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Meiotic Induction of the Yeast *HOP1* Gene Is Controlled by Positive and Negative Regulatory Sites

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The process of meiosis and sporulation in the yeast *Saccharomyces cerevisiae* is a highly regulated developmental pathway dependent on genetic as well as nutritional signals. The *HOP1* gene, which encodes a component of meiotic chromosomes, is not expressed in mitotically growing cells, but its transcription is induced shortly after yeast cells enter the meiotic pathway. Through a series of deletions and mutations in the *HOP1* promoter, we located two regulatory sites that are essential for proper regulation of *HOP1*. One site, called $URS1_H$, brings about repression of *HOP1* in mitotic cells and functions as an activator sequence in cells undergoing meiosis. The second site, which we designated UAS_H, acts as an activator sequence in meiotic cells and has similarity to the binding site of the mammalian CCAAT/enhancer binding protein (C/EBP). Both sites are required for full meiotic induction of the *HOP1* promoter. We conclude that in mitotic yeast cells, the $URS1_H$ site maintains the repressed state of the *HOP1* promoter, masking the effect of the UAS_H site. Upon entry into meiosis, repression is lifted, allowing the $URS1_H$ and UAS_H sites to activate high-level transcription.

When cells commit to meiosis, they undergo a single round of replication and then homologous chromosome pairing, meiotic recombination, and reductional segregation of the homologs during the first meiotic division. In yeast cells, the second chromosomal division is followed by the packaging of the four haploid products of meiosis into spores (for a review, see reference 7). A number of genes have been discovered (*SPO11*, *MER1*, *HOP1*, *SGA1*, *SPO16*, *MEI4*, *RED1*, and *SPO13*) that are known to function exclusively during meiosis and whose expression is restricted to a characteristic time in the meiotic pathway (1, 6, 12, 17, 20, 22, 35, 37). It is appealing to imagine that these genes are regulated in a coordinated fashion to ensure that they are expressed at the proper time and level during meiosis.

The signals that control entry into meiosis by the yeast *Saccharomyces cerevisiae* have been well studied and include a specific set of genetic as well as nutritional conditions (for a review, see reference 21). Genetically, a cell must contain both mating-type loci (*MAT*α and *MAT***a**) to express both the α2 and **a**1 proteins. These proteins function together to repress transcription of the *RME1* gene, which encodes a repressor of meiosis (23). The absence of RME1 protein allows expression of the IME1 protein, an inducer of meiosis (14). The IME1 protein, in turn, functions to induce expression of *IME2*, a second inducer of meiosis (29, 30). The nutritional signal required for the induction of meiosis, nitrogen starvation, feeds into the regulatory cascade by serving as an activator for expression of the *IME1* gene product and possibly at other points downstream in the pathway (14). Once these conditions are met, a cascade of regulatory proteins results in the timed expression of meiosis-specific genes. A number of other genes have been identified, named *UME1* to *UME5*, that allow unscheduled expression of meiosis-specific genes (32). It has been postulated that the *UME* genes are acting as repressors in the regulation cascade and appear to function downstream of *IME2* regulation.

In this report, we examine the regulation of *HOP1*, a gene expressed early in meiosis. The *HOP1* gene was isolated from *S. cerevisiae* by using a screen designed to detect mutants specifically defective in homologous chromosome pairing, which occurs during prophase I of meiosis. Therefore, *hop1* diploids produce spores that are chromosomally imbalanced and inviable (11). The observation that a fragment containing the *HOP1* sequence along with 207 bp upstream of the *HOP1* translation start site is able to complement a *hop1* diploid suggested that all the promoter and regulatory sites required for the proper expression of *HOP1* lie within this small upstream region (12). In this report, we describe the isolation of two sites within the 207-bp promoter region that are required for proper transcriptional regulation and full expression of the *HOP1* gene. One of these sites, which we call $URS1_H$, has also been found to regulate genes involved in nitrogen metabolism (34) as well as other meiosis-specific genes (3). The second site, called UAS_H, is similar to the site of action of the mammalian C/EBP factor and has not been previously implicated in the control of meiosis-specific genes.

MATERIALS AND METHODS

Yeast strains and media. The genotypes of the strains used in this study are presented in Table 1. Strain AJ87 is the diploid product of a mating between strains EG123 and 246-1-1 (28). Strains dip17-2(2-26), 2273-1-2, and 5762-6-2 are from Hollingsworth and Byers (11). 7234 is from T. Formosa. The diploid YV16 containing the various *hop1-lacZ* test constructs was generated by transforming one of the haploid parents (either 5762-6-2 or 2273-1-2) with the appropriate plasmid and then mating to the other haploid parent. This was necessary because the selectable marker was *URA3* and the YV16 diploid is heteroallelic at *URA3*. This particular genetic background was chosen to be consistent with that used in previous work (12). Strains CKY11 and CKY54A were provided by Cindy Keleher. Strains

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TABLE 1. Yeast strains

Strain	Genotype
AJ87	a *trp1 leu2 ura3 his4*
	α *trp1 leu2 ura3 his4*
dip17-2(2-26)	a *LEU2 HIS4 trp1 ura3 CAN1 CYH2 ADE2*
	α *leu2 his4 trp1 ura3 can1 cyh2 ade2-1*
	SPO13 hop1-1
	spo13::pNH20-5 *hop1-1*
7234	a *HIS4 TRP1 ura3-x can1 leu1 cyh2 ade2-R8*
	α *his4 trp1 ura3-52 CAN1 LEU1 CYH2 ADE2*
2273-1-2	α *ura3-52 ade2-1 his7*
5762-6-2	a *ura3-x ade2-R8 trp1 can1 cyh2*
YV16	α *trp1 ura3-52 can1 cyh2 ade2-R8 his7*
	a *TRP1 ura3-x CAN1 CYH2 ade2-1 HIS7*
CKY11	a *ura3 leu2 trp1 his4 ssn6Δ9*
CKY54A	α *ura3 leu2 his4 tup1*::*LEU2*
AMP107	a *ura3 leu2 trp1 ho*::*LYS2*
AMP109	a *ura3 leu2 trp1 ho*::*LYS2*
	α *ura3 leu2 trp1 ho*::*LYS2*
AMP115	a *ura3 leu2 trp1 ho*::*LYS2 ime1-12*::*TRP1*
	α *ura3 leu2 trp1 ho*::*LYS2 ime1-12*::*TRP1*
AMP118	a *ura3 leu2 trp1 ho*::*LYS2 rme1-5*::*LEU2*
AMP245	a *ura3 leu2 trp1 ho*::*LYS2 ime2-2*::*LEU2*
	α *ura3 leu2 trp1 ho*::*LYS2 ime2-2*::*LEU2*
RSY10	a *ura3 leu2 trp1 his3 ade2 ade6 can1*
RSY82	a *ura3 leu2 trp1 his3 ade6 can1 ume1-1*
RSY94	α *ura3 leu2 trp1 his4-C can1 ume2-2*
RSY128	α *ura3 leu2 trp1 his3 ade2 ade6 can1 ume3-1*
RSY143	α *ura3 leu2 his4-C ade6 can1 ume4-1*
RSY104	a *ura3 leu2 his3 ume5-2*

AMP107, AMP109, AMP115, AMP118, and AMP245 were provided by Aaron Mitchell, and strains RSY10, RSY82, RSY94, RSY104, RSY128, and RSY143 were provided by Randy Strich.

Sporulation medium (SPM) is 3% potassium acetate and 0.02% raffinose. Yeast extract-peptone-dextrose (YEPD) medium consists of 2% bacto-peptone, 1% yeast extract, and 2% dextrose. Synthetic medium (5) was made by the addition of the appropriate drop-out powders (26) to 0.7% yeast nitrogen base without amino acids. SD contains 2% dextrose. SG contains 2% galactose.

Oligonucleotides. Oligonucleotides were synthesized at the Biotechnology Resource Center at University of California, San Francisco. The following oligonucleotides were used to site direct mutations in the *HOP1* promoter:
Underlined bases indicate changes introduced in the mutagenesis of the *HOP1* promoter. The parentheses indicate the restriction site created by the mutation.

The following double-stranded oligonucleotides were used in construction of plasmids pAV138, pAV140, pAV178, and pAV179.

URS1H-20: tcgacTTAACCTGGGCGGCTAAATTc
gAATTGGACCCGCCGATTTAAgagct

URS1H-8: tcgacTGGGCGGCg
gACCCGCCGcagct

UASH-13: tcgaCGTGTGAAGTGATg
GCACACTTCACTAcagct

Lowercase letters indicate bases that differ from the *HOP1* promoter sequence and were introduced for purposes of cloning.

Site-directed mutagenesis of *HOP1* promoter. Plasmid pNH44-1 contains the 2.7-kb *Eco*RI-*Eco*RV fragment of the *HOP1* gene cloned into the *Eco*RI-*Hin*cII backbone of pVZ1, a derivative of pBS+ (Stratagene) given to N.H. by Steve Henikoff. The *HOP1 Eco*RI-*Eco*RV fragment contains 1 kb of DNA upstream from the start of the protein along with the first 519 codons of the *HOP1* coding sequence. Specific mutations in the *HOP1* promoter which create unique restriction sites were constructed in plasmid pNH44-1 by site-directed oligonucleotide mutagenesis with the *dut*$^-$ *ung*$^-$ system and verified by restriction digests (31). Plasmids pNH71-3 ($URS1_H$), pNH74-7 ($SP1_H$), and pNH76-3 (UAS_H) (sites in parentheses indicate the mutated site) were constructed by using $URS1_H$ (*Xho*I), $SP1_H$ (*Xho*I), and UAS_H (*Eco*RV) mutagenic oligonucleotides, respectively. Plasmids containing double mutations, pNH78-1 ($URS1_H$, UAS_H), pNH82-18 ($SP1_H$, UAS_H), and pNH86-2 ($URS1_H$, $SP1_H$), were constructed by site-directed mutagenesis of plasmid pNH71-3 or pNH76-3. The *HOP1* promoter mutations were used to generate two sets of plasmids described below. One set contains the mutations upstream of a *hop1-lacZ* gene fusion. These plasmids were used to monitor *HOP1* expression by assaying β-galactosidase activity. In the second set of plasmids, the mutant sites were placed upstream of the intact *HOP1* gene and used to monitor complementation.

Construction of *hop1-lacZ* fusion plasmids. The vector pAV71 was used for construction of the *hop1-lacZ* fusion plasmids. pAV71 is a variant of pLGΔ312S (a 2μ, *URA3* vector containing the *cyc1-lacZ* promoter fusion [9]) that contains a *Bgl*II site in place of the *Sma*I site at bp −312 of the *CYC1* promoter. *hop1-lacZ* fusion plasmids were constructed by inserting the 552 bp *Bgl*II-*Xba*I fragment from either pNH44-1 or the mutant variants (pNH71-3, pNH74-7, pNH76-3, pNH78-1, and pNH82-18) into the *Bgl*II-*Bam*HI backbone of pAV71. The *Bgl*II-*Xba*I fragment of pNH44-1 contains 207 bp of the *HOP1* promoter and the first 115 codons of the *HOP1* coding sequence. pAV71 was cleaved with *Bam*HI, which cleaves in codon 3 of the *cyc1-lacZ* protein fusion, and the ends were filled in with Klenow polymerase. The DNA was then digested with *Bgl*II, and the backbone fragment containing genes coding for β-lactamase, *URA3*, the remainder of the *lacZ* gene, and the 2μ origin of replication was gel purified. pNH44-1 or its mutant derivatives were digested with *Xba*I, and the ends were filled in with Klenow polymerase and then digested with *Bgl*II. The 552-bp fragment containing the *HOP1* promoter region was gel purified and ligated into the backbone fragment of pAV71. Transformants were screened by restriction digest, and the plasmids were sequenced (25) to verify the presence

$URS1_H$ (*Xho*I): GTTTTTAACCTCTCGAGCTAAATTGTAC
$SP1_H$ (*Xho*I): GAAACAACAGAGAAACTCGAGGATAAAGAAAGAAATTGACTGCAG
UAS_H (*Eco*RV): GTAAAGGGAGATCTACGCAGATATCTATATATGTTTTTAACCTGGGCGG

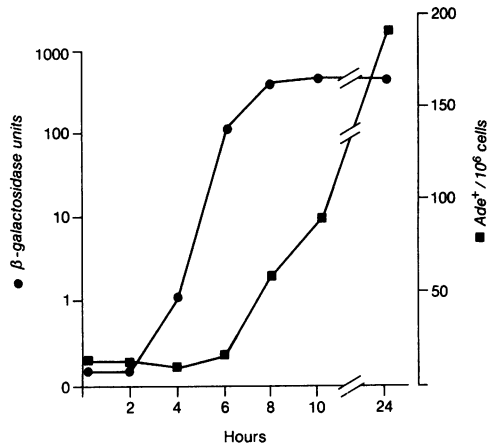

FIG. 1. Meiotic induction of the *hop1-lacZ* fusion. Transformants of strain YV16 were shifted from YEPD to SPM as described in Materials and Methods. Aliquots were assayed at various time points after the shift for expression of β-galactosidase activity and meiotic recombination. The values represent an average between two independent colonies. A total of 75.3% of cells contained pAV79 at the time of transfer to SPM, and 24.7% of the cells had formed asci by 30 h.

of the promoter mutations and to ensure that the junction between *HOP1* and *lacZ* retained the proper reading frame. The following plasmids were cloned by this procedure: pAV79 (wild type), pAV124 ($URS1_H$), pAV127 ($SP1_H$), pAV130 (UAS_H), pAV131 ($URS1_H$, UAS_H), and pAV133 (UAS_H, $SP1_H$). Plasmid pAV132 was constructed by cloning the 450-bp *Xho*I-*Bam*HI fragment from pAV127 ($SP1_H$), which contains the region of the *HOP1* promoter downstream of the $SP1_H$ site along with the first 115 codons of the *HOP1* gene, into the *Xho*I-*Bam*HI backbone of pAV124 ($URS1_H$). pAV125 was constructed by cleaving pAV124 ($URS1_H$) with *Bgl*II and *Xho*I, filling in the ends with Klenow polymerase, and ligating.

Complementation tests of *HOP1* promoter mutations. *HOP1* complementation test plasmid pNH72-2 and variants containing site-directed mutations in the *HOP1* promoter were constructed by cloning the 1.4-kb *Eco*RI-*Bam*HI fragment from pNH44-1 or its mutant derivatives into the *Eco*RI-*Bam*HI backbone of pNH42-2, a *CEN4 URA3 ARS1* shuttle vector that contains a 4.0-kb *Pst*I *HOP1* fragment (12). Plasmids pNH72-2 (wild type), pNH73-26 ($URS1_H$), pNH75-3 ($SP1_H$), pNH80-7 (UAS_H), pNH81-7 ($URS1_H$, UAS_H), and pNH89-4 ($SP1_H$, UAS_H) were transformed into dip17-2(2-26), a homozygous *hop1* strain. Complementation assays for Hop^+ function were performed as described by Hollingsworth et al. (12).

Sporulation and β-galactosidase assays. Assays of the various *hop1-lacZ* fusion constructs were performed in transformants of strain YV16. For the time course shown in Fig. 1, colonies were picked off selective plates and grown to saturation in YEPD. The cells were washed once with water and diluted 1:10 in SPM and incubated at 30°C with aeration. Aliquots were taken every 2 h to assay for *hop1-lacZ* expression, meiotic recombination, and plasmid stability. Cells were plated on SD-ade plates to assay meiotic recombination by scoring for the presence of Ade^+ prototrophs arising from recombination between the *ade2-1* and *ade2-R8* alleles. Sporulation efficiency was assayed by first diluting the cells 1:1 in 3.7% formaldehyde to fix them and then using a light microscope to detect the formation of asci. Plasmid stability was measured by titering cells on SD-ura and YEPD. β-Galactosidase assays were performed as described by Keleher et al. (15) without the addition of glucose. A similar protocol was used to obtain the expression data in Fig. 3 to 5 except that the cells were first grown to saturation in selective medium, diluted 10-fold into YEPD, and then grown again to saturation at 30°C before being transferred to SPM for 24 h.

Heterologous promoter constructs and assays. Plasmid pAV73 is a derivative of pLGΔ312S (9) that contains a *cyc1-lacZ* fusion under the control of the *CYC1* promoter, the yeast 2μ origin, the f1 origin of replication for production of single-strand DNA, and genes encoding *URA3* and β-lactamase. Plasmid pAV128-1 contains the blunt-ended 103-bp *Bgl*II-*Pst*I (−206 to −103) fragment of the *HOP1* promoter from pNH44-1 inserted into the blunt-ended *Xho*I sites in the *CYC1* promoter of pAV73. Mutant derivatives of pAV128-1, plasmids pAV128-15 ($URS1_H$), pAV134 (UAS_H), and pAV135 ($URS1_H$, UAS_H), were constructed in a similar manner except that the 103-bp *HOP1* promoter fragments containing the mutations were cloned from plasmids pNH71-3, pAV130, and pAV131, respectively. Plasmid pAV148 contains the wild-type blunt-ended 103-bp *Bgl*II-*Pst*I fragment from pNH44-1 cloned into the *Sma*I site upstream of the *CYC1* promoter in plasmid pAV72. pAV157 was constructed in a similar manner except that the *HOP1* promoter fragment contains the $URS1_H$ mutation from plasmid pAV124.

Plasmids pAV138-1, pAV138-2, and pAV138-3 contain one, two, and three copies, respectively, of the URS1H-20 double-stranded oligonucleotide cloned into the *Xho*I site of pAV73. Plasmids pAV140-1, pAV140-2, and pAV140-5 contain one, two, and five copies, respectively, of the URS1H-8 oligonucleotide cloned into the *Xho*I site of pAV73. Plasmids pAV141-1, pAV141-2, and pAV141-3 contain one, two, and three copies, respectively, of URS1H-20 cloned into the *Xho*I site of pLR1Δ21, a vector that contains a *gal1-lacZ* fusion under the control of the *GAL1* promoter (38). Plasmids pAV178-1 and pAV179-1 contain one copy each of the URS1H-20 and UASH-13 sites, respectively, inserted into the *Sal*I site of pΔSS, a *cyc1-lacZ* vector that is missing the endogenous *CYC1* UAS sites. All constructs were screened by restriction digest and verified by sequencing.

β-Galactosidase assays on the *CYC1* and *GAL1* heterologous test promoter constructs were performed in strain AJ87. Cells transformed with *CYC1* constructs were grown on SD-ura to late-log growth, and β-galactosidase assays were performed as described previously (15). *GAL1* constructs were grown on SG-ura medium instead of SD-ura. $URS1_H$ repression in *ssn6* and *tup1* mutant strains was tested by β-galactosidase assays of CKY11 and CKY54A (Table 1) transformed with plasmids pAV73, pAV138, pAV148, and pAV157. Assays were performed as described above except that since these strains are clumpy, the cell density was measured in assay buffer with the addition of 5 mM EDTA. $URS1_H$ repression in the *ume* mutant strains was assayed by comparing the levels of β-galactosidase expression in cells transformed with plasmids pAV73, pAV128-1, and pAV148. Meiotic induction of the *hop1-lacZ* fusion in the *rme1*, *ime1*, and *ime2* mutant strains was monitored by β-galactosidase filter assays of pAV79 transformants grown on SD-ura or SPM plates.

RESULTS

The 207 bp upstream of the *HOP1* ATG are sufficient for meiotic regulation and expression. The *HOP1* gene is expressed in yeast cells upon entry into meiosis (12). Analysis of RNA prepared from diploid cells at various stages after meiotic induction by Northern (RNA) blots indicates that the *HOP1* transcripts are induced 3 to 6 h after the cells have been transferred to SPM. To easily assay expression of the *HOP1* promoter, we constructed a plasmid, pAV79, which contains a *hop1-lacZ* gene fusion. A fragment encoding the first 115 residues of the HOP1 protein along with 207 bp of the promoter region was joined in frame to the *lacZ* gene. We tested whether the *hop1-lacZ* fusion was induced during meiosis and whether it followed the same temporal pattern of expression as *HOP1*. The diploid strain, YV16 (Table 1), was transformed with pAV79, transferred to SPM at 30°C, and assayed at various times for β-galactosidase activity. In addition, plasmid stability, meiotic recombination, and ascus formation were monitored. YV16 is heteroallelic at *ade2*, and therefore, meiotic recombination can be detected by the generation of Ade^+ prototrophs in return-to-growth experiments (27). Expression of the fusion protein first appears between 2 and 4 h after switching to SPM (Fig. 1), a time slightly earlier than was previously reported for the detection of the *HOP1* transcript (12). This difference is probably due to the higher sensitivity of β-galactosidase detection when compared with Northern analysis. Induction of Ade^+ prototrophs occurs between 6 and 8 h as was previously reported for this strain (11). Therefore, the *HOP1* gene is expressed approximately 2 to 4 h before the onset of meiotic recombination. Expression of the *hop1-lacZ* fusion protein is induced at least 400-fold, and the level of β-galactosidase activity appears to be maintained from 12 to 24 h after the cells are switched to SPM. The *hop1-lacZ* fusion protein is not expressed in normal haploid cells under sporulation conditions or in diploid cells during vegetative growth (data not shown). This indicates that the fusion protein has the same genetic and nutritional requirements for meiotic expression as endogenous *HOP1* (12). These experiments show that the 207-bp region immediately upstream of the *HOP1* translation start site is sufficient for normal regulation of expression of the *HOP1* gene. The sequence of this region of the *HOP1* promoter is shown in Fig. 2 (from reference 12).

Two regulatory sites in the *HOP1* promoter are required for proper timing and levels of expression. A series of deletions and site-directed mutations in the *HOP1* promoter were constructed to identify the regulatory sites in this region that control expression of *HOP1*. To test whether any transcriptional regulatory sites reside within the *HOP1* coding region itself, as has been reported for the meiotically induced gene *SPO11* (C. Atcheson, cited in reference 3), a *hop1-lacZ* fusion was constructed containing the 207-bp promoter sequence and only the first six residues of *HOP1* fused to *lacZ* (pAV119). The mitotic and meiotic levels of β-galactosidase expression of pAV119 are the same as for pAV79, indicating that there are no required regulatory sites between amino acids 6 and 115 of *HOP1* (Fig. 3). Plasmid pAV126 contains a deletion of the promoter from the *Bgl*II site (−207) to the *Pst*I site (−102), leaving only 102 bp of the promoter. This construct fails to express the *hop1-lacZ* fusion in either mitotic or meiotic cells, indicating that the 105-bp region between −207 and −102 of the *HOP1* promoter contains sequences that are required for *HOP1* transcription.

Inspection of the *HOP1* promoter shows a high content of

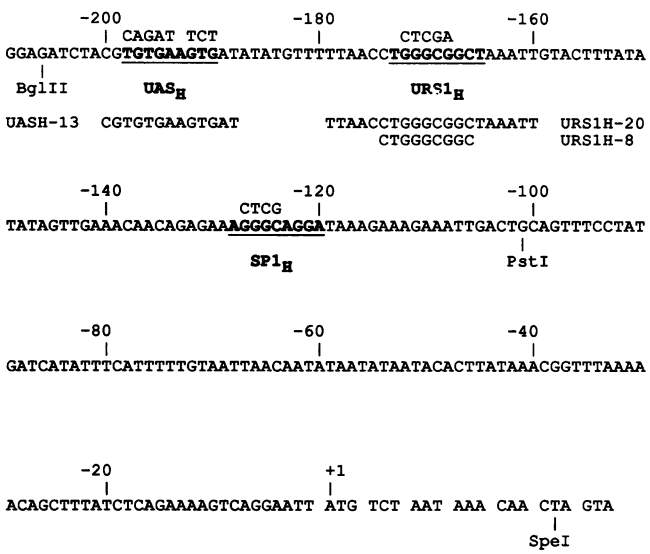

FIG. 2. *HOP1* promoter sequence. The $URS1_H$, $SP1_H$, and UAS_H sites are underlined. Sequences above the sites indicate site-directed mutations. Sequences below the sites indicate oligonucleotides used in construction of various test plasmids. Relevant restriction sites used in promoter deletion studies are indicated. Coordinates are relative to the ATG of the *HOP1* coding sequence.

A+T base pairs. However, Hollingsworth et al. (12) noticed a region, which we call $URS1_H$ (−172 to −166), that is G+C-rich, and it was hypothesized that this site may be important for meiotic regulation of *HOP1*. The $URS1_H$ site is identical to the consensus mammalian Sp1 binding site, GGGCGG (5, 8). The $URS1_H$ site also shows strong similarity (11 of 12 bp) to the yeast upstream repression sequence, URS1, first identified in the *CAR1* promoter (33). The URS1 site is also found upstream of genes involved in nitrogen metabolism and other meiosis-specific genes (3, 34). In one case, it was shown to be required for meiotic expression (3). To determine whether $URS1_H$ is involved in the regulation and/or expression of the *HOP1* promoter, we altered the site by directed mutagenesis. The resulting mutant promoter was then cloned either upstream of the *hop1-lacZ* fusion to assay for expression or upstream of the intact *HOP1* gene to test its ability to complement a *hop1* diploid. A 5-bp mutation in the $URS1_H$ site (Fig. 2) affects expression of *hop1-lacZ* in both mitotic and meiotic cells (pAV124, Fig. 3). In mitotic cells, the mutation causes a 140-fold increase in expression of the fusion protein; that is, the gene is derepressed. In cells undergoing meiosis, this mutant fails to show any induction of expression above the mitotic level; this level is eightfold lower than that shown by the wild-type promoter during meiosis. In complementation tests, the mutant fails to complement a diploid *hop1* strain (pNH73-26, Fig. 3), suggesting that the eightfold decrease in expression of HOP1 protein in meiotic cells is sufficient to cause a mutant phenotype. Therefore, it appears that the $URS1_H$ site functions as a repressor site in mitotic cells and as an activator site for *HOP1* transcription in sporulating cells.

The fact that mutation of the $URS1_H$ site shows a 140-fold increase in the level of expression in mitotic cells suggests that $URS1_H$ is the site of action of a repressor in mitotic cells and that mutation of this site reveals the presence of an activator sequence located elsewhere in the promoter. A series of deletions of the *HOP1* promoter were constructed

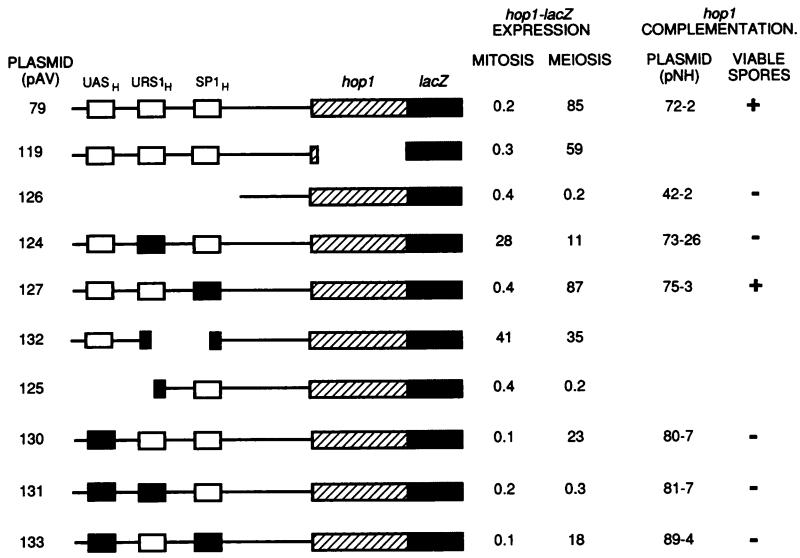

FIG. 3. Complementation and expression of deletions and point mutations in the *HOP1* promoter. Deletions and point mutations were constructed as described in Materials and Methods. The upstream sequences were placed in front of either a *hop1-lacZ* gene fusion (for expression studies) or the wild-type *HOP1* gene (for complementation analyses). β-Galactosidase assays were performed in strain YV16, and the reported values are an average of at least three independent transformants. Complementation assays were performed in the homozygous *hop1* diploid, dip17-2(2-26), as described previously (12). Site-directed mutations are designated by solid boxes in the $URS1_H$, $SP1_H$, and UAS_H sites.

in the $URS1_H$ mutant background to locate such an activation site. A deletion between the mutant $URS1_H$ site and bp −125 shows the same level of *HOP1* expression as the $URS1_H$ mutant, ruling out the possibility that the activation site is located in this region (pAV132, Fig. 3). However, a deletion of the region between −207 and the mutant $URS1_H$ site at −170 abolishes expression in mitotic cells (pAV125, Fig. 3). Inspection of this region revealed a sequence, GTGTGAAGTG (−199 to −190), that resembles (six of nine match) the recognition sequence of the mammalian transcriptional regulatory factor CCAAT enhancer binding protein (C/EBP) (13). A site-directed mutation which changes 8 bp (Fig. 2) at this site in a $URS1_H$ mutant background failed to express the fusion protein in mitotic or meiotic cells (pAV131, Fig. 3). This result suggests that this second regulatory site, which we call UAS_H, functions as an activator sequence in mitotic cells.

We next tested whether the UAS_H mutation has any effect on an otherwise wild-type promoter. As expected, the UAS_H mutation in a wild-type promoter background does not express the fusion protein in mitotic cells. Upon being shifted to sporulation conditions, the mutant promoter is induced to a level fourfold below that of wild type (pAV130, Fig. 3). When the UAS_H-mutated promoter is placed upstream of the intact *HOP1* gene, this construct can no longer complement a *hop1* diploid (pNH80-7, Fig. 3). We presume, therefore, that the fourfold decrease in expression resulting from the UAS_H mutation prevents sufficient HOP1 protein from being made. Note that the level of *HOP1* expression observed in UAS_H mutants during sporulation is similar to the unregulated level of transcription in $URS1_H$ mutants during both mitosis and sporulation. The fact that both mutants fail to complement rules out the possibility that failure of the $URS1_H$ mutant was due to premature expression. These results indicate that the UAS_H site acts as an activator sequence and is required for full meiotic induction of the *HOP1* promoter.

There exists another G+C-rich region within the HOP1 promoter, which we call $SP1_H$ (−127 to −120), that is similar to the variant Sp1 site (GGGAGG) found in the human cardiac α-actin promoter (10). A *HOP1* promoter that contains a 4-bp mutation of the $SP1_H$ site (Fig. 2) exhibited no phenotype in mitotic or meiotic cells (pAV127, Fig. 3). This mutation also fully complemented *hop1* diploid cells (pNH75-3, Fig. 3). Therefore, we conclude that the $SP1_H$ site is not important for expression of *HOP1*.

$URS1_H$ site is able to repress heterologous promoters. The derepression of *HOP1* expression caused by the $URS1_H$ mutant suggests that this sequence functions as a repressor site in mitotic cells. We wanted to test whether this site would repress transcription when placed in a nonmeiotic promoter. Plasmid pAV128-1 contains a 103-bp fragment (−206 to −103) from the wild-type *HOP1* promoter cloned between the UAS and the TATA of the *CYC1* promoter. This fragment contains the *HOP1* $URS1_H$ and UAS_H sites (Fig. 2). The *CYC1* promoter in this construct directs transcription of a *cyc1-lacZ* fusion protein (9). Transformants containing this plasmid were assayed for *cyc1-lacZ* expression by monitoring β-galactosidase activity under mitotic and meiotic conditions. When compared with a plasmid without an insert, the wild-type *HOP1* fragment repressed transcription approximately 1,400-fold (pAV73 versus pAV128-1, Fig. 4). A similar construct with the $URS1_H$ mutation repressed transcription 36-fold (pAV128-1 versus pAV128-15, Fig. 4). Therefore, a large part of the observed repression (40-fold) appears to be dependent solely on the $URS1_H$ site. The 1,400-fold repression of the *CYC1* promoter by the wild-type *HOP1* fragment cannot be attributed to an alteration of the spacing between the *CYC1* UAS and TATA, since this fragment repressed transcription 30-fold when placed upstream of the *CYC1* UAS (pAV148, Fig. 4). Furthermore, this upstream repression was completely eliminated by the presence of the $URS1_H$ mutation (pAV157, Fig. 4).

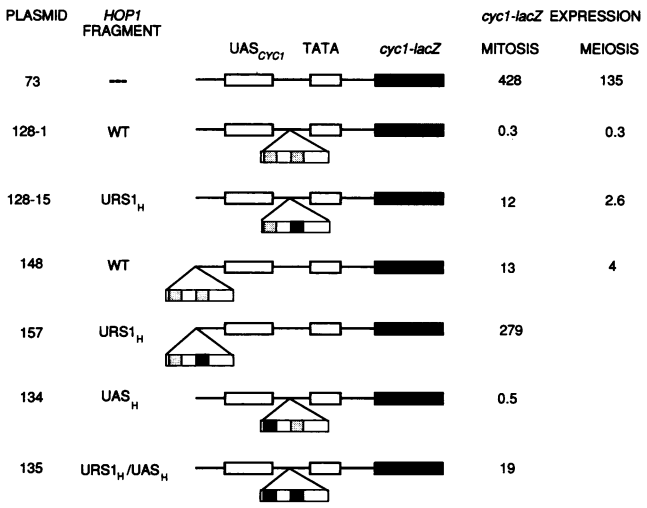

FIG. 4. *HOP1* repression of the heterologous *CYC1* promoter. Constructs were assayed for β-galactosidase expression in the strain AJ87 as described in Materials and Methods (see Table 1 for genotype). β-Galactosidase activity, expressed in Miller units, represents the average of at least three independent transformants. Hatched boxes represent wild-type URS1$_H$ and UAS$_H$ sites. Blackened boxes indicate mutations in the URS1$_H$ or UAS$_H$ site. In addition, the genotype of the 103-bp *HOP1* fragment is given next to the plasmid name.

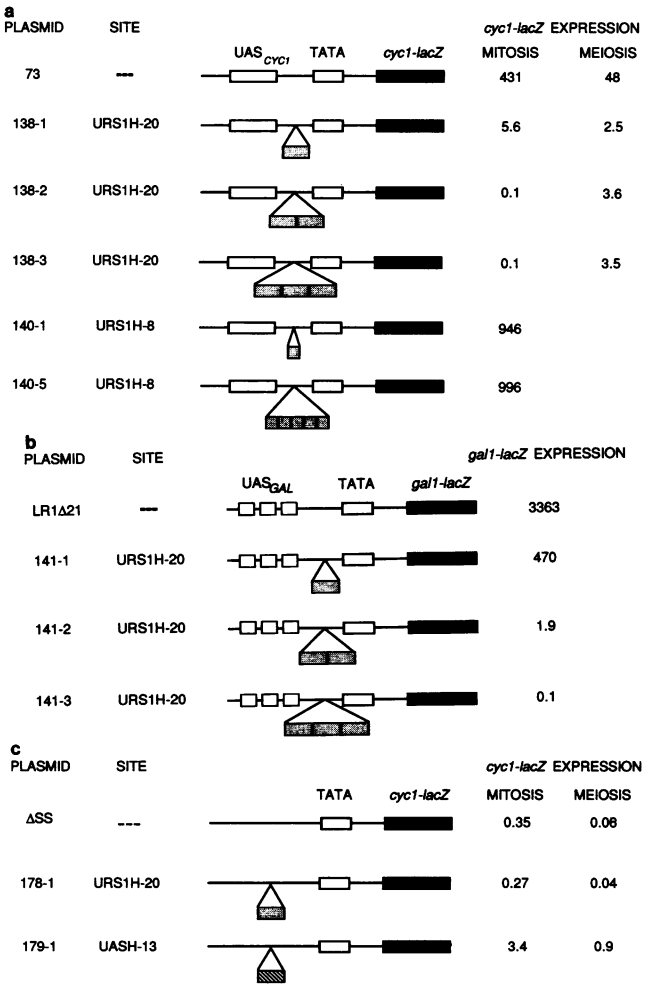

FIG. 5. (a) *HOP1* URS1$_H$ site alone can repress a heterologous promoter. Experiments were performed as detailed in the legend to Fig. 4. The hatched boxes indicate the number of URS1H-20 or URS1H-8 sites inserted into the vector. URS1H-20 and URS1H-8 are synthetic oligonucleotides containing 10 or 4 bp, respectively, in either direction from the center of the URS1$_H$ site. (b) *HOP1* URS1$_H$ site can repress the *GAL1* promoter. The hatched boxes indicate the number of URS1H-20 sites inserted into plasmid pLRΔ21. Experiments were performed as described in the legend to Fig. 4 except that cells were grown on galactose instead of glucose. (c) *HOP1* UAS$_H$ site functions as an activator sequence. URS1H-20 and UASH-13 sites were inserted into a *CYC1-lacZ* vector missing the *CYC1* UAS sites, and constructs were assayed as described in the legend to Fig. 4.

URS1$_H$ appears to function as both an activator and a repressor site, while UAS$_H$ only serves as an activator site. Therefore, we predicted that UAS$_H$ mutants should have no effect on the ability of URS1$_H$ to repress transcription in the heterologous system. This was indeed the case. The plasmid pAV134, carrying the UAS$_H$ mutation in the *HOP1* promoter fragment, repressed transcription 850-fold (Fig. 4).

To roughly define the boundaries of the URS1$_H$ site in the *HOP1* promoter, we synthesized oligonucleotides centered around the URS1$_H$ site and cloned them between the UAS and TATA sites of the *CYC1* test promoter. A construct that contains one copy of the 20-bp URS1$_H$ site, called URS1H-20 (see Material and Methods for the sequence), repressed *CYC1* transcription 77-fold (pAV138-1, Fig. 5a). Constructs that contain two or three copies of the URSH-20 oligonucleotide repressed transcription 4,000-fold, suggesting that multiple repressor sites function cooperatively. In contrast, plasmids constructed with one or five copies of an oligonucleotide containing only eight bases centered around the URS1$_H$ site, URS1H-8, failed to show any repression of the *CYC1* promoter (pAV140-1 and pAV140-5, Fig. 5a). This indicates that in addition to the URS1H-8 site, sequences within the 20-bp site are required for repression (Fig. 2).

The ability of URS1$_H$ to repress a heterologous promoter is not limited to the *CYC1* gene; similar experiments were done placing URS1$_H$ within the *GAL1* promoter with the same results (Fig. 5b). The observation that the URS1$_H$ site can effect repression of several different yeast promoters raises the question of whether URS1$_H$-mediated repression requires some of the same proteins as are used in other yeast repression systems. For example, it has been shown that the SSN6 and TUP1 proteins are required for repression of genes involved in determining cell mating type as well as the response to glucose availability (4, 16, 24, 36). URS1$_H$-mediated repression of the *cyc1-lacZ* promoter was tested in *ssn6* and *tup1* mutant strains and was found to be unaffected (data not shown). This result indicates that URS1$_H$-mediated repression utilizes a set of proteins at least some of which are distinct from those required by other repression systems.

What proteins are responsible for the mitotic repression of *HOP1*? The *UME1-5* genes were identified from mutants that allow expression of meiotic genes during vegetative growth (32). It therefore seemed reasonable that some of these proteins might be required to mediate repression at the *HOP1* URS1$_H$ site. However, we found less than a twofold difference in the level of URS1$_H$ repression in *ume1*, *ume2*, *ume3*, *ume4*, and *ume5* strains when compared with wild type (data not shown).

HOP1 UAS$_H$ site functions as an independent activator

TABLE 2. Effects of meiosis regulatory mutants on *hop1-lacZ* expression

Strain	Relevant genotype	β-Galactosidase expression[a]	
		Mitosis	Meiosis
AMP107	*MAT***a** *RME1*	–	–
AMP118	*MAT***a** *rme1*	–	+
AMP109	*MAT***a**/*MAT*α *IME1/IME1 IME2/IME2*	–	+
AMP115	*MAT***a**/*MAT*α *ime1/ime1 IME2/IME2*	–	–
AMP245	*MAT***a**/*MAT*α *IME1/IME1 ime2/ime2*	–	+

[a] Expression of the *hop1-lacZ* fusion was monitored by β-galactosidase filter assays of cell patches grown on SD-ura or SPM.

sequence. In the context of the *HOP1* promoter, both the $URS1_H$ and UAS_H sites function as activator sites during meiosis. In addition, the UAS_H site appears to work as an activator sequence in mitotic cells when $URS1_H$ has been inactivated. These results raise the question of whether these sites function as activator sequences in a different context or whether they require additional sequences specific to the *HOP1* promoter. To address this question, we cloned the *HOP1* $URS1_H$ and UAS_H sites into a *cyc1-lacZ* construct that was missing the endogenous UAS (Fig. 5c). The $URS1_H$ site failed to show any activation of transcription above background levels during mitotic or meiotic growth. This result suggests that there are other sequences within the *HOP1* promoter that are required for activation by the $URS1_H$ site. The UAS_H site in pAV179-1 activates transcription of the promoter about 10-fold above background levels (compare pAV179-1 to pΔSS in Fig. 5c) in both mitotic and meiotic cells, indicating that it can function as an independent activator site.

Genetic control of *HOP1* expression. The genetic cascade of proteins involved in regulating meiosis has been extensively studied, and it seemed likely that some of these proteins directly or indirectly regulate expression of *HOP1*. The genetic requirement for initiation of meiosis in yeast cells is the presence of the *MAT*α2 and *MAT***a**1 genes. The **a**1 and α2 proteins work together to repress transcription of *RME1*, which encodes a repressor protein that in turn allows the expression of *IME1* (14, 23). The IME1 protein functions as an activator protein to express *IME2*, another activator protein, as well as possibly other meiosis-specific proteins (29, 30). Previous work demonstrated that *HOP1* is under the control of the *MAT* locus (12). This finding was confirmed by the observation that the *hop1-lacZ* fusion was expressed in a haploid strain (AMP118) under sporulation conditions if the strain is mutant in *RME1* (Table 2). A diploid *ime1* strain (AMP115), however, failed to express the fusion protein under sporulation conditions, indicating that this protein is essential for *HOP1* induction. The *ime2* strain (AMP245) had a normal pattern of *hop1-lacZ* expression. These results suggest that *HOP1* is regulated by *RME1* and *IME1* but is independent of *IME2*. Our observations are consistent with those of A. Mitchell (22a) and Smith et al. (30), who monitored *HOP1* transcripts by Northern blots in each of these mutant strains.

DISCUSSION

HOP1 expression is tightly repressed in mitotically growing cells, but upon entry into meiosis, expression is induced nearly 1,000-fold. Two sites were identified in this region, $URS1_H$ and UAS_H, that are essential for proper regulation and expression of the promoter. The $URS1_H$ site functions as a repressor site to turn off transcription of the *HOP1* promoter during mitotic growth of the cell. When the cell enters meiosis, the $URS1_H$ functions as an activator site and is required for full expression of the *HOP1* promoter. The UAS_H site functions as an activator sequence in meiotic cells; however, UAS_H is repressed by the $URS1_H$ site during mitotic growth of the cell. When cells enter meiosis, the promoter is derepressed and both $URS1_H$ and UAS_H are required for full *HOP1* expression.

The mechanism of repression by $URS1_H$ is not specific for the *HOP1* promoter since it functions as a very strong repressor site in heterologous test promoters. Moreover, Sumrada and Cooper (34) previously proposed that URS1 is a common repression element that regulates expression of a number of different genes. However, repression by the $URS1_H$ site is distinct from the mechanism used by other known yeast repressors since it does not require the function of SSN6 or TUP1, proteins required for negative regulation by other repressor proteins.

The strong repression mediated by the $URS1_H$ site raises the question of why *HOP1* is so tightly regulated. Electron micrographs of cells stained with HOP1 antibodies indicated that the HOP1 protein is associated with the chromosomes during prophase I, and it is thought to be a component of the synaptonemal complex, the structure formed when homologous chromosomes pair during meiosis (12). If the HOP1 protein is an integral part of the synaptonemal complex, then a specific stochiometric ratio of HOP1 to the other proteins in the complex might be required. For example, underexpression or overexpression of a given component of the complex might block productive assembly of the structure. It may therefore be essential that *HOP1* is coordinately expressed with other components at the proper time and level to allow formation of the synaptonemal complex. Consistent with these ideas, overexpression of *HOP1* during sporulation was found to block cells from completing meiosis (8a). Upon entering meiosis, it may be important that HOP1 be absent so that proper levels of the protein are present during meiosis.

The model of a specific stoichiometry of HOP1 in the synaptonemal complex would also predict that underexpression of *HOP1* would have a detrimental effect during meiosis. We have shown that mutations in the $URS1_H$ and UAS_H sites result in a four- to eightfold decrease in the level of expression of the *hop1-lacZ* fusion. This decrease appears to be functionally significant since these mutants fail to complement a diploid *hop1* mutant strain when placed upstream of the intact *HOP1* gene.

A third prediction stemming from this model is that other components of the synaptonemal complex, as well as other genes involved in early meiotic events, may contain regulatory sites similar to those of *HOP1* to ensure that the levels of these proteins would be coordinately expressed. In fact, sequences similar to the *HOP1* $URS1_H$ site have been found within the upstream regions of several other meiosis- and sporulation-specific genes (3). We noticed that many of these genes contain sequences with some similarity, although weak, to the *HOP1* UAS_H site (Fig. 6). It is noteworthy that *DMC1*, a meiosis-specific gene which is required for formation of the synaptonemal complex, contains an 11 of 13 match to the UAS_H site in its promoter (2). With the exception of the *SPO13* URS1 site, which has been shown to be required for meiotic induction (3), it is not currently known which of these sites are important for meiotic regulation or expression. However, it seems likely that many of

GENE	UAS_H	HOMOL.	$URS1_H$	HOMOL.
HOP1	TGTGAAGTG -198 -190		TGGGCGGCT -173 -165	
DMC1	TGTGGAGAG -168 -160	7/9	TGGGCGGCT -129 -137	9/9
SPO16	TGTGATGTA -198 -190	7/9	TGGGCGGCT -90 -82	9/9
SPO12	TGTGGAAAA -345 -337	5/9	AGGGCATCT -220 -212	6/9
SPO11	TGTGTAGTG -252 -244	8/9	TTGGCGGCT +163 +171	8/9
SPO13			TCGGCGGCT -88 -96	8/9
RED1			TCAGCGGCT -166 -158	7/9
MER1	AGTGAAATA -270 -262	6/9	TCGGCGGCT -103 -111	8/9
MEK1			TCGGCGGCT -135 -127	8/9
RME1			CCGGCGGCC -103 -111	6/9
MEI4			TGGGCGGCT -98 -89	9/9
C/EBP	TGTGGAAAG	6/9		
CAR1 URS1			TCGGCGGCT	8/9

FIG. 6. Homologies of the *HOP1* $URS1_H$ and UAS_H sites with sequences in other meiosis-specific genes.

these genes are regulated in a manner similar to that of *HOP1* and use the same transcriptional regulatory proteins that function through the $URS1_H$ and UAS_H sites.

Why are two activator sites required for normal expression of *HOP1*? We have shown that the $URS1_H$ site is required to ensure that transcription is turned off during mitotic growth, and it seems likely that the signal for meiotic induction works at least partially through this site. The UAS_H site, on the other hand, appears simply to increase the induced level of transcription of the *HOP1* promoter. It is possible that meiotic genes that are not as highly expressed as is *HOP1* may not require a second activator site like UAS_H. In these cases, activation from the URS1 site alone would be sufficient.

The $URS1_H$ site was first identified by sequence inspection: it is homologous to the binding site for the mammalian transcription factor, Sp1 (12). The full repression site is contained within the 20-bp synthetic oligonucleotide URS1H-20. This site bears a striking similarity (eight of nine match) to the upstream repression sequence URS1 of *CAR1*, a gene involved in arginine metabolism (19, 33). Furthermore, Luche et al. (19) have shown that the URS1 site consists of 9 bp, consistent with our finding that the 20-bp URS1H-20 oligonucleotide brings about repression, but the 8-bp oligonucleotide does not. The URS1 site functions in vivo as both a repressor and activator site for *CAR1* expression and has been shown to function as a repressor site in heterologous test promoters (18). Sites homologous to URS1 have subsequently been located upstream of a large number of genes involved in cellular metabolism (34). The results from this study suggest a model of *HOP1* regulation that resembles the mechanism of regulation of *CAR1* expression and may even involve some of the same transcriptional regulatory factors (18).

In the context of the *HOP1* promoter, we showed that upon sporulation, $URS1_H$ mediated repression is inactivated and together with the UAS_H site functions to activate transcription. Since the $URS1_H$ site switches from a repressor site to an activator site, it might be expected that when the $URS1_H$ site is cloned into a heterologous test promoter, transcription could be derepressed under sporulation conditions. We have not, however, observed any derepression of either the *CYC1* or *GAL1* promoter constructs containing either the $URS1_H$ site alone or the *Bgl*II-*Pst*I fragment (−206 to −103) containing the $URS1_H$, $SP1_H$, and UAS_H sites (Fig. 4). In addition, it appears that $URS1_H$ does not function as an activator sequence on its own (Fig. 5b). These results show that the *HOP1* $URS1_H$ site is not sufficient for activation of transcription by itself and suggest that other sequences are required for meiotic induction of the *HOP1* promoter. This postulates the presence of an inducer site, similar in function to the UAS_I site in the *CAR1* promoter, that would inactivate the $URS1_H$-mediated repression under sporulation conditions (18). This site apparently is not present in the 103-bp *HOP1 Bgl*II-*Pst*I fragment used in the heterologous promoter constructs, and it seems likely that if this site exists, it is contained within the −102 to +18 region of the *HOP1* promoter.

It has been shown that both the *CAR1* and *HOP1* genes are repressed by a similar regulatory site, URS1. Why do genes involved in two apparently different cellular processes use the same regulatory site? Both of these classes of genes respond to complex metabolic signals. In particular, both classes are directly or indirectly regulated by the level of nitrogen in the cell. It is therefore possible that genes involved in nitrogen metabolism in addition to meiosis-specific genes use some of the same regulatory proteins to control expression in response to the level of nitrogen. Although it has been shown that expression of *HOP1* is indirectly regulated by nitrogen starvation, through the control of *IME1* expression (30), it has not been shown whether there is any direct regulation of *HOP1*. If *HOP1* is directly regulated by nitrogen starvation, then it seems likely that the URS1 site is the site of action of these common factors.

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