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Genetics

Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast

(*UME* genes/*Saccharomyces cerevisiae*/transcription/regulation/sporulation)

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ABSTRACT **Mutations in *Saccharomyces cerevisiae* have been identified that derepress early meiotic genes functioning in separable pathways required for normal meiotic development. The phenotypes of these *ume* (*u*nscheduled *m*eiotic gene *e*xpression) mutations suggest that their wild-type alleles encode negative regulators acting downstream of both the cell-type and nutritional controls of meiosis. These newly defined loci do not affect either general transcription or transcription of meiotic genes expressed later in meiosis and spore formation.**

Initiation of meiosis in *Saccharomyces cerevisiae* is under the control of two independent, convergent regulatory pathways, one responding to cell type and the other sensing nutritional status (1–3). The cell-type pathway operates through a transcriptional regulatory cascade in which the products of the *MAT***a** and *MATα* loci combine to form a negative regulator (4, 5) that inhibits the expression of *RME1* (6, 7), which encodes a repressor of meiosis. *RME1*, in turn, negatively regulates *IME1*, an inducer of meiosis (8), which positively regulates *IME2* (9). Overexpression of either *IME1* or *IME2* allows meiotic functions to be expressed during mitosis (8, 9).

The nutritional pathway senses glucose and nitrogen deprivation and involves a number of well-characterized genes, e.g., *ARD1* (10), *BCY1*, *CYR2*, and *CYR3* (11), and *RAS2* (12, 13). Evidence that the nutritional and cell-type pathways are initially independent is based on the observation that *rme1* mutants still require starvation conditions to enter meiosis (6) and, conversely, mutants that interfere with nutritional control, allowing meiosis in rich media, still require both *MAT***a** and *MATα* expression (14). *IME1* is regulated by both cell type and nutritional conditions and represents the first known point at which these pathways converge.

The process of meiosis and gamete formation in yeast includes DNA replication, recombination, chromosome segregation at meioses I and II, and spore formation. A number of genes required for these events have been cloned and found to be developmentally regulated; i.e., they exhibit elevated message levels only during sporulation (15–17). Among these are *SPO13*, a gene required for chromosome segregation at meiosis I (18), *SPO11*, a gene involved in recombination (19), and *SPO16*, a gene that affects the efficiency of early prophase events (R. T. Elder and R.E.E., unpublished results). The purpose of this study was to identify trans-acting regulators that directly control the expression of these genes. Our approach was to use a fusion reporter gene to recover regulatory mutations that derepress the mitotic expression of these meiosis-specific genes. Here we report the successful application of this method to meiotic control and the identification of five such trans-acting genes.

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MATERIALS AND METHODS

Strains and Plasmids. Mutants were isolated in RSY10 (S. Frackman, University of Wisconsin-Milwaukee), an *ade6* derivative of W303-1A (R. Rothstein, Columbia University College of Physicians and Surgeons): *MAT***a** *ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*. Dominance and segregation were examined in crosses to RSY75: *MATα his4 leu2-3,112 ura3-1*. Plasmids were transformed into yeast by a lithium acetate procedure (20). The *spo11–lacZ* fusion in p(spo11)153 was derived from p(spo11)152, provided by C. Atcheson (University of Chicago).

Media. Growth and sporulation media were as described (19). Top agar in the β-galactosidase plate assays was 0.7% agar/1 mM $MgCl_2$/0.1 M sodium phosphate, pH 7.0, with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside at 140 μg/ml.

Nuclease S1 Analyses. RNA was isolated from 50-ml samples of late-logarithmic-phase cultures ($6–8 \times 10^6$ cells per ml) grown in glucose medium and S1 reactions were performed as described (21). Twenty micrograms of total RNA or 5 μg of poly(A)$^+$ RNA was used per hybridization. The *SPO13* and *SPO11* probes contained the 3′ end of the coding region ("probe B" in refs. 16 and 17, respectively); the *SPO12* probe contained a *Hin*dIII–*Eco*RI fragment including the 5′ mRNA start site (R. T. Elder and R.E.E., unpublished results); the *SPS2* probe was a 760-base-pair *Bgl* II fragment covering the 5′ end of the gene (22) and was constructed in our laboratory by R. T. Surosky; the *SPO16* probe consisted of a *Bgl* II–*Hin*dIII 5′ probe (R. T. Elder and R.E.E., unpublished results) constructed by R. T. Surosky. A 3′ rather than 5′ probe was used to detect *SPO13* RNA, since the latter generated a smear due to heterogeneity of the 5′ ends of the RNA. A 3′ probe was also used for *SPO11*, due to proximity of an upstream transcript expressed in mitosis (16). S1 analysis of actin message levels in total and poly(A)$^+$ samples was used to calculate mRNA enrichment of the poly(A)$^+$ fractions.

Plasmid Copy-Number Determinations. The copy number of p(spo13)28 was determined as described (23). A *URA3* probe was used to measure the copy number of *URA3* on the plasmid relative to the chromosomal gene.

RESULTS

Recovery of Nine Nuclear Recessive Mutant Alleles. To identify trans-acting regulators of meiosis, mutants were sought that expressed *SPO13* in haploid cells on medium containing both glucose and nitrogen. Under these conditions, we anticipated the recovery of lesions in genes that operate downstream of *IME1*, a gene through which both the cell-type and nutritional controls have been proposed to exert their regulation of meiosis (8). Since relaxation of either one of these regulatory pathways alone will not allow *SPO13* expression, mutations upstream of *IME1* were not expected to be detected.

Haploid strain RSY10 was mutagenized by exposing cells carrying p(spo13)28, a multicopy plasmid containing a *spo13*–

lacZ fusion, to ethyl methanesulfonate. Among 12,000 independent colonies screened, 31 isolates were obtained that expressed the *spo13–lacZ* fusion under mitotic growth conditions. Variants with plasmid rather than nuclear lesions were eliminated from further study (Fig. 1). Nine isolates continued to demonstrate β-galactosidase activity that segregated in a 2:2 Mendelian fashion, indicating that these mutations are in nuclear genes (Table 1). The dominant or recessive nature of these mutations was determined by assaying the ability of the heterozygous diploids to express β-galactosidase. All nine diploids failed to exhibit β-galactosidase activity, demonstrating that the mutant alleles are recessive.

Enhanced β-Galactosidase Expression Is Dependent on *SPO13* Promoter Sequences. To determine whether mitotic β-galactosidase expression resulted from overreplication of the fusion plasmid during vegetative growth, the copy number of p(spo13)28 was measured in the mutant strains. Copy number was the same in both the wild type and the mutants (18–21 per cell), indicating that plasmid overreplication was not responsible for the mutant phenotype (data not shown). To confirm that the mutant genes exerted their effect(s) through the *SPO13* promoter and not via general promoter and/or *lacZ* sequences as reported elsewhere (25), the production of β-galactosidase was assayed from a disabled *cyc1–lacZ* fusion carried on plasmid pZJ (24) in both wild-type and mutant strains. This plasmid lacks *CYC1* upstream activation sequences but retains a functional TATA box, allowing low-level constitutive expression (26, 27). Two independent transformants of each strain were grown to late logarithmic stage and assayed for β-galactosidase activity. Differences in β-galactosidase activity in eight of the nine mutants (20–147 units/mg) and the wild-type strain (88 units/mg) were within the limits of strain variation for this assay (Table 1). Thus, these mutants do not affect transcription in a general way. One of the nine mutants (m4) exhibited a 10-fold increase in specific activity (1147 units/mg) over the wild type and was excluded from further analysis since it enhanced β-galactosidase activity regardless of the promoter sequences present. The eight mutants that displayed the expected phenotypes for altered transcriptional regulators were examined in greater detail.

Table 1. β-Galactosidase activity of the *spo13–lacZ* and *cyc1–lacZ* fusion genes in spore segregants and haploid mutants

Mutant*	Tetrad segregation *UME:ume*,† no. of tetrads						Activity of *cyc1–lacZ*,‡ units/mg
	4:0	3:1	2:2	1:3	0:4	Total	
m1 (*ume1-1*)	0	10	23	1	0	34	65
m2 (*ume2-1*)	0	0	22	1	0	23	ND
m3 (*ume2-2*)	1	0	23	2	0	26	147
m4	1	0	25	3	0	29	1147§
m5 (*ume5-2*)	1	1	29	1	0	32	142
m6 (*ume2-3*)	0	1	31	0	0	32	ND
m7 (*ume4-1*)	0	2	44	3	0	49	42
m8 (*ume3-1*)	0	2	26	1	0	29	20
m9 (*ume5-1*)	1	1	43	4	0	49	ND

*The *ume* gene assignments are based on results shown in Fig. 2.

†Mutants were crossed to wild type (RSY75) and spore colonies were assayed for β-galactosidase activity (*UME*, absence of activity; *ume*, presence of activity). The high number of 3:1 tetrads for m1 (*ume1-1*) may have resulted from false negative segregants due to the weak response of this mutant in plate tests.

‡One representative haploid mutant from each complementation group was transformed with pZJ (24) and two independent transformants were assayed in duplicate; each sample was assayed at two extract concentrations and the measurements were averaged. Values are given as total β-galactosidase activity (units) per mg of soluble protein. Average activities ranged ±5–30%. ND, not done.

§The m4 isolate exhibited a 10-fold increase in β-galactosidase activity from the *cyc1–lacZ* fusion and was not studied further.

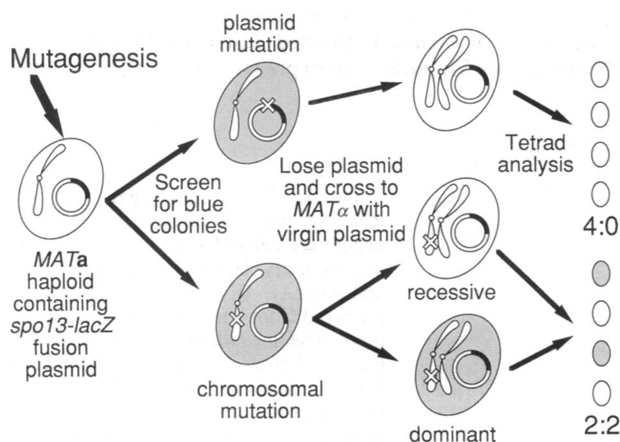

FIG. 1. *SPO13* regulatory mutant screen. The X depicts an ethyl methanesulfonate-induced mutation that allows β-galactosidase expression (shaded cells) from the *spo13–lacZ* fusion. Colonies to be assayed for β-galactosidase activity were lifted or spotted onto Whatman paper, frozen in liquid nitrogen to promote cell lysis, and overlaid with top agar containing 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside at 140 μg/ml. Plasmid-borne mutations will result in 4^+ (wild type): 0^- (mutant) segregation (top pathway) and chromosomal mutations, 2^+:2^- segregation (bottom pathway) for β-galactosidase activity, following plasmid loss and reintroduction of an unmutagenized plasmid through mating.

Complementation groups were determined by mating outcross segregants from the eight mutants in all pairwise combinations; diploids containing p(spo13)28 were selected and assayed on plates for β-galactosidase activity (Fig. 2). The results revealed the presence of five complementation groups, which we have designated *ume1*, *ume2*, *ume3*, *ume4*, and *ume5* (*u*nscheduled *m*eiotic gene *e*xpression). Three alleles of *ume2* and two alleles of *ume5* were recovered. The gene assignments were verified by segregation analysis of the double-mutant diploids (data not shown).

The Ume Phenotype Is Not Dependent on *IME1* or *IME2* Expression. The *ume* mutant screen was designed to identify regulators downstream of the *IME1* and *IME2* meiotic activators (see above). To directly test this assumption, disruptions were made of *IME1* or *IME2* in all *ume* mutant strains and the double mutants were tested for the ability to express

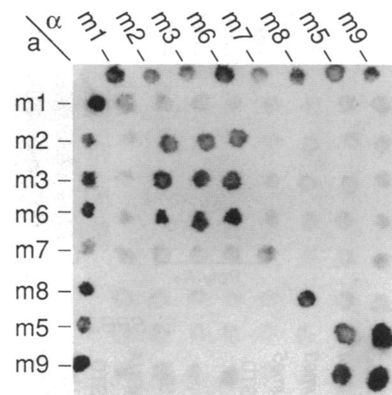

FIG. 2. Complementation analysis of *SPO13* regulatory mutants. Haploid mutant parents (left column and top row) were mated in all combinations, and diploids containing the *spo13–lacZ* fusion gene were selected and assayed on plates for β-galactosidase expression (see Fig. 1 legend). Five complementation groups were identified from the eight alleles tested and designated *ume1* (m1), *ume2* (m2, m3, and m6), *ume3* (m8), *ume4* (m7), and *ume5* (m5 and m9).

the *spo13–lacZ* fusion gene. Null alleles of *IME1* and *IME2* were constructed by integrating plasmids YIpK26 (8) and pAM412-2 (9), respectively, into the *ume* strains. The disruptions were confirmed by Southern blot analysis. All *ime ume* double mutants behaved as single *ume* mutants and continued to express the *spo13–lacZ* fusion gene (data not shown). Moreover, no *IME1* message was detected by S1 analysis in *ume* mitotic RNA samples. These results demonstrate that the *UME* genes function downstream or independently of *IME1* and *IME2*.

***SPO13* mRNA Abundance Is Increased in *ume* Mutants During Mitotic Growth.** To verify that the *ume* mutations affect the expression of the chromosomal *SPO13* gene as indicated by the *spo13–lacZ* reporter gene, S1 analysis of the *SPO13* message was performed on both mutants and wild-type cells (Fig. 3*A*). As expected, no *SPO13* signal was detected from total RNA preparations isolated from mitotic cultures in the *UME* parental strain. Mutants *ume1* and *ume4* exhibited a reproducible but low level of mitotic expression, <5% of the fully induced levels observed during sporulation (≈3 mRNA molecules per cell, ref. 17). No detectable signal was observed in the *ume2, -3*, and *-5* lanes.

To amplify the signal of *SPO13* message for S1 analysis, poly(A)$^+$ RNA was isolated from vegetative cultures of wild-type (*UME*), *ume2, ume3*, and *ume5* strains. The enrichment provided by the poly(A)$^+$ isolation (≈40-fold) revealed the presence of *SPO13* message in these mutants but not in the wild type. Since poly(A)$^+$ mRNA preparations contain varying amounts of rRNA contamination, the amount of poly(A)$^+$ mRNA used in each hybridization was normalized relative to actin message. The levels of actin mRNA in *ume* mutant and wild-type strains were similar as determined by S1 analysis of total RNA preparations (Fig. 3*F*). The presence of *SPO13* chromosomal mRNA clearly demonstrates that the *UME* gene products function to regulate the level of *SPO13* transcript. Furthermore, the similar actin message levels in wild type and *ume* mutants support the conclusion that the *UME* genes are not general transcriptional regulators, as indicated by the *cyc1–lacZ* expression experiments (see above). Since a 3′ probe was used to detect *SPO13* RNA, primer extension experiments were performed to map the 5′ ends of the *SPO13* message and determine whether the normal start sites were utilized. When compared to the meiotic *SPO13* mRNA from SK1, the major start sites observed appeared identical (data not shown).

Unscheduled *SPO11* and *SPO16* Expression in *ume* Mutants. About 200 genes are thought to be involved in meiosis and spore formation (1). It seems unlikely that each of these genes is independently regulated, but rather that one, or a few, regulatory pathways coordinate their expression. Given this, the *ume* mutations might be expected to have a more global effect on the regulation of meiosis-specific genes. The mitotic expression of another fusion gene, *spo11–lacZ*, was therefore examined. *SPO11* is regulated similarly to *SPO13*; mRNA levels are at the limits of detection during vegetative growth and are induced ≈70-fold during meiosis (16). All *ume* mutants expressed this fusion gene during mitotic growth, whereas the wild type did not (Fig. 4).

As with *SPO13*, S1 analysis of mitotic poly(A)$^+$-enriched RNA preparations revealed increased levels of *SPO11* mRNA in *ume1, ume4*, and, to a lesser extent, *ume3* strains (Fig. 3*B*). These results were reproducible in both total and poly(A)$^+$ RNA preparations. Surprisingly, the *ume2* and *ume5 SPO11* RNA levels were not above basal wild-type levels even though these mutants exhibited β-galactosidase activity from the *spo11–lacZ* fusion. This may have been due to sensitivity of the S1 assay, decreased lability of the *spo11–lacZ* message, and/or the high copy number of the fusion gene in the β-galactosidase assays. It should be noted that the level of *SPO11* mRNA in the *UME* strain appeared similar to the *SPO13* transcript levels in *ume2* and *ume5* strains (Fig. 3 *A* and *B*); however, *UME* strains containing the *spo11–lacZ* fusion gene did not show β-galactosidase activity. One possible explanation for this observation is a translational role for *UME2* or *UME5*.

The mRNA levels of the *SPO16* gene, similar to *SPO11* and *SPO13*, are barely detectable in vegetative cells but increase dramatically early in meiosis (R. T. Elder and R.E.E., unpublished results). Sporulating *spo16* diploids exhibit a reduced ability to complete early prophase, resulting in an increased level of mononucleate cells. S1 analyses of mitotic *SPO16* mRNA levels in wild type and the *ume* mutant strains revealed an increase in *SPO16* message similar to *SPO11* and *SPO13* (Fig. 3*C*). To quantify the increase in message levels, the bands corresponding to the *SPO16* S1-protected probe were excised and the radioactivity was determined by liquid scintillation counting. Values ranged from 10% (for *ume3* and *ume5*) to 200% (for *ume4*) over wild type.

Regulation of Genes Expressed Later in Meiosis Is Not Affected in *ume* Mutants. The expression pattern observed in

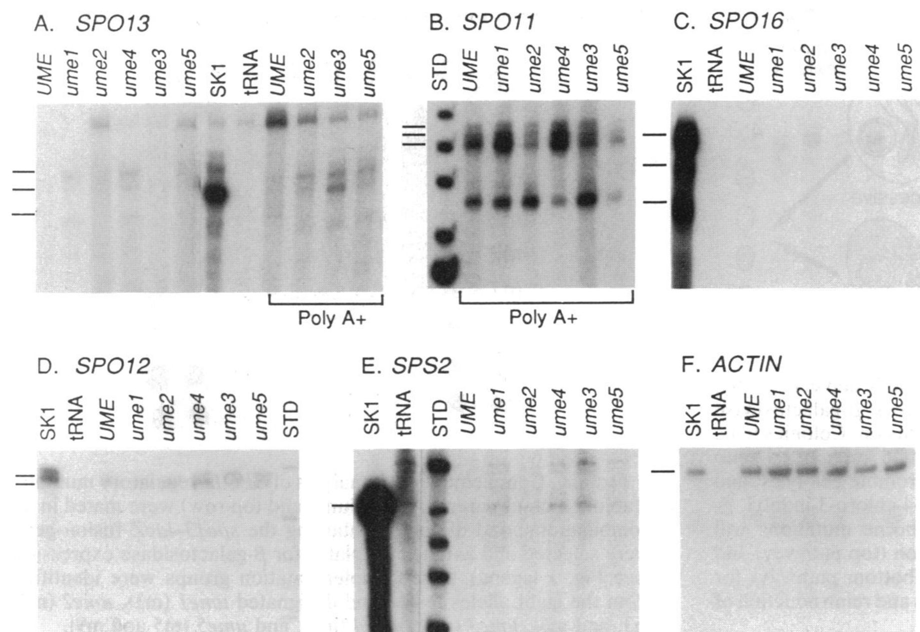

FIG. 3. Nuclease S1 analysis of total RNA or poly(A)$^+$-enriched RNA from vegetative cells of wild-type (*UME*) and *ume* mutants. Total RNA (20 μg per lane) and poly(A)$^+$ RNA (5 μg per lane) were prepared from late-logarithmic-stage cultures. The lines identify probe sequences protected from S1 digestion by mRNA. Size standards (STD) were derived from *Hin*fI-digested YRp7 DNA. *UME*, wild-type parent; tRNA, control for nonspecific probe self-annealing; SK1, total RNA from *UME* diploids in sporulation medium at the time of maximum mRNA accumulation; alleles used for these experiments were *ume1-1, ume2-2, ume3-1, ume4-1*, and *ume5-2*. (*A*) *SPO13* probe. (*B*) *SPO11* probe. (*C*) *SPO16* probe. (*D*) *SPO12* probe. (*E*) *SPS2* probe. (*F*) Actin probe. Lower band in *B* is the result of probe self-annealing. The tRNA control lane contained the same band (data not shown).

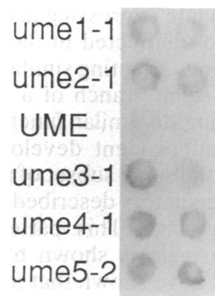

FIG. 4. β-Galactosidase activity of a *spo11–lacZ* fusion gene in *ume* mutants. Mutant (*ume*) and wild-type (*UME*) strains were transformed with p(spo11)153, a high-copy plasmid carrying a *spo11–lacZ* gene. Two individual transformants were picked and assayed on plates for β-galactosidase activity as described in Fig. 1.

yeast during meiosis and spore formation has been divided into early, middle, and late periods (3). *SPO11*, *SPO13*, and *SPO16* belong to the early expression group (refs. 16 and 17; R. T. Elder and R.E.E., unpublished results) and appear to be coregulated by the *UME* genes. To determine whether the *UME* genes also regulate genes expressed in the later two classes, S1 analysis was performed with probes for *SPO12* and *SPS2* transcripts. *SPO12*, like *SPO13*, is involved in the meiosis I segregation process (18). This gene is expressed at a low basal level during vegetative growth, with message abundance increasing 15-fold during sporulation. *SPO12* message levels peak about 3 hr after the appearance of *SPO13* message, and thus *SPO12* represents a "middle" expressing gene (S. Frackman, R. T. Elder, and R.E.E., unpublished data). The *SPS2* gene is postulated to be involved in spore maturation and is transcribed late in meiosis (15). The S1 analysis of *SPO12* and *SPS2* message levels during mitotic growth is depicted in Fig. 3 *D* and *E*. No significant difference in message levels can be seen between the wild type and the *ume* mutant strains. The apparent enhancement of *SPO12* mRNA in the *ume4* lane is not reproducible, and we therefore conclude that there is no effect on *SPO12* expression. Our data thus far indicate that the *ume* mutants only affect the regulation of genes belonging to the early expression class.

Sporulation Efficiency Is Reduced in *ume4* Mutants. Alterations in genes that regulate a number of meiosis-specific functions might be expected to affect normal sporulation. To examine this possibility, ascus formation was examined in *ume* diploids. Heterozygous *UME/ume* diploids produced wild-type levels of asci (50–70%) consistent with the recessive nature of the mutations. The *ume4* homozygotes exhibited a substantial reduction in ascus production (11%). The *ume2*, *ume3*, and *ume5* homozygotes also displayed a reduction in ascus formation (28–38%), though not to the same extent as the *ume4* diploid.

In wild-type strains, the appearance of "binucleate" and "tri- and tetranucleate" cells reflects the completion of meiosis I and meiosis II, respectively. To monitor the progress of *ume4* diploids through the two meiotic divisions, the mutant cells were examined by staining with 4′,6-diamidino-2-phenylindole (DAPI). The *ume4* mutant accumulated cells at the "mononucleate" stage (Fig. 5 *Lower*) compared to wild-type cells (*Upper*), indicating an early block prior to the completion of meiosis I. These data argue that the *UME4* gene is essential for normal meiotic development.

DISCUSSION

This report describes the identification and characterization of genes that regulate the expression of a specific set of meiotic genes. The recovered mutations, *ume1–ume5*, define five new trans-acting regulatory genes that control the transcript levels of *SPO11*, *SPO13*, and *SPO16* during mitotic growth. The *UME* genes appear to function either downstream or independently of *IME1* and *IME2*, two meiotic activators. Overproduction of the *IME1* or *IME2* product has been shown to stimulate *SPO11*-dependent meiotic recombination in vegetative cells (9), indicating a direct effect of *IME1* and *IME2* on early meiotic genes. These results are compatible with the hypothesis that *IME2* acts as an activator of meiosis by negatively regulating the function of *UME* genes in a transcriptional cascade (Fig. 6). This model assumes that the cell-type and nutritional controls exert their effects in regulating meiosis exclusively through the *IME1* and *IME2* genes. Evidence against a separate pathway of cell-type control is the similar level of β-galactosidase activity in *ume* haploid and diploid strains (Fig. 2). In addition, *SPO13* message levels in *ume3* and *ume4* diploids were similar to haploid levels (data not shown).

Although message levels are <5% of the maximum observed during meiosis, the mutations clearly permit expression of early meiotic genes during vegetative growth and are consistently highest in *ume1* and *ume4* mutants. An earlier report showed that low levels of *SPO13* message are induced when *MAT***a**/*MAT***a** or *MATα/MATα* diploids are starved for nitrogen (17). The *UME* genes are unlikely to be involved in this starvation response, since they are epistatic to *IME1* and no similar induction was seen in glucose-grown RSY10. Moreover, another gene, *UME6*, was identified whose mutant alleles give vegetative mRNA levels comparable to fully induced meiotic levels (R. T. Surosky and R.E.E., unpublished results). The low level of mRNA accumulation observed with the alleles recovered here may be due to the isolation of leaky alleles for these genes; i.e., strong *ume*

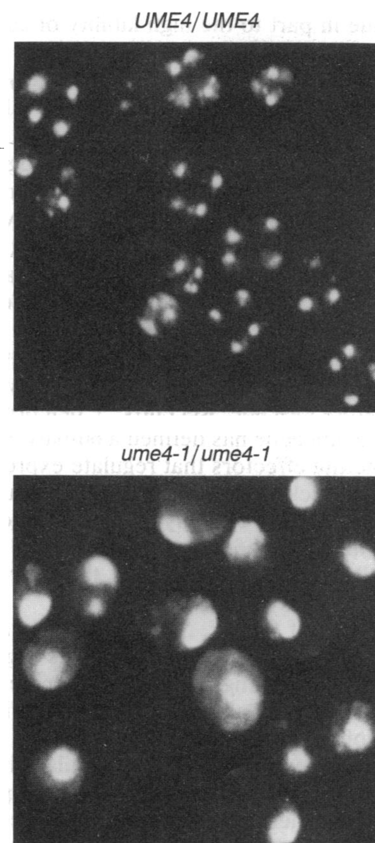

FIG. 5. Sporulated wild-type (*UME4/UME4*) and *ume4-1/ume4-1* diploids stained with 4′,6-diamidino-2-phenylindole (DAPI). Selected diploids were incubated on sporulation medium for 5 days at 30°C. Cells were fixed in 70% ethanol, stained with DAPI (0.025 μg/ml) and photographed at ×100 magnification. (×1500.)

GENE	MEIOTIC EVENT AFFECTED	TIME OF EXPRESSION	*UME* REGULATION
SPO16	MI Early Prophase	Early	Yes
SPO11	Recombination	Early	Yes
SPO13	MI Segregation	Early	Yes
SPO12	MI Segregation	Middle	No
SPS2	Spore Wall Formation	Late	No

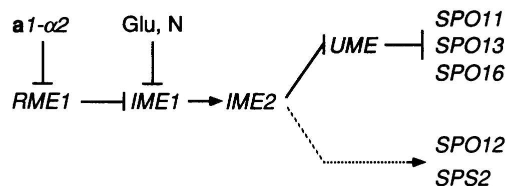

FIG. 6. Model for regulation of genes expressed early in meiotic development. Bars and arrows, negative and positive regulation, respectively. The relationships between cell-type regulation, nutritional control, *IME1*, and *IME2* have been demonstrated (8, 9). The independence of middle (*SPO12*) and late (*SPS2*) meiotic genes from *UME* control (dotted arrow) and a presumptive pathway of the regulation of the *UME* genes by the *IME2* product are shown.

alleles of these loci may be lethal or impair vegetative growth, particularly if they allow the unscheduled expression of a bank of meiotic genes during mitosis. Since mitotic growth was required in the mutant screen, mutants with growth defects would not have been detected. An additional screen to identify temperature-sensitive conditional mutants was performed but no allele of this type was recovered. The low levels of these early-gene mRNAs observed in the mutants may also be due in part to the high lability of early meiosis-specific RNAs. Some evidence for this has been provided by recent experiments demonstrating an extremely rapid turnover of meiotic *SPO11*, *SPO13*, and *SPO16* mRNA (R. T. Surosky and R.E.E., unpublished results).

All eight mutants isolated are recessive, suggesting that they either define negative regulatory functions or define positive functions that ultimately act via a negative regulator. In either case, it is necessary to postulate that these early meiotic genes are subject to some degree of negative control (Fig. 6). Two possible explanations can be considered for how these genes execute their effect(s) on the target gene. They may each act alone or in combination at one or several cis-acting sites upstream of *SPO13*, or they may function as part of a regulatory cascade. Recently, 5′ deletion analysis of a *spo13–lacZ* fusion gene has defined a 60-base-pair putative site for trans-acting effectors that regulate expression of the fusion gene (L. Buckingham and R.E.E., unpublished observations). We believe it unlikely that the products of all five *UME* genes bind independently to one or more sites in this relatively small region but rather that they participate in a regulatory cascade.

The coregulation of *SPO11*, *SPO13*, and *SPO16* shown by *UME* genes provides further insight into the organization of the mechanisms controlling meiotic development. Meiosis-specific genes have been assigned to different classes based on their time of expression (3, 16). While the *SPO11* and *SPO13* products are expressed at a similar time early in meiosis, they act in the genetically separable pathways of recombination and meiosis I segregation, respectively. The regulation of *SPO12*, which is also involved in meiosis I segregation but belongs to a later transcription class (S. Frackman, R. T. Elder, and R.E.E, unpublished results), is not affected in the *ume* mutant backgrounds. Similarly, the expression of *SPS2*, a late expressing gene involved in ascus formation (15), is also unaffected in the *ume* mutants. It is therefore clear that genes affecting similar functions need not be controlled by the same branch of a regulatory cascade, whereas genes affecting dissimilar functions, which participate in genetically independent developmental processes, may be controlled by the same subset of regulators. Our data show that the *UME* regulators described here affect genes in the early expression class. This pattern of control may explain the defect in meiosis shown by the *ume4* mutant strain. Recent studies have shown that mitotic expression of either *SPO11* (C. Atcheson and R.E.E., unpublished results) or *SPO13* (R. T. Surosky and R.E.E., unpublished results) does not disrupt meiosis. However, the premature expression of a larger class of early meiotic genes may lead to an arrested state either because several functions are executed out of sequence or because feedback mechanisms are affected that in turn halt meiotic development.

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