MOLECULAR AND CELLULAR BIOLOGY, Apr. 1993, p. 2172–2181
0270-7306/93/042172-10$02.00/0

Vol. 13, No. 4

Bipartite Structure of an Early Meiotic Upstream Activation Sequence from *Saccharomyces cerevisiae*

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Received 28 July 1992/Returned for modification 15 September 1992/Accepted 8 January 1993

Diploid a/α *Saccharomyces cerevisiae* cells cease mitotic growth and enter meiosis in response to starvation. Expression of meiotic genes depends on the *IME1* gene product, which accumulates only in meiotic cells. We report here an analysis of the regulatory region of *IME2*, an IME1-dependent meiotic gene. Deletion and substitution studies identified a 48-bp IME1-dependent upstream activation sequence (UAS). Activity of the UAS also requires the *RIM11*, *RIM15*, and *RIM16* gene products, which are required for expression of the chromosomal *IME2* promoter and for meiosis. Through a selection for suppressors that permit UAS activity in an *ime1* deletion mutant, we identified recessive mutations in three genes, *SIN3* (also called *RPD1*, *UME4*, and *SDI1*), *RPD3*, and *UME6* (also called *CAR80*), that were previously known as negative regulators of other early meiotic genes. Mutational analysis of the *IME2* UAS reveals two critical sequence elements: a G+C-rich sequence (called URS1), previously identified at many meiotic genes, and a newly described element, the T_4C site, that we found at a subset of meiotic genes. In agreement with prior studies, URS1 mutations lead to elevated *IME2* UAS activity in the absence of IME1. However, the URS1 mutations prevent any further stimulation of UAS activity by IME1. Repression through URS1 has been shown to require the *UME6* gene product. We find that activation of the *IME2* UAS by IME1 also requires the *UME6* gene product. Thus, UME6 and the URS1 site both have dual negative and positive roles at the *IME2* UAS. We propose that IME1 modifies UME6 to convert it from a negative regulator to a positive regulator.

The yeast *Saccharomyces cerevisiae* has many large sets of genes that are expressed only in response to particular external, internal, or genetic signals. For many sets of genes, key regulators that recognize or transmit each signal have been identified, as have the target DNA sites through which these regulators ultimately act (reviewed in references 34 and 38). Our studies have focused on a group of genes, called meiotic genes or sporulation-specific genes, that are expressed only in sporulating cells (reviewed in references 14 and 15). Expression of these genes increases in response to nitrogen starvation and is restricted to one type of cell, the a/α cell. Several regulatory genes have been identified that govern meiotic gene expression. The focus of this study is the site that responds to these regulatory genes and unusual functional interactions that lead to meiosis-specific gene expression.

IME1 and IME2 (also called SME1) are positive regulators of meiotic genes that are expressed at high levels only in starved a/α cells (8, 17, 28, 44). SIN3 (also called UME4, RPD1, and SDI1) and RPD3 have both positive and negative effects on many genes unrelated to meiosis, but appear to be primarily negative regulators of early meiotic genes (19, 31, 33, 40, 41). RIM11, RIM15, and RIM16 are required for expression of *ime2-lacZ* fusions and, presumably, *IME2* (16, 35). UME6 (also called CAR80) has genetic properties of a negative regulator of meiotic and nonmeiotic genes (21, 33). Epistasis studies have indicated that SIN3 may act independently or as a target of IME1 or IME2 (33).

The functional roles of these regulators are clear, but their precise mechanisms of action are not. IME1 is a nuclear protein that can activate transcription when bound, as a lexA fusion protein, to an upstream site (27). However, IME1 has no sequence features common to DNA binding proteins (29), so whether and how IME1 may interact with regulatory sequences is unknown. IME2, a protein kinase homolog (44), probably stimulates meiotic genes indirectly by phosphorylating a target protein. Such an IME2 substrate has yet to be identified. SIN3 is a large nuclear protein with four putative paired amphipathic helices (40, 42). These paired amphipathic helices are thought to be involved in protein-protein interactions, but the protein that interacts with SIN3 to regulate meiotic genes is not known at present. The deduced RPD3 product has no informative homologies (40). RIM11 is a protein kinase homolog (3) that lacks a known substrate. Molecular properties of UME6, RIM15, and RIM16 have thus far not been reported.

Although *IME2* specifies a regulator of early meiotic genes, we have found that *IME2* expression is regulated in parallel with other early meiotic genes: it is positively regulated by IME1 and negatively regulated by SIN3. We report here that an IME1-dependent UAS at *IME2* includes both a sequence implicated in regulation of many early meiotic genes, URS1 (4, 7, 39), and a previously unrecognized sequence that we call the T_4C site. (We note that the name URS1 has also been used for a segment of the *HO* upstream region [see reference 31].) Homology to the T_4C site is found near the URS1-like sequences of several other early meiotic genes. Our studies indicate that SIN3 acts as a negative regulator at the *IME2* upstream activation sequence (UAS) and that it acts independently of IME1 and IME2. We found that both URS1 and the UME6 gene product have dual positive and negative roles at the *IME2* UAS: in the absence of IME1, they repress activation by the T_4C site; in the presence of IME1, they augment activation.

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TABLE 1. Yeast strains used

Strain	Genotype[a]
107	a *GAL80*
109	a/α *GAL80/GAL80*
115	a/α *ime1-12::TRP1/ime1-12::TRP1 GAL80/GAL80*
256	α *ime1-12::TRP1 IME2-6::LEU2 arg6*
275	a *ime1-12::TRP1 IME2-6::LEU2 his1*
722	a *ime1-12::TRP1 his1*
1170	α P_{GAL1}-*IME1-14::TRP1 rim16-12 his4*
KB32	α *ime1-12::TRP1 ime2-7-HIS3::LEU2 his3ΔSK arg6 GAL80*
KB36	a *ime1-12::TRP1 ime2-7-HIS3::LEU2 his3ΔSK met13 GAL80*
KB37	a P_{GAL1}-*IME1-14::TRP1 his1*
KB38	a *his3ΔSK GAL80*
KB202	α P_{GAL1}-*IME1-14::TRP1 ime2-7-HIS3::LEU2 his3ΔSK*
KB203	a P_{GAL1}-*IME1-14::TRP1 ime2-7-HIS3::LEU2 sin3::LEU2 his3ΔSK*
KB217	α *ime1-12::TRP1 ime2-7-HIS3::LEU2 arg6 his3ΔSK*
KB218	a *ime1-12::TRP1 ime2-7-HIS3::LEU2 sin3::LEU2 arg6 his3ΔSK*
KB264	a P_{GAL1}-*IME1-14::TRP1 rim11::LEU2 met4 his4*
KB339	α *ime1Δ12::TRP1 ime2-7-HIS3::LEU2 ume6-239 arg6 his3ΔSK GAL80*
KB396	α *his3ΔSK*
1408-2B	α P_{GAL1}-*IME1-14::TRP1 rim15-1*

[a] All strains have the genotype *ura3 leu2::hisG trp1::hisG lys2 ho::LYS2 gal80::LEU2* except as noted. Diploids 109 and 115 are homozygous for these markers.

MATERIALS AND METHODS

Strains and media. Yeast strains are derivatives of SK1 and are described in Table 1. Mutations previously described include *ura3*, *leu2::hisG*, *lys2*, *ho::LYS2*, *gal80::LEU2*, *arg6*, *his1*, *his3ΔSK*, *his4*, *met13*, P_{GAL1}-*IME1*, *IME2-6::LEU2*, *rim16-12*, *ime1-12::TRP1* (see references 17 and 20), and *rim15-1* (35). The *sin3::LEU2* mutation (42), provided by David Stillman, was an insertion of the *LEU2* gene between the *Nsi*I and *Sal*I sites of *SIN3*; it was introduced by transformation from plasmid CS117. The *ume6::LEU2* mutation, provided by Randy Strich, was an insertion of the *LEU2* gene into the *UME6 Bam*HI site; it was introduced by transformation from plasmid pPL5914. The *ime2-HIS3::LEU2* fusion is described in detail elsewhere (27); its structure is essentially similar to that of an *ime1-HIS3* fusion described previously (20).

The *rim11::LEU2* deletion/insertion mutation was a replacement of a 350-bp *RIM11 Bgl*II fragment with a 3-kbp *Bgl*II fragment containing the *LEU2* gene. The deletion removes 278 bp of coding region, including the *RIM11* initiation codon, and causes a recessive *rim11* defect (3).

Yeast and bacterial media, including YEPD, YEPAc, SD, SC, and galactose indicator plates, followed standard recipes (23). Liquid sporulation medium, potassium acetate–5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (KAc-X-Gal) plates, and Spo plates have also been described previously (16). SAG plates, for suppressor isolation and complementation tests, had the same composition as SC plates except that 2% potassium acetate and 2% glycerol replaced glucose as the carbon source.

Plasmid constructions. Plasmids for tests of *IME2* promoter and UAS function all derived from the multicopy *CYC1-lacZ* plasmid pLGΔ312S (6). Custom oligonucleotides used in plasmid construction and analysis are described in Table 2. *IME2* promoter deletions were initially analyzed in plasmid pLGΔ312SΔRS, a pLGΔ312S derivative in which annealed oligonucleotides CYC1B and CYC1T replace the pLGΔ312S *Xho*I-*Bam*HI interval. Unidirectional deletions of the *IME2* upstream region were constructed by exonuclease III and S1 digestion from the *Xho* I site that lies ca. 1 kb upstream of *IME2* (44). Endpoints were determined through dideoxy sequencing. Transfer of the deletions through polylinkers resulted in inclusion of the sequence CCCGGGCT GCAGGAATTCGATATCAAGCTTGCATGCCTGCAG at the 5′ end of each deletion and the sequence ATCGATAC CGTCGACCTCGAG 3′ of the *IME2 Hin*dIII site at position −18 (relative to the AUG codon). The deletions were moved to plasmid pLGΔ312SΔRS as either *Sma*I-*Xho*I or *Sma*I-*Bgl*II fragments. Plasmid pKB984, used for the experiment shown in Fig. 1, had an upstream endpoint at position −984.

Plasmid pKB100 was constructed by polymerase chain reaction (PCR) amplification of the region between −584 and −443 in pKB650, which contains IME2 sequences −584 to −18 in the pBluescript (SK−) polylinker, with primers IME2-1 and IME2-2, which contain *Sma*I and *Sal*I sites, respectively. The amplified fragment was cleaved with *Sma*I and *Sal*I and ligated to *Sma*I- and *Sal*I-digested pLGΔ312S. The pKB102 insert was derived from PCR amplification from plasmid pKB630, which contains *IME2* sequences from −540 to −18 cloned into the pBluescript SK(−) polylinker with the SK primer and oligonucleotide IME2-2. The pKB103 insert was derived, similarly, from plasmid pKB580, which contains *IME2* sequences −493 to −18. Amplified products were digested with *Eco*RV and *Sal*I and ligated to *Sma*I-*Sal*I-cleaved pLGΔ312S. pKB110 was constructed by PCR amplification of the pKB650 template with primers IME2-1 and IME2-4, digesting with *Sma*I and *Sal*I, and ligating to *Sma*I- and *Sal*I-digested pLGΔ312S. pKB110K was constructed by PCR amplification of pKB650

TABLE 2. Oligonucleotides

Name	Sequence
CYC1B	5′-GATCCGGTCATTATTAATTTAGATC-3′
CYC1T	5′-TCGAGATCTAAATTAATAATGACCG-3′
CYC1-P	5′-GAATATTTAGAGAAAAGAAG-3′
IME2-1	5′-CATCCCGGGTCCTTTTCTCCGGTTGTCC-3′
IME2-2	5′-ACTGTCGACGCTTTTTGCCGCCGAAGTCT-3′
IME2-4	5′-ACTGTCGACCTCAAATAGCCGCCGTAAC-3′
IME2-5	5′-CTTGGTACCTCCTTTTCTCCGGTTGTCC-3′
IME2-6	5′-CTTTATGTTACGGCGGCTATTTGAGG-3′
IME2-7	5′-TCGACCTCAAATAGCCGCCGTAACATAAAGGTAC-3′
KPN1-P	5′-ATGGTACCAT-3′
URA3-1P	5′-GCAGGCTGGGAAGCATATTTG-3′

with primers IME2-4 and IME2-5, which contain *Kpn*I and *Sal*I sites, respectively. The amplified fragment was cloned into pKB112, a modified version of KmditpLGΔ312S that contains *Nco*I and *Kpn*I sites in place of the *Sma*I site. pKB112 was constructed by cleaving pLGΔ312S with *Sma*I and reclosing with *Kpn*I linker KPN-P. pKB142 was constructed by annealing oligonucleotides IME2-6 and IME2-7 and then ligating to plasmid pKB112 that had been digested with *Kpn*I and *Sal*I.

Integrating derivatives of plasmids related to pLGΔ312S were constructed by deletion of the 2.2-kb *Hin*dIII fragment, which includes almost all 2μ plasmid sequences. Integration at the chromosomal *ura3* allele, directed by digestion with *Stu*I, was confirmed by Southern analysis. Various strains carrying each integrated plasmid were constructed by standard genetic manipulations (23).

Mutant UAS library. A library of mutant *IME2* −584/−537 fragments was produced by PCR amplification of the UAS insert in plasmid pKB110K. The reaction used primers CYC1-P and URA3-1, which prime on each side of the insert, and followed mutagenic conditions (11) except that the $MnCl_2$ concentration was reduced to 0.1 mM. PCR products were digested with *Kpn*I and *Sal*I and ligated to pKB112. The mutant plasmid library was amplified in *Escherichia coli* and transformed into yeast strain KB202, and colonies with reduced β-galactosidase activity were identified as light blue or white colonies on X-Gal plates. Plasmids were retrieved in *E. coli*, sequenced with primer CYC1-P, and retransformed into yeast cells for further characterization.

Isolation and characterization of *ime1* suppressors. Haploid yeast strains KB32 and KB36, which have an *ime1* null mutation, *ime2-HIS3*, and the *his3ΔSK* deletion at the *HIS3* locus, were transformed with either pKB100 or pKB110. Single colonies from two transformants each were grown as patches on SC medium lacking uracil, replica plated to SAG-His-Ura, and mutagenized by UV with a Stratalinker set to 45 to 75 μJ × 100. One papilla was picked per patch and transferred to SC-Ura and then tested for *CYC1-lacZ* expression by replica plating to KAc-X-Gal. Dominance was tested by mating mutants to the *ime1 ime2-HIS3* parent strain of opposite mating type. Diploids were selected on SC-Arg-Met and replica plated to SAG-His-Ura. Complementation tests for *sin3* and *ume6* were accomplished by examining *ime2-HIS3* expression of diploids obtained from crosses to *ime1 sin3::LEU2* or *ime1 ume6::LEU2* strains on SC-His. *rpd3* mutations (40) were identified by complementation with the *RPD3* plasmid pMV34 (provided by M. Vidal and R. Gaber).

β-Galactosidase assays. Quantitative β-galactosidase assays were performed on permeabilized cells as described previously (29). Cells were grown to the exponential phase in YEPAc and shifted to sporulation medium for various times, as noted for each figure or table. For *ime1ΔIME2-6* strains, assays were conducted after 21 h in sporulation medium based on previous studies that showed delayed meiotic gene expression in these strains (17). Numbers are the average of determinations with at least three cultures. β-Galactosidase plate tests, involving either X-Gal plates or filter lifts, were done as described elsewhere (35).

T_4C site homology search. Sequences similar to the *IME2* T+C-rich UAS region were identified by searching for TTTTCTCCG and allowing up to two mismatches. We further demanded identity to bases critical for *IME2* UAS activity (underlined), as determined by our mutational analysis. We confined the search to 100 bp on either side of URS1 at the meiotic genes *SPO11*, *SPO13*, *SPO16*, *HOP1*, *MEI4*, *RED1*, *MER1*, *MRE4*, *MEK1*, and *DMC1* and searched entire 5′-flanking sequences of the *REC102*, *SPS4*, and *SGA1* genes, which lack URS1. We note that the T+C-rich region shared between the later meiotic genes *SPS4* and *SGA1* (9) does not match the T_4C site. We also searched URS1-flanking regions of the nonmeiotic genes *CAR1*, *CAR2*, *GAL1*, *HSF1*, *ILV2*, *PYK1*, and *TOP1*.

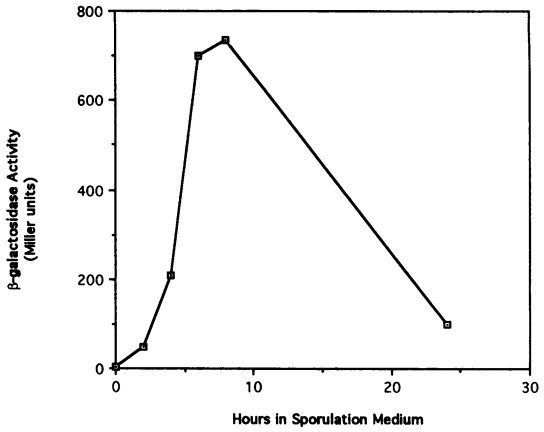

FIG. 1. Regulation of *CYC1-lacZ* expression from the *IME2* promoter. Wild-type a/α strain 109 carrying pKB984 was grown in YEPAc and transferred to sporulation medium (0 h). At various times after the shift, samples were removed for β-galactosidase determinations.

RESULTS

Deletion analysis of *IME2* 5′ sequences. We set out to identify *IME2* 5′-flanking sequences that are necessary for its expression and regulation by IME1. We replaced the promoter and upstream sequences of a *CYC1-lacZ* fusion with a fragment containing *IME2* sequences from −984 to −18. *CYC1-lacZ* was expressed from this promoter at low levels in nonmeiotic (vegetative) a/α cells and 200-fold-higher levels in meiotic (starved) a/α cells (Fig. 1). No expression was detectable in an a/α *ime1/ime1* mutant diploid (Fig. 2). Kinetics of *CYC1-lacZ* expression paralleled accumulation of *IME2* RNA (29). We conclude that this *IME2* 5′ segment is an IME1-dependent promoter.

We examined deletion derivatives of this fragment that retained the −18 downstream endpoint to identify sequences required for promoter activity (Fig. 2). Deletions to −820 or −584 caused only a twofold decrease in *CYC1-lacZ* expression and did not alter dependence on IME1. Deletions to −493 or to −459 caused a further 3- to 10-fold decrease in expression and, again, did not alter dependence on IME1. Deletions to −442 or −336 essentially abolished expression. We conclude that sequences between −584 and −442 play a strong positive role in *IME2* promoter activity.

To determine whether sequences that confer *IME1*-dependence lie 3′ to these positive sequences, we placed the *CYC1* UAS region adjacent to the *IME2* −442 to −18 region. This hybrid promoter, like the intact *CYC1* promoter, was expressed at comparable levels in *IME1/IME1* and *ime1/ime1* diploids (Fig. 2, compare pCYCΔ442 and pLGΔ312S). These results suggest that *IME1*-dependence is conferred by sequences upstream of −442.

To further delineate *IME2* upstream regulatory sequences, we replaced only the *CYC1* UAS region with *IME2* nucle-

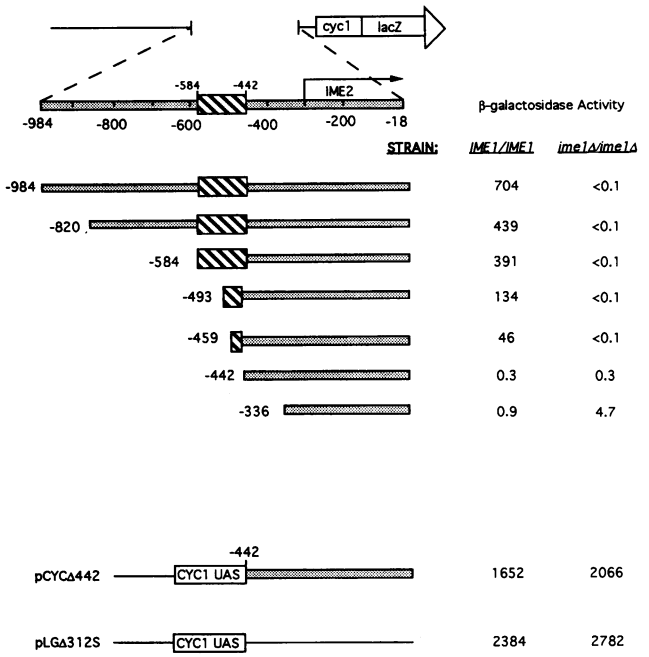

FIG. 2. Deletion analysis of the *IME2* promoter. Portions of the *IME2* promoter were used to replace the *CYC1* promoter and upstream region of *CYC1-lacZ* fusion plasmid pLGΔ312S. For plasmid pCYCΔ442, the *IME2* promoter fragment replaced only the region between the *CYC1* UASs and initiation codon. Strains 109 (*IME1/IME1*) and 115 (*ime1Δ/ime1Δ*) carrying each plasmid were grown in YEPAc and transferred to sporulation medium for 8 h prior to assay for β-galactosidase. *IME2* 5′ sequences are symbolized by the gray bar and cross-hatched rectangle, which corresponds to the region analyzed in Fig. 3.

otides −584 to −443. Expression of the *CYC1-lacZ* reporter was measured in a haploid strain that expressed high levels of IME1 (P_{GAL1}-*IME1*). The −584/−443 region stimulated *CYC1-lacZ* expression 500-fold relative to the control plasmid with no UAS (Fig. 3, column 1). Further subcloning revealed that this region contains at least two UASs: a stronger one between −584 and −537 (plasmid pKB110K) and a weaker one between −493 and −443 (plasmid pKB103). All these upstream fragments had 100-fold-less UAS activity in an *ime1Δ* strain than in the P_{GAL1}-*IME1* strain (Fig. 3, column 2). Thus, the *IME2* upstream region contains at least two IME1-dependent UASs.

Autoregulation of *IME2*. IME1 activates early meiotic genes through two pathways (17; reviewed in reference 15). In one pathway, IME1 acts indirectly by stimulating *IME2* expression and IME2 stimulates meiotic gene expression. This IME2-dependent pathway is active in an *ime1Δ* strain that expresses IME2 from a hybrid *GAL1-IME2* promoter (*ime1Δ* P_{GAL1}-*IME2* strain). In the other pathway, IME1 acts independently of IME2. This IME2-independent pathway is active in a strain that expresses IME1 and not IME2 (P-$_{GAL1}$-*IME1 ime2Δ*). Studies of a chromosomal *ime2-lacZ* fusion indicate that both pathways can stimulate IME2 expression (data not shown). To determine which pathway activates the *IME2* UASs, we measured UAS activity in strains that use individual pathways (Fig. 3, columns 3 and 4). Both strains expressed *CYC1-lacZ* from the −584 to −443 and −540 to −443 regions; only the P_{GAL1}-*IME1 ime2Δ* strain expressed *CYC1-lacZ* from the −584 to −537 or −493 to −443 regions. We conclude that the IME2-dependent pathway acts through the −540 to −443 interval. The IME2-independent pathway acts through both the −584 to −537 and −493 to −443 intervals; neither of these is stimulated by the IME2-dependent pathway.

Sequence determinants of *IME2* UAS activity. We characterized UAS-defective mutant derivatives of the −584 to −537 fragment to refine our definition of IME1-dependent UAS sequence requirements. We chose the −584 to −537 fragment because it responds only to one activation pathway (the IME2-independent pathway). In addition, it is a stronger UAS than the −493 to −443 fragment. To isolate UAS-defective mutations, we transformed a population of (−584/−537)-*CYC1-lacZ* plasmids containing mutagenized UAS inserts into a P_{GAL1}-*IME1 ime2Δ* strain and screened for transformants that failed to express *CYC1-lacZ*. Ten mutant plasmids were isolated and retransformed into yeast cells to quantitate their defects. Two of the plasmids had 5-fold expression defects; eight of the plasmids had 10- to 80-fold expression defects (Fig. 4). Sequences of these UAS inserts revealed two clusters of mutations. Six mutations lay within

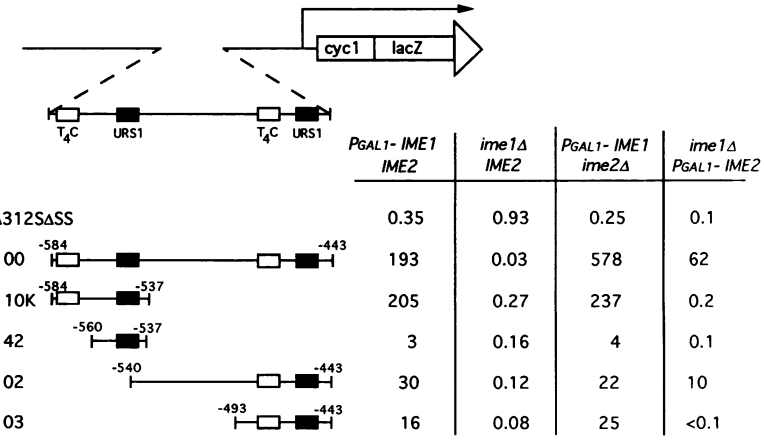

FIG. 3. Deletion analysis of the *IME2* UAS region. The *IME2* 5′ fragments indicated were used to replace the UAS region of *CYC1-lacZ* fusion plasmid pLGΔ312S. Control plasmid pLGΔ312SΔSS had no UAS region. Filled and open boxes indicate URS1 homology and T+C-rich sequences, respectively. Strains KB37, 722, KB202, and 256 × 275 carrying each plasmid were grown in YEPAc and transferred to sporulation medium for 4 h (or 21 h for strain 256 × 275) prior to assay for β-galactosidase.

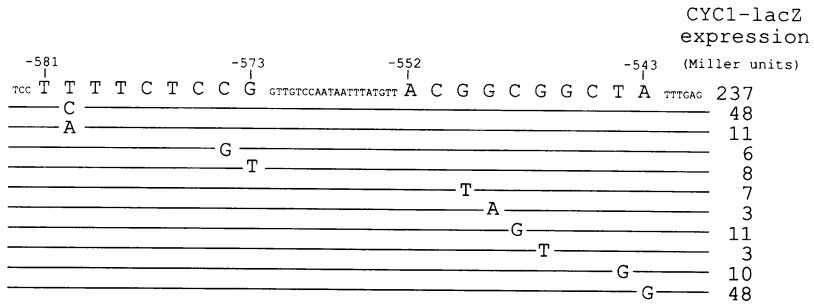

FIG. 4. Point mutations that inactivate the *IME2* UAS. The *IME2* −584 to −537 sequence is printed on the top line; single mutations that reduce UAS activity are indicated on successive lines. Numbers in the right-hand column are the *CYC1-lacZ* levels in strain KB202 (P_{GAL1}-*IME1 ime2*) carrying each plasmid after 4 h in sporulation medium.

the URS1-like sequence in the −552 to −543 interval. The other four mutations lay in a T+C-rich sequence in the −581 to −573 interval. Nine additional expression-defective plasmids were double mutants: three had one mutation between −550 and −543, two had one mutation between −581 and −573, three had mutations in both of these intervals (data not shown), and one had two mutations in the −581 to −573 interval (−576, T→C; −581, ΔT; see Table 3). We conclude that a URS1-like sequence and a T+C-rich sequence are both required for UAS activity.

To see whether the residual activities of mutant *IME2* UASs remained IME1 dependent, we compared expression of mutant (−584/−537)-*CYC1-lacZ* plasmids in P_{GAL1}-*IME1* and *ime1*Δ strains (Table 3). We first examined three mutations in the T+C-rich sequence. These mutant UASs had 10-fold-greater residual activity in the P_{GAL1}-*IME1* strain than in the *ime1*Δ strain. Similar results were obtained with a deletion that eliminates the T+C-rich sequence (plasmid pKB142; Fig. 3). We conclude that mutations in the T+C-rich sequence reduce the overall level of UAS activity, but do not eliminate stimulation of the UAS by IME1.

We also examined two mutations in the URS1-like sequence. Each mutant UAS was at least as active in the *ime1*Δ strain as in the P_{GAL1}-*IME1* strain (Table 3). This reduced level of UAS activity was considerably greater than activity of the wild-type UAS in the *ime1*Δ strain. Thus, URS1 mutations lead to an intermediate level of UAS activity that is independent of IME1. These results suggest that URS1 has dual positive and negative roles at the *IME2* UAS: in the presence of IME1, URS1 has properties of a positive site; in the absence of IME1, URS1 has properties of a negative site.

We sought to confirm that both sequence elements were required for UAS activity under conditions in which IME1 is expressed from its own promoter, rather than the *GAL1* promoter. Therefore, we examined UAS activity in **a**, α, and **a**/α cells, expressing only the natural *IME1* gene, after incubation in sporulation medium (Table 3). **a** and α cells express *IME1* at levels too low to activate *IME2* and other meiotic genes; **a**/α cells express *IME1* at sufficient levels to activate these genes (29). We found that the −584 to −537 UAS was 10-fold less active in **a** or α cells than in **a**/α cells. UAS activity was reduced by a mutation in either the T+C-rich sequence or the URS1-like sequence. These results indicate that both sequences contribute to UAS activity when *IME1* is expressed at its natural level.

We sought to confirm that meiosis-specific UAS activity did not depend on some artifactual property of multicopy plasmids. Therefore, we integrated a derivative of the (−584/−537)-*CYC1-lacZ* reporter plasmid at the *URA3* locus and monitored its expression through plate assays (Table 4). We found that *CYC1-lacZ* was expressed in **a**/α cells, not in **a** or α cells, and in sporulation medium, not in vegetative growth medium. Therefore, the −584 to −537 fragment stimulates meiosis-specific expression when present either in single-copy or multicopy plasmids.

TABLE 3. Activity of mutant *IME2* UAS fragments

−584 to −537 fragment	(−584/−537)-*CYC1-lacZ* expression[a]					
	Expt 1 (YEPAc medium)			Expt 2 (sporulation medium)		
	P_{GAL1}-*IME1*	*ime1*Δ	*ime1*Δ *sin3*::*LEU2*	**a**/α	**a**	α
Wild type	129	2.0	57	15	0.3	0.2
TC-region mutations						
−580 (T→A)	11	0.3	3.0	ND[b]	ND	ND
−574 (C→G)	6.0	0.3	4.0	0.5	0.08	0.07
−576 (T→C), −581 (ΔT)	6.0	0.3	3.0	ND	ND	ND
URS1 mutations						
−548 (C→G)	11	32	60	ND	ND	ND
−547 (G→T)	6.0	28	45	0.6	1.0	1.0

[a] β-Galactosidase was measured in log-phase YEPAc cultures of strains KB202 (P_{GAL1}-*IME1*), KB32 (*ime1*Δ), and KB197 (*ime1*Δ *sin3*::*LEU2*) or after 8 h in sporulation medium for strains AMP107 × KB396 (**a**/α), AMP107 (**a**), and KB396 (α). Each strain carried the (−584/−537)-*CYC1-lacZ* plasmid derivatives indicated.

[b] ND, not determined.

TABLE 4. Activity of the *IME2* −584 to −537 UAS when integrated at a chromosomal location

Cell type	(−584/−537)-*CYC1-lacZ* expression[a]	
	Vegetative medium	Sporulation medium
a	−	−
α	−	−
a/α	−	+

[a] Expression of an integrated (−584/−537)-*CYC1-lacZ* reporter plasmid was assayed with filter lift assays (35). Cells were incubated overnight on filters placed on vegetative medium (SC plates) or sporulation medium (Spo plates) as indicated. Filters were then removed for permeabilization and assay (35). +, patch of cells turned blue within 24 h; −, patch remained white after 24 h.

TABLE 5. Effect of *rim11*, *rim15*, and *rim16* mutations on *IME2* UAS activity

Plasmid	UAS insert	*CYC1-lacZ* expression[a]			
		P_{GAL1}-*IME1*	P_{GAL1}-*IME1 rim11Δ*	P_{GAL1}-*IME1 rim15-1*	P_{GAL1}-*IME1 rim16-12*
ΔSS[b]	None	0.34	0.45	0.27	0.56
pKB100	−584 to −443	120	0.16	1.2	0.19
pKB110K	−584 to −537	122	0.79	7.7	0.06

[a] β-Galactosidase activity was measured in log-phase YEPAc cultures of strains KB37, KB264, 1408-2B, and 1170 carrying the plasmids indicated. UAS inserts are diagrammed in Fig. 3.

[b] Plasmid pLGΔ312SΔSS, which lacks any UAS, served as a negative control.

Positive regulators that act at the *IME2* UAS. Several gene products are required in addition to IME1 for *IME2* expression. Recessive mutations in *RIM11*, *RIM15*, and *RIM16* cause defects in expression of a chromosomal *ime2-lacZ* fusion gene despite expression of IME1 (16, 35). These gene products may act in the same pathway as IME1 or in a different pathway. If these gene products act in the same pathway as IME1, they should act through the IME1-dependent UAS. If they act in an independent pathway, they may act elsewhere in the *IME2* promoter. Thus, we measured UAS activity of the −584 to −443 fragment and of the −584 to −537 fragment in P_{GAL1}-*IME1* strains carrying each *rim* mutation (Table 5). Both fragments provided little UAS activity in *rim11*, *rim15*, and *rim16* mutants. We conclude that RIM11, RIM15, and RIM16 are required for UAS activity of the −584 to −537 fragment. Accordingly, they may act in the same pathway as IME1.

Negative regulators that act at the *IME2* UAS. We developed a selection to identify mutations in negative regulators of *IME2*. Our selection made use of an *ime2-HIS3* hybrid gene, integrated at the chromosomal *IME2* locus, in which the *HIS3* coding region is transcribed from the *IME2* promoter. Strains that carry this fusion (and a deletion mutation at the *HIS3* locus) are His^+ only if they express IME1. We selected for suppressors that bypass the need for IME1 by selecting His^+ derivatives of an *ime1Δ ime2-HIS3* strain. To enable us to identify *trans*-acting mutations that affect −584 to −537 UAS activity, we included the (−584/−537)-*CYC1-lacZ* plasmid pKB110 in the strain and screened among His^+ mutants for those expressing *CYC1-lacZ* (Lac^+ phenotype). We identified 73 recessive His^+ Lac^+ mutations. Most of the mutations fell into two complementation groups, with 46 and 22 members. We confirmed that several mutations in each group segregated as single gene traits.

Mutations in *SIN3*, *RPD3*, and *UME6* permit expression of the early meiotic gene *SPO13* in the absence of starvation or **a**/α cell-type signals (33, 40). *sin3* mutations bypass the need for IME1 to activate *SPO13* (33); *rpd3* and *ume6* mutations may have similar effects. We found that both *sin3*::*LEU2* and *ume6*::*LEU2* mutations permit *ime2-HIS3* expression in strains with a deletion of *IME1*. Therefore, we used complementation and segregation tests to determine whether we had isolated *sin3*, *rpd3*, or *ume6* mutations.

All 46 mutants in the largest complementation group failed to complement a *sin3*::*LEU2* null mutant for suppression of an *ime1* deletion. We confirmed linkage of one of the mutations and *sin3*::*LEU2* (23PD:0NPD:0T). We conclude that these mutants have *sin3* defects.

All 22 mutants in the second largest complementation group failed to complement a *ume6*::*LEU2* null mutant for suppression of an *ime1* deletion. We attempted to test linkage of five of the mutations to *ume6*::*LEU2*, but four of the mutations gave very poor spore viability (<20%) in crosses to *ume6*::*LEU2* strains. The fifth mutation (*sup32-7*), which had the least severe effect on *ime2-HIS3* expression, yielded 19 tetrads with four viable spores from over 60 tetrads dissected. All 19 tetrads were PDs, indicating tight linkage between *sup32-7* and *ume6*::*LEU2*. These results indicate that *sup32-7* is a *ume6* allele. Our results suggest that all 22 mutants in the *sup32-7* complementation group have *ume6* defects and that UME6 is required for production of viable spores. We suggest that spores from the *sup32-7*/*ume6*::*LEU2* diploid were viable because the *sup32-7* allele (which we now designate *ume6-327*) is only mildly defective.

Two of the remaining mutants were complemented by a low-copy-number *RPD3* plasmid. This result suggests that these mutations are *rpd3* alleles. However, these mutants were not characterized further.

Role of SIN3 in *IME2* UAS activity. We considered two explanations for suppression of an *ime1* deletion by *sin3* mutations. One is that SIN3 represses the UAS through which IME1 normally stimulates *IME2* expression; IME1 may activate the UAS, for example, by antagonizing or modifying SIN3. Alternatively, SIN3 may repress a second UAS within the −584 to −537 fragment that is not used by IME1 to activate *IME2* expression; this cryptic UAS may be used by a different activation pathway. The first model predicts that the same UAS point mutations will impair both IME1-dependent UAS activity and SIN3-repressible UAS activity; the second model predicts that different UAS point mutations will impair IME1-dependent UAS activity and SIN3-repressible UAS activity. We tested these predictions by examining activity of several UAS point mutants in *sin3* strains.

We first determined effects of three mutations in the T+C-rich sequence (Table 3). These mutations had caused 10- to 20-fold *CYC1-lacZ* expression defects in P_{GAL1}-*IME1* and *ime1Δ* strains. Similarly, the mutations caused 10- to 20-fold *CYC1-lacZ* expression defects in an *ime1Δ sin3*::*LEU2* strain. We conclude that the T+C-rich sequence contributes to UAS activity in the *sin3* mutant.

We also determined effects of two mutations in the URS1-like sequence (Table 3). We had found that these mutations lead to elevated UAS activity in the absence of IME1. In the *ime1Δ sin3*::*LEU2* strain, the URS1 mutations had no effect on UAS activity. We conclude that the URS1-like sequence does not contribute to UAS activity in the *sin3* mutant.

TABLE 6. Effects of *IME1* expression in *sin3* and *ume6* mutants

SIN3 or *UME6* allele	(−584/−537)-*CYC1-lacZ* expression[a]	
	*ime1*Δ strain	P_{GAL1}-*IME1* strain
SIN3 UME6	1.2	120
sin3::*LEU2*	39	210
ume6::*LEU2*	6.0	1.0
ume6-22	5.3	7.6
ume6-327	1.5	12.0
ume6-329	2.3	3.3
ume6-3212	2.8	2.5
ume6-3227	8.0	2.8

[a] β-Galactosidase activity from the plasmid-borne (−584/−537)-*CYC1-lacZ* fusion was determined in log-phase YEPAc cultures. Strains had the *SIN3* or *UME6* allele listed in the first column and either *ime1*Δ or P_{GAL1}-*IME1*, as indicated in each column heading.

Instead, our results suggest that SIN3-dependent repression may require the URS1-like sequence, because both *sin3* and URS1 mutations have similar, nonadditive effects.

Our results thus far are compatible with a model in which IME1 stimulates the *IME2* −584 to −537 UAS, indirectly, by inhibiting SIN3 (33). This model predicts that the UAS will be insensitive to presence of IME1 in the absence of SIN3. Therefore, we compared (−584/−537)-*CYC1-lacZ* expression in *SIN3* and *sin3*::*LEU2* strains that express or lack IME1 (Table 6). We observed that expression of P_{GAL1}-*IME1* stimulated UAS activity by essentially the same increment (ca. 150 β-galactosidase units) in both *SIN3* and *sin3*::*LEU2* strains. These results indicate that IME1 cannot act simply through inhibition of SIN3. These findings suggest that IME1 and SIN3 act independently at the *IME2* UAS.

Role of UME6 in *IME2* UAS activity. Our view of UME6 as a negative regulator of the *IME2* −584 to −537 UAS is based on the observation that *ume6* mutations permit *ime2-HIS3* expression in vegetative cells that lack IME1. These observations are consistent with a previous study (21) showing that UME6 is required for repression through URS1. Quantitative assays of −584 to −537 UAS activity support this interpretation but suggest a more diverse role for UME6 (Table 6). A comparison of *ime1*Δ *UME6* and *ime1*Δ *ume6*::*LEU2* strains indicates that the *ume6*::*LEU2* mutation causes a slight increase in UAS activity, as expected if UME6 is a negative regulator. However, a comparison of *ime1*Δ *ume6*::*LEU2* and P_{GAL1}-*IME1 ume6*::*LEU2* strains indicates that IME1 cannot stimulate UAS activity in the absence of UME6. Similar results were obtained with five *ume6* alleles that we had isolated as suppressors of an *ime1*Δ mutation (*ume6-22*, *-327*, *-329*, *-3212*, and *-3227*; Table 6). These findings indicate that UME6 has a positive role in UAS activity in the presence of IME1. Immunoblots confirmed that *ume6* mutations do not affect IME1 protein accumulation (data not shown). We conclude that UME6 is required for IME1 to stimulate the *IME2* UAS.

DISCUSSION

Meiotic UAS regions and meiotic promoters. This study is the first to identify an early meiosis-specific UAS. Studies of the early meiotic genes *SPO13* (4) and *HOP1* (39) have failed to identify meiotic UAS regions. Instead, these genes appear to have meiosis-specific promoters, in which sites that direct initiation of transcription (and, possibly, translation) are necessary for properly regulated expression. What accounts for the apparent difference between *IME2* and these other genes?

One answer might be methodology. Our studies were facilitated by comparing P_{GAL1}-*IME1* and *ime1*Δ strains, which clearly give a greater differential signal for early meiotic gene expression than the more standard comparison of different cell types or growth conditions. However, we showed that the *IME2* −584 to −537 UAS does respond properly to cell type and nutritional signals. The vectors we used are derived from the *CYC1-lacZ* fusion (6), which has become the standard for defining UASs and was used in analysis of *HOP1* (39). (We note that the *CYC1* promoter has several nonproductive translational initiation codons between the UAS and RNA start region, so that replacement of the *CYC1* UAS with a complete promoter does not stimulate expression of the *CYC1-lacZ* reporter.) Again, our studies were facilitated by using multicopy reporter plasmids, but we demonstrated that the −584 to −537 UAS is active when the reporter plasmid is integrated. Thus, there is no obvious flaw in our methodology.

A second way to account for our findings is to postulate that *IME2* is regulated by a fundamentally different system from that regulating other early meiotic genes. However, our analysis reveals otherwise. We have previously shown that two regulatory pathways, IME2 dependent and IME2 independent, can activate *SPO11*, *SPO13* (17), and *HOP1* (18), just as we found here for the initial *IME2* UAS (−584 to −443) derivative. Prior studies have shown that SIN3, RPD3, and UME6 are required for repression of *SPO13* and other early meiotic genes in vegetative cells (33, 40); we report here analogous findings for the *IME2* −584 to −537 UAS. Prior studies have pointed to the widespread distribution and functional importance of URS1 sites in meiotic regulatory regions (4, 39); the two IME1-dependent UASs we identified contain URS1 sites. Furthermore, the results from our unbiased mutagenesis showed clearly that −584 to −537 UAS activity requires a functional URS1 site. These parallels between *IME2* UAS activity and expression of other early meiotic genes argue convincingly that the same regulatory systems govern their expression.

A third way to account for our findings is to suppose that the *IME2* regulatory region is constructed slightly differently from the other regulatory regions analyzed. One indication that this answer is correct comes from the finding that the *IME2* regulatory sites lie much farther upstream than *SPO13* or *HOP1* regulatory sites (−400 to −600 for *IME2* versus less than −200 for the other genes, relative to the initiation codon). Too few early meiotic genes have been analyzed in detail to known whether separable meiotic UASs will be the rule or the exception. In addition, it may be premature to conclude that *SPO13* and *HOP1* do not have meiotic UASs, because UAS and promoter sequences may be interdigitated. We believe that it would be informative to exchange key sites from the *IME2* meiotic UASs and meiotic promoters in order to delineate the functional differences.

Implications of *IME2* autoregulation. The present studies have shown that IME2 can stimulate its own expression. This observation explains how cells with low levels of IME1, such as *mck1* or *rim1* mutants (20, 35) and even many wild-type strains, can sporulate with fidelity. The problem is to ensure expression of all early meiotic genes, some at high levels and some at low levels, in every cell that initiates meiosis. The consequence of failure to express one recombination gene, for example, is inviability of spore progeny (see reference 5 for a discussion). The solution now appears to be a simple one: if a cell makes enough IME1 to stimulate

IME2 expression, then IME2 can amplify the signal to ensure balanced expression of early meiotic genes.

Although our observations simplify one problem, they complicate another, namely, the question of how early meiotic genes are shut off. Our previous studies suggested a simple explanation: IME2 shuts *IME1* off and thus leads to its own down-regulation and down-regulation of other early meiotic genes (17, 28). This scenario cannot be so simple if IME2 stimulates its own expression. We suggest that IME2 kinase activity or the level of a substrate may be altered to permit early meiotic genes to be shut off.

Sequence requirements for an IME1-dependent UAS. Our mutational analysis identified two regions of an IME1-dependent UAS that are required for activity: a URS1-like sequence (−552 to −543) and a T+C-rich sequence (−581 to −573). URS1-like sequences have been found in the regulatory regions of many genes expressed in vegetative cells (36) and, notably, in demonstrated or presumed regulatory regions of almost all early meiotic genes (4, 7, 10, 39). The canonical URS1, found in the *CAR1* upstream region, was defined as a negative site through deletion and substitution experiments (13, 36). Substitution experiments with URS1-like sequences from other genes revealed a wide range of negative activities and argued that similarity of a sequence to the *CAR1* URS1 was not a precise indicator of function (13). Our analysis indicates that the URS1-like sequence at the *IME2* UAS is functionally related to the *CAR1* URS1 for three reasons. First, the sequences are identical at eight of nine positions. Second, identical point mutations abolish both activation through the *IME2* UAS and repression through the *CAR1* URS1: the URS1 point mutations that we isolated correspond to several of the most severe repression-defective substitutions found in a saturation mutagenesis of the *CAR1* URS1 (13). Third, the same diffusible factor, UME6, is required for repression through the *CAR1* URS1 (21) and for activation through the *IME2* UAS. Our results indicate clearly that the *IME2* URS1 plays a positive role in IME1-dependent, meiosis-specific UAS activity. The parallels between the *CAR1* and *IME2* URS1 functional requirements argue against a model in which there are two competing URS1 binding proteins, one for repression and another for activation. Thus, we propose that a single URS1-protein complex can play positive or negative roles in transcription depending on context of the site or presence of interacting proteins, as is known for MCM1, RAP1, YY1, and glucocorticoid receptor (24–26, 30).

The T+C-rich sequence has not previously been identified at an early meiotic gene. We have found similar sequences at the early meiotic genes *DMC1*, *MEK1*/*MRE4*, and *RED1* (Fig. 5). All these 5′ regions have URS1-like sequences, as well. The spacing between T+C-rich sequences and URS1 homology varies from 8 to 48 bp. The relative orientations of the two elements vary, but the T+C-rich sequence always lies upstream of the URS1 homology, relative to the meiotic transcription unit. These comparisons yield the consensus sequence TTTTCXXCG, in which X is either T, C, or A. We designate this sequence the T_4C site.

One might expect to find the T_4C site as widely distributed among early meiotic genes as URS1. However, we found no convincing T_4C site within 100 bp of URS1 sequences at *SPO11*, *SPO13*, *MER1*, *MEI4*, or *HOP1*. It is noteworthy that *IME2* and *DMC1* are expressed at high levels (this study and reference 2), whereas *SPO11* and *SPO13* are expressed at low levels (1, 43). In fact, our T_4C site point mutations reduce (−584/−537)-*CYC1-lacZ* activity to the range we have seen for *SPO13-lacZ* under similar assay conditions

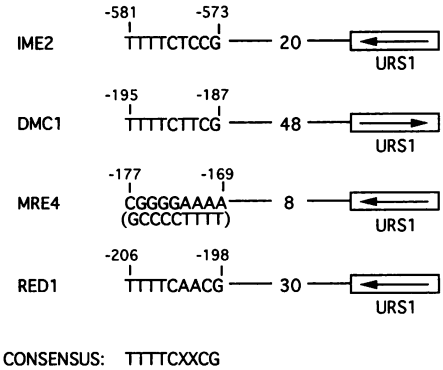

FIG. 5. Occurrence of the T_4C site and URS1 in early meiotic gene upstream regions. The 5′ regions of *IME2*, *DMC1*, *MRE4*, and *RED1* (2, 10, 37, 44) are diagrammed with T_4C sites aligned. The orientation of T_4C site homology at *MRE4* is opposite that of the other genes. The locations of the closest URS1-like sequences and their orientations are indicated by the boxed arrows. The bottom line shows a consensus T_4C site sequence derived from this comparison. We note that *MRE4* is the same gene as *MEK1* but that an apparent sequence polymorphism results in weaker T_4C site homology (TTTTCCCGG) and URS1 homology at *MEK1* (10, 22).

(29). Perhaps sequence variability of the T_4C site is a means to adjust the relative activities of early meiotic UASs. Support for this idea comes from our observation that T_4C site mutations reduce *IME2* UAS activity but do not affect IME1 dependence. In addition, we note that the weak *IME2* UAS, which lies in the −493 to −443 interval, includes both URS1 homology and a nearby T+C-rich sequence (TTTTC CC*T*G) with a departure from the T_4C site consensus at a critical base pair (italicized). Therefore, we suggest that some weak meiotic UASs may include T_4C sites with base substitutions that lead to reduced activity.

A recent study of the meiotic *HOP1* regulatory region suggests a second reason for the lack of T_4C sites at some meiotic genes (39). The *HOP1* promoter region contains a URS1-like sequence that is essential for meiosis-specific expression. Upstream of the URS1-like sequence is a site, designated UAS_H, that is required for maximal levels of *HOP1* expression. Homology to UAS_H was found at several other meiotic genes; like the T_4C site, UAS_H homology was always upstream of URS1-like sites. The UAS_H sequence differs considerably from the T_4C site sequence (TGT GAAGTG and TTTTCXXCG, respectively), although critical base pairs for UAS_H activity have not been defined. Five of the six regulatory regions with UAS_H homology have no T_4C site homology, and three of the four regulatory regions with T_4C site homology have no UAS_H homology (the exception being *DMC1* with UAS_H and T_4C site homologies). Thus, UAS_H and the T_4C site may play interchangeable roles.

If the T_4C site plays a specific role in meiotic gene regulation, it might not be present at nonmeiotic genes. We searched for the T_4C site at seven nonmeiotic genes that contain URS1 (13). We found a T_4C site within 100 bp of URS1 at only one gene, *ILV2*. This 5′ region differed from the meiotic genes in that URS1 was upstream of the T_4C site. However, this observation suggests that distribution of T_4C sites (and, by implication, their function) is not restricted to meiotic genes.

Relationships between positive and negative regulators of *IME2* UAS activity. *sin3* mutations have been identified

previously through selections for increased expression of many different genes. Strich et al. (33) identified *sin3* mutations (called *ume4*) that permitted expression of several early meiotic genes irrespective of nutritional and cell type signals. The finding that these genes were expressed in *sin3 ime1* and *sin3 ime2* double mutants indicated that SIN3 must act downstream or independently of IME1 and IME2. Our present findings refine the understanding of these relationships. First, we found that the *IME2* −584 to −537 UAS is negatively regulated by SIN3 but is not activated by IME2. We infer that IME2 does not stimulate a UAS simply by inactivating SIN3. Second, we found that IME1 stimulates the −584 to −537 UAS in a *sin3* null mutant. We conclude that IME1 does not activate the UAS simply by inactivating SIN3. Third, we found that T_4C site point mutations that reduce IME1-dependent activity of the −584 to −537 UAS also reduce its IME1-independent activity in *sin3* mutants. Thus, *sin3* mutations do not cause activation of an adventitious UAS. We conclude that SIN3 and IME1 act independently at the *IME2* UAS.

Despite some similarities between *sin3* and *ume6* mutant phenotypes, our findings support a different relationship between UME6 and IME1. UME6 has properties of a negative regulator of the *IME2* UAS in the absence of IME1: the *ume6::LEU2* mutation permits low-level, IME1-independent UAS activity. On the other hand, UME6 has properties of a positive regulator of the *IME2* UAS in the presence of IME1: all *ume6* mutations tested, including the *ume6::LEU2* insertion allele, reduce or abolish the stimulation of UAS activity by IME1. One interesting possibility is that a UAS-UME6 complex is required to recruit both negative and positive regulators (such as SIN3 and IME1, respectively). The observation that *ume6* mutations cause reduced sporulation (3, 32) and spore viability is consistent with a positive role for UME6 in meiotic UAS activity. A second possibility is that UME6 acts only as a negative regulator but that physiological defects of *ume6* mutants indirectly impair IME1 activity. In that context, we note that the *ume6::LEU2* mutation causes a mild growth defect in YEPAc medium which is more pronounced when P_{GAL1}-*IME1* is expressed. We favor a more direct role for UME6 in repression and activation, though, because the URS1 site also has alternate positive and negative activities.

Model for early meiotic gene activation. Our results are consistent with a simple working model for control of *IME2* −584 to −537 UAS activity. We propose that UAS activity is governed by the combined action of two regulatory proteins or complexes, one acting at URS1 and a second acting at the T_4C site (Fig. 6). In nonmeiotic cells, the T_4C site is the site at which a positive regulator acts. Activity of this positive regulator is blocked through interaction of SIN3 and UME6 with the URS1 site. Under conditions that favor meiosis, *IME1* is expressed. IME1 then modifies the URS1 complex so that it amplifies, rather than impedes, the positive signal from the T_4C site complex.

The identity of proteins in the URS1 repression-activation complex is unclear at present. A heteromeric URS1 binding protein has recently been purified (12), but its function has yet to be established. UME6 is not required for formation of the major URS1-protein complex identified by gel retardation assays (21). Because a *sin3* null mutation has little effect on repression of the *CYC1* UAS through URS1 (21, 39), it seems unlikely that SIN3 is an obligate complex constituent. Our finding that URS1 point mutations abolish IME1-dependent UAS activity but that *sin3* mutations do not suggests a similar conclusion: URS1 point mutations may prevent

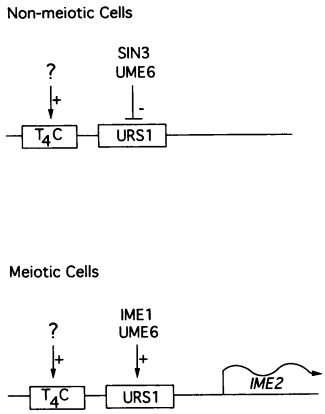

FIG. 6. Model for early meiotic gene regulation. Functional interactions of gene products and sites that lead to *IME2* −584 to −537 UAS activity are shown. Upper panel: In nonmeiotic cells, a positive regulator acts through the T_4C site and a negative regulator acts through URS1. Negative activity of URS1 requires UME6 and SIN3. Repression by the URS1 complex overcomes activation by the T_4C site complex. Lower panel: When cells enter meiosis, IME1 accumulates and alters the URS1 complex so that it amplifies the T_4C complex positive signal and stimulates *IME2* UAS activity. Positive activity of URS1 requires both IME1 and UME6, but not SIN3.

functional complex formation, but *sin3* mutations do not. If UME6 does not bind directly to URS1, then UME6 may serve as an adaptor to permit interaction of the URS1-protein complex with SIN3 or IME1.

The protein that binds to the T_4C site is also unknown. Studies of lexA-IME1 fusions suggest that IME1 may play a direct role in activation (27). However, we consider direct binding of IME1 to the T_4C site unlikely because the T_4C site is a weak UAS in *sin3* or *ume6* mutants that lack IME1. In addition, we have thus far not detected DNA binding by IME1 (3). We found that three gene products, RIM11, RIM15, and RIM16, are all required for UAS activity of the *IME2* −584 to −537 fragment. Sequence analysis of *RIM11* indicates that it specifies a protein kinase homolog (3). Thus, RIM15 and RIM16 are candidates for binding proteins that recognize the T_4C site or URS1.

ACKNOWLEDGMENTS

We thank Suzanne Driscoll for her preliminary studies on the *ime2-HIS3* fusion, Sophia Su for creating deletions of the *IME2* 5′ region, and Marc Vidal, Rick Gaber, David Stillman, Randy Strich, and Shelly Esposito for providing plasmids and unpublished information. We are grateful to David Shore for comments on this manuscript and to Marian Carlson for many discussions and insights.

This work was supported by Public Health Service grant GM3951 and by funds from the Irma T. Hirschl Charitable Trust and the Searle Scholars Program/The Chicago Community Trust. K.S.B. was supported, in part, as a predoctoral trainee in the Cellular, Molecular, and Biophysical Studies training program (GM 08224).

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