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Genetics

UME6 is a central component of a developmental regulatory switch controlling meiosis-specific gene expression

(sporulation/yeast/transcription/URS1 promoter element)

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ABSTRACT **The *UME6* gene of *Saccharomyces cerevisiae* was identified as a mitotic repressor of early meiosis-specific gene expression. It encodes a Zn_2Cys_6 DNA-binding protein which binds to URS1, a promoter element needed for both mitotic repression and meiotic induction of early meiotic genes. This paper demonstrates that a complete deletion of *UME6* causes not only vegetative derepression of early meiotic genes during vegetative growth but also a significant reduction in induction of meiosis-specific genes, accompanied by a severe defect in meiotic progression. After initiating premeiotic DNA synthesis the vast majority of cells (≈85%) become arrested in prophase and fail to execute recombination; a minority of cells (≈15%) complete recombination and meiosis I, and half of these form asci. Quantitative analysis of the same early meiotic transcripts that are vegetatively derepressed in the *ume6* mutant, *SPO11, SPO13, IME2*, and *SPO1*, indicates a low level of induction in meiosis above their vegetative derepressed levels. In addition, the expression of later meiotic transcripts, *SPS2* and *DIT1*, is significantly delayed and reduced. The expression pattern of early meiotic genes in *ume6*-deleted cells is strikingly similar to that of early meiotic genes with promoter mutations in URS1. These results support the view that *UME6* and URS1 are part of a developmental switch that controls both vegetative repression and meiotic induction of meiosis-specific genes.**

Diploid yeast cells undergo meiosis and spore formation in response to starvation for glucose and nitrogen. Many meiosis-specific genes required for the coordination of these events have been cloned and classified by their time of expression during sporulation (1, 2). The activation of meiotic genes requires expression of *IME1*, an inducer that is among the first meiosis-specific genes expressed. *IME1* expression is regulated by both cell type and nutrition: only cells expressing both *MAT***a***1* and *MATα2* can down-regulate the repressor *RME1* and induce *IME1* in response to starvation. Previously, we reported the identification of six *UME* genes (*u*nscheduled *m*eiotic *e*xpression) that negatively regulate early meiotic gene expression in the absence of *IME1*. Mutations in any of these genes result in derepression of early meiotic genes in haploids during vegetative growth on medium rich in glucose and nitrogen (3, 4). Since derepression of meiosis-specific genes in these mutants does not require *IME1*, we proposed that during wild-type meiosis the Ime1 protein acts upstream by inhibiting Ume repression functions (3, 4). Loss of *UME6* permits the highest level of derepression, suggesting it is a key repressor of meiotic gene expression.

The *UME6* gene represses early meiosis-specific genes, including *SPO13* (4), *SPO11* (4), *HOP1* (5), and *IME2* (6), which require the cis-acting element URS1 for both their vegetative repression and meiotic induction (6–11). While URS1 is sufficient for repression in heterologous promoters (6–10), other enhancer elements act in conjunction with it to promote meiotic induction. These include the T_4C box [present in *IME2* (6), *SPO13* (9), and *HSP82* (11)], and the UAS_H element [in *HOP1* (10)]. Several trans-acting factors are known to bind these meiotic promoter elements: Ume6 (4, 12) and RPA-1,2,3 (13, 14) bind to URS1, and Ubf binds UAS_H (15). The Ume6 protein, which contains a canonical Zn_2Cys_6 DNA-binding domain similar to that of the activator Gal4, specifically binds to the G+C-rich core of URS1 in the *SPO13* and *IME2* promoters (4, 12). This paper shows that *UME6* is required for sporulation, and it provides evidence that *UME6*, like URS1, is part of a developmental switch that regulates both mitotic repression and meiotic induction of meiosis-specific genes.

MATERIALS AND METHODS

Strains. All yeast (*Saccharomyces cerevisiae*) haploid strains were derived from W303-1A and W303-1B (*MAT***a** and *MATα*, respectively, *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*; R. Rothstein, Columbia University, New York). SFY59 is an *ade6* variant of W303-1A (S. Frackman, this laboratory). yC67 is an *ADE2* derivative of yBS65 (*MATα ade2-1 cyh2 his3-11,15 leu1-c met13-c trp1-1 tyr1-2 ura3-1*) made from crosses between K264-10D (16) and W303-1B (B. Schutte, this laboratory). yC105 and yC107 are isogenic derivatives of SFY59 and yC67, respectively, which have undergone a complete deletion of the *UME6* open reading frame, constructed using plasmid pCS4 (4). Intercrosses resulted in the isogenic diploids *UME6/UME6* (SFY59/yC67 = Cx36) and *ume6-D1/ume6-D1* (yC105/yC107 = Cx37). A marker to assay recombination was provided by crossing yC105 to another W303-1B derivative containing a *trp1* duplication (*trp1-1:URA3:trp1-3'Δ*), made by integrating pRS19 (17) at *trp1-1* (L. Henninger, this laboratory). The resulting diploid was sporulated and dissected to obtain *UME6* wild-type (yC120) and *ume6-D1* (yC122) strains from the same tetrad and of the same genotype (*MAT***a** *ade2-1 ade6 can1-100 leu2-3,112 trp1-1:URA3:trp1-3'Δ ura3-1*). These strains were then crossed to a produce near isogenic *ume6-D1/ume6-D1* (yC122/yC107 = Cx57) and *UME6/UME6* (yC120/yC67 = Cx56) diploids. Segregation of *ume6-D1* and the recombination marker was monitored by phenotype and Southern analysis. All transformations were performed by the lithium acetate method (18).

Growth and Sporulation. Growth and sporulation were conducted at 30°C, using methods described in refs. 19–21. Briefly, late logarithmic phase diploids (≈8×10^6 cells per ml) from YPA (1% potassium acetate/2% Bacto-peptone/1% Bacto-yeast extract) were washed, suspended in SPII (2% potassium acetate supplemented with required amino acids at 75 μg/ml) at 5×10^7 cells per ml, and sampled every 2 hr over

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Abbreviations: FACS, fluorescence-activated cell sorter; DAPI, 4',6-diamidino-2-phenylindole; DS, DNA synthesis; C, haploid DNA content; cfu, colony-forming unit.

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24 hr for fluorescence-activated cell sorter (FACS) analysis (22), 4′,6-diamidino-2-phenylindole (DAPI) staining (23), recombination and survival platings, and RNA preparation (24).

S1 Nuclease Protection Assays. S1 nuclease protection assays (24) were performed using 15 μg of total RNA isolated from 20-ml sporulation samples for each hybridization. The following probes have been previously described: *SPO11* (25), *SPO13* (26), *SPS2* (3, 27), and *IME1* (26). The *DIT1* probe (R. Surosky, this laboratory) contains the 0.5-kb *Bam*HI–*Cla* I fragment of *DIT1* (28). The *DED1* probe (pGT31; G. Tevzadze, this laboratory), which contains a 250-bp fragment of 5′ *DED1*, was included in all RNase protection reactions as an internal control for fluctuations in recovery and loading of samples. *DED1* is an essential gene located 5′ of *HIS3* (29) encoding a transcript whose levels are constant during sporulation (C. Atcheson, G. Tevzadze, and R.E.E., unpublished results). All probes were used at concentrations that yielded a linear relationship between hybridization signal and amount of RNA in the protection assay (data not shown). PAGE gels in which S1 nuclease protection assays were conducted were scanned by using a Molecular Dynamics PhosphorImager, and the data were quantitated with IMAGEQUANT (IQ) version 3.2 for Windows.

RESULTS

***UME6* Is Required for Sporulation.** Complete deletion of *UME6* results in a >90% reduction in ascus formation, similar to the disruption allele (4). The *ume6-D1/ume6-D1* diploid (Cx37) produces ≈5% asci at 23°C or 30°C, and <1% asci at 34°C, while the isogenic wild-type strain (Cx36) yields 55%, 80%, and 62% asci, respectively. To determine the nature of the *ume6* sporulation defect, meiotic landmarks were monitored in isogenic wild-type (Cx56) and deletion (Cx57) strains containing a marker to assay recombination.

***UME6* Is Dispensable for Initiation of Premeiotic DNA Synthesis.** Premeiotic DNA synthesis (DS) was measured by FACS analysis of propidium iodide-stained cells (Fig. 1). Cells harvested into sporulation medium from asynchronous growth initially have both 2C and 4C peaks characteristic of logarithmic phase. By 6 hr of sporulation, an intermediary ≈6C peak appears consistent with DS in mother cells, but not attached daughter cells, of a 50–65% budded population. This peak in both wild type and deletion diploids indicates that *UME6* is not required for initiation of DS. In the deletion, the appearance of 6C cells coincides with that of cells containing <1C DNA content. The presence of a clear valley between the 2C and <1C peaks suggests that the <1C peak results either from highly synchronous DNA degradation or from the liberation of buds containing little or no chromosomal DNA from their mother cells by sonication.

***UME6* Is Required for Meiotic Recombination.** Initiation of meiotic recombination was monitored by counting tryptophan-independent (Trp^+) recombinants per colony-forming unit (cfu) resulting from exchange between repeated genes of a heterozygous gene duplication at the *trp1* locus (*trp1-1/trp1-1:URA3:trp1-3′*Δ). Trp^+ recombinants arise from either intra- or interchromosomal exchange (gene conversion or reciprocal recombination) between the *trp1-1* and the *trp1-3′*Δ alleles on a single chromosome or between homologues. These events are induced 30-fold by 16 hr in the wild type, while in the deletion mutant recombination is delayed and reduced, reaching only ≈16% of the wild type by 24 hr (Fig. 2). Mating-type tests indicate that ≈50% of Trp^+ recombinants recovered from the deletion strain after 24 hr of sporulation are maters, suggesting that these cells have segregated their *MAT* alleles by progression at least through the reductional meiotic division. Viability assays indicate that the deletion strain also undergoes a loss of viability after 24 hr of sporulation, with only 25% of the population surviving by 48 hr (the 2-fold increase in

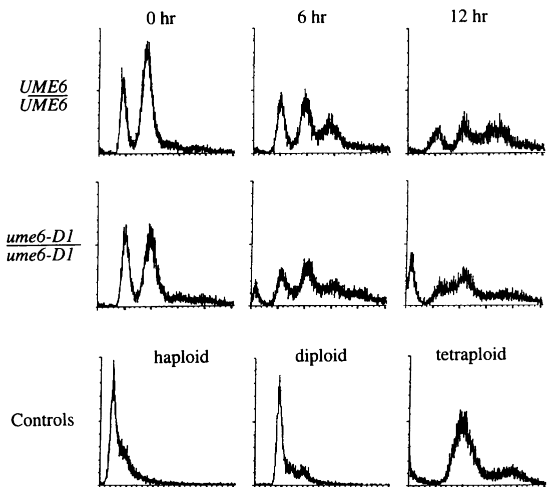

FIG. 1. DS measured by FACS analysis of wild-type and *ume6-D1* diploids. Cells were stained with propidium iodide and mildly sonicated (18) to reduce clumping without causing cell lysis. Flow cytometry on a Becton Dickinson FACScan was analyzed by LYSISII version 1.1 software. The *x*-axis is DNA content and the *y*-axis is cell count at 0, 6, and 12 hr of sporulation, using stationary-phase haploid (1C), diploid (2C), and tetraploid (4C) controls.

wild-type survival is due to cells dropping their buds during sporulation). These results demonstrate that deletion of *UME6* causes a failure in meiotic recombination in most of the sporulating cells accompanied by an eventual loss in viability; a minor population can proceed beyond this block, presumably by a *UME6*-independent pathway.

***UME6* Is Necessary for Meiosis I, Meiosis II, and Ascus Formation.** DAPI staining of nuclei reveals that the vast majority of cells in the deletion strain become arrested in G_2 during meiotic prophase prior to the meiosis I division: ≈85% of cells remain mononucleate, with only ≈15% undergoing meiosis I and <5% completing both the meiosis I and meiosis II divisions (Fig. 3). Light microscopy indicates that by 48 hr only a small fraction of cells form asci (≈8%), most of which (98%) are dyads. Since fewer cells complete both meiotic divisions than form asci, some dyads may result from packaging of meiosis I products. In those cells completing meiosis I or meiosis I and meiosis II, the divisions are substantially

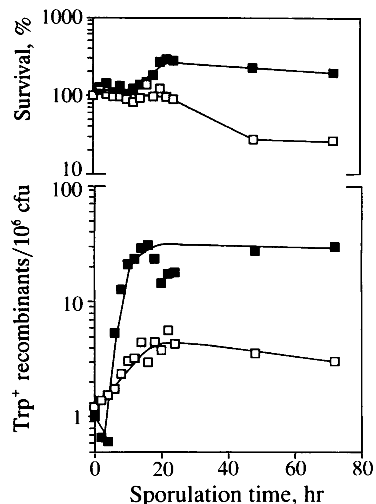

FIG. 2. Survival and recombination in wild-type (■) and *ume6-D1* (□) diploids. (*Upper*) Percent survival = (cfu on nonselective medium at time *x* divided by cfu at time zero) × 100. (*Lower*) Number of Trp^+ recombinants per 10^6 cfu.

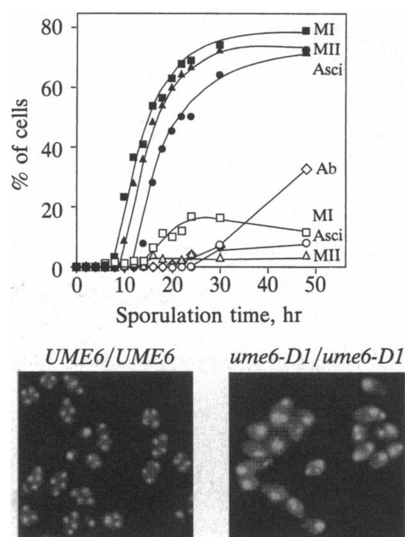

FIG. 3. (*Upper*) Kinetics of the meiotic divisions. Cells completing meiosis I (□, ■) and meiosis II (△, ▲), asci (○, ●), and cells with more than four staining bodies (◇) are shown; filled symbols = wild type, open symbols = *ume6-D1* deletion; 300 cells per sample were counted. (*Lower*) DAPI-stained nuclei after 48 hr of sporulation. (×40.) The mutant contains a cell with more than four staining foci.

delayed, with multinucleate cells first appearing at 16 hr compared to 6 hr in the wild type. A class of abnormal cells containing more than four DAPI-staining foci is detected at 22 hr, reaching nearly 30% of the population by 48 hr. Similar abnormal cells have been reported in other meiotic mutants and appear to be due to nuclear fragmentation (20, 30, 31). The presence of abnormal cells, the loss of cell viability, and the presence of a <1C population may all result from a single deleterious event during *ume6-D1* meiosis (see *Discussion*).

UME6 Is Critical for Proper Early Meiotic Gene Induction. To determine if *ume6-D1* meiotic defects correlate with altered gene expression, representative meiosis-specific transcripts were quantitated during sporulation. Results from isogenic strains lacking the recombination assay marker (data not shown) are similar to those below.

UME6 *is needed for reestablishment of* IME1 *repression during meiosis.* Unlike URS1-regulated early meiosis-specific genes, vegetative levels of the *IME1* transcript remain repressed in *ume6* mutants during growth in either glucose (4) or acetate medium (lane v, Fig. 4). Moreover, the *IME1* transcript accumulates to an ≈3-fold higher level than wild type, failing to decrease even after 10 hr of sporulation (Fig. 4). Thus, while *UME6* is dispensable for vegetative repression of *IME1*, it appears to be required to reestablish *IME1* repression during meiosis.

UME6 *is needed for induction of early meiotic genes.* Analysis of URS1-regulated early meiotic genes induced after *IME1* confirms that *UME6* is essential for their vegetative repression (refs. 4 and 6; G. Tevzadze and R.E.E., unpublished results; and see below). Fig. 4 provides evidence that *UME6* is also required for activation of these early meiotic genes (see also ref. 5). During the first 12 hr of *ume6-D1* sporulation, *SPO11* and *SPO13* transcripts are induced to a relatively low level (0–25%) above vegetative derepressed levels (similar data for *IME2* are not shown; C.M.S., unpublished results; ref. 30). Whereas reestablishment of repression of these genes occurs within 24 hr in the wild-type strain, no evidence of rerepression is seen in the deletion. Thus, *UME6* participates in vegetative repression, meiotic activation, and reestablishment of repression of early meiotic genes.

UME6 *is needed for appropriate timing of mid/late meiotic gene expression. SPS2* induction, which normally occurs at ≈7 hr and reaches a maximum at ≈10 hr of sporulation, is delayed by ≈6 hr in the absence of *UME6*, reaching maximum expression (≈65% of the wild type) after 20 hr in sporulation medium. A similar effect was observed for *SPO12*, another gene normally induced at 8–10 hr (data not shown). *DIT1* expression is even more severely affected: while its transcript is normally induced at ≈10 hr, reaching maximum at 12 hr, it accumulates to only 12% of the wild-type peak after 48 hr of *ume6* sporulation.

Cell-Type Control of *SPO13* Vegetative Repression Is Mediated Through *UME6*. Meiotic induction of *SPO13* occurs only in *MAT*a/α diploids starved for glucose and nitrogen (7, 21). However, loss of *UME6* causes *SPO13* derepression regardless of *MAT* genotype or carbon source. While our previous study indicated that glucose-grown logarithmic-phase *ume6-2* haploid cells expressed *SPO13* at levels comparable to those seen during wild-type sporulation (4), Fig. 4 shows that acetate-grown logarithmic-phase *ume6-D1* diploid cells derepress *SPO13* to only ≈25% of the wild-type maximum. Since

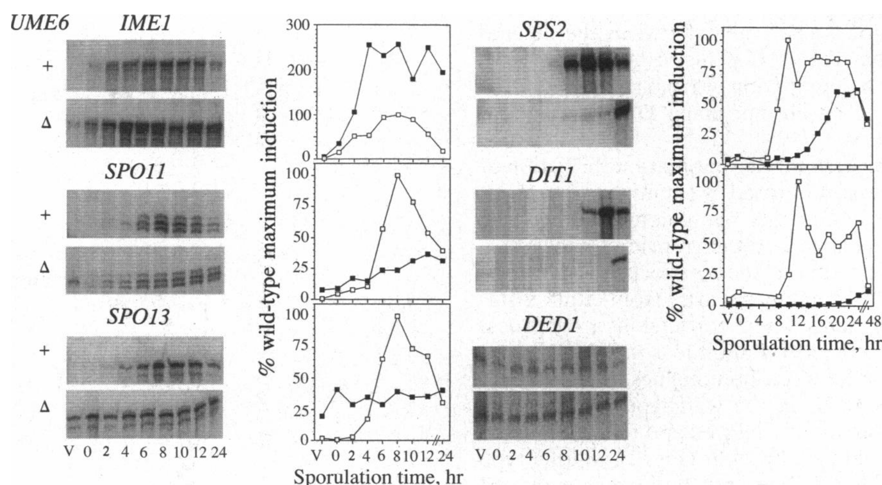

FIG. 4. Meiotic gene expression analyzed by quantitative S1 nuclease protection assays. RNA from wild-type (□) and *ume6-D1* (■) diploids was hybridized to both the control (*DED1*) and a meiosis-specific gene probe in each reaction. The *DED1* transcript from one assay is shown as an example. Reaction mixtures were loaded onto a polyacrylamide gel and electrophoresed for 2.5 hr at 400 V. The gels and corresponding quantitation, normalized to *DED1* expression, are shown for each assay. The *y*-axis = % wild-type maximum induction for each transcript, [(transcript/DED1 at time *x*)/(transcript/DED1 at time of maximum induction)] × 100. The *x*-axis gives hr of sporulation; v (vegetative) is a YPA sample at 5×10^6 cells per ml; 0 hr is a SPII sample taken immediately after transfer to SPII.

a or α cells were examined in the first case and **a**/α cells in the second, this raised the possibility that the a1-α2 repressor regulates *SPO13* through a *UME6*-independent pathway as well as the known dependent pathway. If so, then haploids containing a plasmid bearing the opposite mating type should exhibit reduced expression of *SPO13*. Thus, *SPO13* expression was quantitated in isogenic haploid strains containing different *MAT* genotypes grown in glucose medium (Fig. 5). As expected, *SPO13* expression is fully repressed in wild-type haploids but is derepressed in *ume6-D1* haploids. However, the level of *SPO13* expression in these strains appears to be largely independent of the *MAT* genotype and ploidy (compare yC105 to yC107, and to Cx37). Thus we conclude the a1-α2 repressor does not down-regulate *SPO13* independently of *UME6*, implying that vegetative repression of *SPO13* is primarily mediated by the *RME1/IME1/UME6* pathway. The difference between our current results and those previously reported remains to be determined, and it may be due to differences in strain background or the *ume6* allele used.

DISCUSSION

This study provides evidence that the *UME6* gene, isolated as a negative regulator of meiotic gene expression during vegetative growth, also plays an important role during sporulation in meiotic recombination, the induction of early, middle, and late meiotic gene expression, and the reestablishment of meiosis-specific gene repression. The findings in this paper support the hypothesis that *UME6* acts downstream of the *MAT/RME1/IME1* pathway to mediate the vegetative repression of *SPO13* and acts downstream of, or in conjunction with, *IME1* to mediate induction of meiotic gene expression.

In meiosis, strains deleted for *UME6* undergo premeiotic DNA synthesis but have greatly reduced recombination frequencies and become arrested as mononucleate cells. Several observations indicate that loss of *UME6* is deleterious to sporulating yeast: (*i*) the presence of cells with <1C DNA content, (*ii*) the accumulation of cells with more than four DAPI-stained foci late in meiosis presumably due to nuclear fragmentation, and (*iii*) a significant decrease in viability (to 25%) by 72 hr of sporulation. The similarity in the decline in viability ($\approx$75%) and recombination ($\approx$80%) suggests a connection between these events. As the loss in viability occurs well after induction of recombination, the viability decline is unlikely to be responsible for the reduced number of recombinants. More probable is that a defect prior to or during recombination causes death. This could be due to accumulation of deleterious recombination intermediates or eventual failure of a checkpoint arrest needed when DNA synthesis or recombination is incomplete. The timing of the *ume6* arrest, just prior to or during recombination, correlates well with the reduction and delay in meiosis-specific gene expression. For example, Fig. 4 demonstrates that the induction of *SPO11*, a gene needed for recombination (1, 2, 20), is also significantly delayed and reduced (Fig. 2). Similarly, the delayed induction of both middle (e.g., *SPO12*) and late (e.g., *DIT1*) meiotic genes required for nuclear division and spore formation (1, 2), respectively, correlates with the reduced efficiency and delayed kinetics of meiosis I, meiosis II, and ascus formation (Fig. 3). The initiation of DS in the absence of *UME6*, during reduced expression of several early meiotic genes (*IME2, SPO1, SPO11*, and *SPO13*), implies that wild-type levels of these transcripts are not essential for the transition into S phase. This agrees with other findings that *SPO1, SPO11*, and *SPO13* are dispensable for DS (refs. 1, 20, and 32; G. Tevzadze and R.E.E., unpublished results).

A number of observations support the view that both vegetative repression and meiotic activation signals are transmitted to URS1 directly through Ume6: (*i*) DNA-binding studies demonstrate that the Ume6 binds specifically to the

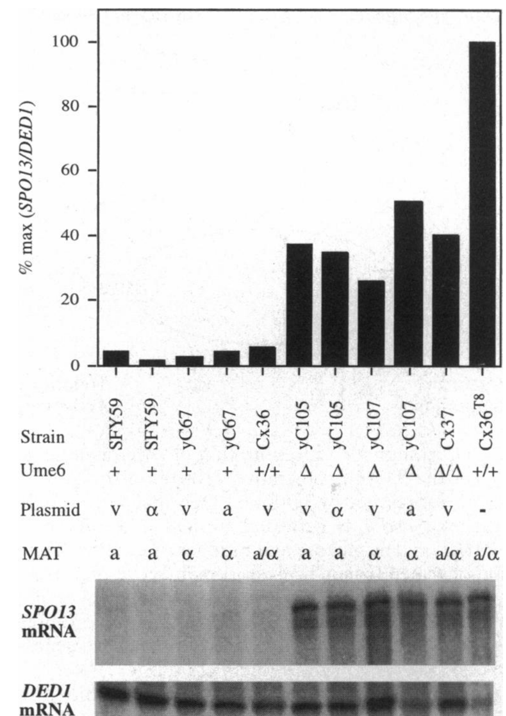

FIG. 5. *MAT* control of *SPO13* expression. RNA samples from either logarithmic-phase cells in synthetic dextrose medium without uracil or cells after 8 hr of sporulation ($Cx36^{T8}$) were hybridized to both *SPO13* and *DED1* probes. Indicated are the chromosomal genotype at *MAT* (**a**, α, or **a**/α), the plasmid genotype [YCp50 vector (v) or YCp50 containing *MAT***a** or *MAT*α], and the *UME6* genotype (*UME6*, +; *ume6-D1*, Δ).

URS1 elements of *SPO13* (4, 12) and *IME2* (12); (*ii*) the pattern of early meiotic gene expression in the absence of *UME6* (Fig. 4, *SPO11* and *SPO13*) resembles that of single base changes in URS1 (6–10); (*iii*) *rim16-12* is a recessive allele of *UME6* which has no defect in vegetative repression but does cause loss of meiotic activation of URS1-regulated genes (5); and (*iv*) a LexA202-Ume6 fusion protein activates transcription at the *lexA* operator in sporulation medium (C.M.S., unpublished results), and a Ume6-LexA87 fusion activates transcription when *IME1* is overexpressed on the *gal1* promoter (5). This last observation also supports the view that the Ime1 protein down-regulates the repression activity of Ume6 either by direct interaction with Ume6 or indirectly by induction of an activator that modifies or associates with Ume6 (Fig. 6). Since Ime1 does not bind DNA directly, but activates transcription when fused to a DNA-binding moiety (33, 34), it may activate early meiotic gene transcription by association with Ume6 bound to URS1.

The gradual rise in early meiotic gene expression during *ume6-D1* sporulation suggests the existence of at least one additional pathway for activating these genes that is partially functional in the absence of Ume6. Although the *IME1* meiotic inducer is overexpressed (3-fold) in *ume6-D1*, late meiotic gene expression is still reduced and delayed. Thus, expression of *IME1* alone is insufficient to induce late expression in the absence of *UME6*, suggesting that *UME6* is also required for the activation of these genes. The consequences of *UME6* loss for meiotic gene expression may thus be twofold: (*i*) failure to reach critical levels of proteins such as Ime2 (5, 6) and Spo1 (G. Tevzadze and R.E.E., unpublished results) needed for middle and late gene induction via interaction with their promoters, and (*ii*) failure of rerepression of *IME1* and of other early meiotic genes which may be inhibitory to meiotic progression and to induction of middle and late genes.

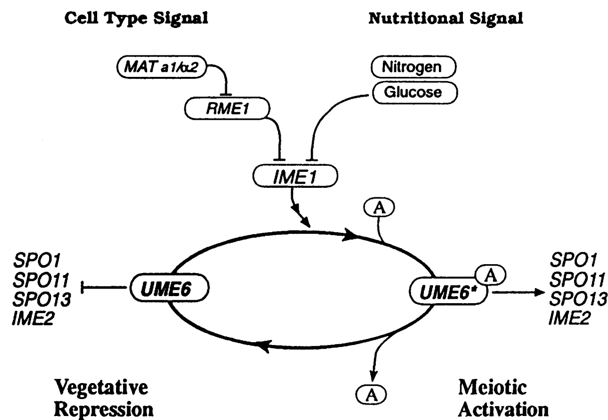

FIG. 6. Diagrammatic representation of early meiotic gene regulation by *UME6*. During vegetative growth *UME6* mediates early meiotic gene repression by both cell-type and nutritional controls. During meiosis, *IME1* is activated by loss of cell-type repression through *RME1*, and by glucose and nitrogen starvation. This results in conversion of Ume6 from a repressor to an activator (*), presumably through interaction with an activating protein (A) or complex of proteins allowing *UME6* to transmit both vegetative repression and meiotic activation signals to URS1-regulated meiotic genes.

The finding that *SPO13* derepression in the absence of *UME6* is independent of *MAT* and ploidy confirms the view (4) that cell type regulates vegetative repression through the *RME1/IME1/UME6* pathway. *UME6* is crucial for the vegetative repression of all URS1-regulated genes tested thus far. Its role in reestablishment of repression in meiosis includes these URS1-containing genes, as well as *IME1*, a non-URS1 gene. Deletion of two other genes, *IME2* (35) and *SIN3/UME4* (A. Helms, R. Strich, and R.E.E., unpublished results) each cause a similar loss of *IME1* rerepression without affecting its vegetative repression, as well as delayed induction of meiosis-specific genes and a severe reduction in ascus formation. We interpret the absence of vegetative derepression of *IME1* in *ume4* and *ume6* mutant strains with caution, as multiple pathways of negative control may obscure the involvement of these genes in such repression. Also, the failure to reestablish repression of meiotic genes in *ume6* mutant strains could be due indirectly to reduced induction of meiotic genes needed for rerepression of *IME1*, such as *IME2*.

Thus far, studies of the cis-acting elements regulating early meiotic gene expression have demonstrated that only URS1 functions in *both* vegetative repression and meiotic activation of these genes (6, 7, 10, 36, 37). We propose that the Ume6 protein is a central component of a URS1-mediated developmental regulatory switch since it directly binds to URS1 (4, 12) and, like URS1, functions in repression and activation of the same genes (this study; ref. 5). In recent years, a growing family of such dichotomous regulators (see ref. 36) functioning in both the repression and the activation of their target genes has been identified in diverse systems. The switch in their function may involve regulation of protein levels, as for *Drosophila* Krüppel, which becomes an activator upon dimerization (37, 38), or association with other proteins, as for YY1 (39), Max (40), and Rb (41).

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1. Esposito, R. E. & Klapholz, S. (1980) in *The Molecular Biology of the Yeast Saccharomyces cerevisiae: Life Cycle and Inheritance*, eds. Strathern, J. N., Jones, E. W. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 211–287.
2. Mitchell, A. P. (1994) *Microbiol. Rev.* **58,** 56–70.
3. Strich, R., Slater, M. R. & Esposito, R. E. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 10018–10022.
4. Strich, R., Surosky, R. T., Steber, C., Dubois, E., Messenguy, F. & Esposito, R. E. (1994) *Genes Dev.* **8,** 796–810.
5. Bowdish, K. S., Yuan, H. E. & Mitchell, A. P. (1995) *Mol. Cell. Biol.* **15,** 2955–2961.
6. Bowdish, K. S. & Mitchell, A. P. (1993) *Mol. Cell. Biol.* **13,** 2172–2181.
7. Buckingham, L. E., Wang, H.-T., Elder, R. T., McCarroll, R. M., Slater, M. R. & Esposito, R. E. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9406–9410.
8. Atcheson, C. L. (1991) Ph.D. thesis (Univ. of Chicago, Chicago).
9. Buckingham, L. E. (1992) Ph.D. thesis (Univ. of Chicago, Chicago).
10. Vershon, A. K., Hollingsworth, N. M. & Johnson, A. D. (1992) *Mol. Cell. Biol.* **12,** 3706–3714.
11. Szent-Gyorgyi, C. S. (1995) *Mol. Cell. Biol.* **15,** 6754–6769.
12. Anderson, S. F., Steber, C. M., Esposito, R. E. & Coleman, J. E. (1995) *Protein Sci.* **4,** 1832–1843.
13. Luche, R. M., Sumrada, R. A. & Cooper, T. G. (1990) *Mol. Cell. Biol.* **10,** 3884–3895.
14. Luche, R. M., Smart, W. C., Marion, T., Tillman, M., Sumrada, R. A. & Cooper, T. G. (1993) *Mol. Cell. Biol.* **13,** 5749–5761.
15. Prinz, S., Klein, F., Auer, H., Schweizer, D. & Primig, M. (1995) *Nucleic Acids Res.* **23,** 3449–3456.
16. Klapholz, S. & Esposito, R. E. (1982) *Genetics* **100,** 387–412.
17. Strich, R., Woontner, M. & Scott, J. F. (1986) *Yeast* **2,** 169–178.
18. Gietz, R. D. & Woods, R. A. (1994) in *Molecular Genetics of Yeast: A Practical Approach*, ed. Johnston, J. R. (Oxford Univ. Press, New York), pp. 121–134.
19. Fast, D. (1973) *J. Bacteriol.* **116,** 925–930.
20. Klapholz, S., Wadell, C. S. & Esposito, R. E. (1985) *Genetics* **110,** 187–216.
21. Wang, H. T., Frackman, S., Kowalisyn, J., Esposito, R. E. & Elder, R. (1987) *Mol. Cell. Biol.* **7,** 1425–1435.
22. Nash, R., Tokiwa, G., Anand, S., Erickson, K. & Futcher, A. B. (1988) *EMBO J.* **7,** 4335–4346.
23. Kassir, Y. & Simchen, G. (1991) in *Methods in Enzymology: Guide to Yeast Genetics and Molecular Biology*, eds. Guthrie, C. & Fink, G. R. (Academic, San Diego), pp. 94–109.
24. Elder, R. T., Loh, E. Y. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2432–2436.
25. Atcheson, C. L., DiDomencio, B., Frackman, S., Esposito, R. E. & Elder, R. T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8035–8039.
26. Surosky, R. T. & Esposito, R. E. (1992) *Mol. Cell. Biol.* **12,** 3948–3958.
27. Percival-Smith, A. & Segall, J. (1984) *Mol. Cell. Biol.* **4,** 142–150.
28. Briza, P., Breitenbach, M., Ellinger, A. & Segall, J. (1990) *Genes Dev.* **4,** 1775–1789.
29. Struhl, K. (1985) *Nucleic Acids Res.* **13,** 8587–8601.
30. Rose, D., Thomas, W. & Holm, C. (1990) *Cell* **60,** 1009–1017.
31. Honigberg, S. M., Conicella, C. & Esposito, R. E. (1992) *Genetics* **130,** 703–716.
32. Klapholz, S. & Esposito, R. E. (1980) *Genetics* **96,** 567–588.
33. Smith, H. E., Driscoll, S. E., Sia, R. A. L., Yuan, H. E. & Mitchell, A. P. (1993) *Genetics* **133,** 775–784.
34. Mandel, S., Robzyk, K. & Kassir, Y. (1994) *Dev. Genet.* **15,** 139–147.
35. Mitchell, A. P., Driscoll, S. E. & Smith, H. E. (1990) *Mol. Cell. Biol.* **10,** 2104–2110.
36. Roberts, S. G. E. & Green, M. R. (1995) *Nature (London)* **375,** 105–106.
37. Sauer, F. & Jäckle, H. (1993) *Nature (London)* **364,** 454–457.
38. Sauer, F., Fondell, J. D., Ohkuma, Y., Roeder, R. G. & Jäckle, H. (1995) *Nature (London)* **375,** 162–164.
39. Lee, J.-S., See, R. H., Galvin, K. M., Wang, J. & Shi, Y. (1995) *Nucleic Acids Res.* **23,** 925–931.
40. Ayer, D. E. & Eisenman, R. N. (1993) *Genes Dev.* **7,** 2110–2119.
41. Weintraub, S. J., Chow, K. N. B., Luo, R. X., Zhang, S. H., He, S. & Dean, D. C. (1995) *Nature (London)* **375,** 812–815.