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Positive Control of Yeast Meiotic Genes by the Negative Regulator UME6

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The yeast meiotic activator IME1 stimulates transcription of many early meiotic genes. These genes share a 5′ sequence called URS1. URS1 sites function as repression sites in cells that lack IME1; we show here that URS1 sites are weak activation sequences in cells that express IME1. Repression through URS1 sites is known to depend upon the URS1-binding protein UME6. We have identified a *UME6* allele (previously called *rim16-12*) that causes a defect in IME1-dependent activation of meiotic genes but not in repression through URS1 sites. In contrast, a *ume6* null mutation causes defects in both IME1-dependent activation and in repression through URS1 sites. A LexA-UME6 fusion protein is an IME1-dependent transcriptional activator, whereas a LexA-UME6 fusion carrying the *rim16-12* substitution cannot activate transcription. These findings argue that IME1 activates meiotic genes by converting UME6 from a negative regulator to a positive regulator; the *rim16-12* mutant protein is defective in conversion to a positive regulator.

The yeast UME6 protein and URS1 site are components of a sequence-specific repression system. URS1 sites are widely distributed in genetic regulatory regions (29), where they function as repression sites in growing, nonmeiotic cells (4, 10, 12, 17, 31). Repression of URS1-containing genes requires the *UME6* gene product (16, 27). UME6 is a zinc cluster protein that binds specifically to a URS1 site in vitro (27). A second protein, the RPA1/2/3 heterotrimer (RP-A), also binds to URS1 sites (11). Binding of RP-A is independent of UME6 (16), and binding of recombinant UME6 is independent of RP-A (27). The functional relationship among RP-A, URS1, and other URS1-binding proteins is presently unclear. However, genetic analysis indicates that UME6 is a repressor or part of a repression complex that acts through URS1 sites in nonmeiotic cells (16, 27).

Three observations made in nonmeiotic cells indicate that the URS1/UME6 system participates in repression of early meiotic genes. First, URS1-like sites are found upstream of almost all early meiotic genes (4; see reference 14 for a compilation). Second, mutations that remove or disrupt these URS1 sites cause increased expression in nonmeiotic cells (1, 4, 31). Third, *ume6* loss-of-function mutations cause increased early meiotic gene expression in nonmeiotic cells (1, 26, 27). Thus, repression by the URS1/UME6 system prevents inappropriate meiotic gene expression in nonmeiotic cells.

Entry into meiosis is accompanied by elevated expression of *IME1* (7); the *IME1* gene product is required for expression of almost all meiotic genes (see reference 14 for a review). Two observations indicate that the URS1/UME6 system may be required for IME1 to activate early meiotic genes. First, mutations in the URS1 sites near the meiotic genes *SPO13*, *HOP1*, and *IME2* cause reduced expression during meiosis (1, 4, 31). At *HOP1* and *IME2*, mutations in nearby positive sites (called the UAS_H [UAS, upstream activating sequence] and T_4C sites) also cause reduced expression during meiosis (1, 31). Second, UME6 is required for the IME1-dependent activation of *IME2* expression (1). Whether UME6 has a positive role in expression of other meiotic genes is unknown (27).

In this study, we provide evidence that URS1 is the IME1 response element and that UME6 protein is an IME1-dependent activator. Our analysis of an unusual *ume6* allele indicates that UME6 has separable positive and negative activities.

MATERIALS AND METHODS

Strains and media. *Saccharomyces cerevisiae* strains (Table 1) are derivatives of strain SK-1 (6). Mutations previously described include auxotrophic markers and *ime2-4-lacZ*::*LEU2*, P_{GAL1}-*IME1*::*TRP1*, *ime2-7-HIS3*::*LEU2* (25), *ume6*::*LEU2* (1), *rim16-12* (15), and *rim11*::*LEU2* (2). The *gal80*::*LEU2* mutation (30) present in several strains permits *GAL1* promoter activity in the absence of glucose, such as in galactose, acetate, and sporulation media. *rim16-12* and *ume6*::*LEU2* were generally followed in crosses through failure to complement *rim16-12* or *ume6*::*LEU2* tester strains for sporulation.

Yeast and bacterial media, including media used for YPD, YPAc, SD, SC, SGal, and galactose indicator plates, were prepared by standard recipes (20). Potassium acetate (KAc) plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) have been described previously (15).

***RIM16*/*UME6* plasmid complementation tests.** Our analysis of *UME6* plasmid function relies on tests for complementation of *rim16-12* and *ume6*::*LEU2* mutations. The *rim16-12* mutation prevents P_{GAL1}-*IME1* strains from expressing *IME2*: P_{GAL1}-*IME1 ime2-lacZ* strains are Lac^+, and P_{GAL1}-*IME1 ime2-lacZ rim16-12* strains are Lac^- (15). Similarly, P_{GAL1}-*IME1 ime2-HIS3* strains are His^+, and P_{GAL1}-*IME1 ime2-HIS3 rim16-12* strains are His^-. Complementation of the *rim16-12* defect by a plasmid is indicated by a Lac^+ phenotype of a P_{GAL1}-*IME1 ime2-lacZ rim16-12* transformant or by a His^+ phenotype of a P_{GAL1}-*IME1 ime2-HIS3 rim16-12* transformant. The *ume6*::*LEU2* mutation prevents repression of *IME2* that occurs in the absence of IME1: *ime1*Δ *ime2-HIS3* strains are His^-, and *ime1*Δ *ime2-HIS3 ume6*::*LEU2* strains are His^+ (1). Complementation of the *ume6*::*LEU2* defect by a plasmid is indicated by a His^- phenotype of an *ime1*Δ *ime2-HIS3 ume6*::*LEU2* transformant. $Lac^{+/-}$ phenotypes were scored on KAc–X-Gal plates; $His^{+/-}$ phenotypes were scored on synthetic galactose medium lacking histidine.

***CYC1-lacZ* plasmids.** DNA manipulations were carried out by standard methods. Oligonucleotides are listed in Table 2. Plasmids pLGΔ312SΔSS, pKB110K, and pKB112 were described previously (1, 5). Plasmid pKB143 was constructed by digesting pKB112 with *Xho*I and ligating to kinase-treated, annealed URS1-A and URS1-B oligonucleotides. Plasmids pKB148 and pKB150 were constructed by digesting plasmid pLGΔ312SΔSS with *Xho*I and ligation to kinase-treated, annealed URS1-A and URS1-B oligonucleotides. Plasmid pKB144 was constructed by digesting pKB112 with *Kpn*I and *Sal*I and ligation to kinase-treated, annealed T4C-A and T4C-B oligonucleotides. pKB160 was constructed by digesting pKB144 with *Xho*I and ligation to kinase-treated, annealed URS1-A and URS1-B oligonucleotides. Inserts in the *CYC1* promoter were sequenced by using the CYC1-P primer described previously (1).

Integrating *CYC1-lacZ* plasmids (used for URS1 repression assays) were con-

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TABLE 1. Yeast strains used in this study

Strain	Genotype[a]
AMP109	**a**/α *GAL80/GAL80*
AMP115	**a**/α *ime1Δ12::TRP1/ime1Δ12::TRP1 GAL80/GAL80*
AMP179	**a**/α
AMP258x1184	**a**/α *ime1Δ12::TRP1/ime1Δ12::TRP1 arg6/ARG6 his3ΔSK/HIS3 met4/MET4*
AMP722	**a** *ime1Δ12::TRP1 his1*
AMP1007	**a** P_{GAL1}*-IME1-14 his3ΔSK*
AMP1008	α P_{GAL1}*-IME1-14 arg6*
AMP1168	**a** *rim16-12* P_{GAL1}*-IME1-14 ime2-4-lacZ met4*
AMP1178	α *ime1Δ12::TRP1 his3ΔSK*
AMP1179	**a** *ime1Δ12::TRP1 his3ΔSK*
AMP1281	**a** P_{GAL1}*-IME1-14 arg6*
KB39	α *rim16-12 ime1Δ12::TRP1 his3ΔSK met13*
KB48	**a** *ume6::LEU2 his3ΔSK arg6 GAL80*
KB202	α P_{GAL1}*-IME1-14 ime2-7::HIS3 his3ΔSK*
KB426x569	**a**/α *rim16-12/rim16-12 arg6/ARG6 his3ΔSK/HIS3*
KB480	α *rim16-12* P_{GAL1}*-IME1-14 ime2-7::HIS3 his3ΔSK met4*
KB538	**a** *rim16-12*
KB539	α *ume6::LEU2 ime1Δ12::TRP1 ime2-7::HIS3 his3ΔSK met4 GAL80*
KB543	**a** *ume6::LEU2 his3ΔSK arg6 met4*
KB557	**a**/α *rim11::LEU2/rim11::LEU2 met4/MET4 GAL80/GAL80*
KB572x129-2A	**a**/α *ume6::LEU2/ume6::LEU2 arg6/ARG6 met4/MET4*
HEY33-4B	α *rim16-12 his3ΔSK arg6*
HEY33-7A	α *rim16-12* P_{GAL1}*-IME1-14 ime2-7::HIS3 his3ΔSK met4*
HEY32-1A	**a** *rim16-12* P_{GAL1}*-IME1-14 his3ΔSK arg6*
1415-1A	α *arg6*
1415-1C	α *RIM16::URA3 arg6*
1416-5A	α *RIM16::URA3 arg6*
1421-1C	α *ume6::LEU2 arg6*

[a] All strains carry additional markers *ura3 leu2::hisG trp1::hisG lys2 ho::LYS2 gal80::LEU2* except as noted. Diploid strains are homozygous for these markers.

structed by deletion of the *Hin*dIII fragment containing 2μm sequences from plasmids pKB112 and pKB143. Resulting plasmids were cleaved within the *URA3* gene with *Stu*I and transformed into strain AMP1281. Transformants were colony purified, analyzed by Southern blotting, and mated to strains 1415-1A, HEY33-4B, 1421-1C, and AMP1178. Meiotic progeny of these crosses were assayed for β-galactosidase activity.

Isolation and manipulation of *UME6* (*RIM16*) and *rim16-12*. YCP50-based genomic libraries (19) were transformed into strain HEY33-7A (α *rim16-12 ime2-HIS3*). Approximately 6,000 Ura^+ colonies were patched onto SC-Ura plates and replica plated to SGal-His to identify His^+ colonies. Plasmid dependence of the His^+ phenotype was determined among 5-fluoro-orotic acid-induced plasmid cures. Plasmid-dependent His^+ colonies were tested for the ability to complement the *rim16-12* sporulation defect by mating to HEY32-1A (**a** *rim16-12*). Plasmid pB3-3II contained a 10-kb fragment that was determined to complement the *rim16-12* mutation.

The *rim16-12* allele was retrieved onto plasmid pHY14-2 by gap repair (21).

TABLE 2. Oligonucleotides used in this study

Name	Sequence
URS1-A	5′-TCGAGGTTACGGCGGCTATTTC-3′
URS1-B	5′-TCGAGAAATAGCCGCCGTAACC-3′
URS1-C	5′-TCGAGGGTAGCCGCCGAGGGC-3′
URS1-D	5′-TCGAGCCCTCGGCGGCTACCC-3′
T4C-A	5′-CTCCTTTTCTCCGGTTG-3′
T4C-B	5′-TCGACAACCGGAGAAAAGGAGGTAC-3′
RIM16-A	5′-CTAGGACACTACCGC-3′
RIM16-B	5′-GCCTGTGCGACATGG-3′
UME6-N6	5′-GCAATGAAAAAAAAAGGCGGCCGCTAAAAGCTCACTGAA-3′
lexA-N1	5′-TAAGAATGGCGGCCGCAAAGCGTTAACGGCCAGG-3′
lexA-N2	5′-TAAGAATGCGGCCGCCTGGTTCACCGGCAGCCAC-3′

Plasmid pHY14-2 was constructed by inserting a *Spe*I-*Sal*I fragment from pB3-3II into plasmid pRS316 (22). This fragment includes the entire *UME6* coding region (27) and extends into YCp50 sequences. We digested pHY14-2 with *Bam*HI and *Cla*I and transformed linearized DNA into *rim16-12* strain AMP1168. The resulting plasmid is pHY16-2. The *rim16-12* mutation was mapped to a 2.0-kb *Bam*HI-to-*Spe*I fragment by exchange of this fragment with wild-type plasmid pHY14-2. The mutation was then identified by sequencing mutant plasmids with oligonucleotides RIM16-A and RIM16-B (Table 2).

To construct pKB189 (*lexA-UME6*), pHY14-2 was first subjected to site-directed mutagenesis using oligonucleotide UME6-N6 (Table 2) to generate a *Not*I site, forming pKB178. A high-copy-number plasmid was generated from pKB178 by subcloning the *Spe*I-to-*Sal*I fragment carrying *UME6* into the high-copy-number plasmid pKB174, generating pKB183. pKB174 was made by digesting pRS426 with *Bss*HII and religation to create a plasmid in which the polylinker is in the reverse orientation, such that toxicity of *UME6* inserts to *Escherichia coli* would be relieved. pKB183 was digested with *Not*I and ligated to a segment encoding the LexA DNA-binding domain derived from pSH2-1 (13) by PCR amplification using primers lexA-N1 and lexA-N2. pKB193 (*lexA-rim16-12*) was constructed by swapping the *Spe*I-*Bam*HI fragment from pHY16-2 with the *Spe*I-*Bam*HI fragment on pKB189 to generate pKB193.

Linkage of the pB3-3II insert to the *RIM16* and *UME6* loci. Integrating plasmid pHY15-3 (Fig. 1) was constructed by inserting a 2-kb *Cla*I fragment from pB3-3II into plasmid YIP5 (18). pHY15-3 was linearized with *Xho*I and transformed into strain AMP1008 (*UME6*); integration was confirmed by Southern analysis. Four transformants were crossed to strain AMP1168 (*rim16-12*); all 64 meiotic tetrads from these crosses displayed 2 Ura^+ Rim^+:2 Ura^- Rim^- segregation. Two Ura^+ Rim^+ meiotic segregants from these crosses, 1415-1C and 1416-5A, were mated to KB48 (*ume6::LEU2*); all 32 meiotic tetrads from these crosses displayed 2 Ura^+ Leu^-:2 Ura^- Leu^+ segregation.

β-Galactosidase assays. β-Galactosidase assays were performed on permeabilized cells as described previously (25). Activities are the averages of at least three independent transformants. For assays of high-copy-number *CYC1-lacZ* reporter plasmids, cells from an SC-Ura overnight culture were inoculated into either YPD or YPAc. Vegetative cells were harvested after at least two generations at a density of 10^7 cells per ml. Sporulating cells were harvested after transfer of YPAc cultures to sporulation medium (2% KAc plus required amino acids at 20 mg/liter) for 4 h. For assays of LexA fusion protein activity, strains AMP1007 and AMP1179 were transformed with pKB189 (*lexA-UME6*) or pKB193 (*lexA-rim16-12*). Strain AMP1178 was transformed with reporter plasmid pHS178, pHS179, or pHS180, containing zero, one, or six operators, respectively, upstream of a *gal1-lacZ* fusion (23). Saturated cultures in SC-His-Ura containing 0.5% glucose were filtered and resuspended in sporulation medium for 4 h.

Assays of integrated *CYC1-lacZ* reporter plasmids were conducted on exponential-phase YPD cultures. Values are the averages of four meiotic segregants for each genotype.

RNA analysis. Procedures for cell growth, preparation of RNA, and Northern (RNA) filters have been described previously (24). Probes for meiotic genes (2) and control probe pC4/2 (9, 28) have been described elsewhere.

RESULTS

Allelism of *RIM16* and *UME6*. We previously identified a recessive mutation, *rim16-12*, that impairs IME1-dependent UAS activation and sporulation (1, 15). We isolated a genomic DNA segment, in plasmid pB3-3II, that complemented *rim16-12* defects in *IME2* expression and sporulation (Fig. 1). To confirm that the complementing DNA segment contained the *RIM16* gene, we showed that the subclone carried in plasmid pHY15-3 could direct plasmid integration to the *RIM16* locus (see Materials and Methods). Three observations indicated that the *UME6* gene was also carried on plasmid pB3-3II. First, the pB3-3II restriction map closely resembled that of the *UME6* locus (27). Second, plasmid pB3-3II complemented a *ume6::LEU2* mutation to permit repression of an *ime2-HIS3* gene (Fig. 1). Third, the site of subclone pHY15-3 integration was tightly linked to the *UME6* locus (see Materials and Methods). These results indicate that the *rim16-12* mutation is tightly linked to the *UME6* locus and that plasmid pB3-3II includes both *RIM16* and *UME6*.

A complementation test indicated that *rim16-12* is a *UME6* allele. The *rim16-12* and *ume6::LEU2* mutations both cause recessive sporulation defects (15, 27). We observed that several *rim16-12/ume6::LEU2* diploids sporulated poorly (<14%), whereas *rim16-12*/+ and *ume6::LEU2*/+ diploids sporulated

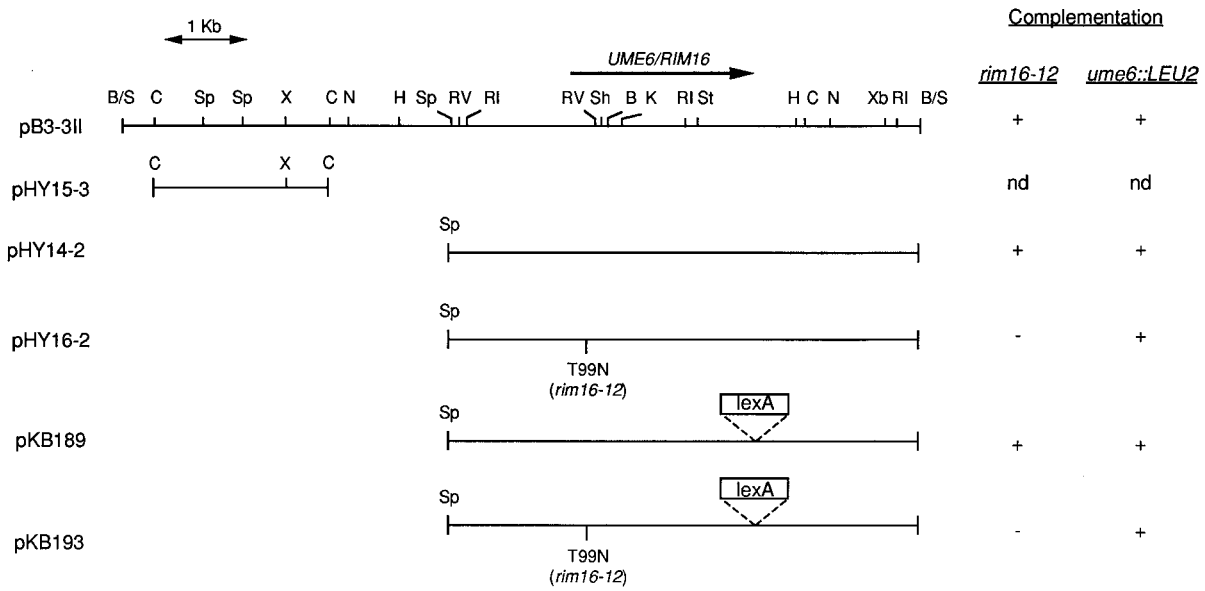


FIG. 1. Maps of *UME6*/*RIM16* plasmids. The arrow denotes the *UME6* coding region (27). Complementation of *ume6*::*LEU2* and *rim16-12* mutants (KB539 and KB480), as described in Materials and Methods, is indicated in the right-hand columns (nd, not determined). Plasmid pB3-3II is the original *UME6* clone carried in plasmid YCp50. Plasmid pHY15-3 is a YIp5 derivative. Plasmid pHY14-2 contains a *Spe*I-*Sal*I fragment in vector pRS316. Plasmid pHY16-2, derived from pHY14-2, contains the *rim16-12* allele retrieved by gap repair. Plasmid pKB189 encodes wild-type UME6 fused to the LexA DNA-binding domain at the last *UME6* codon. Plasmid pKB193 is identical to pKB189 but includes the T99N (*rim16-12*) missense substitution. Restriction site abbreviations: B, *Bam*HI; S, *Sau*3A; C, *Cla*I; Sp, *Spe*I; X, *Xho*I; N, *Nco*I; RV, *Eco*RV; Sh, *Sph*I; K, *Kpn*I; RI, *Eco*RI; St, *Stu*I; H, *Hin*dIII; Xb, *Xba*I.

efficiently (>95%). Therefore, *rim16-12* and *ume6*::*LEU2* fail to complement for sporulation ability.

Sequence determination confirmed that the *rim16-12* mutation lies in *UME6*. We used gap rescue to retrieve the *UME6* gene from the chromosome of a *rim16-12* mutant. The wild-type plasmid, pHY14-2, complemented both *rim16-12* and *ume6*::*LEU2* mutants (Fig. 1). The gap-repaired plasmid, pHY16-2, failed to complement a *rim16-12* mutant but could complement a *ume6*::*LEU2* mutant (Fig. 1). This result indicated the presence of *rim16-12* on the plasmid. The *rim16-12* mutation mapped to a 2-kbp *Bam*HI-*Spe*I fragment. The sequence of this fragment revealed a single nucleotide change from the wild-type *UME6* sequence (27), resulting in a substitution of threonine at *UME6* codon 99 with asparagine. Therefore, *rim16-12* is a missense mutation in *UME6*.

Comparison of *rim16* and *ume6* mutant phenotypes. UME6 is required for repression through the *CAR1* URS1 site (16). To determine whether *rim16-12* causes a repression defect, we compared expression of integrated *CYC1-lacZ* fusion genes with or without a URS1 site in wild-type, *rim16-12*, and *ume6*::*LEU2* strains (Table 3). These experiments used the *IME2* URS1 site inserted between the *CYC1* UAS and TATA regions. In the wild-type and *rim16-12* strains, the URS1 site caused fourfold repression. In the *ume6*::*LEU2* strain, the URS1 site caused no repression. We conclude that the *rim16-12* mutation does not cause a defect in repression through this URS1 site.

UME6 is also required for IME1-dependent activation of the early meiotic *IME2* promoter (1). We examined effects of *rim16-12* and *ume6*::*LEU2* mutations on expression of several early meiotic genes through Northern blot analysis (Fig. 2).

TABLE 3. Repression through URS1 in *ume6* mutants

Plasmid[a]	URS1 site	*CYC1-lacZ* expression[b]		
		UME6	*rim16-12*	*ume6*::*LEU2*
pKB112ΔH	−	390	420	310
pKB143ΔH	+	90	90	380

[a] *CYC1-lacZ* plasmids were integrated at the *URA3* locus in strain AMP1281, and haploid segregants carrying an *ime1Δ12* mutation and the *UME6* allele indicated were constructed through crosses.

[b] Cells were grown to mid-exponential phase in YPD. Numbers are the mean of determinations with at least four segregants of each genotype; standard errors were less than 20% of the mean.

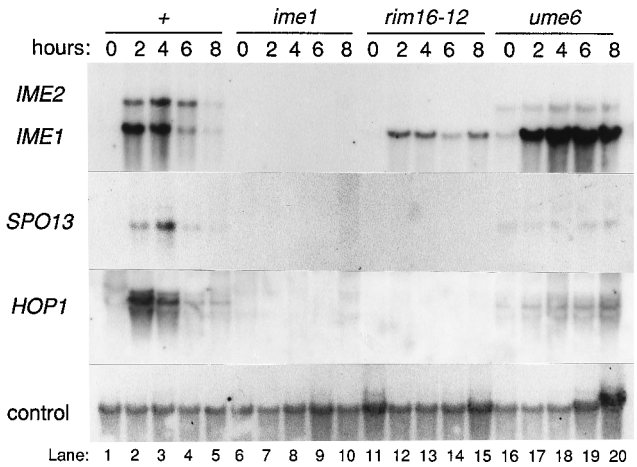
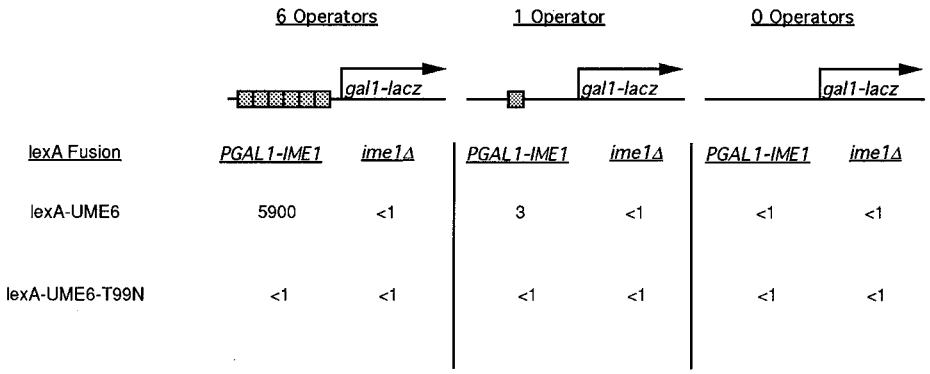


FIG. 2. Meiotic RNA accumulation in *ume6* mutants. Cultures were grown to mid-exponential phase in YPAc and then transferred to sporulation medium. RNA was prepared from YPAc-grown cells (0 h) and after 2, 4, 6, or 8 h in sporulation medium. Northern filters were probed for the indicated meiotic transcripts and with the control probe pC4/2. Strains: AMP1179 (**a**/α; lanes 1 to 5); AMP258x1184 (**a**/α *ime1Δ*/*ime1Δ*; lanes 6 to 10); KB426x569 (**a**/α *rim16-12*/*rim16-12*; lanes 11 to 15); KB572x129-2A (**a**/α *ume6*::*LEU2*/*ume6*::*LEU2*; lanes 16 to 20).



lexA Fusion	PGAL1-IME1	ime1Δ	PGAL1-IME1	ime1Δ	PGAL1-IME1	ime1Δ
lexA-UME6	5900	<1	3	<1	<1	<1
lexA-UME6-T99N	<1	<1	<1	<1	<1	<1

FIG. 3. Transcriptional activation by LexA-UME6. Diploids from either AMP1007 and AMP1178 (P_{GAL1}-*IME1*/*ime1*Δ) or AMP1179 and AMP1178 (*ime1*Δ/*ime1*Δ) carrying one *lexA*-derived plasmid and one reporter plasmid were assayed for β-galactosidase activity after incubation in sporulation medium for 4 h. Numbers are the means of three determinations; standard errors were less than 20% of the mean.

Wild-type and *ime1*Δ/*ime1*Δ diploids served as positive and negative controls, respectively. We examined expression of the early meiotic genes *IME2*, *HOP1*, and *SPO13*, each of which contains a 5′ URS1 site required for high-level, meiotic expression (1, 4, 31). In the wild type, transcript levels of these genes were very low in growing cells (0 h) and increased after incubation for 4 h in sporulation medium. The *ime1*Δ mutation abolished meiotic transcript accumulation. The *rim16-12* mutation also caused a severe reduction in transcript accumulation in sporulation medium. The *ume6*::*LEU2* mutation caused a slight increase in transcript levels in growing cells, but there was little further increase in transcript levels during incubation in sporulation medium. These results indicate that both *rim16-12* and *ume6*::*LEU2* mutations reduce expression of *IME2*, *HOP1*, and *SPO13* in meiotic cells.

We also observed effects of *rim16-12* and *ume6*::*LEU2* mutations on *IME1* transcript accumulation (Fig. 2). In the wild type, *IME1* transcript levels increased until 4 h in sporulation medium and then declined. The *rim16-12* mutation caused a slight reduction in *IME1* RNA accumulation. The *ume6*::*LEU2* mutation caused elevated *IME1* RNA levels. These results indicate that UME6 is formally a negative regulator of *IME1* expression.

IME1-dependent activation by LexA-UME6. Two general models have been proposed for the role of UME6 in early meiotic gene expression. According to one model, UME6 is modified by IME1 to convert it from a negative regulator to a positive regulator (1). A second possibility is that UME6 is strictly a repressor and competes for binding with an activator (27). These models account for the *rim16-12* defect in different ways. The first model argues that the *rim16-12* mutant product has a specific defect in transcriptional activation: it may be insensitive to modification by IME1 or have a defect in a hypothetical UME6 activation domain. The second model argues that the *rim16-12* mutant product is a superrepressor: it may bind to URS1 sites too tightly, or repress too severely, to permit effective competition by a hypothetical positive regulator. These models may be distinguished by examining the transcriptional activation properties of UME6 derivatives with altered DNA binding specificities. The first model predicts that binding of UME6 to a novel site will enable the novel site to be an IME1-dependent UAS; binding of the *rim16-12* mutant product to the novel site will not confer UAS activity. The second model predicts that binding of UME6 or the *rim16-12* mutant product will not confer UAS activity.

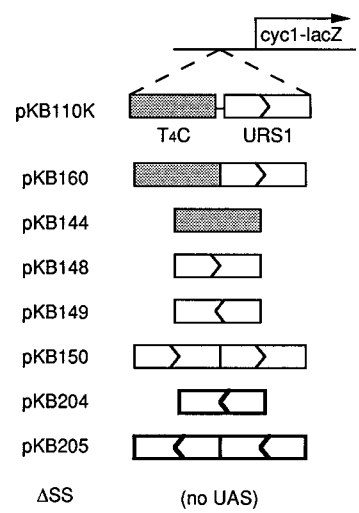
To enable UME6 to bind to a heterologous sequence, we constructed a LexA-UME6 fusion protein. We verified that LexA-UME6 could complement both *ume6*::*LEU2* and *rim16-12* defects (Fig. 1). Promoter activation by LexA-UME6 was examined with *gal1-lacZ* reporter genes containing zero, one, or six *lexA* operators. The reporter gene with no *lexA* operators was expressed at the same low level in the presence and absence of IME1 (P_{GAL1}-*IME1* and *ime1*Δ strains, respectively [Fig. 3]). The reporter gene with one *lexA* operator was expressed at 10-fold-higher levels in the presence of IME1. The reporter gene with six *lexA* operators was expressed at 6,000-fold-higher levels in the presence of IME1. These results indicate that LexA-UME6 can function as an IME1-dependent activator.

We also examined activation by a LexA-UME6-T99N mutant protein, which has the *rim16-12* substitution. We confirmed that LexA-UME6-T99N complemented *ume6*::*LEU2* but not *rim16-12* defects (Fig. 1). We observed that *gal1-lacZ* reporter genes with zero, one, or six *lexA* operators were expressed at the same low level in the presence or absence of IME1 (Fig. 3). These results indicate that the *rim16-12* defect prevents UME6 from becoming an IME1-dependent activator.

Analysis of the *IME2* UAS. The hypothesis that UME6 is an IME1-dependent transcriptional activator predicts that the UME6-binding site, the URS1 site, should be an IME1-dependent UAS. We tested this prediction through comparison of strains that lack IME1 (*ime1*Δ strain; Fig. 4) and express IME1 (P_{GAL1}-*IME1* strain). Cells were grown in glucose medium, in which P_{GAL1}-*IME1* is not expressed, and in acetate and sporulation media, in which P_{GAL1}-*IME1* is expressed.

An IME1-dependent UAS from the *IME2* gene consists of two functional elements: a T_4C site and a URS1 site (1). The T_4C site had low-level UAS activity in both P_{GAL1}-*IME1* and *ime1*Δ strains (compare pKB144 with ΔSS in Fig. 4). Because the T_4C site has less activity in glucose medium than in acetate medium in both strains, it may be a glucose-repressible UAS. The URS1 site had low-level UAS activity in the P_{GAL1}-*IME1* strain and no UAS activity in the *ime1*Δ strain (pKB148 and pKB149). Two URS1 sites together had greater UAS activity than a single site (pKB150). UAS activity of single or double URS1 sites was greater in sporulation medium than in acetate growth medium, as observed for the intact *IME2* UAS. Functional activity of the T_4C and URS1 site oligonucleotides was established by reconstitution of an active, IME1-dependent UAS (compare pKB160 with pKB110K). We conclude that the *IME2* URS1 site transmits an IME1-dependent activation signal. The *IME2* T_4C site augments UAS activity of the URS1 site but does not respond to IME1 on its own.

The URS1 site and its properties were first established



	Glucose		Acetate		Spo	
	ime1Δ	*PGAL1-IME1*	*ime1Δ*	*PGAL1-IME1*	*ime1Δ*	*PGAL1-IME1*
pKB110K	<0.6	0.7	<0.6	150	<0.6	530
pKB160	<0.6	<0.6	<0.6	260	<0.6	760
pKB144	<0.6	<0.6	20	18	12	11
pKB148	<0.6	<0.6	<0.6	1.4	<0.6	6.1
pKB149	<0.6	<0.6	<0.6	2.2	<0.6	7.0
pKB150	<0.6	<0.6	<0.6	9.3	<0.6	50
pKB204	<0.6	<0.6	<0.6	1.3	<0.6	9.4
pKB205	<0.6	<0.6	<0.6	2.6	<0.6	130
ΔSS	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6

FIG. 4. UAS activity of individual URS1 and T_4C sites. Strains KB202 (P_{GAL1}-*IME1 ime2*) and AMP722 (*ime1*Δ) were transformed with the indicated high-copy-number *CYC1-lacZ* reporter plasmids. Plasmids pKB110K and pKB148-160 contain the *IME2* URS1 site; plasmids pKB204 and pKB205 contain the *CAR1* URS1 site. β-Galactosidase assays were conducted on log-phase YPD (glucose) or YPAc (acetate) cultures or after 4 h in sporulation medium (Spo). Numbers are the means for at least three independent transformants; standard errors were less than 30% of the mean.

through studies of the *CAR1* gene (12, 29). The *CAR1* and *IME2* URS1 sites share nine nucleotides (TAGCCGCCG) but differ at flanking nucleotides on both sides. We sought to determine whether the *CAR1* URS1 site also had IME1-dependent UAS activity. We constructed reporter plasmids containing one and two copies of the *CAR1* URS1 site (pKB204 and pKB205, respectively) and examined their expression in P_{GAL1}-*IME1* and *ime1*Δ strains (Fig. 4). These reporters were expressed only in the P_{GAL1}-*IME1* strain; UAS activity was comparable to that of the *IME2* URS1 site. Therefore, the *CAR1* URS1 site can transmit an IME1-dependent activation signal.

DISCUSSION

This report provides evidence that UME6 is converted by IME1 from a negative regulator to a positive regulator and that this conversion is vital for activation of several early meiotic genes. Our arguments are based on the structure of the *IME2* UAS and functional properties of UME6 and LexA-UME6. However, our evidence does not rule out the possibility that IME1 has other roles in meiosis in addition to modifying UME6 activity.

Structure of the *IME2* UAS. Mutational studies first indicated that both T_4C and URS1 sites have positive roles in activation of the *IME2* UAS (1). The T_4C site, in isolation, is a weak IME1-independent UAS that responds to the carbon source. The URS1 site, in isolation, is a weak IME1-dependent UAS. The canonical URS1 site, from the *CAR1* gene (29), also behaves as a weak IME1-dependent UAS. Absence of IME1 from mitotic cells and from haploid **a** and α cells can explain why previous studies have detected only repression activity of isolated URS1 sites (10, 12, 16, 17, 29). Indeed, the *IME2* URS1 site is a negative site in the absence of IME1. These observations support a simple model in which the URS1 site responds to IME1 activity. The T_4C site is not required for URS1 to respond to IME1; rather, the T_4C site amplifies the IME1-dependent activation signal. In addition, the T_4C site may be responsible for glucose repression of *IME2*, which is independent of IME1 expression (8).

Activation through URS1 sites and through the *IME2* UAS is greater in starved cells (incubated in sporulation medium) than in growing cells, despite constant expression of the P_{GAL1}-*IME1* gene. Similarly, levels of several meiotic transcripts increase after transfer to sporulation medium in P_{GAL1}-*IME1* strains (25). This starvation response may reflect starvation responsiveness of IME1 protein itself, because a LexA-IME1 fusion protein is a more potent activator in starved cells than in growing cells (23).

The proposal that URS1 is an IME1 response element accounts well for the presence of URS1 sites in the regulatory regions of almost all known early meiotic genes (4; reviewed in reference 14). Where examined, these URS1 sites have been found to have positive roles in meiotic cells (1, 4, 31). The puzzling observation is that URS1 sites also exist in regulatory regions of genes that are not known to be expressed at elevated levels during meiosis (29). We have observed that two URS1-containing genes, *CAR1* and *INO1*, are expressed at elevated levels in sporulation medium, but elevated expression is independent of IME1 (3). One explanation for the difference in URS1 activity is that our studies of activation through URS1 sites have used strains that overexpress IME1. Lower levels of IME1 in wild-type strains may have less pronounced activity. In addition, we note that URS1 sites are often accompanied by either T_4C sites or UAS_H sites at meiotic genes (1, 31). The ability of such sites to augment IME1-dependent UAS activity may permit preferential activation of functionally important meiotic genes by IME1.

Dual roles of UME6. Two models can explain the finding that URS1 sites can have alternate activities. One is that a single URS1-binding protein is responsible for both activities. A second is that two different proteins with alternate activities compete for binding at URS1 sites. Our findings clearly support the first model.

The idea that binding of UME6 to URS1 sites anchors a repression complex has been well established. That UME6 binds to URS1 sites is indicated by two observations: a null *ume6* mutation alters the spectrum of URS1-protein complexes detectable by gel shift assays (16, 27), and the UME6 C-terminal region forms a specific complex with DNA containing a URS1 site (27). That UME6 binding causes repression is

indicated by the finding that *ume6* mutants are defective in repression through URS1 sites in nonmeiotic cells (16, 27). Although the heterotrimer RP-A also binds to URS1 sites (11), no functional role for the RP-A–URS1 complex has been established.

The idea that UME6 might adopt a positive role in the presence of IME1 stems from the observation that activation of the *IME2* UAS by IME1 is defective in *ume6* loss-of-function mutants (1). The behavior of the *IME2* UAS might have appeared exceptional in light of the report that a *ume6* null mutant expresses several early meiotic genes at elevated levels in cells lacking IME1 (27). We also found that *IME2*, *HOP1*, and *SPO13* RNA levels were elevated during vegetative growth of a *ume6* mutant. This result confirms that repression through URS1 sites is defective in the *ume6* mutant. However, in the wild-type strain, there is a substantial increase in levels of these meiotic transcripts in sporulation medium. In the *ume6* mutant, there was little increase in transcript levels in sporulation medium. These observations clearly argue that UME6 is required for activation of several early meiotic genes.

Studies of LexA-UME6 provide the most compelling evidence that UME6 becomes a transcriptional activator in the presence of IME1. We observed that LexA-UME6 activated gene expression, when bound to upstream *lexA* operators, only in the presence of IME1. The finding that a LexA fusion to the *rim16-12* mutant product does not activate gene expression, despite the presence of IME1, provides a key parallel between the *rim16-12* defects in meiotic gene expression and in the artificial *lexA*-reporter system.

Role of UME6 in *IME1* expression. UME6 is apparently a negative regulator of *IME1* expression: the *ume6*::*LEU2* mutation caused increased *IME1* RNA levels in both vegetative and sporulating cells, and the *rim16-12* mutation reduced *IME1* RNA levels in sporulating cells. (Reduced *IME1* expression is not sufficient to explain the *rim16-12* sporulation defect because P_{GAL1}-*IME1 rim16-12* strains are defective in sporulation and *IME2* expression [1, 15].) We infer that the effect of these *ume6* mutations on *IME1* RNA levels is indirect because *IME1* has no obvious URS1 site. One simple explanation for this phenotype is that UME6 is a negative regulator of one of the many positive regulators of *IME1* expression (see reference 14). In that context, we note that *RIM11* has a URS1 site and functions, in part, as a positive regulator of *IME1* RNA accumulation (2).

Nature of the interaction between UME6 and IME1. We have proposed that IME1 converts UME6 from a repressor to an activator. The observation that LexA-IME1 fusion proteins are transcriptional activators, and that the IME1 activation domain is required for function (23), suggests a simple mechanism for this conversion: that IME1 binds to a UME6 repression complex at URS1 sites and exposes the IME1 transcriptional activation domain. A second possibility is based on the finding that IME1 is detectable in RIM11 immune complexes (2): IME1 may permit RIM11 to phosphorylate UME6; phosphorylation would convert UME6 to an activator. Our biochemical experiments have thus far failed to reveal either type of interaction in solution. We note that the presence of T_4C or UAS_H sites improves the IME1 responsiveness of URS1 sites 10- to 100-fold. Proteins that act at T_4C and UAS_H sites, which are presently unidentified, may be required to stabilize a hypothetical IME1-UME6 complex.

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REFERENCES

1. **Bowdish, K. S., and A. P. Mitchell.** 1993. Bipartite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**2172–2181.
2. **Bowdish, K. S., H. E. Yuan, and A. P. Mitchell.** 1994. Analysis of RIM11, a yeast protein kinase that phosphorylates the meiotic activator IME1. Mol. Cell. Biol. **14:**7909–7919.
3. **Bowdish, K. S., H. E. Yuan, and A. P. Mitchell.** Unpublished results.
4. **Buckingham, L. E., H. T. Wang, R. T. Elder, R. M. McCarroll and R. E. Esposito.** 1990. Nucleotide sequence and promoter analysis of *SPO13*, a meiosis-specific gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:**9406–9410.
5. **Guarente, L., and T. Mason.** 1983. Heme regulates transcription of the *CYC1* gene of *Saccharomyces cerevisiae* via an upstream activation site. Cell **32:**1279–1286.
6. **Kane, S., and R. Roth.** 1974. Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118:**8–14.
7. **Kassir, Y., D. Granot, and G. Simchen.** 1988. *IME1*, a positive regulator gene of meiosis in *S. cerevisiae*. Cell **52:**853–862.
8. **Kawaguchi, H., M. Yoshida, and I. Yamashita.** 1992. Nutritional regulation of meiosis-specific gene expression in *Saccharomyces cerevisiae*. Biosci. Biotechnol. Biochem. **56:**289–297.
9. **Law, D. T., and J. Segall.** 1988. The *SPS100* gene of *Saccharomyces cerevisiae* is activated late in the sporulation process and contributes to spore wall maturation. Mol. Cell. Biol. **8:**912–922.
10. **Lopes, J. M., K. L. Schulze, J. W. Yates, J. P. Hirsch, and S. A. Henry.** 1993. The *INO1* promoter of *Saccharomyces cerevisiae* includes an upstream repressor sequence (URS1) common to a diverse set of yeast genes. J. Bacteriol. **175:**4235–4238.
11. **Luche, R. M., W. C. Smart, T. Marion, M. Tillman, R. A. Sumrada, and T. G. Cooper.** 1993. *Saccharomyces cerevisiae* BUF protein binds to sequences participating in DNA replication in addition to those mediating transcriptional repression (URS1) and activation. Mol. Cell. Biol. **13:**5749–5761.
12. **Luche, R. M., R. Sumrada, and T. G. Cooper.** 1990. A *cis*-acting element present in multiple genes serves as a protein binding site for the yeast *CAR1* gene. Mol. Cell. Biol. **10:**3884–3895.
13. **Magee, P. T.** 1987. Transcription during meiosis, p. 355–382. *In* P. Moens (ed.), Meiosis. Academic Press, Orlando, Fla.
14. **Mitchell, A. P.** 1994. Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. **58:**56–70.
15. **Mitchell, A. P., and K. S. Bowdish.** 1992. Selection for early meiotic mutants in yeast. Genetics **131:**65–72.
16. **Park, H. D., R. M. Luche, and T. G. Cooper.** 1992. The yeast *UME6* gene product is required for transcriptional repression mediated by the *CAR1* URS1 repressor binding site. Nucleic Acids Res. **20:**1909–1915.
17. **Park, H. O., and E. A. Craig.** 1991. Transcriptional regulation of a yeast HSP70 gene by heat shock factor and an upstream repression site-binding factor. Genes Dev. **5:**1299–1308.
18. **Rose, M. D., and J. R. Broach.** 1991. Cloning genes by complementation in yeast. Methods Enzymol. **194:**195–230.
19. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A Saccharomyces cerevisiae genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:**237–243.
20. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Rothstein, R. J.** 1991. Targeting, disruption, replacement, allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:**281–302.
22. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics **122:**19–27.
23. **Smith, H. E., S. E. Driscoll, R. A. L. Sia, H. E. Yuan, and A. P. Mitchell.** 1993. Genetic evidence for transcriptional activation by the yeast *IME1* gene product. Genetics **133:**775–784.
24. **Smith, H. E., and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**2142–2152.
25. **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.
26. **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.
27. **Strich, R., R. T. Surosky, C. Steber, E. Dubois, F. Messenguy, and R. E. Esposito.** 1994. *UME6* is a key regulator of nitrogen repression and meiotic development. Genes Dev. **8:**796–810.
28. **Su, S. S. Y., and A. P. Mitchell.** 1993. Identification of functionally related

genes that stimulate early meiotic gene expression in yeast. Genetics **133:** 67–77.
29. **Sumrada, R. A., and T. G. Cooper.** 1987. Ubiquitous upstream repression sequences control activation of inducible arginase gene in yeast. Proc. Natl. Acad. Sci. USA **84:**3997–4001.
30. **Torchia, T. E., R. W. Hamilton, C. L. Cano, and J. E. Hopper.** 1984. Disruption of regulatory gene *GAL80* in *Saccharomyces cerevisiae*: effects on carbon-controlled regulation of the galactose/melibiose pathway genes. Mol. Cell. Biol. **4:**1521–1527.
31. **Vershon, A. K., N. M. Hollingsworth, and A. D. Johnson.** 1992. Meiotic induction of the yeast *HOP1* gene is controlled by positive and negative regulatory sites. Mol. Cell. Biol. **12:**3706–3714.