 (52) and pAM400 (42). *IME4* was detected by using an isolated 1.0-kb *Eco*RI-*Bam*HI fragment (Fig. 1) or by using a single-stranded riboprobe prepared from *Eco*RI-digested pJS11 (see below). Plasmid pC4 was used as an internal control for the integrity of the RNA preparations. pC4 detects a transcript that is invariant in abundance during sporulation (33).

Growth and sporulation of yeast cells. *S. cerevisiae* strains were grown in YEPD, YEP-acetate, and supplemented minimal medium as described by Sherman et al. (40). Sporulation medium was SPM (1% potassium acetate) supplemented as required by the strain. In some cases, solid sporulation medium was also supplemented with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside; 40 μg/ml [36]). Cells to be sporulated were grown to late exponential phase in liquid YEP-acetate (5×10^7 to 7×10^7 cells per ml) or as patches on plates for 36 to 48 h prior to shift to sporulation medium.

The percentages of sporulated cells were determined by direct microscopic counts using a hemocytometer. In some cases, sporulation was also monitored by staining the cells with the nuclear stain 4,6-diamidino-2-phenylindole (51). Alternatively, cells were lysed onto nitrocellulose filters and assayed for *spr3*::*lacZ* expression (2, 36, 47).

Construction of *S. cerevisiae* strains. The strains used in this study (Table 1) were constructed by standard techniques (14, 19, 37, 40, 44). Strains carrying the *spr3*::*lacZ* reporter were constructed by transplacement as described previously, using pGK11 (20, 21). Haploid segregants carrying the *RES1-1* mutation were detected by their ability to express the *spr3*::*lacZ* fusion at low levels, using a plate assay as described previously (21).

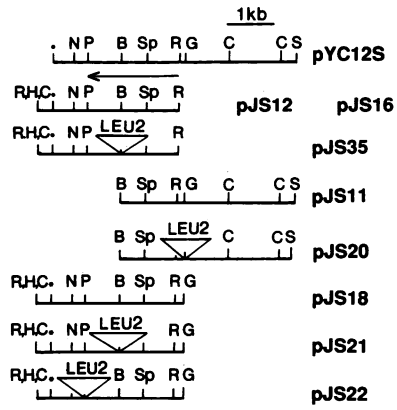

FIG. 1. Maps of plasmid inserts. Insert DNAs of the various *IME4* subclones are shown to scale, except for the *LEU2* inserts in pJS20, pJS21, pJS22, and pJS35. Recognition sites for restriction enzymes are abbreviated as follows: B (*Bam*HI), C (*Cla*I), G (*Bgl*II), H (*Hin*dIII), N (*Nco*I), P (*Pst*I), S (*Sal*I), Sp (*Sph*I), and R (*Eco*RI). The *Bam*HI-*Sau*3A junction of the original clone is indicated with an asterisk. The regions to the left of the *Bam*HI-*Sau*3A junction in pJS12, pJS16, pJS18, pJS21, pJS22, and pJS35 are derived from the original YCp50 vector. The vectors are YCp50 (pYC12S), pBluescript (pJS18, pJS21, and pJS22), pRS316 (pJS11, pJS12, and pJS20), and YEp352 (pJS16 and pJS35).

Strains used for cloning and analysis of *IME4* were isogenic heterothallic derivatives of GKY5 (21). Strains that were defective at the mating-type locus were obtained as described previously, by transplacement of a *mat***a***1*::*LEU2* allele from *Hin*dIII-digested pGK29 (21) into haploid or diploid recipient strains. In cases where the desired event was expected to change the pattern of pheromone production, transformants were assayed by using *sst1* and *sst2* strains (3) as described by Sprague (45).

Strains lacking *RME1* or *IME1* activity were constructed as described previously, using pGK24 (21, 30) or pAM506 (42), respectively, as the source of the null alleles. Strains that contained insertions at the *IME4* locus were constructed by using pJS20, pJS21, pJS22, and pJS34 to generate the *ime4*::*LEU2* (*Bgl*II), *ime4*::*LEU2* (*Bam*HI), *IME4*::*LEU2* (*Pst*I), and *ime4*::*TRP1* (*Eco*RI-*Sph*I) alleles, respectively. pJS20 and pJS34 were digested with *Bam*HI and *Hin*dIII prior to transformation. For pJS21 and pJS22, digestion was with *Nco*I and *Sph*I. The desired rearrangements were detected by Southern blotting. Isogenic diploids were obtained by transforming haploids with YCp50-*HO* (14).

Identification of *IME4*. *S. cerevisiae* JSD125/+ (*mat***a**) was transformed with a yeast genomic library that was constructed by using *Sau*3A-digested DNA derived from strain 125-10, cloned into the *Bam*HI site of YCp50. Uracil prototrophs were patched onto minimal plates, transferred to YEP-acetate plates, and incubated at 30°C for 2 to 3 days. Cells were then transferred to sporulation medium (SPM) that contained X-Gal as well as the amino acid supplements required by the strain. Color was observed after 3 to 4 days of incubation, and isolates that appeared darker in color than

TABLE 1. Strains used[a]

Strain	Genotype	Source
Haploid		
JSH-1	*MATa RES1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
JSH-2	*MATα RES1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
JSH1-mata	*mata1::LEU2 RES1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
125-10	*MATα RES1-1 ho::HIS3 leu2 ura3 trp1 his3 SPR3*	G. Kao
JSH125-1	*MATa RES1-1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
JSH125-2	*MATα RES1-1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
JSH125-mata	*mata1::LEU2 RES1-1 ho::HIS3 leu2 ura3 trp1 his3 spr3::lacZ*	This study
XS144-S19	*MATa leu1 trp5 cyh2 met13 aro2 lys5 ade5*	R. K. Mortimer
Diploid		
GK56	*mata1::LEU2/MATα RES1/RES1 ho::HIS3/ho::HIS3 leu2/leu2 ura3/ura3 trp1/trp1 his3/his3 SPR3/SPR3*	Kao et al. (21)
GK56-mata	*mata1::LEU2/MATα* derivative of GK56	Kao et al. (21)
JSD1	*MATa/MATα RES1/RES1 ho::HIS3/ho::HIS3 leu2/leu2 ura3/ura3 trp1/trp1 his3/his3 spr3::lacZ/spr3::lacZ*	This study
JSD-mata	*mata1::LEU2/MATα* derivative of JSD1	This study
JSD125/+-mata	Cross between JSH125-2 and JSH1-mata	This study
125-12	*MATa/MATα RES1-1/RES1-1 ho::HIS3/ho::HIS3 leu2/leu2 ura3/ura3 trp1/trp1 his3/his3 SPR3/SPR3*	Kao et al. (21)
125-mata	*mata1::LEU2* derivative of 125-12	Kao et al. (21)
JSD125	*MATa/MATα RES1-1/RES1-1 ho::HIS3/ho::HIS3 leu2/leu2 ura3/ura3 trp1/trp1 his3/his3 spr3::lacZ/spr3::lacZ*	This study
JSD125-mata	*mata1::LEU2/MATα* derivative of JSD125	This study
CG783	*MATa/MATα spoT2/spoT2 trp1/trp1 ura1/ura1*	C. Giroux

[a] All other strains used were isogenic to those listed except for disruptions at the *MAT*, *RME1*, *IME1*, and *IME4* loci and are therefore not listed separately.

the controls were retained and characterized further as described below.

The original isolate that defined *IME4* (p15E-15) contained approximately 11.5 kb of *S. cerevisiae* DNA within the YCp50 vector. Subcloning portions of this insert delimited the active region of the original isolate. This was contained within a subclone (pYC12S) that included 4.8 kb of DNA extending from the left border of the insert to a *Sal*I site located 4.8 kb from this junction (Fig. 1). Portions of pYC12S were transferred to the yeast/*Escherichia coli* vectors pRS316 and YEp352 for further analysis (16, 41).

Two constructs (pJS11 and pJS12) were used to determine the direction of transcription of *IME4* and adjacent transcription units. pJS11 contains a 3.5-kb *Bam*HI-*Sal*I fragment cloned between the corresponding sites of pRS316. pJS12 (also called YCp-*IME4*) contains a 2.9-kb *Eco*RI fragment cloned into the *Eco*RI site of the same vector. The probe that detected the *IME4* transcript was synthesized by using T7 polymerase in the *Bam*HI-to-*Eco*RI direction from a pJS11 template. A second probe prepared by using pJS12 for T3 transcription did not hybridize to the sporulation-specific transcript but did detect an apparent haploid-specific transcript originating on the opposite strand (data not shown).

pJS16 (also called YEp-*IME4*) and pJS33 contained the same insert as pJS12, but in YEp352 and pRS314 (41), respectively. pJS35 contained the *LEU2* gene cloned into the unique *Bam*HI site of *IME4*; this was obtained by subcloning a 6.0-kb *Hin*dIII-*Sst*I fragment from pJS21 into YEp352. This *ime4::LEU2* (*Bam*HI) allele was the equivalent of that in pJS21 (below).

Plasmids for disrupting *IME4* were constructed in two steps. pJS18 contained a 3.0-kb *Bgl*II-*Cla*I fragment from pYC12S, cloned into pBluescript SK+ (Stratagene). pJS21 and pJS22 were formed by inserting the wild-type *LEU2* gene (37) into the unique *Bam*HI and *Pst*I sites of pJS18, respectively. pJS20 contained *LEU2* inserted at the unique *Bgl*II site, and pJS34 contained *TRP1* between the *Eco*RI and *Sph*I sites of pJS11. pYE-*IME1* contains *IME1* as a 6.0-kb *Bam*HI-*Xho*I fragment cloned between the *Bam*HI and *Sal*I sites of YEp24 (39).

Mapping of *IME4*. A 1.6-kb *Bam*HI restriction fragment from p15E-15 was hybridized to a commercially available preparation of *S. cerevisiae* chromosomes that had been fractionated by pulse-field electrophoresis (Clontech, Inc.). The *IME4* probe hybridized to a single band at the position of chromosome VII. This identification was confirmed by hybridizing the same blot to a 2.1-kb *Cla*I-*Eco*RI fragment containing the wild-type *RME1* gene; the latter is located near the centromere of chromosome VII. *IME4* was mapped relative to markers derived from XS144-S19. The *IME4* locus was marked in strain JSH α by using a wild-type *URA3* gene inserted at a *Bgl*II site adjacent to the *IME4* transcription unit. This strain (JSH α *IME4::URA3*) was crossed to a segregant from a cross between JSH α and XS144-S19 that was *MATa ura3 ade5 lys5 met13 aro2 cyh2*. Analysis of segregants from the resulting diploid allowed *IME4* to be located on the left arm of chromosome VII, between *ADE5* and *LYS5*.

RESULTS

Isolation of the *IME4* gene. We screened a library of *S. cerevisiae* DNA for plasmids that could enhance the sporulation capability of a *mat*-insufficient diploid strain that was heterozygous for the *RES1-1* mutation. This recipient *mata/MATα RES1-1/+* strain forms pale blue colonies on sporulation medium supplemented with X-Gal, because it contains a sporulation-specific reporter construction (*spr3::lacZ*) integrated at both chromosomal loci. Transformants that sporulated better than the recipient were expected to form darker colonies in accord with the higher levels of β-galactosidase that are produced (17, 20, 21).

Plasmids were rescued from the yeast cells, and those that could confer enhanced activity in a second transformation of

TABLE 2. *IME4* enhancement of sporulation in *mat*-insufficient strains

Relevant genotype[a]	Plasmid[b]	% Sporulation[c]
*MAT***a**/*MATα*	YEp352	52.9
*mat***a***1*::*LEU2*/*MATα*	YEp352	0.0
	YCp-*IME4*	0.3
	YEp-*IME4*	5.5
	YEp-*ime4*::*LEU2*	0.0
*mat***a***1*::*LEU2*/*MATα RES1-1*/*RES1*	YEp352	1.3
	YCp-*IME4*	11.4
	YEp-*IME4*	35.5
	YEp-*ime4*::*LEU2*	0.7
*mat***a***1*::*LEU2*/*MATα RES1-1*/*RES1-1*	YEp352	9.8
	YCp-*IME4*	21.7
	YEp-*IME4*	40.3
*mat***a***1*::*LEU2*/*MATα rme1*::*LEU2*/ *rme1*::*LEU2*	YEp352	7.0
	YCp-*IME4*	14.9
	YEp-*IME4*	57.3
*mat***a***1*::*LEU2*/*MATα RES1-1*/*RES1-1* *rme1*::*LEU2*/*rme1*::*LEU2*	YEp352	27.0
	YCp-*IME4*	29.3
	YEp-*IME4*	49.8

[a] All of the strains were isogenic except at the *MAT*, *RES1*, and *RME1* loci.
[b] YCp-*IME4* is pJS12, YEp-*IME4* is pJS16, and YEp-*ime4*::*LEU2* is pJS35.
[c] Sporulation was determined by 4,6-diamidino-2-phenylindole (51) staining cells that had been incubated in sporulation medium for 48 h. Values are for a typical experiment, and each represents at least 600 cells.

TABLE 3. Evidence that *IME4* is essential for sporulation in *MAT***a**/*MATα* diploids

Relevant genotype[a]	% Sporulation[b]
*MAT***a**/*MATα IME4*/*IME4*	50.1
*MAT***a**/*MATα ime4*::*LEU2* (*Bam*HI)/*ime4*::*LEU2* (*Bam*HI)	0.0
*MAT***a**/*MATα IME4*::*LEU2* (*Bgl*II)/*IME4*::*LEU2* (*Bgl*II)	61.0
*MAT***a**/*MATα IME4*::*LEU2* (*Pst*I)/*IME4*::*LEU2* (*Pst*I)	65.7
*MAT***a**/*MATα ime4*::*TRP1* (*Eco*RI-*Sph*I)/*ime4*::*TRP1* (*Eco*RI-*Sph*I)	0.0

[a] All strains are isogenic except for their genotypes at *IME4*. The *IME4* alleles used were designated according to the placement of the *LEU2* or *TRP1* selectable marker within or adjacent to the *IME4* gene. These constructions are diagrammed in Fig. 1, and the genetic manipulations are further described in Materials and Methods.
[b] Determined microscopically after 48 h of incubation in sporulation medium. At least 400 cells were counted for each value. Results shown are from a typical experiment.

the same recipient were retained. This second test eliminated an expected class of false positives in which the chromosomal *RES1-1* allele had become homozygous during mitotic growth of the transformants. The inserted DNA present in one isolate that scored positively in both tests did not correspond to any of the previously described genes that would be detected in this assay, as determined by restriction mapping and Southern blotting (data not shown). Genes of this class include *IME1*, *IME2* (*SME1*), *MCK1* (*IME3*), *MAT***a**, and *HMR***a** (22, 31, 42, 52). Also, the cloned DNA did not direct integration of plasmid sequences to the *RES1* locus (1 parental ditype:3 tetratype:1 nonparental ditype) and thus did not contain the *RES1* gene. The gene thus obtained was named *IME4* on the basis of the analysis described below.

Enhancement of sporulation by *IME4*. To establish that the enhanced blue color was the result of higher sporulation levels of the *IME4*-containing transformants, rather than altered permeability of the cells to X-Gal or an effect on *spr3*::*lacZ* expression per se, we monitored sporulation levels in transformants that carried portions of the original 11.5-kb insert on single- or multiple-copy vectors. Using the X-Gal assay, we localized the putative activator to a 2.9-kb region that bordered the *Bam*HI cloning site of the original YCp50 vector (Fig. 1 and data not shown). This region was transferred to single-copy (pRS316) and multiple-copy (YEp352) vectors to form pJS12 and pJS16. These plasmids are called YCp-*IME4* and YEp-*IME4*, respectively, in Table 2 and below. These constructions were introduced into *mat*-deficient *S. cerevisiae* strains that were unable to sporulate (*mat***a***1*::*LEU2*/*MATα*) or whose sporulation ability depended on *RES1-1* or *rme1*::*LEU2* bypass mutations.

The data in Table 2 compare the sporulation levels attained by the various strains when they carry plasmid-borne copies of the *IME4* activator. As with *IME1* and *IME2*, excess *IME4* was able to promote sporulation in *mat*-insufficient yeast cells, whether or not additional bypass mutations were also present. As expected from the screen that yielded *IME4*, a single excess copy of the gene enhanced sporulation in strains that were heterozygous for the *RES1-1* mutation (1.3% versus 11.4% in the experiment shown). This effect was also observed in strains whose sporulation ability depended on *rme1*::*LEU2*/*rme1*::*LEU2* or *RES1-1*/*RES1-1* genotypes; approximately twofold stimulation of sporulation occurred in these cases (Table 2).

The multicopy construction (YEp-*IME4*) allowed sporulation by *mat*-insufficient strains of all genotypes. In cells wild type at *RES1* and *RME1*, 5.5% sporulation was achieved, compared with no observed asci for the control carrying the vector alone. Moreover, strains that contained the *RES1-1* and *rme1*::*LEU2* mutations in combination with the YEp-*IME4* construction sporulated almost as efficiently as did isogenic *MAT***a**/*MATα* controls. These data suggest that *IME4* encodes an activator that is limiting in *mat*-insufficient cells, even in cases where the strain background already permits some levels of sporulation.

IME4* is essential for sporulation.** We used gene replacement to examine whether the cloned DNA contains a transcription unit that is essential for sporulation in *MATa**/*MATα* diploid cells (37). We inserted a selectable *LEU2* or *TRP1* marker within the cloned DNA, using the *Bgl*II, *Bam*HI, *Eco*RI, and *Sph*I sites indicated in Fig. 1. Diploid strains that were homozygous for these mutations were constructed and exposed to sporulation medium. The results shown in Table 3 indicate that *MAT***a**/*MATα* strains containing insertions at the *Bam*HI site or between the *Eco*RI and *Sph*I sites in *IME4* were unable to sporulate (no asci in over 10,000 cells examined), whereas strains that contained insertions at the other two positions (*Bgl*II and *Pst*I) could do so normally. We will refer to the inactive alleles of *IME4* as *ime4*::*LEU2* and *ime4*::*TRP1*, respectively. We conclude that the region essential for sporulation lies between the *Bgl*II and *Pst*I sites shown in the restriction maps in Fig. 1.

An additional experiment verified that the region of DNA that is required for sporulation is the same as the one that activates the process when carried on high-copy-number plasmids. We asked, simply, whether the insertion of *LEU2* into the *IME4* region of YEp-*IME4* abolished the ability of this plasmid to activate sporulation in *mat*-insufficient cells. The YEp-*ime4*::*LEU2* construction contained the *ime4*::*LEU2* allele instead of the wild-type *IME4* cloned into the

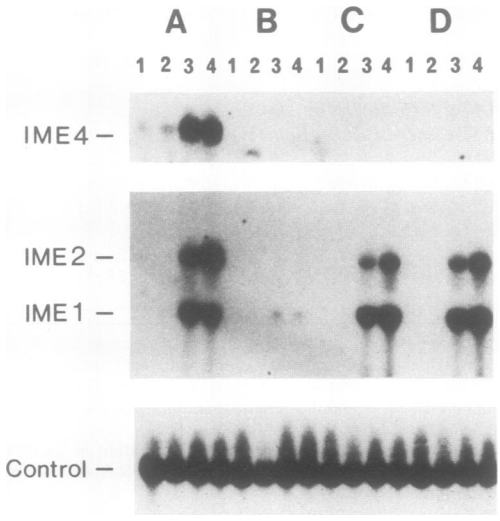

FIG. 2. Cell type dependence of *IME4* expression. RNAs were prepared from *MAT***a**/*MAT*α diploids (group A), *mat***a**::*LEU2*/*MAT*α diploids (group B), *mat***a**::*LEU2*/*MAT*α *rme1*::*LEU2*/*rme1*::*LEU2* diploids (group C), and *mat***a**::*LEU2*/*MAT*α *RES1-1*/*RES1-1* diploids (group D). Cells were grown in YEPD (lanes 1) or YEP-acetate (lanes 2) or were incubated in sporulation medium for 3 (lanes 3) or 8 (lanes 4) h prior to harvest. RNAs were transferred to nitrocellulose filters following fractionation through denaturing formaldehyde agarose gels. Blots were hybridized to probes that detected *IME4*, *IME1*, *IME2*, and a pC4 control (see Materials and Methods). Positions of the corresponding bands are indicated at the left.

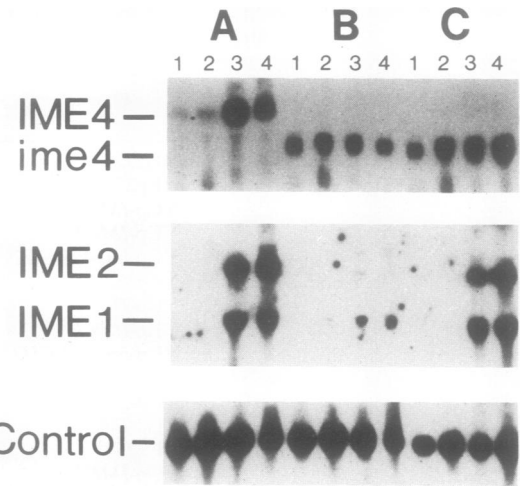

FIG. 3. Activation of *IME1* and *IME2* by *IME4*. RNAs were prepared from *MAT***a**/*MAT*α diploids (group A), *MAT***a**/*MAT*α *ime4*::*LEU2*/*ime4*::*LEU2* diploids (group B), and *MAT***a**/*MAT*α *ime4*::*LEU2*/*ime4*::*LEU2 RES1-1*/*RES1-1* diploids (group C). Cells were grown in YEPD (lanes 1) or YEP-acetate (lanes 2) or were incubated in sporulation medium for 3 (lanes 3) or 8 (lanes 4) h. RNAs were transferred to nitrocellulose following fractionation through denaturing formaldehyde agarose gels. Blots were hybridized to probes that detected *IME4* and its truncated derivative (*ime4*::*LEU2*), *IME1*, *IME2*, and a pC4 control. Positions of the corresponding bands are indicated at the left.

YEp352 vector. This construction did not promote sporulation when introduced into *mat*-insufficient strains (Table 2).

Characterization of the *IME4* transcription unit. To locate the *IME4* transcription unit, we hybridized restriction fragments or riboprobes to RNA from wild-type (*MAT***a**/*MAT*α) and isogenic *mat*-insufficient derivatives of yeast strain JSD1 (Fig. 2 and data not shown). RNAs were prepared from exponentially growing populations of cells (YEPD and YEPA; Fig. 2, lanes 1 and 2, respectively) and cells that had been exposed to sporulation medium for 3 (lanes 3) and 8 (lanes 4) h.

The results of these Northern (RNA) blot experiments showed that the region between the *Eco*RI and *Pst*I sites shown in Fig. 1 hybridized to two RNAs that are transcribed in opposite directions (Fig. 2 and data not shown). The 1.9-kb RNA that corresponds to *IME4* was detected in *MAT***a**/*MAT*α diploids in both growth media examined and accumulated to higher levels in these cells after a shift to sporulation medium.

IME4* activates *IME1*.** We considered two plausible models for the interaction between *IME1* and *IME4*. *IME4* might function to activate *IME1* in response to mating type and nutritional signals. If so, the sporulation defect of *ime4*::*LEU2* strains would be due to inadequate accumulation of the central *IME1* activator. Alternatively, *IME4* might be a crucial downstream target of *IME1* activity. To choose between these possibilities, we first compared *IME1* transcript levels in *MATa**/*MAT*α strains that contained active *IME4* alleles with levels in those that did not. The same blots were also hybridized to probes that were expected to detect *IME4* (and its truncated mutant derivative), *IME2*, and a relatively invariant transcript as controls.

The autoradiographs shown in Fig. 3 demonstrate that *IME1* and *IME2* levels are substantially lower in strains that are homozygous for the *ime4*::*LEU2* allele than in isogenic *IME4* controls. This transcriptional defect was not absolute, as basal levels of *IME1* mRNA were still detectable in the mutant cells. We believe that this defect in *IME1* accumulation is sufficient to explain the sporulation defect of *ime4*::*LEU2* strains, because the levels were comparable to those observed in *mat*-insufficient strains.

To corroborate this result, we examined whether excess copies of *IME1* could suppress the sporulation deficiency of *ime4*::*LEU2* strains; if *IME4* normally activates *IME1*, the increased basal expression of *IME1* from the high-copy-number construction might drive sporulation in the absence of the normal activator. Representative experiments (Table 4) indicate that this is so; excess *IME1* did suppress the sporulation deficiency associated with an inactive allele of *IME4* (*ime4*::*LEU2*). By contrast, excess *IME4* was ineffective in promoting sporulation in cells that contained only inactive *ime1*::*TRP1* alleles. Thus, excess *IME1* obviates the need for *IME4*.

These experiments suggest that *IME4* activates *IME1* in response to signals from the environment and the mating-type locus; *mat*-insufficient cells do not express *IME4* at high levels and consequently fail to activate *IME1*. The resulting sporulation deficiency is mimicked by *MAT***a**/*MAT*α strains that contain defective *ime4*::*LEU2* alleles and can be suppressed by excess or ectopic expression of *IME1*.

***MAT* dependence of *IME4* transcript accumulation.** It was of interest to determine whether the cell type specificity of *IME4* expression is dependent on the *RME1* or *RES1* product. Mutations in either gene lead to *IME1* transcript accumulation in inappropriate cell types, thereby bypassing the usual requirement for both mating-type alleles. One possible explanation for this could be that *rme1*::*LEU2* and *RES1-1* mutations actually lead to *IME4* expression in *mat*-insufficient cells, and that *IME1* expression occurs as a

TABLE 4. Suppression of *ime4*::*LEU2* mutations by *IME1*-containing plasmids

Relevant genotype[a]	Plasmid	% Sporulation[b]
MATa/*MATα ime4*::*LEU2*/*ime4*::*LEU2*	YEp24	0.0
	YEp-*IME4*	41.1
	YEp-*IME1*	24.9
MATa/*MATα ime1*::*TRP1*/*ime1*::*TRP1*	YEp24	0.0
	YEp-*IME4*	0.0
	YEp-*IME1*	32.9

[a] All strains are isogenic except at their *IME1* and *IME4* loci.
[b] Determined after 48 h of incubation in sporulation medium.

secondary consequence of *IME4* activity. If so, we would expect elevated levels of *IME4* mRNA, as well as *IME1* mRNA, in *mat*-insufficient strains that carry *rme1*::*LEU2* or *RES1-1* bypass mutations.

The Northern blots shown in Fig. 2 demonstrate that *IME4* levels remained low in *rme1*::*LEU2* and *RES1-1* strains, whereas *IME1* levels were increased relative to controls that contained neither mutation. *IME2* was also expressed in these strains, indicating that the *IME1* levels attained were sufficient to activate downstream genes. Thus, *rme1*::*LEU2* and *RES1-1* do not activate *IME1* through effects on *IME4*; rather, these mutations bypass the dependence of *IME1* expression on high levels of the *IME4* activator.

Role of *IME4* in *mat*-insufficient cells. To determine whether *IME4* plays any role in sporulation by *mat*-insufficient cells, we deleted *IME4* from the *mat*-insufficient strains that carry *rme1*::*LEU2* or *RES1-1* mutations; if these cells contained a low but functionally significant level of *IME4* transcript, then the *ime4*::*LEU2* allele would cause a reduction of sporulation levels in these cells. If, on the other hand, *IME4* is not expressed at all, then disrupting the gene would have no effect. The results presented in Table 5 indicate that low levels of *IME4*, not detected on our Northern blots, are important for sporulation in *mat*-insufficient cells. Sporulation was greatly reduced, although not completely abolished, when these strains contained the *ime4*::*LEU2* allele in combination with the *rme1*::*LEU2* or *RES1-1* bypass mutations. We conclude that *IME4* does play a role in sporulation in *mat*-insufficient cells, even though the abundance of the RNA is extremely low.

***rme1*::*LEU2* and *RES1-1* partially suppress *ime4* alleles.** The results presented above demonstrate that *IME1* expression does not always require high levels of *IME4* and, indeed, that some low levels of sporulation can occur in the complete absence of *IME4* activity (i.e., in *mat*-insufficient cells containing *rme1*::*LEU2* or *RES1-1* mutations). These results suggest that *IME1* accumulation and sporulation might also be *IME4* independent in *MATa*/*MATα* diploids containing the *rme1*::*LEU2* or *RES1-1* bypass mutations. That is, these mutations might suppress the transcription and sporulation defects that result from the *ime4*::*LEU2* or *ime4*::*TRP1* mutation.

To verify this possibility, we constructed *MATa*/*MATα* strains that were homozygous for the *rme1*::*LEU2* or *RES1-1* alleles in combination with *ime4*::*LEU2* or *ime4*::*TRP1* null alleles and examined whether the double mutants regained the ability to express *IME1* and to sporulate. Autoradiographs that compare the levels of *IME1* mRNA in *ime4*::*LEU2*/*ime4*::*LEU2* strains with and without the *RES1-1* mutation are shown in Fig. 3. Wild-type *MATa*/*MATα* strains that contain neither mutation are included as controls. These results demonstrate that the *RES1-1* mutation suppresses the defect in *IME1* accumulation resulting from the *ime4*::*LEU2* mutation; double-mutant strains accumulated *IME1* RNA to levels that were indistinguishable from wild-type levels. We also examined the final levels of sporulation attained by *MATa*/*MATα* diploids that contained *rme1*::*LEU2* or *RES1-1* mutations. Surprisingly, the sporulation defect of *ime4* strains was very weakly suppressed by the *rme1*::*LEU2* and *RES1-1* mutations (Table 6); that is, the double-mutant strains sporulated to final levels of only 0.5 to 2%, even though *IME1* levels were apparently normal in the *RES1-1* strain. While low, this level of sporulation is significant because *MATa*/*MATα ime4*/*ime4* strains that are wild type for *RME1* and *RES1* did not sporulate at all in many separate experiments. The low-sporulation phenotype was complemented by the addition of the wild-type *IME4* gene on single-copy plasmids and is therefore not due to a secondary mutation in these strains. These results suggest that *IME4* is needed for a second function, in addition to promoting *IME1* accumulation.

TABLE 5. Role of *IME4* in *mat*-insufficient cells

Relevant genotype[a] and plasmid	% Sporulation[b]
MATa/*MATα*	
YCp50	ND
YCp-*IME4*	52.0
mata1::*LEU2*/*MATα*	
YCp50	0.0
YCp-*IME4*	0.2
mata1::*LEU2*/*MATα ime4*::*LEU2*/*ime4*::*LEU2*	
YCp50	0.0
YCp-*IME4*	8.7
mata1::*LEU2*/*MATα RES1-1*/*RES1-1*	
YCp50	20.0
YCp-*IME4*	23.1
mata1::*LEU2*/*MATα RES1-1*/*RES1-1 ime4*::*LEU2*/*ime4*::*LEU2*	
YCp50	0.5
YCp-*IME4*	10.7
mata1::*LEU2*/*MATα rme1*::*LEU2*/*rme1*::*LEU2*	
YCp50	4.3
YCp-*IME4*	13.4
mata/*MATα rme1*::*LEU2*/*rme1*::*LEU2 ime4*::*LEU2*/*ime4*::*LEU2*	
YCp50	0.7
YCp-*IME4*	19.1

[a] The strains were isogenic except at their *MAT*, *IME4*, *RES1*, and *RME1* loci.
[b] Sporulation was assayed microscopically after the cells had been incubated in sporulation medium for 48 h. Results shown are from a typical experiment. At least 600 cells were counted for each determination. ND, not determined.

Mapping of *IME4*. *IME4* was located on chromosome VII by hybridization of the cloned DNA to contour-clamped homogeneous electric field-fractionated *S. cerevisiae* chromosomes. The gene was then mapped relative to other chromosome VII markers as described in Materials and Methods. The data in Table 7 show that *IME4* maps to the left arm of chromosome VII, between *ADE5* and *LYS5*. This position is within 10 centimorgans of the reported location of *SPOT2*, a previously described gene whose activity is required early in the sporulation process (50). To determine whether *IME4* and *SPOT2* were the same gene, we transformed a *spoT2* strain (very kindly provided by Craig Giroux) with pJS33. The wild-type *IME4* gene did not complement the sporulation deficiency of the *spoT2* strain.

TABLE 6. Suppression of *ime4* by *RES1-1* and *rme1::LEU2* in *MATa/MATα* strains

Relevant genotype[a] and plasmid	% Sporulation[b]
MATa/MATα	
YCp50	50.2
YCp-*IME4*	52.0
MATa/MATα rme1::LEU2/rme1::LEU2	
YCp50	88.3
YCp-*IME4*	81.7
MATa/MATα RES1-1/RES1-1	
YCp50	56.8
YCp-*IME4*	61.4
MATa/MATα ime4::LEU2/ime4::LEU2	
YCp50	0.0
YCp-*IME4*	22.8
MATa/MATα ime4::TRP1/ime4::TRP1	
YCp50	0.0
YCp-*IME4*	16.1
MATa/MATα ime4::LEU2/ime4::LEU2 RES1-1/RES1-1	
YCp50	2.5
YCp-*IME4*	33.4
MATa/MATα ime4::TRP1/ime4::TRP1 RES1-1/RES1-1	
YCp50	0.3
YCp-*IME4*	23.2
MATa/MATα ime4::TRP1/ime4::TRP1 rme1::LEU2/ rme1::LEU2	
YCp50	0.4
YCp-*IME4*	53.9
MATa/MATα ime4::TRP1/ime4::TRP1 RES1-1/RES1-1 rme1::LEU2/rme1::LEU2	
YCp50	0.6
YCp-*IME4*	34.0

[a] The strains were isogenic except at the *MAT*, *IME4*, and *RES1* loci. The *IME4* allele (*ime4::LEU2*) contains *LEU2* inserted at the *Bam*HI site in the *IME4* region and is the same as the *ime4::LEU2* (*Bam*HI) construction shown in Fig. 1. For the *ime4::TRP1* (*Eco*RI-*Sph*I) allele, the *TRP1* gene replaces the *Eco*RI-*Sph*I fragment of *IME4* shown in Fig. 1.

[b] Sporulation was assayed microscopically after the cells had been incubated in sporulation medium for 48 h. The results are from a typical experiment. At least 600 cells were counted for each determination.

TABLE 7. Position of *IME4* relative to markers on chromosome VII

Interval	Tetrad analysis[a]			Map distance[b] (centimorgans)
	PD	NPD	TT	
IME4::URA3-ADE5	21	0	17	22
IME4::URA3-LYS5	15	1	22	37
IME4::URA3-ARO2	12	1	25	41
ADE5-LYS5	8	3	27	60
ADE5-ARO2	6	3	29	62
LYS5-ARO2	33	0	5	6.6

[a] PD, NPD, and TT refer, respectively, to parental ditype, nonparental ditype, and tetratype asci. No linkage to *RME1* or *TRP5* was detected, and very loose linkage to *CYH2* was detected.

[b] Calculated according to Perkins' formula (34).

DISCUSSION

IME4 is an activator of sporulation that is expressed at high levels only in *MATa/MATα* cells. The corresponding transcript is detectable during vegetative growth of wild-type diploid cells and is induced substantially upon a shift to the nitrogen-free conditions that promote sporulation. Excess *IME4* drives sporulation in *mat*-insufficient cells, and its absence from *MATa/MATα* diploids causes sporulation to fail at a stage prior to the first meiotic division. This arrest of sporulation is due to a failure of *ime4::LEU2/ime4::LEU2* cells to activate *IME1*.

We have explored the interactions between *IME4* and some other genes involved in the initiation of sporulation, including *MAT*, *RME1*, and *RES1*. The results of these studies reveal new complexities in the pathways that govern the initiation of sporulation in *S. cerevisiae* cells, as diagrammed in Fig. 4. The important features that emerge are that *MAT* and nutritional signals converge on the *IME1* activator by multiarmed and interlocking routes (1, 32, 48). *MAT* controls *IME1* through the activities of *RME1* and, independently, of *IME4*. *IME4* also communicates a nutritional signal to *IME1*, but this is not the only nutritional signal that *IME1* receives; *IME1* responds to nutrition by a pathway that does not require *IME4* activity.

Thus, *MAT* control of sporulation is mediated by two genetically distinct pathways, one requiring the *RME1* product and the second requiring *IME4*. These two pathways act antagonistically on *IME1*, since *RME1* represses *IME1* accumulation, whereas *IME4* activates it. In *MATa/MATα* cells, in which *RME1* levels are low, expression of *IME4* upon starvation leads to efficient accumulation of *IME1* mRNA. In *mata/MATα* cells, by contrast, *RME1* levels are high, and *IME4* is expressed poorly; hence, *IME1* activation fails and sporulation does not occur.

Our results indicate that functional *IME4* product exists at low levels in *mat*-insufficient cells and can promote sporulation in those cells when the *RME1* product is not present. We also confirm that functional *RME1* product is present at low levels in *MATa/MATα* cells (5, 30); we observed partial suppression of *ime4::TRP1* alleles by the *rme1::LEU2* mutation in *MATa/MATα* diploids. Hence, both the *RME1* and *IME4* products can be functionally relevant in inappropriate cell types, in genetic backgrounds that allow their activities to be revealed.

The existence of two pathways guarantees that only *MATa/MATα* cells can normally sporulate. Even though each pathway is leaky on its own, together they place an absolute cell type restriction on sporulation. This bifurcated mechanism resembles that used by the cells to repress the mating response; *MATa/MATα* cells cannot mate, even

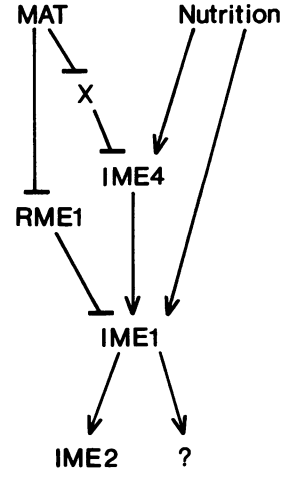

FIG. 4. Model summarizing the interactions described in the text. Positive interactions are indicated by arrows, and negative interactions are indicated by horizontal bars.

when provided with pheromone receptors, because they also lack downstream components of the pheromone response pathways (12, 26).

IME4 is controlled by the *MAT* locus, because the transcript accumulates to high levels only in *MAT***a**/*MAT*α diploids. Thus, *IME4* is ultimately controlled by the **a**1-α2 heterodimer. In the case of *IME4*, however, *RME1* is not the intermediate that couples expression to the *MAT* genotype. We suggest, instead, that an unidentified intermediate, designated X in Fig. 4, functions between the *MAT* locus and the *IME4* gene, to accommodate the established role of **a**1-α2 as a repressor. The suggested intermediate would function in a manner analogous to that of *RME1*, only with *IME4*, rather than *IME1*, as its target.

Our experiments suggest that *IME4* is also required for some function other than activation of *IME1* transcript accumulation. The primary support for this idea is the observation that the *RES1-1* mutation fully suppresses the defect in *IME1* transcript accumulation that occurs in *ime4*::*LEU2* strains but suppresses the sporulation defect very weakly. Excess *IME1*, by contrast, efficiently compensates for the lack of *IME4*. We can reconcile this apparent contradiction in two ways. *IME4* or one of its targets could enhance any one of a number of posttranscriptional steps that may be related to *IME1* activity. Indeed, there is evidence that the *IME1* protein may be modified in vivo (28a) and that the translation of the RNA is regulated by an unusually long 5′ untranslated region (23a). Alternatively, *IME1* and *IME4* activities could overlap such that excess *IME1* can assume the role of *IME4*.

We have not examined any possible interactions between *IME4* and the other genes that are known to influence *IME1* activity. One of these, *MCK1*, encodes a protein kinase that is required for high levels of *IME1* expression and for events associated with ascospore maturation. Strains that lack *MCK1* are able to sporulate but do so inefficiently (31). *IME4* is a potential target for *MCK1*, and it would be of interest to determine whether excess *IME4* can suppress the sporulation defects associated with *mck1*::*URA3* mutations.

IME2 activity also influences *IME1* through a negative feedback mechanism that is not yet understood. The observation that *IME1* transcript levels persist at high levels in cells that lack functional *IME2* suggests that *IME2* or one of its targets is needed to shut down the expression of early genes as cells proceed into sporulation (29, 43). The *IME4* gene and its product are likely targets for this proposed feedback loop, because of the central role of *IME4* in mediating *IME1* accumulation, as outlined above.

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REFERENCES

1. **Cameron, S., L. Levin, M. Zoller, and M. Wigler.** 1988. Cyclic AMP-independent control of sporulation, glycogen metabolism and heat shock resistance in *Saccharomyces cerevisiae*. Cell **53**:555–566.
2. **Casadaban, M. J., A. Martinez-Arias, S. K. Shapira, and J. Chou.** 1983. β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**:293–308.
3. **Chan, R. K., and C. A. Otte.** 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by **a**-factor and α-factor pheromones. Mol. Cell. Biol. **2**:11–20.
4. **Clancy, M. J., B. Buten-Magee, D. Straight, A. Kennedy, R. M. Partridge, and P. T. Magee.** 1983. Isolation of genes expressed preferentially during sporulation in the yeast, *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:3000–3004.
5. **Covitz, P. A., I. Herskowitz, and A. P. Mitchell.** 1991. The yeast *RME1* gene encodes a putative zinc-finger protein that is directly repressed by a1-α2. Genes Dev. **5**:1982–1989.
6. **Cross, F., L. H. Hartwell, C. Jackson, and J. B. Konopka.** 1988. Conjugation in *Saccharomyces cerevisiae*. Annu. Rev. Cell Biol. **4**:429–457.
7. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Dranginis, A. M.** 1990. Binding of yeast a1 and α2 as a heterodimer to the operator DNA of a haploid-specific gene. Nature (London) **347**:682–685.
9. **Fields, S., and I. Herskowitz.** 1987. Regulation by the yeast mating type locus of *STE12*, a gene required for cell type-specific expression. Mol. Cell. Biol. **7**:3818–3821.
10. **Goutte, C., and A. D. Johnson.** 1988. a1 protein alters the DNA binding specificity of α2 repressor. Cell **52**:875–882.
11. **Granot, D., J. P. Margolskee, and G. Simchen.** 1989. A long upstream region of the *IME1* gene regulates meiosis in yeast. Mol. Gen. Genet. **218**:308–314.
12. **Herskowitz, I.** 1988. Life cycle of the budding yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52**:536–553.
13. **Herskowitz, I.** 1989. A regulatory hierarchy for cell specialization in yeast. Nature (London) **342**:749–757.
14. **Herskowitz, I., and R. E. Jensen.** 1991. Putting *HO* to work: practical uses for mating type switching. Methods Enzymol. **194**:132–145.
15. **Herskowitz, I., and Y. Oshima.** 1981. Controlling cell type in *Saccharomyces cerevisiae*: mating type and mating type interconversion, p. 181–209. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), Molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagaloff.** 1986. Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2**:163–167.
17. **Holaway, B. L., G. Kao, M. C. Finn, and M. J. Clancy.** 1987. Transcriptional regulation of sporulation genes in yeast. Mol. Gen. Genet. **210**:449–459.
18. **Hopper, A. K., and B. D. Hall.** 1975. Mating type and sporulation in yeast. I. Mutations which alter mating type control over sporulation. Genetics **80**:41–59.
19. **Ito, H., T. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
20. **Kao, G., D. G. Mannix, B. L. Holaway, M. C. Finn, A. E. Bonny, and M. J. Clancy.** 1989. Dependence of inessential late gene expression on early meiotic events in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **215**:490–500.
21. **Kao, G., J. C. Shah, and M. J. Clancy.** 1990. An *RME1*-independent pathway for sporulation control in *Saccharomyces cerevisiae* acts through *IME1* transcript accumulation. Genetics **126**:823–835.
22. **Kassir, Y., D. Granot, and G. Simchen.** 1988. *IME1*, a positive regulator of meiosis in *S. cerevisiae*. Cell **52**:853–862.
23. **Kassir, Y., and G. Simchen.** 1976. Regulation of mating and meiosis in yeast by the mating type locus. Genetics **82**:187–206.

23a. **Kassir, Y., and G. Simchen.** Personal communication.

24. **MacDonald, R. J., G. H. Swift, A. E. Przybyla, and J. M. Chirgwin.** 1987. Isolation of RNA using guanidinium salts. Methods Enzymol. **152**:219–227.

25. **Malone, R. E.** 1990. Dual regulation of meiosis in yeast. Cell **61**:375–378.
26. **Marsh, L., and I. Herskowitz.** 1988. From membrane to nucleus. The pathway of signal transduction in yeast and its genetic control. Cold Spring Harbor Symp. Quant. Biol. **53**:557–565.
27. **Matsuura, A., M. Treinin, H. Mitsuzawa, Y. Kassir, I. Uno, and G. Simchen.** 1990. The adenylate cyclase/protein kinase cascade regulates entry into meiosis in *Saccharomyces cerevisiae* through the gene *IME1*. EMBO J. **9**:3225–3232.
28. **Miller, A. M., V. L. MacKay, and K. A. Nasmyth.** 1985. Identification and comparison of two sequence elements that confer cell type specific transcription in yeast. Nature (London) **314**:598–603.
28a. **Mitchell, A.** Personal communication.
29. **Mitchell, A. P., S. E. Driscoll, and H. E. Smith.** 1990. Positive control of sporulation-specific genes by the *IME1* and *IME2* products of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**: 2104–2110.
30. **Mitchell, A. P., and I. Herskowitz.** 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. Nature (London) **319**:738–742.
31. **Neigeborn, L., and A. P. Mitchell.** 1991. The yeast *MCK1* gene encodes a protein kinase homolog that activates early meiotic gene expression. Genes Dev. **5**:533–548.
32. **Olempska-Beer, Z., and E. Freese.** 1987. Initiation of meiosis and sporulation in *Saccharomyces cerevisiae* does not require a decrease in cyclic AMP. Mol. Cell. Biol. **7**:2141–2147.
33. **Percival-Smith, A., and J. Segall.** 1986. Characterization and mutational analysis of a cluster of three genes expressed preferentially during sporulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:2443–2451.
34. **Perkins, D.** 1952. Biochemical mutants of the smut fungus *Ustilago maydis*. Genetics **34**:607–626.
35. **Rine, J. D., G. Sprague, and I. Herskowitz.** 1981. *rme1* mutation of *Saccharomyces cerevisiae*: map position and bypass of mating type locus control of sporulation. Mol. Cell. Biol. **1**:958–960.
36. **Rose, M., and D. Botstein.** 1983. Construction and use of gene fusions to *lacZ* (β-galactosidase) that are expressed in yeast. Methods Enzymol. **101**:167–180.
37. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
39. **Schneider, J. C., and L. Guarente.** 1991. Vectors for expression of cloned genes in yeast: regulation, overproduction and underproduction. Methods Enzymol. **194**:373–388.
40. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Sikorski, R. S., and P. Hieter.** 1987. A system of shuttle vectors and yeast host systems designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.
42. **Smith, H. E., and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9**:2142–2152.
43. **Smith, H. E., S. S. Y. Su, L. Neigeborn, S. E. Driscoll, and A. P. Mitchell.** 1990. Role of *IME1* expression in regulation of meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:6103–6113.
44. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **153**:503–517.
45. **Sprague, G. F., Jr.** 1991. Assay of yeast mating reaction. Methods Enzymol. **194**:77–93.
46. **Strathern, J. N., J. Hicks, and I. Herskowitz.** 1981. Control of cell type in yeast by the mating type locus: the α1-α2 hypothesis. J. Mol. Biol. **147**:357–372.
47. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86**:10018–10022.
48. **Tatchell, K., L. Robinson, and M. Breitenbach.** 1985. *RAS2* of *Saccharomyces cerevisiae* is required for gluconeogenic growth and proper response to nutrient limitation. Proc. Natl. Acad. Sci. USA **82**:3785–3789.
49. **Thomas, P. S.** 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol. **100**:255–266.
50. **Tsuboi, M.** 1983. The isolation and genetic analysis of sporulation deficient mutants in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **191**:17–21.
51. **Williamson, D. H., and D. J. Fennel.** 1975. The use of fluorescent DNA-binding agents for detecting and separating yeast mitochondrial DNA. Methods Cell. Biol. **12**:335–351.
52. **Yoshida, M., H. Kawaguchi, Y. Sakata, M. Hirano, H. Shima, R. Akada, and I. Yamashita.** 1990. Initiation of meiosis and sporulation in *Saccharomyces cerevisiae* requires a novel protein kinase homologue. Mol. Gen. Genet. **221**:176–186.