

Multiple and Distinct Activation and Repression Sequences Mediate the Regulated Transcription of *IME1*, a Transcriptional Activator of Meiosis-Specific Genes in *Saccharomyces cerevisiae*

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***IME1* encodes a transcriptional activator required for the transcription of meiosis-specific genes and initiation of meiosis in *Saccharomyces cerevisiae*. The transcription of *IME1* is repressed in the presence of glucose, and a low basal level of *IME1* RNA is observed in vegetative cultures with acetate as the sole carbon source. Upon nitrogen depletion a transient induction in the transcription of *IME1* is observed in *MATa/MATα* diploids but not in *MAT*-insufficient strains. In this study we demonstrate that the transcription of *IME1* is controlled by an extremely unusual large 5' region, over 2,100 bp long. This area is divided into four different upstream controlling sequences (UCS). UCS2 promotes the transcription of *IME1* in the presence of a nonfermentable carbon source. UCS2 is flanked by three negative regions: UCS1, which exhibits URS activity in the presence of nitrogen, and UCS3 and UCS4, which repress the activity of UCS2 in *MAT*-insufficient cells. UCS2 consists of alternate positive and negative elements: three distinct constitutive URS elements that prevent the function of any upstream activating sequence (UAS) under all growth conditions, a constitutive UAS element that promotes expression under all growth conditions, a UAS element that is active only in vegetative media, and two discrete elements that function as UASs in the presence of acetate. Sequence analysis of *IME1* revealed the presence of two almost identical 30- to 32-bp repeats. Surprisingly, one repeat, IREd, exhibits constitutive URS activity, whereas the other repeat, IREu, serves as a carbon-source-regulated UAS element. The RAS-cyclic AMP-dependent protein kinase cAPK pathway prevents the UAS activity of IREu in the presence of glucose as the sole carbon source, while the transcriptional activators Msn2p and Msn4p promote the UAS activity of this repeat in the presence of acetate. We suggest that the use of multiple negative and positive elements is essential to restrict transcription to the appropriate conditions and that the combinatorial effect of the entire region leads to the regulated transcription of *IME1*.**

In the budding yeast *Saccharomyces cerevisiae* the choice between meiosis-sporulation and alternative developmental pathways such as the mitotic cell cycle, pseudohyphae growth, or G₁ arrest depends on the expression and activity of a master regulator, Ime1p. This is deduced from the observation that cells deleted for *IME1* are sporulation deficient and arrest in meiosis at G₁ prior to any meiotic event, i.e., transcription of meiosis-specific genes, premeiotic DNA replication, meiotic recombination, and nuclear divisions (15, 49). *IME1* encodes a transcriptional activator (23, 48) that is recruited to the promoters of early meiosis-specific genes by interacting with a sequence-specific DNA binding protein, Ume6p (41).

Initiation of meiosis depends on two signals: starvation for nutrients and the presence of *MATa1* and *MATα2* gene products (17). The nutrient signal is required at several levels: for the transcription of *IME1* (15), for the translation of *IME1* mRNA (47), for the association of Ime1p with its meiotic target, Ume6p (41), and for entry into the first meiotic division (21). The *MAT* signal is also required in more than one step: for the transcription of *IME1* and for efficient meiosis (15, 47). The purpose of this study has been to identify the elements in *IME1* that are required for its regulated transcription and to

determine the role of the RAS-cyclic AMP-dependent protein kinase (cAPK) pathway in the activity of the regulated upstream activating sequence (UAS) elements. Therefore, we shall summarize below the known information concerning the transcription of *IME1*.

Transcripts of *IME1* are not detected in the presence of glucose, and a low basal level is present in vegetative acetate media (15). Upon nitrogen depletion the level of *IME1* mRNA increases in *MATa/MATα* diploids, reaching a peak at about 6 to 8 h and then declining (15, 49). The transcription of *IME1* is not induced in cells that do not carry both the *MATa1* and *MATα2* alleles (*MAT*-insufficient cells) (15, 49).

Very little is known about the organization of the *IME1* locus. The sequence of *IME1* identifies three putative TATA boxes: TATATTA at -353, TATTTAA at -330, and TATA AAT at -158. Deletions of these TATA boxes revealed that the functional TATA is at -330 (1). Accordingly, the main transcription initiation site of *IME1* RNA was mapped to -229 (1, 47). The complete genomic sequence of *S. cerevisiae* reveals that upstream of *IME1* there is an extremely large region, 4,122 bp long, that is devoid of open reading frames, tRNA, or rRNA. This suggests the possibility that a large region may be involved in the transcriptional regulation of *IME1*. Indeed, previous reports have pointed to this phenomenon (5, 12). Covitz and Mitchell reported that the region between -2243 and -1743 upstream of *IME1* ATG carries a negative element that prevents the expression of *IME1* in *MATa/MATa* cells (5). Furthermore, a 21-bp element (RRE) located at base pair -2024 to -2044 binds Rme1p (5), a zinc finger protein that

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represses the transcription of *IME1* in MAT-insufficient cells (15, 16, 37). The regulated region may extend even further, since multiple copies of *IME1* sequences from -3166 to -3762 promote sporulation in both the presence of nutrients and in *MATa/MATa* diploids (12).

Except for Rme1p, the transcriptional activators and repressors that directly affect the transcription of *IME1* are unknown. Nonetheless, several genes that affect the transcription of *IME1* have been identified. *IME4* encodes a positive regulator that is absolutely required for the transcription of *IME1* (44). The transcription of *IME4* is induced only in *MATa/MATa* diploids that are shifted to nitrogen-depleted medium (44), suggesting that Ime4p transmits both *MAT* and nitrogen signals. *IME4* does not encode a DNA binding protein, and its mode of action is not known. The third gene that mediates *MAT* regulation to *IME1* is *RES1*; a dominant mutation, *RES1-1*, promotes sporulation of MAT-insufficient diploids (14). *RES1* has yet to be cloned, but epistasis tests suggest that it acts in a pathway distinct from either Ime4p or Rme1p (14, 44). The nitrogen depletion signal seems to be transmitted to *IME1* via the RAS-cAPK pathway: mutations that cause lower activity of cAPK, such as *cdc25*, *ras2*, and *cyr1*, lead to the expression of *IME1* and to meiosis in the presence of nitrogen (references 26, 27, and 49 and references therein). On the other hand, mutations that cause constitutive activity of cAPK, such as *RAS2-val19* and *bcy1*, are sporulation deficient and are suppressed by overexpression of *IME1* (27). Mutations in several genes lower the level of *IME1* RNA; these include the serine-threonine protein kinase *MCK1* (34) and the DNA binding protein *RIM1* (50) and its proteolytic cleavage regulators *RIM8*, *RIM9*, and *RIM13* (22, 51).

In this paper we report a systematic analysis of the 5' untranslated region of *IME1* and identify the elements that are required for its regulated transcription. We show that *IME1* is regulated by an unusually large region that is composed of alternate negative and positive elements. Our analysis reveals the presence of distinct elements responding to *MAT*, carbon, and nitrogen regulation. We demonstrate that the RAS-cAPK pathway transmits a glucose signal to one of the regulated UAS elements, IREu. Moreover, gel-shift and expression assays show that Msn2p and Msn4p (Msn2/4p) function as the transcription factors mediating the UAS activity of IREu in the presence of acetate as the sole carbon source.

MATERIALS AND METHODS

Plasmids. Since in many cases multiple steps were involved in plasmid construction, here we describe only the structure of the plasmids, and details are available upon request. YEpl636 carries *MSN2* on a 2 μ m URA3 vector. In this paper we have used two types of single-copy shuttle vectors: ARS CEN and 2 μ m CEN. As previously reported, both vectors are relatively stable and are maintained at a copy number of about one per genome (53).

(i) *IME1*. The following plasmids carry the entire *IME1* gene with various portions of its 5' region on the yeast shuttle vector YCp50 (40): YCp51, *IME1* (-621 to +2132); YCp214, *IME1* (-4401 to +2132); YCpAS169, *IME1* (-915 to +2132); and YCpAS210, *IME1* (-1444 to +2132). The following plasmids carry the entire *IME1* gene with various portions of its 5' region on the yeast shuttle vector YCpLac33 (10): YCpAS174, *IME1* (-1641 to +2132); YCp1348, *IME1* (-1369 to +2132); and YCp1704, *IME1* (-2112 to +2132). The following plasmids carry portions of *IME1* on pUC119: YIpAS360, *IME1* (-4401 to +2132 with a deletion between -1641 to -1369 that was replaced by the *URA3* gene); and P2010, *IME1* (-1122 to -789).

(ii) *ime1-lacZ*. The following plasmids carry the *ime1-lacZ* gene with various portions of its 5' region. In these chimeric genes *IME1* at bp +202 was in-frame fused to the eighth amino acid of the *Escherichia coli lacZ* gene. The following constructs are carried on a CEN derivative of E357 (2 μ m vector) (30): YCpAS128, *IME1* (-4401 to +202); YCpAS133, *IME1* (-621 to +202); YCpAS134, *IME1* (-3015 to +202); YCpAS135, *IME1* (-1369 to +202); YCpAS146, *IME1* (-2112 to +202); YCpAS148, *IME1* (-915 to +202); YCpAS149, *IME1* (-1641 to +202); YCpAS152, *IME1* (-1444 to +202);

YCpAS198, *IME1* (-915 to 621 and -346 to +202); YCpAS340, *IME1* (-1369 to -1202 and -621 to +202); YCpAS341, *IME1* (-1369 to -1122 and -621 to +202); YCpAS344, *IME1* (-915 to -621 and -480 to +202); and YCp1457, *IME1* (-4401 to -1641 and -1369 to +202). The following constructs are carried on pUN75 (7): YCp1333, *IME1* (-756 to +202); YCp1376, *IME1* (-1122 to +202); YCp1379, *IME1* (-1369 to +202); YCp1427, *IME1* (-1153 to +202); YCp1476, *IME1* (-1202 to +202); YCp1477, *IME1* (-621 to +202); and YCp1956, *IME1* (-788 to +202).

(iii) *HIS4uas-ime1-his4-lacZ*. In the following plasmids various portions of the *IME1* 5' region were inserted in the *his4-lacZ* chimeric gene by using the X-1 construct carrying the *HIS4uas-his4-lacZ* gene with a deletion from -181 to -202, leaving 2 Gcn4p binding sites upstream of a *XhoI* site (31). The following chimeric genes are carried on the shuttle vector YCp50: YCp1138, carries no *IME1* information; and YCpAS334, *IME1* (-449 to -227). The following plasmids are carried on YIpLac128 (10): YIp2006, carries no *IME1* information; YIp2032, *IME1* (-915 to -788); YIp2055, *IME1* (-788 to -756); and YIp2067, *IME1* (-1202 to -1153).

(iv) *ime1-his4-lacZ*. In the following plasmids various portions of the *IME1* 5' region were inserted in the *his4-lacZ* chimeric gene by using the X-52 construct carrying the *his4-lacZ* gene (without a UAS) with a deletion from -144 to -316 and a *XhoI* linker at -144 (31). The following chimeric genes are carried on the shuttle vector YCp50: YCp1139, carries no *IME1* information; YCp336, *IME1* (-915 to -621); YCp1497, *IME1* (-1641 to -1369); YCp1689, *IME1* (-1639 to -915); YCp1692, *IME1* (-1641 to -1202); YCp1696, *IME1* (-1641 to -1122); YCp1975, *IME1* (-1641 to -1153); and YCp1981, *IME1* (-1122 to -915). The following construct is carried on pUN75 (7): YCp1391, *IME1* (-756 to -621). The following plasmids are carried on YIpLac128 (10): YIp2007, carries no *IME1* information; YIp1979, *IME1* (-1641 to -1122); YIp1980, *IME1* (-1641 to -1202); YIp1990, *IME1* (-1641 to -1153); YIp1994, *IME1* (-1153 to -1122); YIp2020, *IME1* (-1122 to -788); YIp2023, *IME1* (-915 to -788); and YIp2083, *IME1* (-788 to -756).

Yeast strains. The following yeast strains were used: Y419G, *MATa/MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112 ade2/ade2-R8 lys2/LYS2 his7/HIS7 can1-11/CAN1 trp5- Δ /TRP5*; Y419G1, isogenic to Y419G but *MATa/MATa*; Y419G- Δ UCS3 and Y419G1- Δ UCS3, isogenic to Y419G and Y419G1, respectively, but heterozygous for a deletion of upstream controlling sequence 3 (UCS3) (a one-step replacement of a portion of the *IME1* 5' region was accomplished following transformation of parental strains with an *EcoRI-ClaI* fragment carrying *IME1* [-4401 to -1641]-*URA3-IME1* [-1369 to -621] from YIpAS360); Y422, *MATa/MATa ura3-52/ura3-52 trp1- Δ /trp1- Δ leu2-3,112/leu2-3,112 ade2-1/ade2-R8 his4-519/HIS4 his6-1/HIS6 gal/GAL⁺ can1/CAN1*; Y424, isogenic to Y422 but *ime1::TRP1/ime1::TRP1* (9); Y1065, *MATa ura3-52 trp1- Δ leu2-3,112 his3::hisG ade2-R8 GAL⁺ CAN⁺ gal80::hisG gal4::hisG*; Y1093, isogenic to Y1065 but *cdc25-2::URA3* (a one-step replacement of *CDC25* was accomplished following transformation of Y1065 with a *SalI-PvuII* fragment carrying *cdc25-2::URA3* [27]); Y1132, isogenic to Y1065 but *msn2::HIS3 msn4::URA3* (a one-step replacement of *MSN2* and *MSN4* was accomplished by transformation with a *BamHI-SphI* fragment from pt32- Δ XB::HIS3 [8] and *EcoRI* fragment from pZfh45-GEX3X [8], respectively); Y1133, isogenic to Y1065 but *bcy1::URA3* (a one-step replacement of *BCY1* was accomplished by transformation with a *BamHI* fragment carrying *sral-20::URA3* [51a]). Correct replacements were checked by Southern blotting (data not shown). In addition, various *ime1-his4-lacZ* chimeric genes were integrated at the *LEU2* loci in various haploid and diploid strains. Integrative plasmids used were digested with *PpuMI* prior to transformation.

Media and genetic techniques. PSP2 (minimal acetate medium), and SPM (sporulation medium) have been described previously (18). Synthetic dextrose (SD) medium has also been described previously (46). Meiosis was induced as follows: cells were grown in PSP2 supplemented with the required amino acids to 10⁷ cells/ml, washed once with water, and resuspended in SPM (time zero; also designated SA). Yeast transformation with lithium acetate was done as described by Ito et al. (13). Proteins were extracted from at least three independent transformants and assayed for β -galactosidase activity as previously described (28, 39). Results are given in Miller units.

Gel-shift assays. The following complementary oligonucleotides were annealed to create the 38-bp IREd double-stranded DNA: UROdsal, 5' AATTC TTTCCGCTCTTCGAGGGAAAGGATCAAAGGCGCG, and UROdrI, 5' GAAAGGCAGAAAGCTCCCTTTCTAGTTCCGCGCAGCT. The following complementary oligonucleotides were annealed to create the 38-bp IREu double-stranded DNA: IREulr1, 5' AATTC TTTTCGCTCTTCGAGGGAAAGGATCAAAGGCGCG, and IREuls 5' TCGACGCGCTTTGATCCTTCCCCTCG AAGACGAAAAG. The UASrm DNA was isolated as a 127-bp *XhoI-BglII* fragment from P2010. These DNA fragments were end labeled with [α -³²P]dATP by using the Klenow enzyme. Gel-shift assays were performed essentially as described previously (2). The probe (300 ng) was incubated with 10 μ g of whole yeast cell extracts (prepared as described in references 2 and 44). The reaction mixture was applied to a 5% polyacrylamide gel. The gel was dried and exposed to both a phosphorimager and to X-ray film.

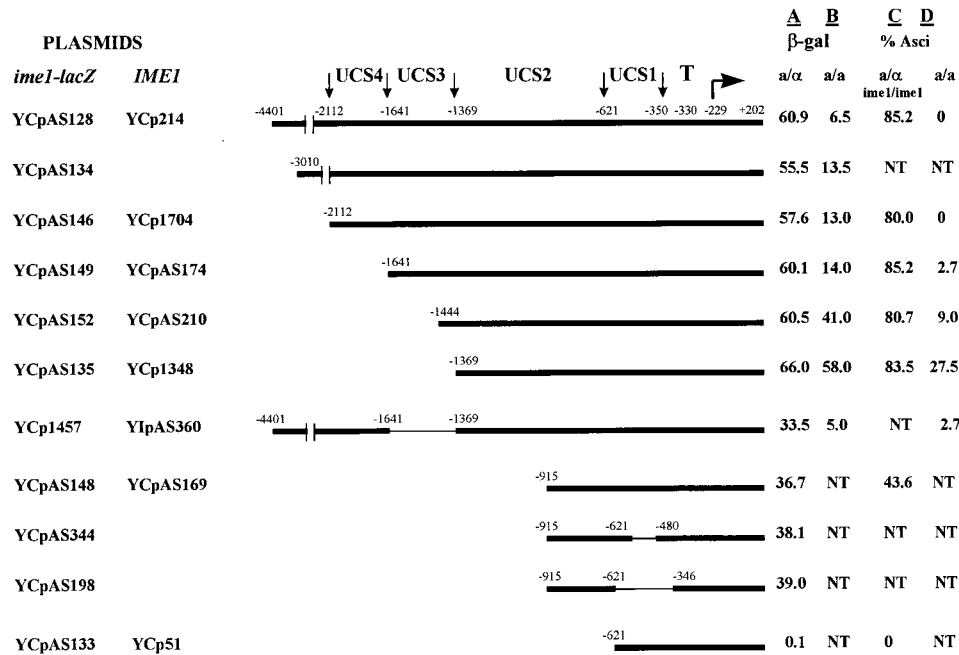


FIG. 1. The transcription of *IME1* is mediated by at least four UCSs (UCS1 to UCS4). Yeast strains carrying on a CEN plasmid either *ime1-lacZ* (columns A and B) or *IME1* (columns C and D) with various portions of the 5' upstream region were grown in PSP2 to 10^7 cells/ml. Cells were washed once in water and were resuspended in SPM. At 6 h in SPM proteins were extracted and β -galactosidase activities were determined. The levels of β -galactosidase are given in Miller units. The results are averages of three or four independent transformants. Standard deviations were less than 10%. At 72 h the percentages of asci were determined. NT, not tested. The following strains were used: Y419G (*MATa/MAT α*) (column A); Y419G1 (*MATa/MATa*, isogenic to Y419G (columns B and D); and Y424 (*MATa/MAT α* *ime1-0/ime1-0*) (column C). The borders of the designated UCS and the TATA box are designated UCS1 to UCS4 and T, respectively. The transcription initiation site is indicated.

RESULTS

At least four UCS (UCS1 to UCS4) mediate the regulated transcription of *IME1*. In a previous report (47) we compared the expression in β -galactosidase units of an *ime1-lacZ* chimeric gene present on a single-copy vector to the expression determined by Northern blot analysis of a genomic copy of *IME1*. The reported analysis showed that both methods gave similar results, i.e., the same pattern of regulation. This allowed us to make use of this *ime1-lacZ* chimeric gene for a deletion analysis aimed at identifying positive and negative elements that regulate the transcription of *IME1*. The validity of this approach, i.e., using *lacZ* chimeric genes on a single-copy vector rather than integrating the chimeric genes at a specific place in the genome, was further examined by additional methods. First, using Northern blot analysis we found that the transcription of an *ime1-lacZ* chimeric gene present on a single-copy vector was regulated in the same manner as the genomic copy of *IME1*, although the level of the *IME1* RNA was higher when expressed from the genomic copy (reference 1 and data not shown). Second, the levels of expression of various *lacZ* chimeric genes present on a single-copy vector were compared to the levels of expression of the same genes integrated in the genome. Although in the genome the chimeric genes gave rise to higher levels of expression, their regulation was identical in both cases (see Fig. 6 and data not shown). We assume that the lower levels of expression observed when the chimeric genes are placed on a single-copy vector is due to either plasmid loss or repression effects from the centromere.

Various portions of the 5' upstream region of *IME1* were fused in-frame to *lacZ* by using naturally occurring restriction sites. In these chimeric genes *IME1* was fused at amino acid 69

to the eighth amino acid of the *E. coli lacZ* gene. Proteins were extracted from *MATa/MAT α* diploids (derivatives of strain 419G) carrying these fusion genes, at 0 and 6 h in SPM, and from cells grown in SD to 10^7 cells/ml. The activity of β -galactosidase was determined. Figure 1 shows a schematic representation of the various plasmids used. In vegetative medium with either glucose or acetate as the sole carbon source the chimeric genes were not expressed, giving rise to less than 0.1 Miller units of β -galactosidase (data not shown). At 6 h in SPM, an *ime1-lacZ* fusion that extends to -1369 gave rise to essentially the same level of β -galactosidase as the one that extends to -4401 (Fig. 1, column A, compare YCpAS135 to YCpAS128, YCpAS134, YCpAS146, YCpAS149, and YCpAS152). On the other hand, an *ime1-lacZ* fusion that extends to -621 (Fig. 1, column A, YCpAS133) was not expressed. These results suggest that the region between -621 to -1369, designated UCS2, exhibits UAS activity. In order to confirm this conclusion we measured the abilities of plasmids carrying the entire *IME1* gene with various portions of the 5' upstream region to complement an *ime1* disruption allele, i.e., to promote sporulation to a *MATa/MAT α* *ime1-0/ime1-0* diploid. An *IME1* gene that extends to -1369 and one which carries *IME1* information up to -4401 bp gave rise to almost identical levels of sporulation (Fig. 1, column C, YCp214, YCp1704, YCpAS174, YCpAS210, and YCp1348), whereas an *IME1* gene that extends to -621 did not promote sporulation (Fig. 1, column C, YCp51). Partial deletion of UCS2 led to a decrease in both the level of expression and the efficiency of sporulation (Fig. 1, columns A and C, YCpAS148 and YCpAS169).

The induced level of expression of *IME1* that occurs upon nitrogen depletion depends on the presence of both *MATa*/*IME1*

and *MAT α 2* gene products and is absent in *MATa/MATa* diploids (15). In order to identify the site(s) that mediates this regulation, the above-described *ime1-lacZ* and *IME1* plasmids were transformed into an isogenic *MATa/MATa* strain. The level of β -galactosidase at 6 h in SPM and the percentage of asci following 72 h of incubation in SPM were measured. *MATa/MATa* diploids that carry the entire *IME1* gene with 5' regions extending up to at least -2112 are properly regulated, i.e., they do not sporulate (Fig. 1, column D, YCp214 and YCp1704). Lack of sporulation in these diploids correlates with a low level of expression of the corresponding *ime1-lacZ* chimeric gene in this *MATa/MATa* strain in comparison to the *MATa/MAT α* diploid (Fig. 1, column B, YCpAS128, YCpAS134, and YCpAS146). However, *MATa/MATa* cells that carry an *IME1* or an *ime1-lacZ* gene with a 5' region extending to -1369 do sporulate, and they show almost the same level of β -galactosidase as their isogenic *MATa/MAT α* diploids (Fig. 1, columns B and D, compare YCpAS128 to YCpAS135 and YCp214 to YCp1348). The discrepancy between an almost complete level of expression of *IME1* and a low efficiency of sporulation (66 versus 60 U of β -galactosidase and 27.5 versus 83.5% asci) suggests that the *MATa1* and *MAT α 2* gene products might be required in meiosis not only for the expression of *IME1* but also for an additional meiotic event. A similar conclusion was drawn when the level of *IME1* RNA was compared in *MATa/MAT α* and *MATa/MATa rme1 Δ* diploids or when *Ime1p* was overexpressed in *MATa/MATa* diploids. In both cases a high level of expression did not lead to a high percentage of asci (15).

The results presented in Fig. 1 suggest that upstream of -1369 reside a site or sites that repress the transcription of *IME1* in *MAT*-insufficient diploids. Further deletion analysis revealed the existence of two such sites, UCS3, which resides between -1369 and -1641, and UCS4, which resides between -1641 and -2112. Deletion of either UCS3 or UCS4 promotes low, inefficient sporulation in *MATa/MATa* diploids (Fig. 1, column D, compare YCp1704 to YCpAS174, YCpAS210, and YIpAS360). Note that deletion of UCS4 was checked in a *MATa/MATa* diploid carrying *IME1* on a CEN plasmid, whereas deletion of UCS3 was determined in a *MATa/MATa* diploid heterozygote for a genomic deletion of this element. A *MATa/MAT α* diploid heterozygote for the same deletion (strain Y419G Δ UCS3) sporulated with high efficiency (80.1% asci), suggesting that the low levels of sporulation obtained in the *MATa/MATa* diploid (Y419G1 Δ UCS3) are specific. Interestingly, the effect of UCS3 or UCS4 deletion could be observed only when sporulation was assayed, and the levels of β -galactosidase from *MATa/MATa* diploids carrying comparable *ime1-lacZ* genes were not affected (Fig. 1, column B compare YCpAS146 to YCpAS149 and YCp1457). These results indicate that very low levels of *Ime1p* suffice for initiation of meiosis and that deletion of both UCS3 and UCS4 elements relieves the requirement for *MATa* and *MAT α* for the expression of *IME1* and for meiosis.

Identification and function of UCS1. Figure 1 demonstrates that the region between the TATA box at -330 (1) and -621 does not carry any UAS element. Accordingly, two deletions in this region, between -480 and -621 and between -346 and -621, did not reduce the level of expression of these fusion genes in comparison to that of the control plasmid (Fig. 1, column A, compare YCpAS148 to YCpAS344 and YCpAS198). However, it is possible that this region is required to repress rather than activate the transcription of *IME1*. Indeed, *MATa/MAT α* diploids carrying an *ime1-lacZ* gene with a deletion between -346 and -621 (YCpAS198) gave rise to low, but elevated levels of β -galactosidase in vegetative cul-

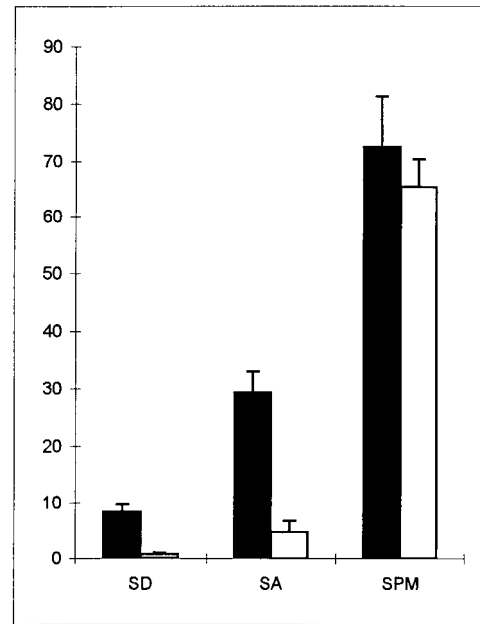


FIG. 2. UCS1 possesses URS activity in the presence of nitrogen. Strain Y419G carrying the *UASHis4-lacZ* (filled bars) or *UASHis4-UCS1-lacZ* (open bars) chimeric gene on a CEN vector (YCp1138 or YCpAS334, respectively) was grown as described in the legend for Fig. 1, and at 0 (SA) and 6 (SPM) h in SPM samples were taken to extract proteins and measure lacZ levels. In addition, proteins were extracted from cells grown in glucose medium (SD) to 10^7 cells/ml. The levels of β -galactosidase are given in Miller units. The results are averages of three or four independent transformants. Error bars indicate standard deviations.

tures: 2.5 U when glucose was the sole carbon source and 15.4 U in the presence of acetate as the sole carbon source. On the other hand, plasmids carrying the entire region gave less than 0.1 U in both glucose and acetate vegetative media. This region, designated UCS1, appears to play a role in nutrient repression of *IME1*.

In order to confirm the proposed negative role of UCS1, we inserted it downstream of *HIS4uas* in a *his4-lacZ* fusion and determined the level of *lacZ* in *MATa/MAT α* diploids (Y419G) carrying either this plasmid (YCpAS334) or the parental plasmid bearing the *HIS4uas-his4-lacZ* chimeric gene (YCp1138). Figure 2 shows that the level of expression of *UASHis4-lacZ* is increased in the presence of acetate as the sole carbon source in comparison to that in the presence of glucose as the sole carbon source. Moreover, as previously reported (6), the transcription of *HIS4* is further induced upon nitrogen depletion. Insertion of UCS1 upstream of a *HIS4* TATA box prevents the transcriptional activation function of *HIS4* UAS in vegetative culture with either glucose or acetate as the sole carbon source (Fig. 2, SD or SA, respectively) but has no effect upon nitrogen depletion (Fig. 2, SPM). UCS1 was also inserted upstream of a *his4-lacZ* fusion (plasmid YCp1139) that lacked its own UAS. The resulting chimeric gene (on plasmid YCpAS331) remained silent under all growth conditions, confirming that UCS1 does not possess any UAS activity and that it serves only as an upstream repression sequence.

UCS2 contains positive and negative elements. In order to identify the elements within UCS2 that are responsible for its regulated expression, 5' serial deletions of UCS2 were constructed in the *ime1-lacZ* fusion. The level of β -galactosidase following 6 h in SPM was determined for *MATa/MAT α* diploids carrying these chimeric genes. Figure 3 demonstrates that

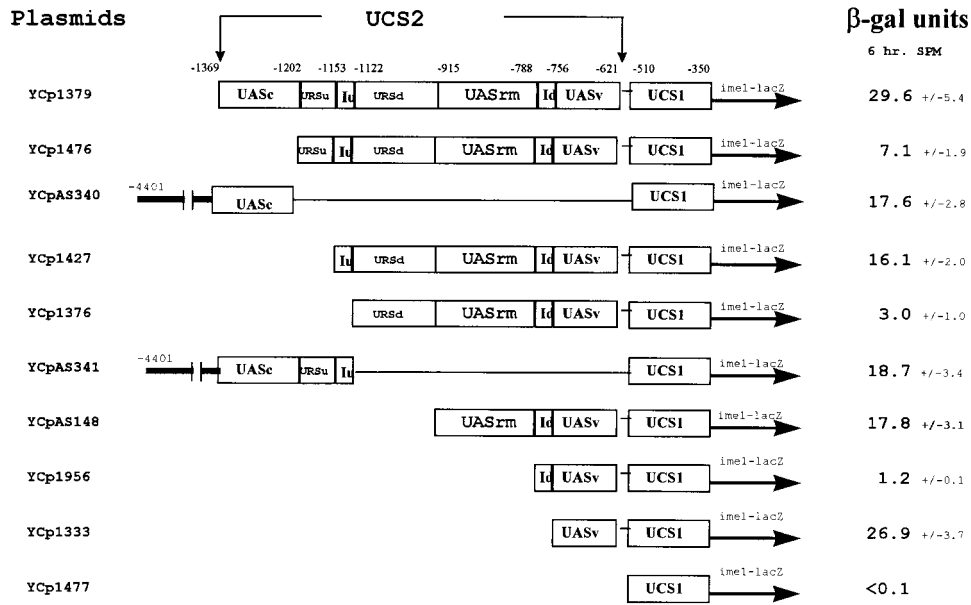


FIG. 3. UCS2 consists of positive and negative elements. *MATa/MAT α* diploids (Y422) carrying on a CEN vector various *ime1-lacZ* chimeric genes were grown in PSP2 to 10^7 cells/ml. Cells were washed once in water and were resuspended in SPM. At 6 h in SPM proteins were extracted and β -galactosidase activities were determined. The levels of β -galactosidase are given in Miller units. The results are averages of three or four independent transformants, and standard deviations are indicated.

UCS2 is made of alternate positive and negative elements. YCp1379 gave rise to 29.6 U of β -galactosidase. Deletion of 167 bp in YCp1476 led to only 7.1 U and a further deletion of 50 bp, as in YCp1427, led to 16.1 U, and so on.

The *ime1-lacZ* chimeric genes cannot be used to determine whether the UAS or URS activity of the various elements is subject to nutrient regulation. This is due to both the presence of UCS1 and translational regulation (47) that prevents expression in the presence of nitrogen. Therefore, characteriza-

tion of UCS2 was achieved following the insertion of these elements in a *his4-lacZ* gene lacking or carrying its own UAS. The level of β -galactosidase under various growth conditions was determined for *MATa/MAT α* diploids carrying these chimeric genes either integrated at the *LEU2* locus or on a CEN plasmid (Fig. 4 and 6). For simplicity, the various identified elements will be separately described, starting from the 3' element.

(i) **The UASv element.** An *ime1-lacZ* gene that extends to -756 was almost completely expressed (Fig. 3, compare

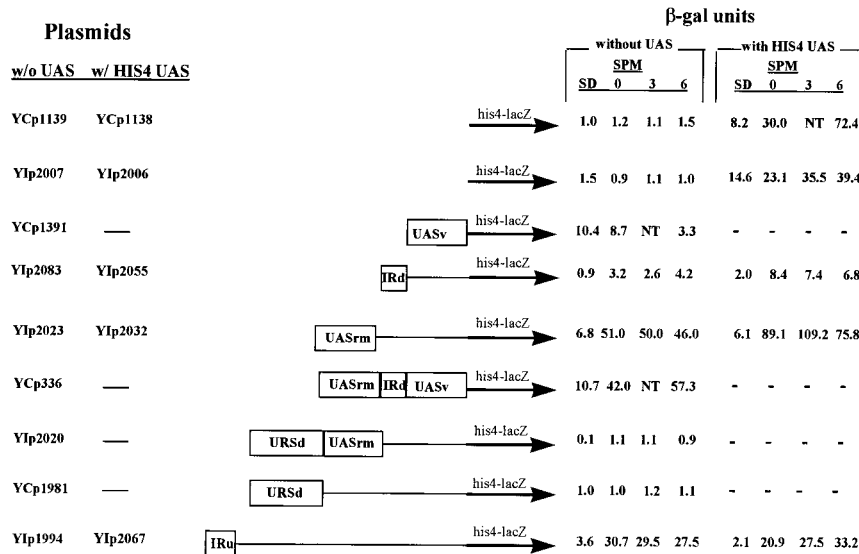


FIG. 4. UCS2 contains positive and negative elements that mediate expression of *his4-lacZ* and *HIS4uas-his4-lacZ*. Various elements from UCS2 were inserted into either the *HIS4uas-his4-lacZ* or *his4-lacZ* chimeric gene. Y422 strains carrying these genes on either a CEN vector (YCp plasmid) or integrated in the genomic *LEU2* gene (YIp plasmid) were grown as described in the legend for Fig. 1. Samples were taken to extract proteins and measure lacZ levels at 0, 3, and 6 h in SPM. In addition, proteins were extracted from cells grown in SD medium to 10^7 cells/ml. The levels of β -galactosidase are given in Miller units. The results are averages of three or four independent transformants. Standard deviations were less than 10%.

IREd -788 **TTTCCG TCTTCGAGGGAAAGGATCAAAGGCGC**
 ||| || ||||| ||||| ||||| ||||| ||||| ||||| |||||
IREu -1153 **TTTTTC TCTTCGAGGGGAAGGATCAAAGGCGC**
 || | || | || | || | || | || | || | || | || | || | || |
STRE CGATTTTCCAGGGGCTGT
SCBo **TTTTTCG TG**

FIG. 5. Sequence alignment of the two IRE elements in *IME1* to known UAS elements. The sequence of the STRE element is underlined. Given is the sequence used in band-shift assays as a probe for the binding of Msn2/4p (25). The SCB element (3) is in opposite orientation.

YCp1333 to YCp1379), whereas an *ime1-lacZ* gene that extends to -621 (YCp1477) was not expressed. These results suggest that the region between -621 to -756 exhibits a UAS activity, designated UASv (for UAS activity in vegetative cultures). Figure 4 demonstrates that insertion of this element upstream of the *his4-lacZ* chimeric gene promotes its expression in vegetative culture with either glucose or acetate as the sole carbon source (compare YCp1391 to YCp1139). Upon nitrogen depletion (6 h in SPM) lower levels of β -galactosidase are observed, suggesting that either UASv is less active in this condition or that it is totally inactive in this condition and that the activity observed is due to the stability of the β -galactosidase protein that is transcribed and translated in the vegetative cultures.

(ii) **The IRE elements.** Computer analysis of UCS2 revealed the presence of two almost identical 32-bp repeats designated IRE (*IME1* repeated element) (Fig. 5). The presence of such a large repeat suggests that it may recruit identical regulators. Therefore, using designed oligonucleotides and PCR, we constructed *ime1-lacZ* fusion genes whose 5' ends terminated either upstream to each one of the IRE repeats or downstream to these elements. Figure 3 demonstrates that the upstream element, IREu, serves as a UAS element: an *ime1-lacZ* chimeric gene that extends to the IREu element shows an about fivefold increase in expression compared to that of a chimeric gene that does not include this element (Fig. 3, YCp1427 YCp1376). Insertion of the IREu element upstream of the silent *his4-lacZ* gene promotes its expression (Fig. 4, compare YIp2007 to YIp1994, and Fig. 6, compare YIp1990 to YIp1979 and YCp1975 to YCp1696). Moreover, the UAS activity of the

IREu element is subject to nutrient regulation: low UAS activity is observed in vegetative medium with glucose as the sole carbon source, and an increase in UAS activity is observed in vegetative medium with acetate as the sole carbon source and in SPM upon nitrogen depletion. The lower level of expression in the presence of glucose may be due either to a lack of UAS activity or to specific repression of the UAS activity in this medium. In order to distinguish between these two possibilities we inserted the IREu element downstream of a *HIS4* UAS in a *his4-lacZ* chimera and determined its effect on the function of the *HIS4* UAS under various growth conditions. Figure 4 shows that the resulting *HIS4uas-IREu-his4-lacZ* chimeric gene (YIp2067) gives levels of β -galactosidase similar to that given by the *IREu-his4-lacZ* chimeric gene (YIp1994). Thus, under all growth conditions the *HIS4* UAS seems silent, suggesting that the regulatory protein(s) that binds to the IREu element interferes with the binding of Gcn4p to *HIS4* UAS. This analysis could not determine, therefore, if the IREu element also serves as a URS.

Surprisingly, the downstream element, IREd, exhibits URS activity: a chimeric gene carrying UASv and UCS1 is expressed, whereas the addition of the IREd element causes a 22-fold reduction in expression (Fig. 3, compare plasmid YCp1956 to YCp1333). Insertion of this element upstream of the silent *his4-lacZ* gene promotes low levels of expression, about 10-fold less than the ones observed for the *IREu-his4-lacZ* chimera (Fig. 4, compare YIp2083 to YIp1994). Insertion of IREd downstream to *HIS4* UAS in a *his4-lacZ* fusion results in a three- to sevenfold reduction in its activity under all growth conditions (Fig. 4, compare YIp2055 and YIp2006). However, since the positive element, IREu, also prevents the UAS activity of *HIS4*, further analysis is required to determine if IREd can repress any UAS or, specifically, UASv.

Gel-shift assays using either the IREu or IREd elements and total proteins extracted from yeast cells grown under the same conditions described above for the lacZ experiments, revealed the formation of two specific DNA-protein complexes (Fig. 7). Nonspecific competition with 10 \times and 100 \times plasmid pUC19 DNA did not reduce the formation of these complexes (data not shown), whereas specific competition showed such a decrease (Fig. 7A, compare lanes 8 and 9 to lane 3, and Fig. 7B,

Plasmids	β -galactosidase units							
	SD	plasmid			integrative			SD
		0	3	6	0	3	6	
 P1497	0.4	0.4	0.5	0.6	-	-	-	-
 P1692 P1980	16.0	17.1	17.1	13.9	54.3	45.3	40.4	41.5
 P1975 P1990	1.0	0.8	1.2	0.8	8.8	8.4	16.5	10.2
 P1696 P1979	8.0	13.4	26.3	29.8	21.1	30.8	39.8	48.2
 P1689	0.7	0.6	1.0	1.1	-	-	-	-
 P1139 P2007	1.0	1.2	1.1	1.5	1.5	0.9	1.1	1.0

FIG. 6. Effects of various elements present in *IME1* UCS2 on the expression of *his4-lacZ*. Various elements from UCS2 were inserted into the *his4-lacZ* chimeric gene. Y422 strains carrying this gene on either a CEN vector (YCp plasmid) or integrated in the genomic *LEU2* gene (YIp plasmid) were grown as described in the legends for Fig. 1 and 4. The levels of β -galactosidase are given in Miller units. The results are averages of three or four independent transformants. Standard deviations were less than 10%.

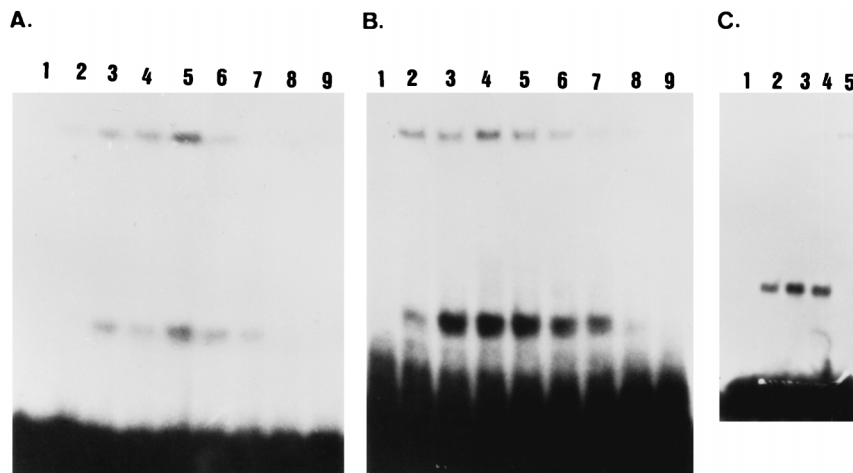


FIG. 7. Two protein-DNA complexes are formed on the IRE elements. Gel retardation assays were performed with 300 ng of ^{32}P -end-labeled 38-bp double-stranded oligonucleotides IREulr1 and IREuls, creating IREu (A and C), or UROdsal and UROdr1, creating IREd (B), and 30 ng of cold plasmid pUC19 DNA. Lane 1 is free probe. Proteins were extracted from cells grown in minimal glucose medium to 10^7 cells/ml (panels A and B, lane 2; panel C, lanes 2 and 4) or from cells pregrown in PSP2 to 10^7 cells/ml, washed once in water, and resuspended in SPM for 0 (panels A and B, lanes 3 and 6 to 9; panel C, lanes 3 and 5), 3 (panels A and B, lane 4), and 6 (panels A and B, lane 5) h. For lanes 6 and 7 cold IREd was added in excess molar ratios of 0.1 and 0.5, respectively. For lanes 8 and 9 cold IREu was added in excess molar ratios of 0.1 and 0.5, respectively. The strains used were as follows: Y419G, a wild-type diploid (panels A and B); Y1065, a wild-type haploid (panel C, lanes 2 and 3), and Y1132, an *msn2Δ msn4Δ* haploid (panel C, lanes 4 and 5).

compare lanes 6 and 7 to lane 3). Moreover, since the addition of the nonspecific competitor increases the level of these complexes (data not shown), in the experiment reported in Fig. 7 each lane also included cold plasmid pUC19 DNA. Figure 7A demonstrates that the formation of the IREu-protein complexes is subject to nutrient regulation: in the presence of glucose very low levels are observed, and about a twofold increase is observed in vegetative medium with acetate as the sole carbon source (phosphorimager calculations). Upon nitrogen depletion the intensity of the complexes is further increased. Thus, the regulated UAS activity of IREu, i.e., low in glucose and high in acetate and upon nitrogen depletion, mirrors the formation of the two DNA-protein complexes on this element. This analysis suggests that IREu serves only as a UAS element (but see below).

Figure 7B demonstrates that the same DNA-protein complexes that are formed on IREu are also formed on IREd. However, for IREd, the lower-molecular-weight complex seems constitutive in the presence of acetate (compare intensities in lanes 3 to 5). The different affinity of the regulatory proteins to each element can be revealed by competition analysis: Fig. 7 demonstrates that the IREu element is a better competitor than the IREd element when the probe is made either of IREu (Fig. 7A, compare lanes 6 and 7 to 8 and 9) or IREd (Fig. 7B, compare lanes 6 and 7 to 8 and 9). The difference in binding affinity pinpoints the two mismatched regions as the binding sites for the regulatory proteins and explains the lower UAS activity of this element compared to that of the IREu element (Fig. 4 and 5).

(iii) **The UASrm element.** Figure 3 identifies the presence of a UAS element, designated UASrm (regulated middle), from bp -788 to -915 . An *ime1-lacZ* gene whose 5' end extends to this element is expressed, whereas a chimeric gene ending prior to this element is not expressed (Fig. 3, compare YCpAS148 to YCp1956). The nature of this element, i.e., regulated or constitutive, was revealed by inserting it in the *his4-lacZ* reporter gene. Figure 4 demonstrates that UASrm exhibits nutrient-regulated UAS activity: it promotes low expression in the presence of glucose and exhibits high activity in acetate and sporulation media (Fig. 4, compare YIp2023 to

YIp2007). In vegetative medium with acetate as the sole carbon source, and upon nitrogen starvation, the presence of UASrm does not interfere with the activity of *HIS4* UAS, and additive levels of β -galactosidase are observed (Fig. 4, compare YIp2032 to YIp2023 and YIp2006). Gel-shift assays confirm these conclusions. Figure 8 shows the binding of a specific protein(s) to UASrm. The level of the bound protein is regulated by nutrients: a low level is present in the presence of glucose, and an eightfold increase (calculated by phosphorimager) is observed in acetate-containing medium. These results reflect the regulated UAS activity of this element. Figure 4 shows a slight reduction in the activity of UASrm at 6 h in SPM (46 versus 50 U); however, a more pronounced reduction (fourfold) is observed for the formation of the DNA-protein complex (Fig. 8, compare SPM to SA). We suggest that this discrepancy is due to the stability of the *lacZ* protein.

(iv) **URSd.** Upstream of UASrm, the region between -915 and -1122 possesses URS activity. An *ime1-lacZ* chimeric gene extending to -915 is expressed, whereas an *ime1-lacZ* chimeric gene extending to -1122 shows about sixfold reduction in expression (Fig. 3, compare YCpAS148 to YCp1376). An *his4-lacZ* gene carrying this element is not expressed (Fig. 4, YCp1981), suggesting that it exhibits no UAS activity. The URS activity of URSd is not subject to regulation and is observed under all growth conditions: it lowers the UAS activity of *IME1* UASs in glucose and acetate media and in SPM (Fig. 4, compare YIp2020 to YIp2023, and Fig. 6, compare YCp1689 to YCp1696).

(v) **URSs.** Figure 3 indicates that the region between -1153 and -1202 exhibits URS activity. Addition of this element 5' to an *ime1-lacZ* gene that extends to -1153 caused twofold repression (Fig. 3, compare expression from YCp1476 to that from YCp1427). Addition of this element 3' to *IME1* UASc in an *his4-lacZ* chimeric gene caused between 2- and 20-fold repression under all growth conditions (Fig. 6, compare expression from YIp1990 to that from YIp1980 and expression from YCp1975 to that from YCp1692).

(vi) **UASc.** Figure 3 indicates that the region between -1202 to -1369 exhibits UAS activity. A fourfold increase in expression was observed when this element was added to a plasmid

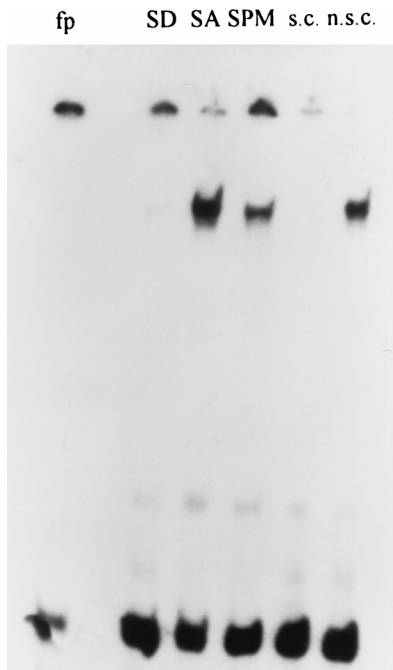


FIG. 8. A single protein-DNA complex is formed on UASrm. Gel retardation was performed with 300 ng of a 32 P-end-labeled UASrm element present on a 127-bp *XhoI-BglIII* fragment derived from p2010. Proteins were extracted from cells of strain Y419G grown in minimal glucose medium (SD) to 10^7 cells/ml or from cells pregrown in PSP2 to 10^7 cells/ml, washed once in water, and resuspended in SPM for 0 (SA) and 5 h (SPM). For competition, cold plasmid pUC19 DNA (n.s.c) or UASrm DNA (s.c) was added in excess molar ratios of 10 to protein samples extracted at 5 h in SPM. fp, free probe.

lacking it (Fig. 3, compare expression from YCp1379 to that from YCp1476 and expression from YCpAS340 to that from YCp1477). The UAS activity of this element is constitutive under all growth conditions. This is demonstrated when UASc (constitutive) is inserted upstream of *his4-lacZ* (Fig. 6, compare YCp1692 and YIp1980 to YCp1497 and YIp2007).

The glucose signal is transmitted to IREu via the RAS-cAPK pathway and the Msn2/4p transcription factors. An active RAS-cAPK pathway represses the transcription of *IME1* (27). We determined if this effect is mediated via any of the regulated UAS elements of *IME1* by examining the effect of a temperature-sensitive mutation in *CDC25* (RAS exchange factor [4]) on their UAS activity. Table 1 demonstrates that the level of expression of a *UASrm-his4-lacZ* chimeric gene is iden-

tical in wild-type and *cdc25-2* haploids grown at either the permissive or restrictive temperature. Thus, UASrm is not the target for the RAS-cAPK pathway. On the other hand, the UAS activity of IREu is increased in *cdc25-2* isogenic strains; in SD medium, a 6- to 10-fold increase is observed. This increase is already evident at the permissive temperature, and higher levels are observed at the nonpermissive temperature. At 34°C the level of expression of *IREu-his4-lacZ* is higher than the level observed for the wild-type strain in SA medium (30.8 versus 10.4 U), suggesting that Cdc25p transmits a glucose signal that prevents the UAS activity of IREu. Cdc25p is required to activate Rasp; however, Rasp activates two signal pathways, that of the cAPK and the pheromone-induced mitogen-activated protein kinase cascade (8a, 11). In order to establish that for IREu Cdc25p modulates the activity of cAPK, we examined the effect of deletion of the regulatory subunit of cAPK, *BCY1*, on the level of expression of *IREu-his4-lacZ*. Table 1 demonstrates that in *bcy1Δ* strains IREu does not activate transcription. We conclude that the RAS-cAPK pathway transmits a glucose signal to IREu.

What DNA binding protein serves as a target for the RAS-cAPK pathway and binds to and activates IREu? In order to identify positive regulators, we screened for multicopy plasmids that increase the expression of an *IREu-cyc1-lacZ* chimeric gene (38). Several genes were identified, including *MSN2*. Table 1 demonstrates that when *MSN2* is carried on a multicopy plasmid a twofold increase in the UAS activity of IREu is observed in glucose medium. Deletion of *MSN2* has no effect (data not shown). Nonetheless, this negative result may be due to the presence of its close homolog, *MSN4* (8). Indeed, in the isogenic, *msn2Δ msn4Δ* double mutant, IREu shows no UAS activity (Table 1). *MSN2-MSN4* encode a transcriptional activator (8) that binds to and activates STRE elements (25, 43). Sequence analysis reveals that IREu carries such an element (Fig. 5). We used gel-shift assays to determine if Msn2/4p bind to IREu. As described above, Fig. 7 demonstrates that two DNA-protein complexes are formed on IREu. The levels of these DNA-protein complexes are higher in Fig. 7A and B than in Fig. 7C. This is probably due to the fact that for the results shown in the former panels proteins were extracted from diploid cells, whereas for the results in the latter panel protein were extracted from haploid cells. In vegetative medium with acetate as the sole carbon source the lower-molecular-weight DNA-protein complex disappears when proteins are extracted from the *msn2Δ msn4Δ* double mutant (Fig. 7C, compare lanes 3 to lane 5). Interestingly, in glucose medium deletion of both *MSN2* and *MSN4* has no effect (Fig. 7C, compare lane 2 to 3), suggesting that in glucose and acetate

TABLE 1. The RAS/cAPK pathway prevents the UAS activity of IREu in the presence of glucose

Strain genotype ^a	Temp (°C)	Level of β-galactosidase in ^b :			
		IREu		UASrm	
		SD	SA	SD	SA
Wild type	25	0.8 ± 0.14	8.2 ± 0.56	10.7 ± 1.18	46.1 ± 4.69
	30	0.88 ± 0.22	8.55 ± 0.25	7.4 ± 2.34	51.2 ± 9.0
	34	3.4 ± 0.42	10.4 ± 0.85	21.3 ± 6.71	54.4 ± 6.69
<i>cdc25-2</i>	25	5.4 ± 0.57	55.3 ± 3.89	8.1 ± 1.48	64.2 ± 11.5
	34	30.8 ± 2.19	163.0 ± 8.41	4.2 ± 0.8	56.0 ± 7.07
<i>bcy1Δ::URA3</i>	30	0.4 ± 0.18	0.82 ± 0.28		
<i>msn2Δ msn4Δ</i>	30	0.15 ± 0.03	0.1 ± 0.01		
[<i>MSN2</i>]	30	1.82 ± 0.13	8.21 ± 1.77		

^a The following isogenic strains were used: Y1065 (wild type), Y1093 (*cdc25-2*), Y1133 (*bcy1Δ::URA3*), Y1132 (*msn2Δmsn4Δ*), Y1065 carrying YEP1636, ([*MSN2*]).

^b Levels of β-galactosidase (± standard deviations) are given in Miller units.

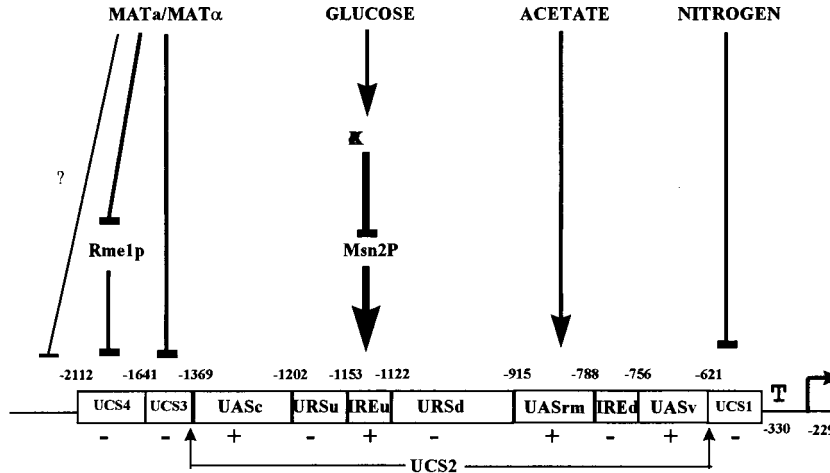


FIG. 9. Schematic structure of *IME1* 5' untranslated region. The elements that respond to the various meiotic signals, *MAT*, glucose, acetate, and nitrogen, are illustrated. A positive role is marked by an arrow, whereas a negative role is marked by a line. ?, preliminary result; +, UAS; -, URS.

media this band consists of different proteins. We assume that, normally, in SA medium only Msn2/4p bind the STRE element, whereas the protein that binds IREu DNA in glucose medium may be either degraded or excluded from binding. Further work is required to establish if this protein functions in glucose medium as a transcriptional activator or repressor.

DISCUSSION

Using systematic deletion analysis of an *ime1-lacZ* fusion we demonstrate that the transcription of *IME1* is regulated by a remarkably large region, over 2,100 bp long, which is the largest region known in *S. cerevisiae*. In comparison, the *HO* gene, another gene with a long and complex promoter, is made of only 1,400 bp (32). Preliminary data suggest that 5' of the 2,100-bp upstream region of *IME1* there are yet additional elements that control its transcription: Granot et al. (12) showed that multiple copies of an 0.5-kb *XhoI-BglIII* fragment from positions -3166 to -3762 of *IME1* promote low levels of sporulation to a *MATa/MATa* diploid. They proposed that a titration of a negative regulator that binds to this region is responsible for derepression of the transcription of *IME1* in this mater diploid (12). In agreement, in this report we demonstrate that deletion of this region promotes a twofold increase in the expression of *ime1-lacZ* in *MATa/MATa* diploids (Fig. 1 column B, compare YCpAS128 and YCpAS134).

A schematic map of *IME1* is illustrated in Fig. 9. For simplicity, the 5' region of *IME1* was divided into four UCS, UCS1 to UCS4. UCS1, UCS3, and UCS4 function as negative elements, whereas UCS2 functions as a positive element that is absolutely required for the transcription of *IME1*. UCS1 and UCS2 mediate nutrient regulation: UCS1 prevents the transcription of *IME1* in the presence of nitrogen, whereas UCS2 promotes its transcription in the presence of a nonfermentable carbon source, i.e., acetate versus glucose. UCS3 and UCS4 repress the transcription of *IME1* in *MAT*-insufficient cells. This organization of the *IME1* 5' region explains its mode of transcription. In vegetative medium with glucose as the sole carbon source, *IME1* is silent, since two of its UAS elements, IREu and UASrm, are not active. On the other hand in vegetative medium with acetate as the sole carbon source, the UAS activity of these elements leads to the low levels of *IME1* RNA. Complete activation requires lack of repression from

UCS1, UCS3, and UCS4, a condition that occurs only in *MATa/MATa* diploids upon nitrogen depletion.

UCS4 (-1641 to -2112) represses the transcription of *IME1* in cells that do not carry both *MATa1* and *MATa2* gene products. This result is in agreement with that reported by Covitz and Mitchell (5) showing that a region between -2243 and -1743 consists of a negative element that transmits the *MAT* signal. UCS4 carries the binding site for Rme1p (5), a negative regulator that mediates *MAT* repression to *IME1* (15). In this report we show that the *MAT* signal is also transmitted via UCS3. Sequence analysis reveals that UCS3 does not carry the Rme1p binding site. Further analysis is required to identify the gene(s) whose product(s) binds to and regulates this element. Here we demonstrate that deletion of both UCS3 and UCS4 is required for complete derepression (Fig. 1). In agreement, Covitz and Mitchell (5) have shown that an *IME1* gene that extends to -2001 (deletion of only UCS4) gives rise to 18% asci in *MATa/MATa* cells, whereas an *IME1* gene that extends to -1202 (deletion of both UCS3 and UCS4) gives rise to a higher level, i.e., 34%. Covitz and Mitchell (5) suggested that only the UCS4 region transmitted a *MAT* signal, since an *IME1* gene that extends to -2243 and carried a deletion between -1122 and -1743 gave only 1.3% asci in *MATa/MATa* cells. Two reasons may explain their inability to identify UCS3. (i) The *IME1* gene used in that study was not efficiently expressed, since two positive regulators, designated here as UASc and IREu, were also deleted. (ii) Deletion of only UCS3 is not sufficient for complete derepression.

Deletion analysis reveals that UCS2 consists of alternate positive and negative elements (Fig. 9). Previously, a less detailed deletion analysis divided UCS2 into only two positive elements, UASd and UASu (45), which consist of UASv, IREd, and UASrm and of URSd, IREu, and UASc, respectively. The presence of UCS1 prevents the characterization of these elements in the intact *IME1* or *ime1-lacZ* gene. Moreover, since nutrients also regulate the translation of *IME1* (47), the expression of the various *ime1-lacZ* chimeric genes could be determined only under sporulation conditions. Therefore, the various elements were inserted in a heterologous reporter gene, *his4-lacZ*, carrying or lacking its own UAS. This analysis reveal that UCS2 is made of the following elements: a constitutive UAS element, UASc, that promotes expression of a reporter gene under all growth conditions; three constitutive

URS elements, URSu, URSd, and IREd; a UAS element, UASv, that promotes expression in vegetative media; and two regulated UAS elements, IREu and UASrm, that promote expression of reporter genes in the presence of acetate. Accordingly, in the presence of acetate as the sole carbon source the abundance of specific DNA-protein complexes, formed on these regulated UASs, is higher than in the presence of glucose (Fig. 7 and 8).

One of the striking features of the analysis reported here is the presence of two, almost identical repeats, IREu and IREd (Fig. 5), that in the context of *IME1* sequences are not identical in function. This difference is also reflected by both the UAS activity of these elements in the heterologous *his4-lacZ* gene (Fig. 4) and the different affinities in the binding of specific proteins to these elements (Fig. 7). Figure 5 shows that an A to G mutation in the IREu element creates an AGGGG STRE. STRE elements function as UAS that respond to various stresses including nitrogen depletion (24, 42). STRE elements are activated by the binding of the transcriptional activator Msn2p or its homolog Msn4p (25, 43), suggesting that these proteins also bind to and regulate the function of IREu. Indeed, cells deleted for both *MSN2* and *MSN4* show the elimination of a specific DNA-protein band from IREu (Fig. 7C) and a substantial reduction in the UAS activity of IREu (Table 1). The IREd element carries the AGGGA PDS element to which *in vitro*-made Msn2p and Msn4p do not bind (25), explaining why *in vivo*, by itself, the IREd element serves as only a weak UAS.

The second mismatch between the IRE elements creates, in IREu, the sequence TTTTCGTC, which is almost identical (7 of 8 nucleotides) to the known cell cycle box (SCB) CACGA AAA (3) (Fig. 5). SCBs serve as UAS elements upon exit from G₁ arrest and are present in the *HO* gene as well as in the G₁ cyclins *CLN1* and *CLN2* (3, 33, 35). We suggest that the upper DNA-protein complex on the IRE elements (Fig. 7) is formed on this sequence. Transcription mediated by SCB elements is accomplished by the binding of the Swi4p and Swi6p transcription factors (20, 33, 35). We determined, therefore, the role of these proteins in regulating the expression of both the *ime1-lacZ* and *IREu-his4-lacZ* chimeric genes. Deletion of either *SWI4* or *SWI6* resulted in a three- to fourfold increase in the expression of both genes (38). The Swi4p-Swi6p complex activates transcription only upon progression from G₁ to the mitotic S phase (19, 20), explaining why the transcription of *IME1* is induced upon G₁ arrest (47). Thus, the Swi4p-Swi6p complex is a positive regulator for initiation of the mitotic cell cycle and a negative regulator for initiation of meiosis. Similarly, Rme1p is a positive regulator for the transcription of the G₁ cyclin *CLN2* and a negative regulator for the transcription of *IME1* (15, 29, 52).

In this report we demonstrate that the RAS-cAPK pathway prevents the UAS activity of IREu in the presence of glucose (Table 1), whereas the transcriptional activators Msn2/4p are required for both the formation of a specific DNA-protein complex on IREu and its UAS activity in acetate medium (Fig. 7C and Table 1). Since *in vitro*-made Msn2p binds to the STRE element (25, 43) that is also present in IREu (Fig. 5), we suggest that cAPK mediates its effect on IREu via Msn2/4p (Fig. 9). Accordingly, in a recent meeting it was reported (12a) that nuclear localization of Msn2p is modulated by cAPK: in the presence of high cAPK activity, or in nonstress conditions, Msn2p is confined to the cytoplasm, whereas upon stress it is translocated to the nucleus.

In this report we show that the RAS-cAPK pathway transmits a glucose signal to IREu. On the other hand, previous reports have shown that this pathway transmits a nitrogen

rather than a glucose signal to both *IME1* and meiosis (references 26 and 27 and references therein). Since in the latter case the entire *IME1* 5' region was examined, it is possible that this pathway, with a different element (UCS1?), also transmits a nitrogen signal to *IME1*. However, biochemical evidence suggests that the RAS-cAPK pathway transmits a glucose rather than a nitrogen signal (54) and that the level of cyclic AMP is not affected by nitrogen (36). We suggest, therefore, that the complex organization of the *IME1* 5' region is responsible for this discrepancy. We propose that nitrogen repression through UCS1 can be relieved only upon full activation of both IREu and UASrm. Thus, in the presence of glucose, activation of the IREu element by low cAPK activity does not suffice to overcome UCS1 repression and cells do not initiate meiosis in glucose media. However, in acetate media, when UASrm is also activated, UCS1 is neutralized and *IME1* is expressed.

IME1 encodes the master regulator of the developmental pathway, meiosis. It is not surprising, therefore, that the transcription of *IME1* is mediated by an extremely large and complex region. The *IME1* 5' region consists of constitutive as well as regulated positive and negative elements. The combinatorial effect of the entire region leads to the regulated transcription of *IME1*, i.e., silent in vegetative media with glucose as the sole carbon source, low levels of mRNA in vegetative acetate media, and increased levels upon nitrogen depletion. We suggest that the use of many negative elements is essential to restrict transcription to the appropriate conditions, since any deviation may lead to the initiation of meiosis at the wrong time or condition, resulting in cell death.

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