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Induction of Meiosis in *Saccharomyces cerevisiae* Depends on Conversion of the Transcriptional Repressor Ume6 to a Positive Regulator by Its Regulated Association with the Transcriptional Activator Ime1

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The transcription of meiosis-specific genes, as well as the initiation of meiosis, in the budding yeast *Saccharomyces cerevisiae* depends on *IME1*. *IME1* encodes a transcriptional activator which lacks known DNA binding motifs. In this study we have determined the mode by which Ime1 specifically activates the transcription of meiotic genes. We demonstrate that Ime1 is recruited to the promoters of meiotic genes by interacting with a DNA-binding protein, Ume6. This association between Ime1 and Ume6 depends on both starvation and the activity of a protein kinase, encoded by *RIM11*. In the absence of Ime1, Ume6 represses the transcription of meiotic genes. However, in the presence of Ime1, or when Ume6 is fused in frame to the Gal4 activation domain, Ume6 is converted from a repressor to an activator, resulting in the transcription of meiosis-specific genes and the formation of asci.

The major mode by which organisms regulate entry into and progression through developmental pathways is through both positive and negative transcriptional regulation. Precise control of gene expression may be accomplished by any one of the following mechanisms: modulating the availability of the regulators, their ability to bind DNA, their ability to interact with a recruiting protein, or their ability to function or converting their activity from transcriptional repression to activation or vice versa (11, 19). Here we describe the mechanism by which the developmental pathway, meiosis, is executed in the budding yeast *Saccharomyces cerevisiae*. Specifically, we elaborate the roles of the three main transcriptional regulators of meiosis-specific genes (MSG): two positive regulators, Ime1 and Rim11, and a DNA-binding protein, Ume6, which is a negative regulator in vegetative cultures and has a positive role during meiosis.

UME6 encodes a DNA-binding protein which binds to URS1, a specific sequence present in the 5′ untranscribed regions of many genes, including early MSG (2, 47). Insertion of a URS1 element upstream or downstream of a heterologous UAS results in transcriptional repression, suggesting that URS1 is the binding site of a repression complex (49, 52). Moreover, deletion of *UME6* results in the transcription of MSG in vegetative cultures (46, 47), indicating that Ume6 is a negative regulator. This vegetative transcription is independent of the meiosis-specific positive regulator, Ime1 (47), suggesting the existence of additional activators, whose activity might be masked by Ume6. In agreement, 5′ to the URS1 element, UAS elements have been detected: UAS_H in the *HOP1* gene (34, 52), T_4C in the *IME2* gene (4, 6), and HSE in the *HSP82* gene (49). Proteins which are required for the function of these positive elements in both mitosis and meiosis have been identified (34, 49). Conversely, under meiotic conditions, full induction of MSG depends on the presence of both the URS1 element and Ume6 (4, 6, 46, 47, 49, 52). Furthermore, diploids homozygous for *ume6*Δ alleles show very low levels of sporulation (6, 46, 47). These results suggest that under meiotic conditions Ume6 serves as a positive regulator (6, 46). It seemed possible, therefore, that under meiotic conditions, Ume6 might be converted from a repressor to an activator. Support for this view comes from the observation that a LexA-Ume6 fusion protein is an *IME1*-dependent transcriptional activator (6). Two alternative models were suggested to explain these results: (i) Ume6 is converted into a transcriptional activator following its phosphorylation by Rim11, a process which is mediated by Ime1, or (ii) Ume6 is converted into an activator by binding to Ime1 (6). Precedent for the first model would be the conversion of c-fos from an activator to a repressor by specific phosphorylation (33). The second model predicts that a URS1 DNA probe should exhibit an Ime1-dependent mobility shift and that physical interaction between Ime1 and Ume6 should be detected. However, it has been reported that these two types of experiments have not yielded positive results (4, 6). Therefore, the first model, which assumed a nondirect effect of Ime1, seemed more probable (6, 34, 46).

Ime1 is required for initiation of and progression through meiosis, as well as for the transcription of MSG (23, 31, 32). By fusing various portions of Ime1 to heterologous DNA-binding (28, 44) or transcriptional activation (28) domains, it was demonstrated that Ime1 functions as a transcriptional activator. Deletion analysis revealed that Ime1 is composed of at least two domains, a transcriptional activation domain (referred to in designations as ad) which is localized to amino acids 164 to 229 and an interaction domain (referred to in designations as id) which is localized to amino acids 270 to 360 (28). The interaction domain of Ime1 is required for the transcription of MSG but does not activate transcription on its own. However, when this domain is fused to a heterologous transcriptional activation domain, the hybrid protein can drive the transcription of MSG (28). It was suggested, therefore, that this domain is required for specific interaction with meiotic targets (28). The predicted amino acid sequence of Ime1 does not show any

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homology to known DNA-binding proteins (40, 45). This raised the possibility that Ime1 binds to DNA through a novel DNA binding motif. Alternatively, Ime1 might be recruited to the promoters of MSG through interaction with a sequence-specific, DNA-binding protein. Precedent comes from the viral activator protein VP16, which is recruited to the DNA by the Oct-1 protein (48); from the association of the coactivator OCA-B/Bob1/OBF-1, which has no intrinsic DNA-binding activity, with either Oct-1 or Oct-2 (16, 27, 48); and from the recruitment of the coactivator CBP to various promoters following interaction with different transcription factors (3, 26).

The *RIM11* (*MDS1*) gene encodes a serine/threonine protein kinase which phosphorylates Ime1 and is required for the transcription of MSG (5, 35). Transcriptional activation by a LexA-Ime1 fusion protein depends on Rim11 (44); thus, it was suggested that Rim11 is required for the function of Ime1's activation domain (5, 6, 31, 44).

In this report we demonstrate that Ime1 interacts with Ume6. This interaction requires starvation for both glucose and nitrogen. Using the two-hybrid screen, we identified *RIM11* as a clone whose product also associates with the interaction domain of Ime1. We show that Rim11 is required not for transcriptional activation by Ime1 but rather for the association of Ime1 with Ume6 and for self-association of Ime1. Our results demonstrate a new mode of transcriptional regulation: the transcriptional activation domain of Ime1 is tethered to the promoters of MSG by association with a transcriptional repressor, Ume6, resulting in the specific transcription of these genes.

MATERIALS AND METHODS

Plasmids. pAV79 carries the *hop1-lacZ* gene on a pBR322 2μm *URA3* vector (52). pAS135 carries the *ime1-lacZ* gene on a pBR322 2μm *CEN6 URA3* vector (40). pGK21 carries the *spr3-lacZ* gene on a pBR322 2μm *URA3* vector (21). pGAD2F carries the *pADH1-GAL4(ad)* gene on a pBR322 2μm *LEU2* vector (7) (a lowercase *p* before a gene designation indicates a promoter). pMA424 carries the *pADH1-GAL4(bd)* gene (where *bd* represents the DNA-binding domain) on a pBR322 2μm *HIS3* vector (7). YEp53 carries *IME1* from position −621 to position +2100 on a 2μm *URA3 LEU2* vector. The *LEU2* gene on a *Bgl*II fragment was inserted into YEpK26-7 (40). YEp1202 carries the *pADH1-GAL4(bd)-IME1(6–360)* gene (containing positions 6 to 360 of *IME1*) on a pBR322 2μm *URA3* vector (28). YEp1240 carries the *pADH1-IME1* gene on a pUC119 2μm *LEU2* vector. This plasmid was constructed by ligating a *Hin*dIII *IME1* fragment (positions −31 to +2100) to pADNS (37). YEp1302 carries the *IME1* gene (positions −1368 to +2100) on a pBR322 2μm *LEU2* vector. This plasmid was constructed by ligating an *Nhe*I *IME1* fragment from pDG11 (15) to YEplac181 (14). YEp1338 carries *pADH1-GAL4(1–147)-IME1(270–360)* on a pBR322 *HIS3* 2μm vector (28). YCp1357 carries *pADH1-GAL4(1–147)-IME1(270–360)* on a pBR322 *HIS3* 2μm *CEN* vector (28). YCp1404 carries *pADH1-GAL4(768–881)-IME1(270–360)* on a pBR322 *LEU2* 2μm *CEN11* vector (28). YCp1487 carries *pADH1-GAL4(1–147)-IME1(270–360)* on a Bluescript *ARSH4 CEN6 TRP1* vector. This plasmid was constructed by ligating an *Eco*RV-*Sal*I fragment from YEp1338 (28) into pRS414 (44). YEp1551 carries the *pADH1-GAL4(768–881)-UME6(159–836)* gene on a pBR322 *LEU2* 2μm vector. This plasmid was constructed by three-piece ligation between a vector, YE plac181 (12), cut with *Hin*dIII and *Pst*I; *Bam*HI-*Nsi*I-cut *pADH1-GAL4(768–881)* from pGAD2F (7); and a *Hin*dIII-*Bam*HI *UME6* fragment from plasmid 5905 (47). In order to obtain a Gal4(768–881)-Ume6 fusion protein, the resulting plasmid was digested with *Sac*I and *Sal*I, treated with T4 polymerase, and self-ligated. Then the plasmid was digested with *Bam*HI, treated with Klenow fragment, and self-ligated. P1582 carries *RIM11* on a pUC119 vector. An *Eco*RI-*Sph*I *RIM11* fragment derived from PCR using yeast DNA as a template and oligonucleotides MDS1+1 and MDS1 1380R was ligated to pUC119 digested with the same enzymes. P1583 carries *ume6*::*URA3-hisG* on a Bluescript vector. A *Bam*HI-*Bgl*II-cut *URA3-hisG* gene from pNKY51 (1) was inserted into the *Bam*HI site of *UME6* (*Xba*I-*Xho*I *UME6* fragment from 5905 [47] in Bluescript), creating a disruption at amino acid 158. P1607 carries *gal80*::*URA3-hisG* on a Bluescript vector. A *Bam*HI-*Bgl*II-cut *URA3-hisG* gene from pNKY51 (1) was inserted into the *Bgl*II site of *gal80* in pTT801 (51), creating a deletion between positions −15 and +596, as well as gene disruption. P1611 carries *rim11*::*LEU2* on a pUC119 vector. This deletion allele was constructed in two steps. First, a *Bgl*II *LEU2* fragment was inserted into the *Bgl*II site of *RIM11* in P1582, creating a disruption of *RIM11* at amino acid 93. Then, the 3′ end of RIM11, from amino acid 157, was deleted by digestion with *Sna*BI and *Nru*I and self-ligation. YEp1616 carries *pADH1-RIM11* on a pBR322 *LEU2* 2μm vector. This plasmid was constructed by three-piece ligation between *Bam*HI-*Eco*RI-cut *pADH1* from pADNS (38); an *Eco*RI-*Hin*dIII *RIM11* fragment from P1582; and YEplac181 (12) cut with *Bam*HI-*Eco*RI. YEp1758 carries the *IME1(1–308)-lacZ* gene on a pBR322 2μm *URA3* vector. This plasmid was constructed by inserting a *Hin*dIII-*Sal*I *IME1* fragment from position −31 to position +925 into E365R (25). A *Sal*I site at position +925 was inserted by PCR using oligonucleotides IME1−244 and IME1+942R. YEp1761 carries the *IME1 (1–360)-lacZ* gene on a pBR322 2μm *URA3* vector. This plasmid was constructed by inserting a *Hin*dIII-*Bam*HI *IME1* fragment from position −31 to position +2100 into E365R (25). YEp1788 carries the *pUME6-UME6(1–232)-GAL4(768–881)* gene on a pBR322 2μm *LEU2* vector. This plasmid was constructed by three-piece ligation between a vector, YEplac181 (12), cut with *Sac*I and *Sal*I; a *Kpn*I-*Sal*I *GAL4(768–881)* fragment from pGAD2F (7); and a *Kpn*I-*Sac*I *UME6* fragment from plasmid 5905 (47). YEp1790 carries the *pIME1-IME1(1–308)-lacZ* gene on a pBR322 2μm *URA3* vector. This plasmid was constructed by inserting the *IME1* promoter (positions −1357 to −31) on a *Hin*dIII fragment into YEp1758. YEp1821 carries the *pIME1-IME1(1–360)-lacZ* gene on a pBR322 2μm *URA3* vector. This plasmid was constructed by in vivo recombination between YEp1761 and a *bla-pIME1-IME1 Sca*I fragment derived from YEp1302. YEp1827 carries the *pUME6-UME6(1–232)-GAL4(768–881)* gene on a pBR322 2μm *TRP1* vector. This plasmid was constructed by ligating a *Sac*I-*Sal*I *pUME6-UME6(1–232)-GAL4(768–881)* fragment from YEp1788 into YEplac112 (12) cut with the same enzymes. YCp1844 carries the *pIME1-GAL4(768–881)-UME6(159–836)* gene on a pBR322 *LEU2 ARS1 CEN4* vector. This plasmid was constructed by three-piece ligation between a vector, YCplac111 (12), cut with *Hin*dIII and *Eco*RI; the *IME1* promoter from position −1368 to position −31 on an *Eco*RI-*Sal*I fragment; and a *Xho*I-*Hin*dIII fragment from YEp1551. YEp1862 carries *IME1* from position −621 to position +2100 on a 2μm *TRP1 LEU2* vector. YCp1865 carries *UME6* on an *ARS1 CEN4 URA3* vector. Three-piece ligation between a vector, YC plac33 (12), cut with *Bgl*II-*Sal*I; a *Bgl*II-*Kpn*I *UME6(1–232)* fragment derived from PCR using oligonucleotides UME6-1 and UME6-809C; and a *Kpn*I-*Xho*I *UME6(232–836)* fragment from 5905 (47) was performed. YEp1921 carries *pADH1-GAL4(ad)-UME6(1–836)* on a pBR322 2μm *LEU2* vector. This plasmid was constructed by a three-piece ligation between a vector, YEplac112 (12), cut with *Pst*I-*Hin*dIII; a *Bam*HI-*Nsi*I *pADH1-GAL4(768–881)* fragment from pGAD2F (7); and a *Bgl*II-*Hin*dIII *UME6* fragment from plasmid YCp1865.

Yeast strains. Yeast strains are described in Table 1. For Y919 a one-step deletion protocol was used to replace the *UME6* allele in the haploid parent (Y707) with a DNA fragment (from plasmid P1583) which carries the *ume6*::*URA3-hisG* gene. Ura$^+$ transformants were patched on 5-FOA plates to select for derivatives which had recombined out the *URA3* gene. For Y952 and Y1010 a *rim11*::*LEU2* fragment from plasmid P1611 on a *Pvu*II fragment replaced *RIM11* in Y546 and GGY::171, respectively. Southern analysis (data not shown) confirmed the correct replacement of *RIM11* by *rim11*::*LEU2*. Y1017 is a haploid segregant (tetrad analysis) of a diploid resulting from mating of Y952 to GGY::171. Y456, Y457, Y469, and Y470 represent diploidization of strains Y707, Y919, Y546, and Y952, respectively, by the *HO* gene on a YCp50 plasmid (plasmid pHO-c6 [38]). For Y476 a one-step deletion protocol was used to replace the *GAL80* allele in the haploid parent (Y707) with a DNA fragment (from plasmid P1607) which carries the *gal80*::*URA3-hisG* gene. Ura$^+$ transformants were patched on 5-FOA plates to select for derivatives which had recombined out the *URA3* gene. A *gal80*::*hisG* strain which expressed *GAL1-lacZ* in the presence of glucose was made diploid by transformation with a plasmid carrying the *HO* gene (plasmid pHO-c6 [38]). Strain Y476 is a derivative which has lost the plasmid.

Oligonucleotides. Oligonucleotides used were as follows: MDS1+1, 5′ CG CGCGGAATTCATGAATATTCAAAGCAAT; MDS1-1380, 5′ GCGGGGCC CGCATGCCGATGATGTAGTATTATA; GAL4E, 5′ ATGATGAAGATAC CCCAC; IME1-244, 5′ GGGCCCGGCCGGCTGCAGATTTAAACTACCGT ATAC; IME1+942R, 5′ GGCCGGCCGGGTCGACCTCTTTTGAATTTAAT TT; UME6-1, 5′ GGCCCGGAGATCTTGCTAGACAAGGCGCGCT; and UME6-809C, 5′ TCAGTATCGACATAGGCG.

Media and genetic techniques. SPM (sporulation medium) and SPO (for sporulation on plates) have been described previously (24). Synthetic dextrose (SD), yeast extract-peptone-dextrose (YEPD), and synthetic acetate (SA) have been described previously (39). SDP (SD with phosphate buffer) has been described previously (36). Meiosis and sporulation were induced in the following manner: stationary-phase cells (about 7×10^7) grown in minimal glucose media were harvested, washed once in water, and resuspended in SPM at a titer of 10^7.

Standard methods for DNA cloning and transformation were used (29). Yeast transformation with lithium acetate (LiOAc) was done as described previously (18). Proteins were extracted from at least three independent transformants and assayed for β-galactosidase activity as described previously (30, 36). Results are given in Miller units. For determination of β-galactosidase expression on plates (i.e., the formation of white or blue colonies), 100 μl of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; 20 mg/ml) was spread on top of either SA, SDP, or SPM plates.

Preparation of yeast protein extracts and Western blot (immunoblot) analysis. Protein extracts were prepared from trichloroacetic acid-treated cells as described previously (10). Western blot analysis was performed as described

TABLE 1. Yeast strains

Strain	Relevant genotype	Reference
GGY::171	*MAT*α *URA3*::*GAL1-lacZ gal80*Δ *gal4*Δ *his3-200 ura3-52 leu2-3, 112*	7
Y153	*MAT***a** *URA3*::*GAL1-lacZ LYS2*::*GAL-HIS3 gal80*Δ *gal4*Δ *his3-200 leu2-3, 112 trp1-901 ura3-52*	8
Y187	*MAT*α *URA3*::*GAL1-lacZ gal80*Δ *gal4*Δ *his3-200 leu2-3, 112 trp1-901 ura3-52*	17
Y546	*MAT***a** *leu2-3, 112 ura3-52 trp1*Δ *ade2-1 his4-519*	
Y707	*MAT*α *leu2-3, 112 ura3-52 trp1*Δ *ime1*::*TRP1 ade2-101 his3*Δ	
Y742	*MAT***a** *leu2-3, 112 ura3-52 trp1*Δ *ade2-1 his3*Δ	
Y919	*MAT***a** *leu2-3,112 ura3-52 trp1*Δ *his3*Δ *ime1*::*TRP1 ume6*::*hisG*	
Y952	*MAT***a** *leu2-3, 112 ura3-52 rim11*::*LEU2 trp1*Δ *ade2-1 his4-519*	
Y1010	*MAT*α *URA3*::*GAL1-lacZ gal80*Δ *gal4*Δ *his3-200 ura3-52 leu2-3, 112 rim11*::*LEU2*	
Y1017	*MAT*α *gal4*Δ *URA3*::*GAL1-lacZ his3-200 leu2-3, 112 ura3-52 rim11*::*LEU2 trp1*Δ	
Y1020	*MAT*α *gal4*Δ *his3-200 leu2-3, 112 ura3-52 rim11*::*LEU2 trp1*Δ	
Y456	*MAT***a**/*MAT*α *ime1*::*TRP1*/*ime1*::*TRP1 trp1*Δ/*trp1*Δ *his3*Δ/*his3*Δ *ura3-52*/*ura3-52 leu2-3, 112*/*leu2-3, 112*	
Y457	*MAT***a**/*MAT*α *ime1*::*TRP1*/*ime1*::*TRP1 ume6*::*hisG*/*ume6*::*hisG trp1*Δ/*trp1*Δ *his3*Δ/*his3*Δ *ura3-52*/*ura3-52 leu2-3, 112*/*leu2-3, 112*	
Y469	*MAT***a**/*MAT*α *trp1*Δ/*trp1*Δ *ura3-52*/*ura3-52 leu2-3, 112*/*leu2-3, 112*	
Y470	*MAT***a**/*MAT*α *rim11*::*LEU2*/*rim11*::*LEU2 trp1*Δ/*trp1*Δ *ura3-52*/*ura3-52 leu2-3, 112*/*leu2-3, 112*	
Y476	*MAT***a**/*MAT*α *ime1*::*TRP1*/*ime1*::*TRP1 trp1*Δ/*trp1*Δ *his3*Δ/*his3*Δ *ura3-52*/*ura3-52 leu2-3, 112*/*leu2-3, 112* *gal80*::*hisG*/*gal80*::*hisG*	

previously (9). The Gal4(bd)-Ime1 protein was detected by using antibodies directed against GAL4(bd) (sc-510; Santa Cruz Biotechnology).

Isolation of clones which interact with Ime1(270–360). The two-hybrid method (7) was used to isolate genes whose products interact with Ime1 (the protocol used was essentially the one described previously [8]). Strain Y153, which carries the *GAL4(bd)-IME1(270–360)* chimeric gene on plasmid YCp1487, was transformed with a yeast cDNA-Gal4(ad) expression library (kindly provided by S. Elledge). Transformants were selected on SD media lacking tryptophan, leucine, and histidine but including 100 mM 3-aminotriazole. The selected 3-aminotriazole-resistant transformants were screened for production of blue colonies on SA plates freshly spread with 100 μl of X-Gal (20 mg/ml). Using such plates has two advantages. (i) Plates are easy to make, the assay is simple, and low levels of β-galactosidase are detectable. (ii) SA plates may serve as permissive conditions for meiosis (23), enabling the isolation of genes whose products interact with Ime1(270–360) only under meiotic conditions. Putative blue 3-aminotriazole-resistant colonies were examined for dependence on Ime1(270–360) as follows: trp^- derivatives which had lost the *TRP* plasmid carrying the *GAL4(bd)-IME1(270–360)* gene were examined for their ability to express *GAL1-lacZ* on SA–X-Gal plates. Elimination of false-positive white colonies was further accomplished by mating to a *MAT*α haploid strain (Y187) expressing any of the following nonrelated chimeric genes: *Gal4(bd)-Lamin*, *Gal4(bd)-Cdk2*, and *Gal4(bd)-p53* (for a detailed description see reference 17). Colonies which did not express the *GAL1-lacZ* gene were taken for further analysis.

RESULTS

A Gal4(ad)-Ume6 fusion protein bypasses *ime1*Δ for the transcription of MSG and meiosis. The meiosis-specific gene *HOP1* is silent in vegetative cultures, and its transcription is induced during meiosis (52). We wanted to determine whether this vegetative silencing depends on *UME6*, as was previously reported for other early MSG (6, 46, 47).

Using a filter assay (28), we found that a wild-type strain (Y456) did not express the *hop1-lacZ* gene (present on plasmid pAV79) in vegetative cultures, while disruption of *UME6* (strain Y457) resulted in the formation of blue colonies. The strains used also had deletions of the *IME1* gene. Thus, in order to determine if Ume6 is required for sporulation, the *ume6* and *UME6* isogenic diploids (strains Y457 and Y456, respectively) were transformed with *IME1* on a multicopy plasmid (YEp53) and assayed for sporulation. The resulting *ume6* transformants were sporulation deficient (0% asci), whereas the isogenic *UME6* transformants gave rise to 86% asci. This result is in agreement with previous reports suggesting that, depending on nutrients, Ume6 is either a negative or a positive transcriptional regulator (6, 46, 47).

The positive role of Ume6 in meiosis led us to speculate that Ume6 may serve as the platform molecule which tethers Ime1 to specific DNA sites. We postulated that if Ume6 mediates the interaction of Ime1 with meiotic promoters, then an in-frame fusion of a transcriptional activation domain (ad) to *UME6* might relieve the requirement for Ime1 in initiation of meiosis. Thus, *ime1*Δ diploids which express the Gal4(ad)-Ume6(159–836) fusion protein were examined both for their ability to express the meiosis-specific gene *HOP1* (52) under vegetative and meiotic conditions and for their ability to complete the meiotic pathway, i.e., to produce asci. Table 2 shows that deletion of *IME1* (second row), as previously reported (23, 31), results in only low levels of *HOP1* expression and 0% asci. Introduction of the *IME1* gene on a 2μm vector (first row) complements *ime1*Δ, permitting the expression of *hop1-lacZ* only under starvation conditions and giving rise to 86% asci. The chimeric protein Gal4(ad)-Ume6(159–836), expressed from the meiosis-specific *IME1* promoter, bypasses the requirement for Ime1 for the transcription of *HOP1* and for sporulation. The expression of *HOP1* is induced 81-fold in SPM, and a high percentage of asci is observed (Table 2, third row). When either Ime1 or Gal4(ad)-Ume6(159–836) protein is expressed from the vegetative promoter *ADH1*, meiosis is inefficient, and low levels of asci are produced in both cases

TABLE 2. Suppression of *ime*1Δ by a Gal4(ad)-Ume6(159–836) chimeric protein

Plasmid[a]	Promoter	Protein	% Asci[b]	*hop1-lacZ* expression in[c]:	
				SD	SPM
YEp1302	IME1	Ime1	86	0.08	45.40
pGAD2F	ADH1	Gal4(ad)	0	0.06	0.45
YCp1844	IME1	Gal4(ad)-Ume6(159–836)	40	0.03	2.45
YEp1240	ADH1	Ime1	25	0.02	2.18
YEp1551	ADH1	Gal4(ad)-Ume6(159–836)	1.2	2.10	1.38

[a] Strain Y476 which carried any one of the listed plasmids was used.

[b] Production of asci was examined microscopically following at least 48 h of incubation in SPM.

[c] The examined strains also carried the *hop1-lacZ* gene on a 2μm plasmid (pAV79). Proteins were extracted from 10^7 cells grown in minimal glucose media (SD) or following 14 h of incubation under meiotic conditions (SPM). The level of β-galactosidase in Miller units is the average of those for two independent transformants. The standard deviation was less than 10%.

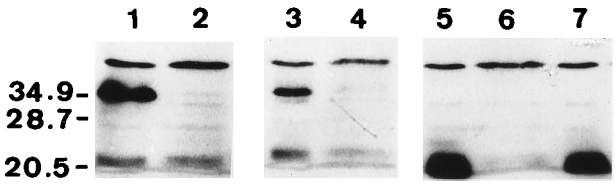

FIG. 1. Western analysis of Gal4(bd)-Ime1(id) in *RIM11* and *rim11*Δ strains. Proteins were extracted from 10^7 cells grown in minimal glucose media (lanes 1, 3, 5, and 7) or from starvation cultures following 6 h in SPM (lanes 2, 4, and 6). Two strains were used: a *RIM11* strain (Y742) (lanes 3, 4, and 7) and a *rim11*Δ strain (Y1017) (lanes 1, 2, 5, and 6). These strains carried either Gal4(bd)-Ime1(id) (YEp1338; lanes 1 to 4) or Gal4(bd) (pMA424; lanes 5 to 7). Western blot analysis was performed with antibodies directed against Gal4(bd). The expected molecular mass of the control Gal4(bd) protein is 19.9 kDa, whereas the molecular mass of the Gal4(bd)-Ime1(id) protein is 28.3 kD.

(Table 2, rows 4 and 5). We propose that the *IME1* promoter is more potent under meiotic conditions than is the *ADH1* promoter and that it thus provides higher levels of sporulation. In agreement with this notion, we found that when a truncated Ime1 protein is expressed from the *ADH1* promoter, it is readily detected in vegetative cultures but is not detectable in meiotic cultures (Fig. 1; compare lanes 3 and 4). Apparently, the molecular mass of Gal4(bd)-Ime1(id) is higher than expected (about 34 kDa), suggesting a posttranslational modification, as was previously reported (44). Conversely, when Ime1 is expressed from its own promoter, it is detected only under starvation conditions (data not shown).

The expression of Ime1 in vegetative cultures is not sufficient for the expression of meiosis-specific genes (e.g., *hop1-lacZ* as shown in Table 2, fourth row; similar data obtained for *ime2-lacZ* and *spr3-lacZ* by filter assays are not shown). These results are in agreement with the observation that meiosis is not induced in logarithmic-phase cells overexpressing Ime1 (40). The detection of Ime1 protein but not MSG expression in vegetative cultures (Fig. 1) implies that starvation is required for sporulation not only for the expression of Ime1 but also for an additional event (see below and reference 40). In contrast, the *pADH1-GAL4(ad)-UME6(159–836)* construct promoted the expression of *HOP1* in both vegetative and starvation cultures (Table 2, fifth row). We conclude that a DNA-binding protein which represses transcription can be artificially converted into a potent activator by being fused to a heterologous activation domain.

Ime1 interacts with Ume6. The observation that fusion of a transcriptional activation domain to Ume6 results in meiotic gene expression and meiosis raised the possibility that Ume6 may actually serve as a platform for Ime1 binding. We therefore examined whether Ume6 and Ime1 interact by using the two-hybrid system (7). This system can identify proteins which interact either directly or indirectly via a mediator. As the full-length Ime1 protein carries its own transcriptional activation domain, we used only a portion of Ime1, the last 90 C-terminal amino acids [Ime1(id), which does not include ad]. Expression of the reporter gene *GAL1-lacZ* was assayed in cells carrying both the *GAL4(bd)-IME1(id)* and *GAL4(ad)-UME6* chimeric genes. Two *GAL4(ad)-UME6* fusions were examined: the first was truncated by removal of the 158 N-terminal amino acids, and the second included only the 232 N-terminal amino acids. Interaction was observed only for the latter fusion (Tables 3 and 4), suggesting that the N terminus of Ume6 is sufficient for interaction with Ime1(id). In the first construct Gal4(ad) was fused to the N terminus of Ume6, suggesting the possibility that the lack of interaction might be due to the position of Gal4(ad). However, since a Gal4(ad)-Ume6(1–836) chimeric protein also interacts with Ime1(id) (data not shown), this possibility is ruled out.

TABLE 3. Rim11 regulation of interaction between Ime1 and Ume6

Fusion protein(s)	*GAL1-lacZ* expression in the following medium[a]:			
	RIM11[b]		*rim11*Δ[b]	
	SD[c]	SPM[d]	SD[c]	SPM[d]
Gal4(bd)-Ime1(id) + Ume6(1–232)-Gal4(ad)	2.60	1,777	0.32	1.15
Gal4(bd)-Ime1(id) + Gal4(ad)-Ume6(159–836)	0.05	7.20	NT	NT
Gal4(bd)-Ime1(id)	0.07	5.00	0.42	1.37

[a] Interaction between Ime1 and Ume6 was determined by the ability of a Gal4(bd)-Ime1(270–360) to activate transcription of a *GAL-lacZ* reporter gene by itself or in the presence of various Ume6-Gal4(ad) chimeras. The level of β-galactosidase is given in Miller units. The results are the averages of those for three or four independent transformants. The standard deviation was less than 10%. NT, not tested.

[b] The *RIM11* strain was GGY::171, and the *rim11* strain was Y1017. These strains carried YCp1357, which expressed Gal4(1–147)-Ime1(270–360) from the *ADH1* promoter. These strains also carried the following individual plasmids: YEp1551 [*pADH1-GAL4(ad)-UME6(159–836)*], YEp1788 or YEp1827 [*pUME6-UME6(1–232)-GAL4(ad)*], and YEplac181 or YEplac11 vectors (12).

[c] Vegetative cultures. Proteins were extracted from 10^7 cells grown in minimal glucose media.

[d] Starvation cultures. Stationary-phase cells were shifted to SPM. Proteins were extracted following 18 h of incubation at 30°C.

Ime1 induces the transcription of MSG and meiosis only under starvation conditions (Table 2) (40). Therefore, the above-described interaction was assayed in both vegetative and meiotic cultures. In logarithmic-phase cells growing on glucose, a low level of interaction, giving rise to an approximately 35-fold increase in the level of β-galactosidase activity in comparison with the control, which carries only the *GAL4(bd)-IME1(id)* fusion, was detected (Tables 3 and 4). However, upon starvation, interaction was very strong, giving rise to a 350-fold increase in the level of β-galactosidase activity (Tables 3 and 4). The level of β-galactosidase increases upon longer incubation in starvation media (compare Tables 3 and 4, in which samples were taken following 6 and 18 h of incubation in SPM, respectively). These results suggest that the interaction between Ime1 and Ume6 either is inhibited by glucose or depends on nitrogen depletion. In order to distinguish between these two possibilities, we determined the levels of β-galactosidase activity (expressed from the *GAL1-lacZ* gene)

TABLE 4. Nutrient-dependent interaction between Ime1 and Ume6

Fusion protein(s)	*GAL1-lacZ* expression in[a]:			
	SD[b]	SPM[c]	SPM+D[c]	SPM+NH_4[c]
Gal4(bd)-Ime1(id) + Ume6(1–232)-Gal4(ad)	0.96	143.9	6.98	16.39
Gal4(bd)-Ime1(id)	0.05	0.4	0.4	0.42

[a] Interaction between Ime1 and Ume6 was determined by the ability of Gal4(bd)-Ime1(270–360) (plasmid YCp1357) to activate transcription of a *GAL-lacZ* reporter gene (in strain GGY::171) by itself or in the presence of a Ume6(1–232)-Gal4(ad) chimera (plasmid YEp1788). The level of β-galactosidase is given in Miller units. The results are the averages of those for three or four independent transformants. The standard deviation was less than 10%.

[b] Vegetative cultures. Proteins were extracted from 10^7 cells grown in minimal glucose media.

[c] Starvation cultures. Stationary-phase cells were shifted to one of the following media: SPM, SPM+D, or SPM plus 15 mM $(NH_4)_2SO_4$ (SPM+NH_4). Proteins were extracted following 6 h of incubation at 30°C.

TABLE 5. Requirement of Rim11 for expression of meiotic genes

Plasmid	Gene examined[a]	β-Galactosidase level (Miller units) in the following medium:			
		RIM11/*RIM11*[b]		*rim11*Δ/*rim11*Δ[b]	
		SD[c]	SPM[d]	SD[c]	SPM[d]
pAS135	*ime1-lacZ*	0.05	19.30	0.08	31.55
pAV79	*hop1-lacZ*	0.21	42.90	0.11	0.37
pGK21	*spr3-lacZ*	0.46	10.98	0.27	0.77

[a] The level of expression of each MSG is inferred from the level of β-galactosidase.
[b] The *RIM11* strain was Y469, and the isogenic *rim11*Δ strain was Y470.
[c] Vegetative cultures. Proteins were extracted from 10^7 cells grown in minimal glucose media.
[d] Starvation cultures. Stationary-phase cells were shifted to SPM. Proteins were extracted after 6 h for *ime1-lacZ* and at 12 h for both *spr3-lacZ* and *hop1-lacZ*.

in cells incubated in either SPM, SPM containing 2% glucose (SPM+D), or SPM containing 15 mM $(NH_4)_2SO_4$. Table 4 demonstrates that the presence of either glucose or ammonia reduces the ability of Ime1 to interact with Ume6.

Unlike Ume6, Ime1 is normally expressed only under meiotic conditions. Thus, it is possible that in vegetative cultures Ime1 protein is unstable. If such was the case here, the diminished interaction observed between Ime1 and Ume6 in vegetative cultures might be due to insufficient levels of Ime1. However, Western analysis rules out this possibility (Fig. 1). These results suggest that low levels of Ime1 are sufficient for the association of Ime1 with Ume6 and that nutrients inhibit this interaction.

Rim11 interacts with Ime1. As mentioned above, URS1 elements, the binding sites for Ume6, are present in the promoters of most MSG (for a detailed list see reference 31). However, adjacent to the URS1 site in various MSG, there are additional positive elements: T_4C in the *IME2* promoter and UAS_H for *HOP1* (4, 52). Moreover, differential expression of MSG was observed in cells expressing the *ime1-3*(Ts) allele: at the nonpermissive temperature, *IME2* was expressed whereas *HOP1* was not (39). We proposed, therefore, that Ime1 may also interact with additional auxiliary proteins.

In order to clone additional genes whose products interact with the interaction domain of Ime1, we used the two-hybrid method. Several clones which specifically interact with Ime1 (id) were identified (see Materials and Methods for details). In this paper we report the characterization of clone 17. In the presence of both clone 17 and the *GAL4(bd)-IME1(id)* chimera, *GAL1-lacZ* was expressed, giving rise to 280 U. When the full-length gene present on clone 17 (plasmid YEp1616) was not fused to Gal4(ad), overexpression of this gene did not promote expression of *GAL1-lacZ*, giving rise to only 1.2 U. This result allows the following two conclusions: (i) the interacting protein encoded by clone 17 does not contain its own transcriptional activation domain, and (ii) this protein cannot endow the Ime1 interaction domain with a transcriptional activation potential.

Partial sequence analysis, using a primer from the *GAL4(ad)* portion (GAL4E), as well as restriction enzyme mapping, revealed the cloning of a known gene, *MDS1*/*RIM11*, which encodes a serine/threonine protein kinase (5, 35). The Gal4(ad)-Rim11 fusion protein expressed from the isolated clone is truncated by removal of the first 8 N-terminal amino acids of Rim11. We constructed a deletion allele of the *RIM11* open reading frame (see Materials and Methods) and found, as reported by others (5, 35), that *RIM11* is not an essential gene, although *rim11*Δ cells do exhibit slow growth. A diploid strain, Y470, which is homozygous for this deletion is sporulation deficient, but the presence of the *pADH1-RIM11* gene on plasmid YEp1616 restores its ability to sporulate.

Table 5 demonstrates that Rim11 is not required for the expression of *IME1* but is required for the expression of various genes which are regulated by Ime1, e.g., *HOP1* and *SPR3*.

Rim11 is required for the interaction between Ime1 and Ume6. Rim11 phosphorylates Ime1 (5); however, the function of this phosphorylation is not known. Previous reports have shown that phosphorylation of transcription factors can modulate their activity by any one of the following mechanisms: by affecting their ability to enter the nucleus, to bind to DNA, to activate transcription, to dimerize, or to interact with coactivators (11, 22, 42, 50). In addition, phosphorylation might be required to stabilize a transcription factor. All of the possibilities mentioned above are compatible with the requirement of Rim11 for the transcription of MSG and production of asci. Our aim was to determine which possibility applied to the role of Rim11 phosphorylation of Ime1.

In order to determine whether Rim11 is in fact required for Ime1 to activate transcription, as previously suggested (44), we examined the ability of a Gal4(bd)-Ime1 fusion protein to activate the expression of *GAL1-lacZ* in *RIM11* and *rim11*Δ strains. Table 6 demonstrates that this fusion protein activates the transcription of *GAL1-lacZ* in *rim11*Δ cells as well as in *RIM11* strains. Furthermore, the ability of Ime1 to activate transcription is not dependent on starvation. The lower levels of GAL1-LacZ expression in SPM versus SD are most probably due to use of the *ADH1* promoter. As discussed above (see also Fig. 1), this promoter is not efficient under starvation conditions. Furthermore, Fig. 1 (compare lanes 1 and 3) shows that the level of Gal4(bd)-Ime1(id) is not dependent on the presence of Rim11. These results suggest that Rim11 is required neither for the stability of Ime1 nor for the transcriptional activation function of Ime1 and contradict previous results obtained with a LexA-Ime1 fusion (see Discussion for an explanation of this phenomenon).

In order to examine whether Rim11 is required for the localization of Ime1 to the nucleus, we constructed an in-frame fusion between *IME1* and *lacZ* (plasmids YEp1790 and Yep1821). Using antibodies directed against β-galactosidase, we examined by indirect immunofluorescence the localization of the fusion protein. In both *RIM11* and *rim11*Δ diploids, the Ime1-LacZ fusion protein was localized to the nucleus (data not shown). In a strain which expressed the native *lacZ* gene, β-galactosidase was localized in the cytoplasm (data not

TABLE 6. Lack of requirement of Rim11 for transcriptional activation by Ime1

Plasmid	Fusion protein	*GAL1-lacZ* expression in the following medium[a]:			
		RIM11[b]		*rim11*Δ[b]	
		SD[c]	SPM[d]	SD[c]	SPM[d]
YCp1357	Gal4(bd)-Ime1(270–360)	0.07	0.01	0.42	0.14
YEp1202	Gal4(bd)-Ime1(6–360)	6,135	1,702	7,063	844.6

[a] Transcriptional activation by Gal4(bd)-Ime1 was determined by measuring the level of β-galactosidase (Miller units) expressed from the *GAL1-lacZ* gene.
[b] The *RIM11* strain was GGY::171, and the isogenic *rim11*Δ strain was Y1010.
[c] Vegetative cultures. Proteins were extracted from 10^7 cells grown in minimal glucose media.
[d] Starvation cultures. Stationary-phase cells were shifted to SPM. Proteins were extracted after 6 h.

TABLE 7. Ability of Ime1 to self-associate

Genes[b]	*GAL1-lacZ* expression in the following medium[a]:			
	RIM11[b]		*rim11*Δ[b]	
	SD[c]	SPM[d]	SD[c]	SPM[d]
pADH1-GAL4(bd)-IME1(id) plus:				
IME1 on a 2μm plasmid	0.65	30.78	0.42	0.25
pADH1-IME1 on a 2μm plasmid	0.59	3.48	NT	NT
Genomic *IME1*	0.07	5.0	0.42	1.37

[a] Self-association was determined by the ability of Gal4(bd)-Ime1(id) to activate transcription of a *GAL-lacZ* reporter gene in the presence of Ime1. The level of β-galactosidase is given in Miller units. The results are the averages of those for three or four independent transformants. The standard deviation was less than 10%. NT, not tested.

[b] The *RIM11* strain was GGY::171, and the *rim11* strain was Y1017. These strains carried YCp1357, which expresses Gal4(1–147)-Ime1(270–360) from the *ADH1* promoter. These strains also carried the following individual plasmids: YEp53 (*IME1*), YEp1862 (*IME1*), YEp1240 (*pADH1-IME1*), and the vectors YEplac181 and YEplac112 (12).

[c] Vegetative cultures. Proteins were extracted from 10^7 cells grown in minimal glucose media.

[d] Starvation cultures. Stationary-phase cells were shifted to SPM. Proteins were extracted after 8 h.

shown). We conclude that Rim11 is not required for the localization of Ime1 to the nucleus.

Previously, we have shown that fusion of Gal4(ad) to the interaction domain of Ime1 can promote sporulation in cells lacking *IME1* (28). However, this chimera could not suppress *rim11*Δ: strain Y470 which carried YCp1404 did not sporulate. This result provides additional evidence that Rim11 is not required for Ime1's transcriptional activation function and is consistent with the possibility that Rim11 might be required for the interaction of Ime1 with its meiotic targets. Table 3 shows that this is indeed the case: Rim11 is specifically required for the interaction of Ime1(id) with Ume6. In a *rim11*Δ strain the two hybrid proteins Gal4(bd)-Ime1 and Ume6(1–232)-Gal4(ad) can no longer drive *GAL1-lacZ* expression, and only the low basal level is observed. Thus, Rim11 is indirectly required for localization of the Ime1 activation domain to MSG promoters by allowing association between the Ime1 interaction domain and the URS1 binding repressor, Ume6.

The conclusion that Rim11 is required only for the association between Ime1 and Ume6 predicts that a Gal4(ad)-Ume6 fusion protein may bypass the requirement for Rim11 for the transcription of MSG. Indeed, Gal4(ad)-Ume6(1–836) (plasmid YEp1921) permits a vegetative expression (SD media) of *hop1-lacZ* (plasmid pAV79) in a *rim11*Δ strain (Y1020), giving rise to 32.6 U versus 0.2 U in the control strain.

Self-interaction of Ime1. Many transcription factors function only as dimers, and in some cases, their dimerization is regulated by phosphorylation (11, 22). We examined, therefore, whether Ime1 may self-associate according to the following rationale. The intact Ime1 protein contains an endogenous activation domain. Thus, if Ime1 is expressed along with Gal4 (bd)-Ime1(id), expression of the *GAL1-lacZ* reporter gene should be induced if full-length Ime1 can associate with Ime1(id). Table 7 demonstrates that under starvation conditions, cells which coexpress the Gal4(bd)-Ime1(id) fusion and Ime1 also express the *GAL1-lacZ* gene: under meiotic conditions, high levels of β-galactosidase activity are observed in cells which overexpress *IME1* (from plasmid YEp53) whereas low levels are observed in cells which express *IME1* either from the *ADH1* promoter or from the *IME1* promoter in the genome. As described above, under starvation conditions *ADH1* serves as a weak promoter, giving rise, therefore, to only low levels of β-galactosidase activity. However, in vegetative, glucose media, *pADH1* serves as a strong promoter; yet it does not promote the expression of *GAL1-lacZ*. This suggests that the interaction of Ime1(id) with the full-length Ime1 requires starvation. The transcription of *IME1* is repressed by glucose; therefore, we could not determine directly the effect of glucose by incubating cells in SPM+D. However, the *IME1* gene on plasmid YEp53 is constitutively expressed in vegetative cells grown on acetate as the sole carbon source (40). Therefore, we compared the levels of β-galactosidase in cells incubated on glucose, acetate, or SPM plates containing X-Gal. Blue colonies were observed only on SPM plates, suggesting that the interaction of Ime1(id) with the full-length Ime1 is inhibited by glucose and is dependent on nitrogen depletion. Moreover, Table 7 demonstrates that this interaction also depends on Rim11: in cells with deletions of *RIM11*, only the basal level of β-galactosidase activity is observed. This methodology cannot distinguish between self-association of Ime1 and the possibility that Ime1 is required for the expression of a transcriptional activator which associates with Ime1. Nevertheless, because of evidence discussed below, we favor the first possibility.

DISCUSSION

In this report we describe a unique mechanism of transcriptional regulation: in vivo formation of a competent transcriptional activator depends on the association of two distinct polypeptides (one, Ume6, provides the DNA-binding domain and by itself functions as a repressor, and the second, Ime1, provides the transcriptional activation domain) (Fig. 2). Support for this model comes from a recent report showing that a LexA-Ume6 chimeric protein activates the transcription of a *lexAop-lacZ* reporter gene in meiotic cells which overexpress Ime1 (6). Furthermore, the hydrophobicity of Ime1 and Ume6 may facilitate the formation of a heterodimer between these

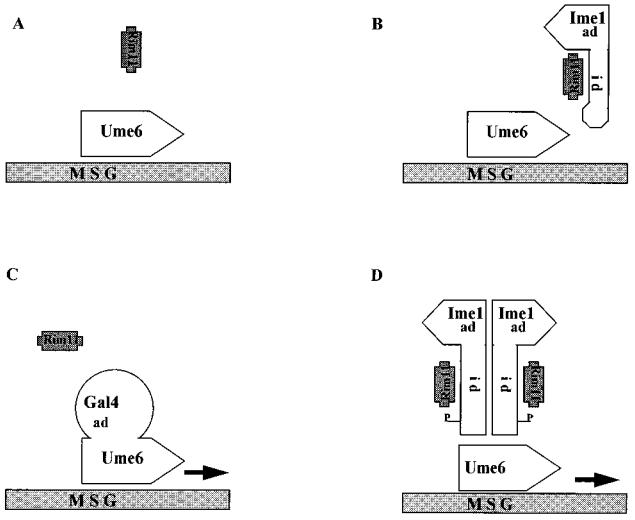

FIG. 2. Schematic model for transcription of MSG. (A) In vegetative cultures, Ume6 binds to the promoter region of MSG. (B) Ectopic expression of *IME1* under vegetative conditions results in association with Rim11. Nevertheless, under these conditions, Ime1 does not interact with Ume6. (C) Fusion of a Gal4 activation domain (ad) to Ume6 changes Ume6's activity from that of a repressor to that of an activator, permitting expression of MSG under both vegetative and meiotic conditions. (D) Under meiotic conditions, Ime1 self-associates and interacts with both Rim11 and Ume6. The interaction of Ume6 with Ime1 localizes the transcriptional activation domain of Ime1 to the promoters of MSG, resulting in their transcription.

two proteins (2). A strategy which uses a single transcriptional activator and various "docking" proteins might explain how Ime1 is separately required for various meiotic functions, i.e., DNA synthesis, meiotic recombination, and nuclear division (39). Similarly, the association of the transcriptional coactivator CBP with various transcription factors, such as CREB and c-JUN, leads to the expression of various genes (3, 26). However, in these reported cases, the proteins which recruit CBP to specific promoters function on their own as transcriptional activators and their association with CBP only increases the level of transcription. In the case reported here, *UME6* does not encode a transcriptional activator; on the contrary, it encodes a negative regulator. However, association with Ime1, or an in-frame fusion of a transcriptional activation domain, converts this repressor into an activator.

A situation in which the DNA-binding domain and the transcriptional activation domain are encoded by two separate genes has an additional advantage: it allows regulation at the level of association of the distinct polypeptides. In fact, in the case reported here, the association between Ime1 and Ume6 is modulated by a protein kinase as well as by glucose and nitrogen depletion. It is interesting that phosphorylation is also required for the interaction of the transcription factors CREB and c-JUN with CBP (3, 26).

Structural domains of Ime1 and Ume6. The last 90 C-terminal amino acids of Ime1 are sufficient to associate with Ume6 (Table 3), suggesting, as was previously proposed (28), that this domain comprises a meiosis-specific interaction domain. Mandel et al. (28) reported that deletion of this domain prevents the expression of MSG, whereas a chimeric protein which is composed exclusively of this domain and the transcriptional activation domain of Gal4 suppresses *ime1*Δ. Interestingly, this domain of Ime1 is required not only for Ime1's interaction with Ume6 but also for its interaction with Rim11 and for either self-association or interaction with a putative meiosis-specific transcriptional activator. In this article we can also report that this domain is not required for the nuclear localization of Ime1: an Ime1-LacZ fusion protein which is truncated by removal of the last 52 C-terminal amino acids (plasmid YEp1790) is localized to the nucleus (data not shown).

The results reported here suggest that Ume6 is composed of at least two functional domains: an N-terminal domain which is sufficient for interaction with Ime1 (Table 3) and a C-terminal, DNA-binding domain, which includes the zinc cluster DNA-binding motif (47) and promotes sporulation in the absence of Ime1 when fused to Gal4(ad) (Table 2). Support for such a domain structure also comes from the observation that a point mutation in the N-terminal domain of Ume6 (*rim16-12*) lowers the level of meiotic expression of *IME2* without affecting the ability of Ume6 to repress transcription in vegetative cultures (6).

The function of Rim11. In this report we show that Rim11 is required for two events to take place: the possible self-association of Ime1 as well as the interaction of Ime1 with Ume6. Our results do not exclude the possibility that Rim11 is directly required for only one of the above-mentioned interactions, which is in turn a prerequisite for the second. For example, stable interaction between Ume6 and Ime1 might require previous dimerization of Ime1. Mutational analysis of Ime1(id) is required to determine whether different amino acid residues participate in the interactions with Ume6 and with itself.

The results described here demonstrate that the interaction between Ime1 and Ume6 requires both starvation and phosphorylation by Rim11. However, it is not known whether Rim11 mediates the starvation signal, i.e., if Rim11 phosphorylates Ime1 only under starvation conditions. Rim11 interacts with Ime1 even under vegetative conditions. It has been suggested that physical interaction between a kinase and its substrate facilitates the specific and rapid phosphorylation of the substrate upon transmission of an appropriate signal (22). Thus, Rim11 may phosphorylate Ime1 only under starvation conditions, this phosphorylation then leading to the association of Ime1 with Ume6 as well as the self-association of Ime1. In agreement with this scenario, Bowdish et al. (5) have shown that Ime1 can be coimmunoprecipitated with Rim11 and phosphorylated by it in vitro. However, it has not been determined if in vivo Rim11-mediated phosphorylation of Ime1 is regulated by nutrients.

Previously, by using a LexA-Ime1 fusion protein, it was concluded that starvation and Rim11 are required for transcriptional activation by Ime1 (5, 44). This conclusion was based on the observation that a LexA-Ime1 fusion protein activated the expression of a *lex(op)-lacZ* reporter gene only under starvation conditions and only in *RIM11*$^+$ strains. This observation contradicts our findings that the Gal4(bd)-Ime1 fusion protein activates the expression of a *GAL1-lacZ* reporter gene in vegetative cultures (28) independently of Rim11 (Table 3). This discrepancy can be explained by the fact that the portion of *lexA* used in the studies described above lacked the dimerization domain which is required for the efficient binding of LexA to DNA (14). Therefore, the inability to detect high-level expression of *lacZ* might be due solely to inefficient binding to DNA. The results reported here suggest that starvation and Rim11 are required for the self-association of Ime1. We propose that LexA-Ime1 dimerizes (presumably because of dimerization of Ime1), binds DNA efficiently, and thus activates transcription only under starvation conditions and only in the presence of an intact *RIM11* gene. These results support, therefore, the model in which Ime1 self-associates rather than the alternative model which assumes interaction between Ime1 and an additional transcription factor.

A model for the induction of meiotic gene expression. *MAT***a**/*MAT*α diploids have three alternative developmental pathways depending on the presence or absence of various nutrients. In the presence of both carbon and nitrogen sources, cells progress through the mitotic cell cycle. Upon nitrogen depletion in the presence of glucose, cells undergo a morphological transition from a yeast form to a pseudohyphal form (13). Upon nitrogen depletion in the presence of a nonfermentable carbon source, cells progress through the meiotic cycle. Previous reports have shown that these nutritional signals, i.e., glucose and nitrogen, determine the availability of the master regulator of meiosis, Ime1 (23, 40). The transcription of *IME1* is repressed by glucose, and a low level is detected in vegetative media when acetate is the sole carbon source (23). Nitrogen depletion induces both the transcription and the translation of *IME1* (23, 40). Nevertheless, when Ime1 is ectopically expressed in vegetative cultures, in the presence of either glucose or acetate, MSG are not transcribed and meiosis is not induced (Table 2) (40). Because of this observation, we have proposed that starvation is required not only for the expression of Ime1 but also for its activation, or for the expression or activation of an additional factor (40). In this study, we have identified a posttranslational event which is modulated by these signals. Self-association of Ime1 and its association with Ume6 are inhibited by the presence of either glucose or nitrogen. Therefore, the transcription of meiosis-specific genes, as well as progression through meiosis, requires starvation not only for the expression of Ime1 but also for its interaction with Ume6 and/or its self-association.

Figure 2 presents a putative model which illustrates how two positive regulators, Ime1 and Rim11, and a negative regulator,

Ume6, control the expression of MSG. In vegetative cultures, both the *UME6* and *RIM11* genes are expressed but *IME1* remains silent. Therefore, under these conditions, the Ume6 repressor binds without Ime1 to the URS1 element present in MSG and silences their transcription (Fig. 2A). Expression of Ime1 under vegetative conditions from a heterologous promoter results in its association with Rim11 but not with Ume6. Thus, transcription of MSG is still repressed (Fig. 2B). However, fusion of the Gal4 activation domain to Ume6 changes Ume6's activity from that of a repressor to that of an activator, permitting expression of MSG under mitotic as well as meiotic conditions (Fig. 2C). Under starvation conditions, Ime1 is expressed, and following association with Rim11, it is phosphorylated. We propose that this phosphorylation modulates Ime1's structure, permitting both its self-association and its interaction with Ume6 (Fig. 2D). The interaction of Ume6 with Ime1 localizes the transcriptional activation domain of Ime1 to the promoters of MSG.

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