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A Bipartite Operator Interacts with a Heat Shock Element To Mediate Early Meiotic Induction of *Saccharomyces cerevisiae HSP82*

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Although key genetic regulators of early meiotic transcription in *Saccharomyces cerevisiae* have been well characterized, the activation of meiotic genes is still poorly understood in terms of *cis*-acting DNA elements and their associated factors. I report here that induction of *HSP82* is regulated by the early meiotic *IME1-IME2* transcriptional cascade. Vegetative repression and meiotic induction depend on interactions of the promoter-proximal heat shock element (HSE) with a nearby bipartite repression element, composed of the ubiquitous early meiotic motif, URS1 (upstream repression sequence 1), and a novel ancillary repression element. The ancillary repression element is required for efficient vegetative repression, is spatially separable from URS1, and continues to facilitate repression during sporulation. In contrast, URS1 also functions as a vegetative repression element but is converted early in meiosis into an HSE-dependent activation element. An early step in this transformation may be the antagonism of URS1-mediated repression by *IME1*. The HSE also nonspecifically supports a second major mode of meiotic activation that does not require URS1 but does require expression of *IME2* and concurrent starvation. Interestingly, increased rather than decreased URS1-mediated vegetative transcription can be artificially achieved by introducing rare point mutations into URS1 or by deleting the *UME6* gene. These lesions offer insight into mechanisms of URS-dependent repression and activation. Experiments suggest that URS1-bound factors functionally modulate heat shock factor during vegetative transcription and early meiotic induction but not during heat shock. The loss of repression and activation observed when the *IME2* activation element, T_4C, is substituted for the HSE suggests specific requirements for URS1-upstream activation sequence interactions.

When starved for nitrogen, diploid **a**/α cells of the yeast *Saccharomyces cerevisiae* can cease their usual budding mode of growth and either begin pseudohyphal growth or undergo meiosis to eventually produce ascospores. The choice of differentiation pathway is operationally determined by the available carbon source. In low-nitrogen medium containing glucose, fermenting cells continue to proliferate as pseudohyphae, whereas in acetate medium depleted of nitrogen and glucose, respiring cells enter meiosis and sporulate. Both pathways entail an initial arrest at the late G_1 cell cycle regulatory checkpoint known as Start, followed by the newly differentiated cell cycle. The single meiotic cell cycle is characterized by the appearance after completion of the premeiotic S phase of a B cyclin, Clb1 (15). Commitment to meiosis occurs at about the time of Clb1 appearance; cells returned to growth medium prior to this time can resume the mitotic cycle (24, 25).

Regulation of entry into the meiotic pathway has been intensively studied and is complex (reviewed in references 43 and 47). Normally only **a**/α cells can enter meiosis because this mating genotype permits the increased transcription of a master transcriptional regulatory gene, *IME1* (inducer of meiosis), in response to nutritional signals transduced by poorly understood mechanisms. *IME1* is not strictly a meiotic gene because its transcription is also stimulated under conditions that do not lead to sporulation, such as in stationary phase or heat-shocked cultures (28, 58). Environmental signals may additionally activate IME1 protein (Ime1p) by posttranslational modification (58). Ime1p has no obvious sequence similarities to known transcriptional regulators and has not been shown to bind to DNA, but it possesses a domain capable of stimulating transcription (44, 61). The carboxyl region of Ime1p is not essential for transcriptional activation but is required for induction by starvation (61) and for the initiation of meiosis (44).

IME1 expression is absolutely required for the transcription of a second transcriptional regulator, *IME2. IME2* is normally transcribed only under sporulation conditions (28, 63) and may be a primary target of glucose repression (28). *IME2* encodes a protein that has homology to the yeast *CDC2* and *CDC28* cell cycle kinases and the signal transduction-associated mitogen-activated protein kinase, but Ime2p also closely resembles the rat mak1 kinase, which is specifically expressed in testis germ cells during meiosis (27, 45). A major function of *IME2* is to stimulate the transcriptional induction of about 20 early meiotic genes, including itself, that encode proteins used for early meiotic functions (reviewed in reference 47). The transcription of all early meiotic genes analyzed to date (*SPO13*, *SPO11*, and *HOP1*) is stimulated by *IME2*, but these genes are also significantly induced by *IME1* in *ime2*Δ cells (48, 63).

The upstream regions of all early meiotic genes contain good sequence matches to the regulatory element URS1 (upstream repression sequence 1), which is also found in many nonmeiotic genes and was first identified as a repression element in an arginine catabolic gene, *CAR1* (42, 70). Deletion of URS1 in *SPO13* and *HOP1* causes inappropriate expression during the mitotic cell cycle (10, 75). *UME6* (*CAR80*) is a regulatory gene essential for maintaining the mitotic repression of early meiotic and other genes (7, 66, 67). In *SPO13*, *IME2*, *CAR1*, *INO1*, and *FOX3*, URS1 has been shown to be a target of Ume6p action because URS1 repression function is compromised in *ume6* disruptants. (7, 13, 39, 52, 67).

Although these URS1-associated regulatory components have been identified, it is not yet well understood how *cis*-acting DNA elements specifically confer early meiotic induc-

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TABLE 1. Yeast strains used in this study

Strain	Genotype[a]
CSGy12	α
CSGy29	**a** *arg4::URA3*
CSGy279	**a** *LEU2 trp1::hisG*
	α *leu2::hisG* TRP1
CSGy34	α YIpCSG11-*LEU2::HSP82*
CSGy86	**a** *HSP82 arg4::URA3*
	α YIpCSG11-*LEU2::HSP82* *ARG4*
CSGy84	α YIpCSG11-*LEU2::HSP82* *ime1-12::TRP1 trp1::hisG arg4::URA3 SMO1 CYH2*^{S}
CSGy87	**a** *HSP82 ime1-12::TRP1 trp1::hisG arg4::URA3 SMO1 CYH2*^{S}
	α YIpCSG11-*LEU2::HSP82* *ime1-12::TRP1 trp1::hisG* *ARG4* *SMO1 CYH2*^{S}
CSGy55[b]	α YIpCSG20-*LEU2::HSP82*
CSGy85[b]	**a** *HSP82 arg4::URA3*
	α YIpCSG20-*LEU2::HSP82* *ARG4*
CSGy194[b]	α YIpCSG20-*LEU2::HSP82* *ume6::URA3*
CSGy267[b]	α YIpCSG20-*LEU2::HSP82* *ime1-12::TRP1 trp1::hisG*
CSGy182[b]	α YIpCSG20-*LEU2::HSP82* pRS P_{ACT1}-*IME1*
CSGy238[b]	α YIpCSG20-*LEU2::HSP82* *ime2-2::LEU2*
CSGy242[b]	α YIpCSG20-*LEU2::HSP82* *ime2-2::LEU2* pRS P_{ACT1}-*IME1*

[a] Unless noted, all strains have the additional markers *ura3 leu2::hisG lys2 ho::LYS2 smo1 cyh2*^{R}*-z.*
[b] YIpCSG20 is the host for mutations that are shown in Fig. 2 but are not listed here.

tion. Deletion analysis of the promoters of *HOP1* and *SPO13* has revealed that meiotically responsive sequences reside close to the start site of transcription, but no element that is capable of meiosis-specific function when placed upstream of a *CYC1-lacZ* promoter fusion has been isolated (10, 75). *HOP1* contains an element (UAS_H [upstream activation sequence of *HOP1*]) about 20 bp upstream of URS1 that can drive unregulated expression of *CYC1-lacZ* in nonmeiotic cells; similar but untested motifs are found 20 to 200 bp upstream of URS1 in nearly half of all early meiotic genes (47). A situation distinct from that for other early meiotic genes prevails for *IME2*: relatively far upstream sequences (−584 to −537) encompass a composite *IME1*-inducible UAS that retains *IME1* regulation in the *CYC1-lacZ* context (7). This UAS also contains an element, T_4C (also with sequence similarities in several early meiotic genes), that nonspecifically stimulates transcription; for *IME1*-mediated activation, T_4C requires the URS1 element that resides 20 bp further downstream within the UAS (7).

HSP82 belongs to a two-member family of yeast heat shock genes, at least one of which is induced transcriptionally in sporulation (32). *HSP82* is an intensively studied heat shock gene that relies primarily on a single heat shock element (HSE) adjacent to the promoter to drive vegetative and heat-induced transcription (18, 19, 35, 46, 72). It is likely that Hsp82p has a phylogenetically conserved function during meiosis or early oogenesis because its homologs are similarly expressed in other fungi (9), the fruit fly (12), and mammals (22, 36).

The experiments described here explore how two entirely different regulatory pathways induce *HSP82* transcription by stimulating a single classical activation element, a nonspecific HSE. Induction of *HSP82* during sporulation is initiated by the early meiotic *IME1-IME2* transcriptional cascade; the initial step may be the antagonism by *IME1* of vegetative repression that is mediated by URS1. In the case of *HSP82*, efficient repression also requires a second novel element. Meiotic stimulation of the HSE is distinct from that of heat shock and utilizes two resolvable mechanisms. (i) The combined action of URS1 and a nonspecific HSE elicits increased transcription; URS1 alone cannot support induction. (ii) *IME2* kinase transmits a starvation signal able to stimulate a generic isolated HSE.

MATERIALS AND METHODS

Strains. All yeast strains are derivatives of SK1. Parent strains into which *HSP82-lacZ* reporter constructs were integrated are listed in Table 1. The progenitor of all CSGy parent strains is Nky274 (1), modified by the inclusion of two additional Mendelian alleles, *smo1* and *cyh2*^{R}*-z*. *smo1* was identified as an allele resulting in a spontaneous smooth-colony isolate distinct from the usual rough SK1 strain. This uncharacterized recessive allele reduces the flocculation that makes handling of SK1 strains difficult. *smo1* does not confer cold-sensitive growth as do several smooth-colony *rim* mutations (69) and does not affect the timing or extent of sporulation. *cyh2*^{R}*-z* was selected on the basis of resistance to cycloheximide by using a batch procedure described elsewhere (71a). Nky290 and Nky291 were crossed with this progenitor to provide *trp1::hisG* and *leu2::hisG* markers (1).

The *ume6::URA3* disruptant was created by chromosomal integration of plasmid pPL5923, provided by Randy Strich (67). Transformation with plasmids pAM412 (48) and pHS113B (62), from Aaron Mitchell, created *ime2-2::LEU2* and *ime1-12::TRP1* chromosomal disruptants. Constitutively expressed Ime1p was transcribed from a Yep24 plasmid bearing the *ACT1* promoter fused to the *IME1* coding sequences (pRS P_{ACT1}-*IME1*, gift of Aaron Mitchell) (69). To allow selection for diploids, the congenic **a** mating partner of CSGy12 was modified by integrating an *arg4::URA3* disruptant plasmid, pMJ92 (gift of M. Lichten).

Plasmid construction. Maps of integrating plasmids that were host to oligonucleotide-directed mutations are shown in Fig. 1A. The plasmids were adapted from pCM81 (gift of C. Moehle) (49), which contained the indicated segments from pRS305 (59) and pLG669Z (20); a short oligonucleotide had been inserted into the *CYC1 Xho*I site of pLG669Z to create a *Bgl*II-*Xho*I polylinker in pCM81. This *CYC1* polylinker was further modified by inclusion of another oligonucleotide to give a *Pst*I-*Bgl*II-*Sal*I-*Xho*I polylinker. PCR amplification of pUTX20 (14) produced *HSP82* DNA mapping from the *Eco*RI site at −1301 to an oligonucleotide-templated *Bam*HI site immediately 3′ to the second codon, designed to give an in-frame fusion at the pLG669Z *Bam*HI site. This *Eco*RI-*Bam*HI fragment was ligated into the polylinker of a modified pBluescript II KS+ (Stratagene, La Jolla, Calif.), deleted of its *Nae*I-*Sma*I fragment. Additional upstream *HSP82* DNA was cloned by chromosomal walking using inverse PCR (51), checked for correspondence to the native locus by indirect end-label mapping (80) of restriction enzyme-digested genomic DNA, and ligated to the pUTX20-derived DNA. The pBluescript *HSP82* insert, spanning 2,200 bp of contiguous upstream sequence, was transferred on a *Sca*I-*Bam*HI fragment to pCM81 to produce YIpCSG11. YIpCSG20 was created by replacing the 1,550-bp *Apa*I-*Bam*HI *HSP82* fragment with the 650-bp *Apa*I-*Bam*HI *CYC1* fragment from the modified pCM81 and then replacing the 260-bp *Sal*I-*Bam*HI *CYC1* sequence with 339 bp of *Hin*dIII-*Bam*HI *HSP82* DNA.

Construction of mutated *HSP82* plasmids. For each mutation, a synthetic oligonucleotide carrying the mutation was used to prime an amplification PCR, using YIpCSG20 as a template and a second primer either 5′ to the *Hin*dIII site or 3′ to the *Bam*HI site. The amplified DNA was isolated on an agarose gel and used as a megaprimer for a second amplification on the YIpCSG20 template (33). DNA from the second amplification was gel purified and restricted with *Hin*dIII and *Bam*HI; the insert was ligated back into YIpCSG20 and transformed into *Escherichia coli* XL1-Blue (Stratagene). After screening on an agarose gel for an insert of the expected size, plasmids from two transformants were linear-

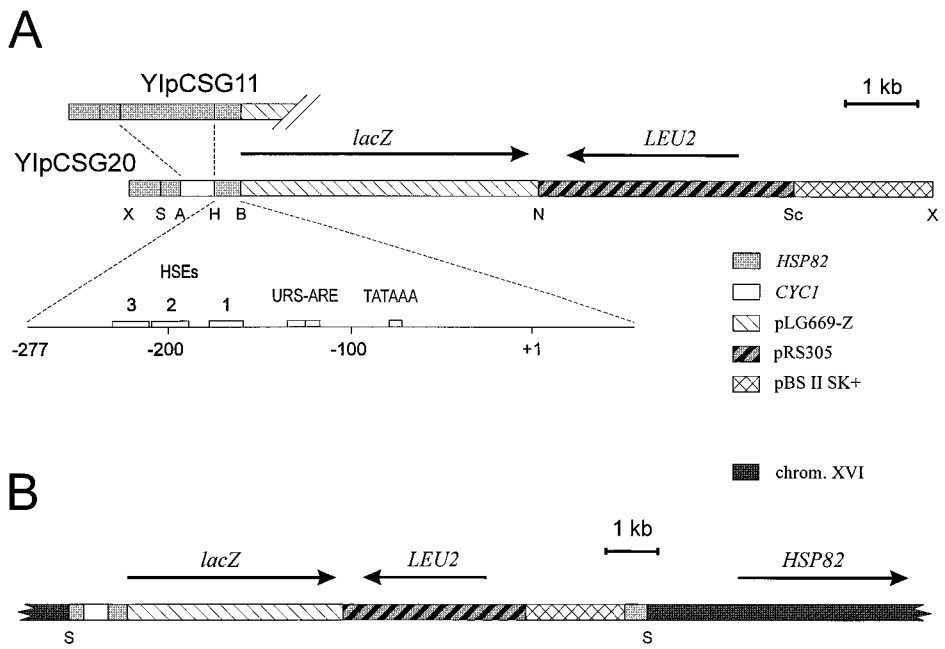

FIG. 1. (A) Design of integrating *HSP82-lacZ* reporter plasmids. Initial deletion experiments used YIpCSG11 as the parental construct. YIpCSG20, the host for the oligonucleotide-directed mutations that are schematicized in Fig. 4, was derived from YIpCSG11 by replacing the *HSP82* sequence between −277 and −1500 with a *CYC1* upstream sequence that has no UAS activity (−700 to −1100). Known *HSP82* regulatory elements within the 339-bp *Hin*dIII-*Bam*HI fragment are shown beneath it; positions are relative to the *HSP82* transcription start (12). The *HSP82* sequence between −1500 and −2150 was retained to target integration of *Spe*I-linearized plasmid to the corresponding region upstream of the chromosomal *HSP82* gene. The key identifies plasmid and gene fragments used to construct *HSP82-lacZ* reporters (see Materials and Methods). Restriction enzyme sites relevant to construction of the plasmids are shown: X, *Xba*I; S, *Spe*I; A, *Apa*I; H, *Hin*dIII; B, *Bam*HI; N, *Nhe*I; Sc, *Sca*I. (B) Structure of the *HSP82* locus in chromosome (chrom.) XVI after integration of YIpCSG20.

ized with *Spe*I and introduced into yeast cells by electroporation (2). Three chromosomal integrants from each yeast transformation were grown in rich acetate medium and assayed for vegetative β-galactosidase activity. Generally four to six transformants gave a single level of activity characteristic of each mutation (3). Genomic DNA corresponding to the integrated construct was PCR amplified by using the secondary primers and sequenced by the dideoxy method. Over 90% of the integrants screened by this technique contained only the desired mutations. Complex mutations targeted to separable sequences or transfer of mutations into a single HSE background was obtained by repeating the mega-primer mutagenesis procedure by using mutated YIpCSG20 as the template.

Sequence analysis. A loose URS1 functional consensus (TNVGCGRCS) used initially to scan *HSP82* was derived from a mutagenesis study of URS1 (42) by scoring all single base pair substitutions that gave 30 or less U of β-galactosidase activity as being members of the functional URS1 set. An 8-of-9-bp match to the functional consensus located the URS82 element. A T nucleotide was noted to frequently occur immediately 5′ to aligned URS1 sequences from nonmeiotic genes (42). When this residue was added to the canonical URS1 motif used to search compiled early meiotic genes, the 5′ T was found in all identified URS1 elements (47) (see Fig. 10). Genes listed in Fig. 10 include all early meiotic genes compiled in reference 47 except *IME4*, *REC102*, and *RIM4*, which were not in the database.

Growth and handling of yeast cells. All media contained 0.001% polypropylene glycol 2000 as an anticlumping agent. For sporulation experiments, yeast cells were grown in filter-sterilized rich potassium acetate (KAc) medium (2% KAc, 0.6% yeast nitrogen base, 0.5% Bacto Peptone, 0.5% yeast extract, 50 mM phthalic acid [pH 5.5]) to an A_{600} of ~0.4 in small (<50 ml) vigorously aerated cultures at 300 rpm and 30°C in a shaking incubator. To induce sporulation, cells were harvested by filtration, washed with H_2O, resuspended at the same density in 2% KAc plus auxotrophic supplements (pH 7.0), and incubated as described above. For β-galactosidase assays, 6-ml samples were removed from cultures at designated times and immediately made 20 mM in NaN_3 to disrupt energy metabolism (34, 72). For experiments comparing vegetative expression, yeast cells were grown in filtered complete synthetic dropout medium (56) plus 2% KAc or glucose and 50 mM phthalic acid (pH 5.5).

β-Galactosidase assays. Quantitative β-galactosidase assays using *o*-nitrophenyl-β-D-galactoside (ONPG) as the substrate were performed on permeabilized cells essentially as described previously (60). In the sporulation experiments shown in Fig. 3 and 5, typical activities of wild-type (WT) −188 *HSP82* constructs ranged from 8 to 10 U in growing rich KAc cultures, were unchanged after 2 h of starvation in 2% KAc, but increased to 80 to 120 U after 10 h of starvation in KAc. Ten hours was chosen as the time to assay induced expression on the basis of initial experiments using centromeric *HSP82-lacZ* reporter plasmids, in which cells grown under selection took 2 h longer to sporulate. To simplify handling of cells, assays performed after 2 h in 2% KAc were taken to represent preinduction expression, and the A_{600} of the 2-h culture, which by this time had ceased proliferation (15), was used to adjust the A_{420} values of ONPG assays from both 2- and 10-h cultures. The sporulation time courses shown in Fig. 7, in which each sample point activity is adjusted with its own A_{600} as described previously (60), confirm the validity of both simplifications.

Within a given experiment, vegetative and induced activities of all reporter constructs varied in concert. To compare activities between independent experiments, all vegetative and induced activities from a given experiment were normalized to the vegetative expression of an appropriate construct that served as an internal standard. When induction was monitored, induced activity values were secondarily scaled to the induced activity value of the internal standard. Normalized expression values are the averages (±2 standard deviation) of two (Fig. 3, 5, and 9) or three (Fig. 6 and 8) separate determinations. All graphs also include a scale calibrated in Miller units based on the average activity (the induced activity where relevant) of the internal standard.

RESULTS

Utility of chromosomal *HSP82-lacZ* reporter constructs. Pilot studies showed that vegetative and meiotic *HSP82* expression levels are high enough to support analysis using *HSP82-lacZ* reporter constructs on centromeric plasmids; these levels of expression are respectively at least 100- and 10-fold greater than those seen for other studied early sporulation genes (7, 10). A set of 5′ deletions spanning sequences from −2200 to the transcription start showed that 277 bp of 5′ sequence was sufficient to fully support sporulation-associated induction (data not shown). However, because of inherent plasmid copy number variability and because presporulation growth in media that maintain selection for plasmids may compromise analyses of nutritional requirements, single-copy *HSP82-lacZ* constructs were subsequently integrated into chromosome XVI immediately upstream of the native *HSP82* gene (Fig. 1B).

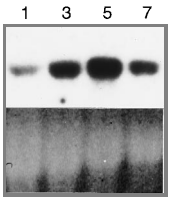

FIG. 2. Accumulation of *HSP82* mRNA during sporulation. Total RNAs from equal numbers of cells were fractionated by agarose gel electrophoresis. The amount of RNA loaded on the gel was monitored by visualizing 18S rRNA with ethidium bromide. The *HSP82* transcript was detected by Northern hybridization as described previously (34). Steady-state mRNA levels after the indicated number of hours in sporulation medium are shown.

Data obtained from integrants paralleled those obtained from plasmids and are in addition highly reproducible; all experiments described here used these chromosomal reporters. The robustness of the assay, in combination with high levels of *HSP82* expression, allows the reliable analysis of relatively small changes in expression.

Sporulation induction requires the minimal heat shock promoter. *HSP82* mRNA accumulation during sporulation was monitored by Northern (RNA) analysis using a probe that does not cross-hybridize to the closely related *HSC82* transcript (34, 72). Message accumulation was significant 3 h after starvation and peaked after 5 h at about 12-fold the level seen at 1 h (Fig. 2). *SPO13*, *SPO11*, and *IME2* mRNAs also attain maximal levels at about this time in strain SK1 (48, 63). These kinetics of accumulation are very similar to that of the B cyclins and their associated kinase activities in sporulating SK1 cells (15).

Integrants containing 2,200 or 277 bp of upstream sequence gave roughly the same levels of vegetative β-galactosidase activity, which were induced more than 10-fold after 10 h in sporulation medium (Fig. 3; compare rows 2 and 5). A further 5′ deletion, which removed two of the three HSEs present in the −277 construct (Fig. 1), reduced vegetative expression twofold but did not alter the extent of induction (Fig. 3, row 4). Deletion of the entire proximal HSE, centered at −167 (Fig. 4), completely abolished both vegetative and induced expression (Fig. 3, row 1).

Maximal β-galactosidase levels for cells grown in rich presporulation medium were generally attained after 8 h of starvation; thus, true maximal activity is about 30% greater than seen at 10 h (see Fig. 7 and Materials and Methods). No significant induction was observed before 4 h. Increased β-galactosidase activity thus lags about 3 h behind the induction of native transcript, similar to the delay observed for an *IME2-lacZ* promoter fusion (7, 63).

***HSP82* is an early meiotic gene rather than a starvation response gene.** Previous work has suggested that that *HSP82* induction during sporulation is the direct consequence of starvation and is independent of the **a**/α genotype required for entry into meiosis (32). Surprisingly, in starved haploid α cells, no induction was observed for a wide variety of constructs (Fig. 3, rows 4 to 13). This apparent contradiction could be due to the presence of the closely related cognate gene, *HSC82*, whose expression was not resolved from that of *HSP82* in the earlier study (6). *HSC82* has a much higher vegetative level of expression than does *HSP82* (6); preliminary experiments indicate that a modest *HSC82-lacZ* induction begins at about 8 h poststarvation (data not shown). The diploid-specific induction of *HSP82* suggested that expression might be regulated by components of the meiotic pathway. I therefore tested whether induction of *HSP82* was dependent on *IME1* function. In an *IME1* null mutant, no *HSP82* induction was seen in either diploids or haploids (Fig. 3, row 3). This result, in combination with the known timing of transcript induction of *SPO11* and *SPO13* in SK1 diploids (48), suggests that *HSP82* is a member of the early meiotic gene cohort.

HSE and TATA mutations preferentially reduce vegetative expression. In a first attempt to characterize elements that might be required for induction, the putative functional cores of the HSE and TATA box were replaced by random sequences. The 6-bp substitution within the HSE (H_6 in Fig. 4) disrupts two of the three intact nGAAn modules of the 20-bp element and would be expected to severely compromise heat shock inducibility (54, 81). Vegetative expression is about 14-fold lower, whereas sporulation-induced expression is diminished about 4-fold (Fig. 3, row 14). Another directed mutation within these HSE modules (H_{P2} in Fig. 4) also preferentially reduces vegetative transcription (Fig. 5, row 3). The ability of mutated HSEs to retain substantial inductive capability may indicate that other sequences within this region provide a similar function during sporulation or that the meiotic machinery is able to stabilize binding to the mutated element. Such stabilization of binding may occur during heat shock because the H_{P2} mutation also greatly diminishes the in vitro binding of heat shock factor (HSF) but only moderately reduces heat shock transcription (46).

When the 6-bp core of the TATA element is replaced, the corresponding reductions in vegetative and meiotic levels are 50- and 5-fold (Fig. 3, row 15). Nucleotides immediately upstream of the TATA box are especially important for maintaining vegetative expression in haploids after prolonged starvation but have comparatively little effect on sporulation expression in diploids (row 13). A perfect match to a yeast thermal response element (29) 7 to 11 bp upstream of the TATA box appears to play no role in early meiosis (row 16). It has been suggested that in *SPO13* and *HOP1*, the meiotic transcription machinery does not utilize a distinct TATA element (10, 47, 75). In the case of *HSP82*, vegetative transcription utilizes basal machinery bound to the TATA box and flanking upstream nucleotides, but meiotic transcription may instead largely rely on alternative or modified basal machinery.

***HSP82* contains a element that represses vegetative expression.** Sequences that might confer specific meiotic regulation were initially sought by constructing four 20-bp substitution mutations spanning sequences between the HSE and TATA box (Fig. 4, Ø I to IV; Fig. 3, rows 6 to 9). A 2.7-fold increase in vegetative expression in both diploids and haploids was noted for Ø II, which replaced sequences between −122 and −141 (Fig. 3, row 7). An analysis using three overlapping 35-bp deletions spanning this region confirmed these results (data not shown). Partial derepression of vegetative transcription can occur in mitotically cycling haploid and diploid cells when the URS1 motif is replaced or deleted (47). A loose functional consensus of the URS1 element (42) (see Materials and Methods) was therefore used to scan the 188-bp upstream region that supports meiotic induction, and an URS1-like sequence was located within the region replaced by the Ø II mutation. Although this sequence (termed URS82) matches the prototypal *CAR1* URS1 element in only 6 of 9 bp, it does contain an 8-of-10-bp match to an extended URS1 motif ($URS1_E$ in Fig. 4) derived by comparing URS1 elements found in early meiotic genes (see Fig. 10). Interestingly, URS82 is embedded in a 25-bp sequence (Fig. 4) that is a good match to a region upstream of *SPO13* (−88 to −113) (10). The *SPO13* sequence is in reverse orientation to that of *HSP82* and contains a perfect *CAR1* URS1 motif (9). The sequence adjacent to URS82 (sequence A) bears a 6-of-7-bp identity to the *SPO13* counter-

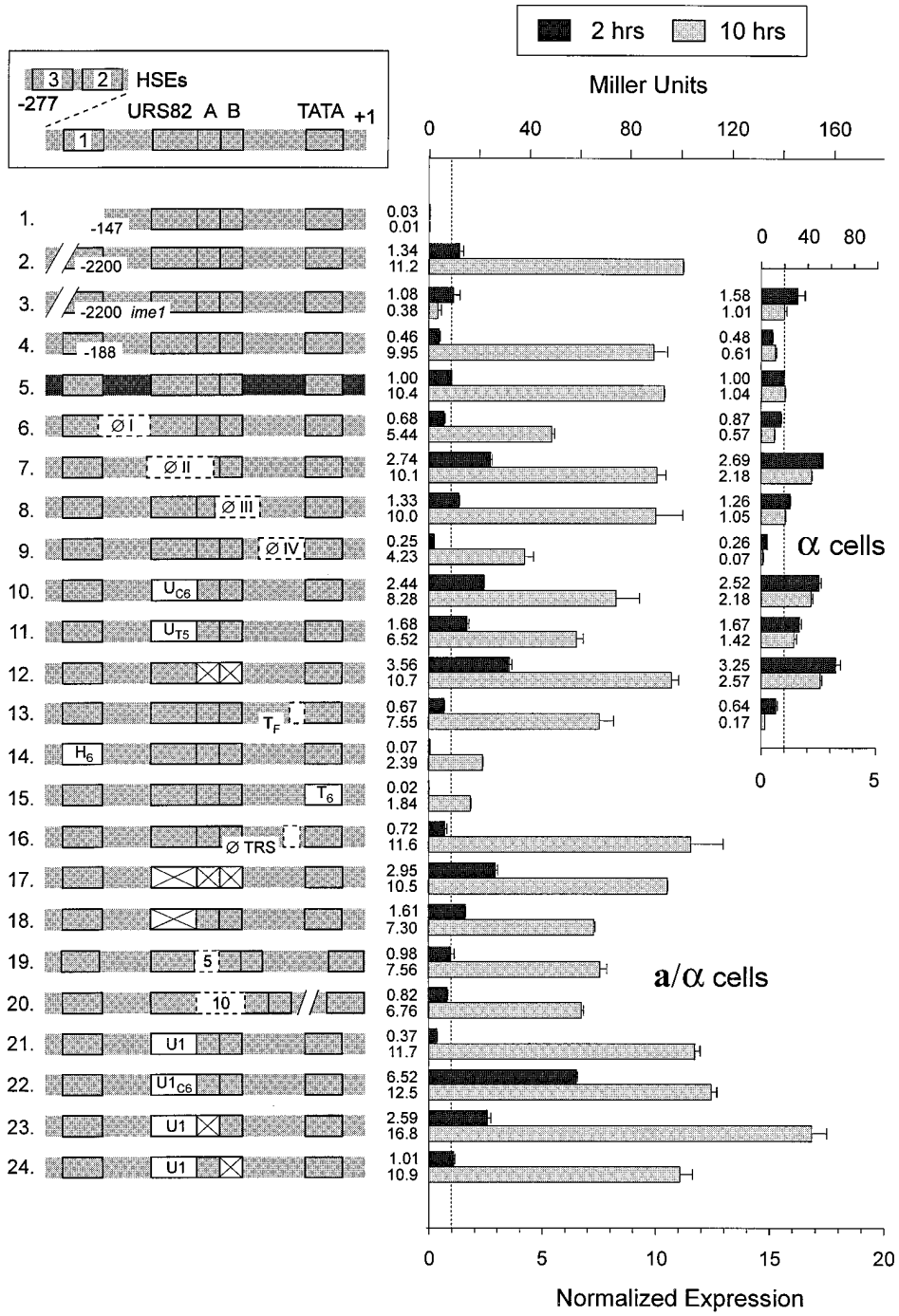

FIG. 3. *HSP82* contains a bipartite element that represses vegetative expression and stimulates early meiotic induction. Normalized β-galactosidase activities of integrated reporter constructs in diploid **a**/α or haploid α cells are graphed, and the corresponding numerical values are displayed. β-Galactosidase activities of uninduced (2 h) and induced (10 h) cultures in sporulation medium were independently normalized for diploid and haploid cells to the uninduced expression levels of a control construct carrying *HSP82* DNA that spans three putative HSEs (−277 to +1; row 5, dark construct). For clarity, the respective reference activities are also represented by dotted lines. Rows 5 to 24 show data from mutations introduced into the 277-bp 5′ sequence; rows 1 to 4 show data from constructs carrying other 5′ deletions of *HSP82* as indicated. All rows show data from WT SK1 cells except for row 3, which shows data from nonsporulating *ime1-12* SK1 cells. In this bar graph and those in Fig. 5, 6, and 9, WT regulatory elements are represented by gray boxes, and specific mutations placed within these elements are named in open boxes according to the key in Fig. 4. Mutations that precisely substitute random sequence for these regulatory elements are represented by crossed-out solid boxes. Mutations that wholly or partially fall outside these regulatory elements are shown as dotted outline boxes and are named according to the key in Fig. 4.

part; a second sequence (sequence B) has a 7-of-8-bp identity to a sequence found 3 bp upstream of the *SPO13* URS1 motif.

On the basis of this analysis, sequences corresponding to URS82, and to the entire homology to *SPO13*, were mutated. Replacement of URS82 with random sequence led to a 60% increase in vegetative activity (Fig. 3; compare rows 5 and 18), whereas replacement of the entire homology tripled the vegetative level (row 17). The repressive capability of URS82 is

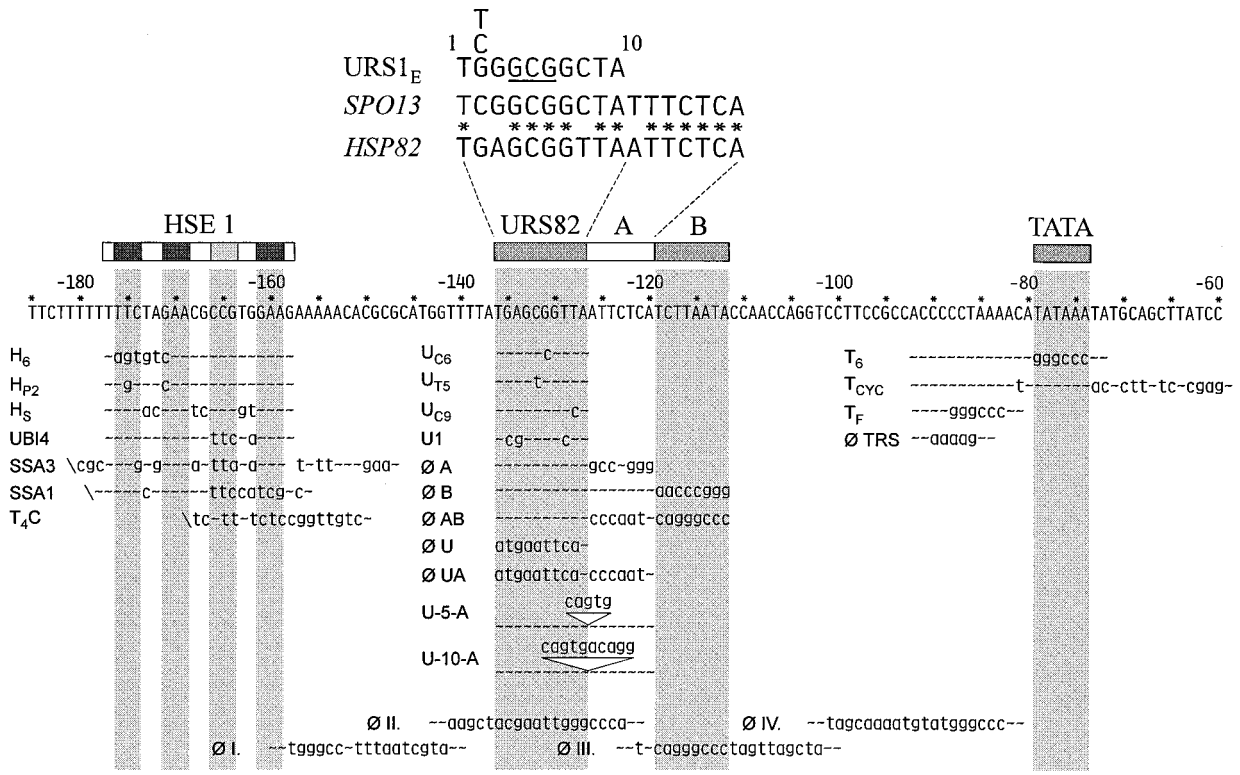

FIG. 4. Functional *HSP82* regulatory elements and mutations used in their characterization. Functional elements are represented by boxes; positions are relative to the initiation of transcription (12). For HSE 1, the consensus GAA modules essential to heat shock function are emphasized in shaded columns. The bipartite repression element, composed of URS82 and the ARE (sequence A), is shown beneath an alignment of the corresponding sequences from *HSP82* and *SPO13*. Immediately above is an extended URS1 consensus sequence ($URS1_E$) rederived from early meiotic genes; underlined letters mark nucleotides especially important to repression function (42). Sequence B corresponds to an analyzed 7-of-8-bp identity between *HSP82* and *SPO13* with little apparent function. Altered or inserted nucleotides are shown in lowercase letters. Specific mutations within elements are designated by subscripts. Sequences replaced by random nucleotides are preceded by Ø, and insertions are represented by numbers (of inserted nucleotides) between dashes. Additional complex mutations created by combining the displayed mutated motifs are not shown. In the case of URS variants (U, URS82; U1, URS1), the mutations indicated by subscripts are numbered from 1 to 10 according to their positions within the motif; for brevity, point mutations are displayed only in the URS82 background. Mutations of HSE 1 include replacements with HSEs from other yeast genes (*UBI4*, *SSA3*, and *SSA1*) and with a meiotic activation element (T_4C) from *IME2*.

obscured in these constructs because of the unregulated contribution of HSEs 2 and 3 to vegetative expression. When assayed in a construct that contains only HSE 1, URS82 repressed vegetative transcription about threefold (Fig. 6, rows 2 and 6).

URS82 behaves like URS1 in repression and activation. *HSP82* constructs containing either URS82 or URS1 behave similarly relative to constructs in which the URS is replaced by a random sequence. URS1 is a stronger repression element than URS82 (ninefold versus threefold; Fig. 6, rows 2, 3, and 6). URS1 is also a less-than-twofold-stronger meiotic activation element than URS82, increasing early meiotic expression by about 55%, compared with 35% for URS82 (Fig. 3; compare rows 5 and 21 with row 18; Fig. 7A and C). The absolute increase in β-galactosidase activity mediated by URS82 (~25 Miller units) during sporulation is about about threefold greater than the total meiotic expression of *IME2* (7) and about sevenfold greater than that of *SPO13* (10). Despite mediating large absolute increases in transcription, URS82 and URS1 contribute only about a third of the total increase in meiotic expression, as a result of the existence of a separate URS1-independent *IME2*-dependent activation pathway that stimulates the HSE (see below). The URS1 element when placed into the *HSP82* context behaves like URS82 (1.55-fold meiotic stimulation, compared with 1.35-fold for URS82) rather than retaining the behavior seen in *SPO13* (~6-fold stimulation [10]). This difference in behavior is especially striking given the identity between the nucleotides that flank URS82 and the *SPO13* URS1 and further argues that URS1 and URS82 are homologous meiotic activation elements of roughly comparable strength. The repressive capability of URS1 is also attenuated by the *HSP82* context because HSE 1 supports relatively high levels of *HSP82* vegetative transcription despite the presence of URS1 (~20-fold greater than in *SPO13* [10]; Fig. 5, row 1).

To further ascertain whether URS82 behaves like URS1, a point mutation characterized in the *SPO13* URS1 was introduced into URS82. In *HSP82*, a C→T transition at −132 gives 1.7-fold-greater vegetative expression (2.4-fold when corrected for contribution of HSEs 2 and 3) but reduces meiotic expression by about 35% relative to that of WT URS82 (Fig. 3, row 11). The corresponding mutation at position −92 of *SPO13* leads to about twofold-greater vegetative activity and a reduction in meiotic activity of about sixfold (10). A second mutation, characterized in an *IME2* URS1 element (7), was also placed into URS82. The G→C transversion at −131 increased vegetative activity 2.4-fold (3.8-fold, corrected) but lowered meiotic expression by about 20% (Fig. 3, row 10). In *IME2*, this transversion at −548 increases vegetative activity 16-fold in an *ime1*Δ background and decreases induced activity 12-fold when *IME1* is overexpressed. The separate *IME2*-dependent mode of activation is relatively weak for *SPO13* (48)

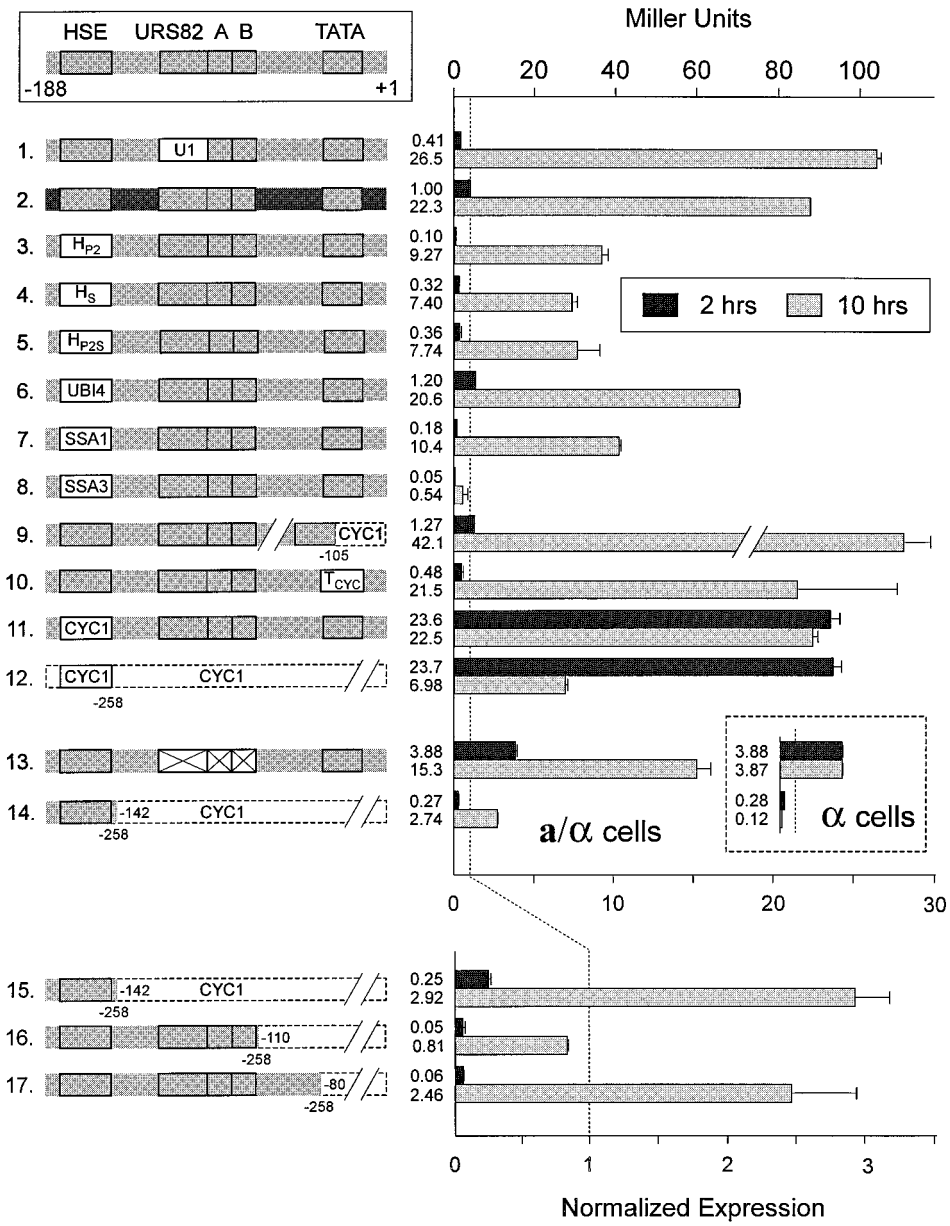

FIG. 5. A generic HSE can by itself confer meiotic induction, but other sequences are also required for efficient meiotic expression. β-Galactosidase activities of reporters in sporulating **a**/α diploid cells were normalized to the activity of a 5′ *HSP82* deletion containing the minimal region that supports meiotic induction (−188 to +1; row 2, dark construct). All integrated reporter constructs contain only a single UAS. Rows 1 to 8, 10, and 13 represent mutations described in Fig. 4. Row 9 shows data from a construct in which all *HSP82* sequence downstream of −66 has been replaced with sequences from downstream of the *CYC1* TATA elements (−105 to +1, including a 3′ *Xho*I site [37]). The mutation shown in row 11 was created by replacing HSE 1 with the 450-bp *Xho*I fragment that carries the *CYC1* UAS (20). Row 12 is a *CYC1-lacZ* construct with the the *CYC1* UAS in its native position at −258; rows 14 to 17 are *CYC1-lacZ* fusions in which the *CYC1* UAS has been replaced by segments of upstream *HSP82* DNA extending from −188 to the indicated 3′ terminus.

and absent for the T_4C-URS1 UAS (7), which likely explains why these URS1 point mutations confer larger changes in induced transcription than are conferred by the analogous mutations in URS82.

A hallmark of *UME6* is its tight linkage to a functional URS1 element: in all tested cases, the deletion of *UME6* relieves URS1-mediated repression (7, 13, 39, 52, 67). Deletion of *UME6* increases vegetative expression of a native URS82-containing construct more than fourfold but has no effect when the URS has been ablated (Fig. 6, rows 2 and 6). If the canonical URS1 is present instead, expression is elevated more than 25-fold to an absolute level twice that supported by URS82 (row 3). The magnitude of the *UME6* phenotype thus correlates with the repressive capability of the element. These data also argue that URS82 is a functional variant of URS1.

The UAS and minimal promoter do not contain simple early meiotic determinants. Since early meiotic expression was only partially reduced by removal of the repression element, it was necessary to more rigorously examine other functional elements for the presence of sequences that might be specific regulatory determinants. For this purpose, all mutations were evaluated in constructs containing only a single proximal UAS

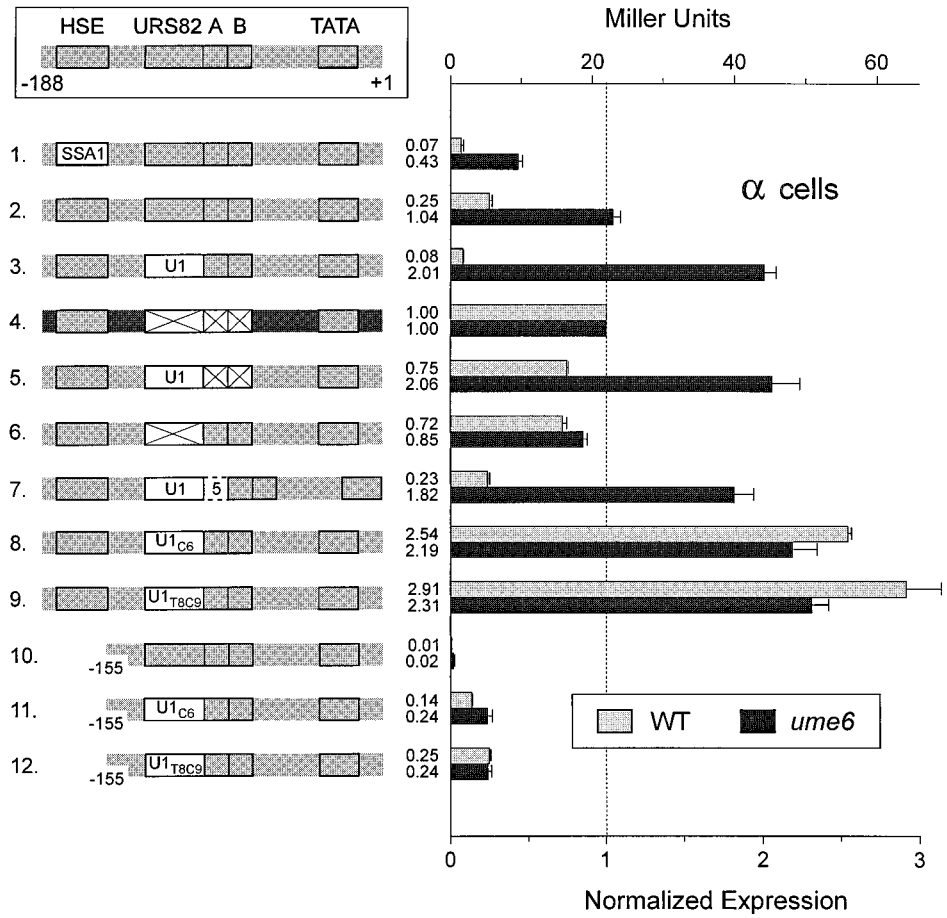

FIG. 6. Loss of *UME6* or rare point mutations enable URS1 to support HSE-dependent activation during vegetative growth. Reporter constructs were integrated into *ume6*::*URA3* (67) or WT α haploid cells. β-Galactosidase activities were assayed for log-phase cultures grown in synthetic acetate medium. To better display the ability of the URS1 element to support either repression or activation, activities from *ume6* or WT cells were separately normalized to those for operatorless reporters (row 4, dark construct). All constructs contain a single or no HSE; mutations are as shown in Fig. 4.

at the position of HSE 1 (Fig. 5). In this background, native *HSP82* displayed a 22-fold early meiotic induction (row 2); replacement of URS82 with URS1 produced slightly higher induced levels and a relative induction of 65-fold (row 1). In yeast HSEs, there are preferred sequences in the 2-bp spacers that separate the GAA modules vital to heat shock induction (4). Conversion of all three of the HSE 1 spacers to rarely found sequences lowered both vegetative and induced expression 3-fold (row 4), whereas point mutations in two of the GAA modules preferentially reduced vegetative expression (10-fold compared with 2.4-fold) (row 3). Combining these mutations gave the spacer mutation phenotype (row 5). The HSE was also replaced by HSEs capable of independent heat-inducible UAS function from three yeast heat shock genes reported to show no (*SSA1*), early (*UBI4*), or middle (*SSA3*) sporulation-associated induction (74, 77). Constructs bearing the transplanted HSEs displayed 68-fold (*SSA1*), 13-fold (*UBI4*), and 10-fold (*SSA3*) early meiotic induction (rows 6 to 8). Low levels of *SSA3* HSE-driven expression reflect the absence of a stimulatory cyclic AMP-responsive element (5). These chimeras induced with the same kinetics (Fig. 7E). The relative levels of early meiotic induction supported by these transplanted HSEs correspond to the relative levels of induction seen during heat shock (data not shown). It is thus unlikely that specific meiotic regulatory elements are interdigitated within these HSEs.

Experiments that replaced *HSP82* promoter sequences with those of the corresponding *CYC1* regulatory region were also conducted. *CYC1* was chosen because it is a standard heterologous environment for evaluating yeast UAS function. Replacement with *CYC1* sequence mapping between its proximal TATA element and the translation start (−105 to −1) (37) or replacement with an extended *CYC1* TATA element (−123 to −102) (37) did not significantly change the magnitude of the meiotic response (Fig. 5, rows 9 and 10). Meiotic induction thus does not require specific sequences downstream of the TATA box. When HSE 1 was replaced by the entire *CYC1* UAS, a high level of constitutive expression was observed (row 11). In its native context, the *CYC1* UAS initially drove expression at a similar level, but upon prolonged starvation, expression declined (row 12). This result may reflect the absence of the *HSP82* sequence that maintains expression in starved haploids.

An HSE can by itself confer meiotic induction, but additional promoter sequences are required for efficient function. The nonspecific requirement for an HSE and the absence of a clear meiotic determinant suggested that an isolated HSE might be capable of conferring meiotic induction. This was tested by replacing the *CYC1* UAS with *HSP82* DNA containing the HSE and adjacent downstream nucleotides (Fig. 5, row 14). The HSE conferred about a 10-fold induction during sporulation, but the induced level was only about 12% of the

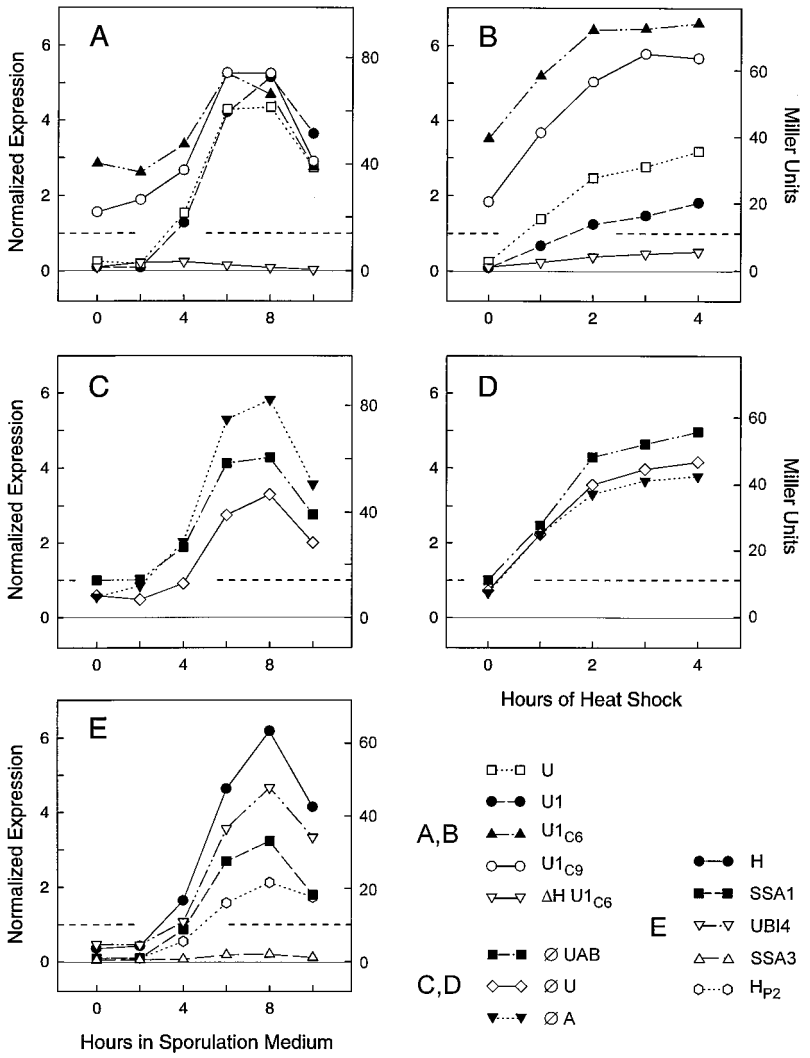

FIG. 7. Interactions of the URS1-ARE and the HSE during sporulation and heat shock. Panels A to D depict data from a single representative experiment; a separate experiment generated the data shown in panel E. Diploid **a**/α SK1 cells were grown in rich acetate medium at 30°C and then either heat shocked at 39°C in the same medium or transferred to 30°C sporulation medium. All reporter constructs are integrants containing a single HSE or no HSE. Mutations are as described in Fig. 4. To emphasize the activating or repressing nature of URS1 variants, β-galactosidase activities are normalized to the vegetative expression of the operatorless *HSP82-lacZ* fusion (0 h; Ø UAB in panel C). The reference activity level is shown by the horizontal dotted lines.

absolute activity seen for the native promoter (row 2). The induction is specific to meiosis because it is not observed in haploids (row 14, inset), and it is not dependent on the nucleotides that flank the HSE (Fig. 3, row 6). These data clearly suggest that a factor binding HSE 1 can be directly or indirectly stimulated during early meiosis. The relative magnitude of induction conferred by the HSE is slightly greater in the *CYC1* context than in an operatorless *HSP82* promoter (Fig. 5, rows 13 and 14 and inset). The higher absolute levels of expression seen in the native *HSP82* context require sequences between URS82 and the TATA box that support both vegetative and meiotic modes of transcription (Fig. 3, row 9). These sequences downstream of URS82 do not support the high native levels of expression when transferred into the *CYC1* reporter (Fig. 5, row 17). However, the operator retains the ability to decrease both vegetative and induced expression about fourfold relative to the solo HSE (rows 15 and 16).

A generic HSE thus appears able to independently confer early meiotic induction, but efficient transcription during sporulation also apparently requires uncharacterized factors that bind 10 to 30 bp upstream of the TATA box. The low overall efficiency of expression in the *CYC1*-promoter *lacZ* reporter, together with the demonstration that the *CYC1* TATA box and sequences further downstream can effectively substitute for their *HSP82* counterparts, suggests that these uncharacterized factors may require a close proximity to TATA-bound basal machinery in order to function.

URS1 utilizes an ARE. The observation that replacement of all 25 bp of the extended homology to *SPO13* derepressed vegetative expression to twice the level seen for a replacement of URS82 alone suggested that the sequences flanking URS82 are functionally important. Mutation of all 15 bp of DNA flanking URS82 (Fig. 3, row 12) gave vegetative expression similar to that resulting from the extended replacement (row 17) and somewhat higher than did mutation of only the core URS element (row 18). To further dissect the flank, replacements were constructed in the context of URS1 because it confers stronger repression. Mutation of sequence A (row 23)

indeed led to nearly the same vegetative expression as did the 25-bp replacement. Replacement of sequence B (row 24) led to a small increase in expression, suggesting that sequence A roughly delimits the flanking sequence important for repression. To better quantitate the relative contributions of URS1 and sequence A to vegetative repression, replacement mutations were evaluated in constructs containing only HSE 1, eliminating the contribution of the distal HSEs. Replacement of either URS1 or sequence A increased vegetative activity about ninefold, to 70 to 75% of the level observed for the 25-bp substitution (Fig. 6, rows 3 to 6). To determine whether URS82 and sequence A together comprise the binding site of a single complex, random spacers of 5 and 10 bp were inserted between the two motifs. Neither insertion derepressed vegetative expression (Fig. 3, rows 19 and 20). A 5-bp insertion between URS1 and sequence A in the context of only HSE 1 gave essentially similar results (Fig. 6, rows 3 and 7). The 7-bp segment contiguous to URS82 thus appears to constitute a separable ancillary repression element (ARE) that binds a factor which cooperates with factors binding URS82 and URS1 to efficiently repress vegetative transcription. If factors binding these sites physically interact as a single functional complex, it must be a flexible association to withstand binding on opposite faces of the DNA helix.

***UME6* deletion converts URS1 from a repression element to an HSE-dependent activation element.** *UME6* has been genetically linked to URS1 function in *IME2*, *SPO13*, and three metabolic genes (7, 13, 39, 52, 67). In haploid cells, a disruption of *UME6* markedly increased the vegetative transcription levels of all *HSP82-lacZ* fusions that carried an intact URS (Fig. 6, rows 1 to 7). Increased expression required the presence of HSE 1 (row 10). The *ume6*Δ mutation elicited the highest levels of expression from those constructs that carried the consensus repression element (rows 3, 5, and 7). Strikingly, the elevated and nearly equal activities conferred by these URS1 promoters were considerably greater than those seen in the control constructs, in which URS1 alone or the entire URS-ARE motif were eliminated (rows 4 and 6). Thus, in a formal sense, the *ume6*Δ allele has converted the URS1-bound factor from a repressor to an activator. The factor binding URS1 is a dependent activator because in order to support increased transcription, it requires an activator that binds the HSE and cannot by itself confer independent UAS activity. The URS1 element itself is crucial to activation function because ablation of the motif eliminates the response (row 6). In contrast, a mutation of the ARE that compromises overall repression function has no effect on activation (row 5). It is noteworthy that in the *ume6*Δ background, the stronger core repression element (URS1) supports higher expression than does the weaker element (URS82) (rows 2 and 3).

Factors bound to URS1 variants can serve dual vegetative roles as independent or HSE-dependent activators. When adjacent to the *CYC1* UAS in a *CYC1-lacZ* reporter, the G→C transversion (Fig. 4, U_{C6}) elevates constitutive expression about 7-fold more than any other of the 52 evaluated URS1 point mutations (42), but it is also unique in that it is able to impart a 14-fold increase in constitutive activity in the absence of the UAS (41). In *HSP82*, this transversion in URS1 increases vegetative activity to 10 times that of URS82 and 30 times that of intact URS1 (Fig. 6; compare row 8 with rows 2 and 3). *HSP82* remains inducible to the same level supported by the intact URS1 (Fig. 3, rows 21 and 22; Fig. 7A). In the absence of the HSE, $URS1_{G\rightarrow C}$ is able to support significant vegetative expression (Fig. 6, row 11) but is no longer able to support induction (Fig. 7A). In these *CYC1* experiments, the $URS1_{G\rightarrow C}$ element has been interpreted to be an artificially generated independent UAS (41). This is clearly also true for *HSP82*, yet it is equally evident that the $URS1_{G\rightarrow C}$ element in collaboration with a neighboring UAS (HSE 1) retains the ability to greatly elevate vegetative expression and not alter meiotic induction. In the course of generating mutations, a T→C transition in URS1 (Fig. 4, U_{C9}) with similar properties was discovered (Fig. 6, rows 9 and 12; Fig. 7A). The transition maps near the junction of the URS and the adjacent ARE. I conclude that certain URS1 variants can function as an independent UAS but that these variants can also interact with the HSE to support greatly increased vegetative transcription.

Upregulation of URS1 by point mutation or *ume6* deletion may share a common mechanism. To test whether the two independent means of achieving URS1-associated increases in vegetative transcription are mechanistically related, point mutations that strongly elevated vegetative transcription were evaluated in *ume6*Δ cells. In the mutant background, both $URS1_{G\rightarrow C}$ and $URS1_{T\rightarrow C}$ variants displayed slightly decreased vegetative expression in the presence of HSE 1 (Fig. 6, rows 8 and 9). The point mutations are therefore epistatic to *ume6*Δ, which argues that both classes of mutation affect the same regulatory components. Since the *ume6*Δ phenotype arises from the loss of a factor linked to URS1 repression function, the activating point mutations may interfere directly or indirectly with Ume6p actions that target the URS1-bound repressor complex.

URS1 and the ARE have different effects during early meiotic induction. As described, meiotic expression of *HSP82* is about 55% higher in the presence of an intact URS1 element than in its absence. In contrast, an intact ARE adjacent to URS1 decreases levels of meiotic expression by as much as 45% (Fig. 3; compare rows 21 and 23; Fig. 7A and C). These data indicate that an intact ARE plays a continuing repressive role throughout early meiosis and offer further evidence that factors bound to the ARE and URS1 may not function as an integral complex. The opposing roles of URS1 and the ARE appear to be specific to meiosis, in distinction to heat shock, in which case URS1 or ARE replacements have equivalent small effects (Fig. 7C and D).

URS1 and the HSE interact during sporulation but not during heat shock. Induction of *HSP82* under the two very different physiological conditions of early meiosis and heat shock requires a generalized HSE. To explore whether the role of an HSE in the developmental response is distinguishable from its role during heat shock, I compared the patterns of induction during sporulation and heat shock for various mutations of the HSE and the URS-ARE operator.

In sporulating cells, *HSP82* was induced with similar kinetics and attained a constant maximal level of transcription for all URS1 variants that either strongly repressed or activated vegetative expression (Fig. 7A). In marked contrast, heat shock of the same URS1 constructs led to widely varying levels of induced transcription that roughly correlated with vegetative expression (Fig. 7B). The increase in expression during sporulation is inversely related to the vegetative transcription level, whereas the level of transcription during heat shock increases in a roughly additive manner. In the case of heat shock, the straightforward interpretation is that constitutively bound HSF is directly stimulated by heat shock through a pathway independent of the URS-ARE repression-activation motif (21, 46).

The pattern observed during sporulation is consistent with *HSP82* induction occurring through the relief of URS-mediated repression. The complex bound to an activating URS1 variant during vegetative growth is the functional equivalent of a complex bound to the canonical URS1 at some point during meiotic induction. This model is supported by the observation

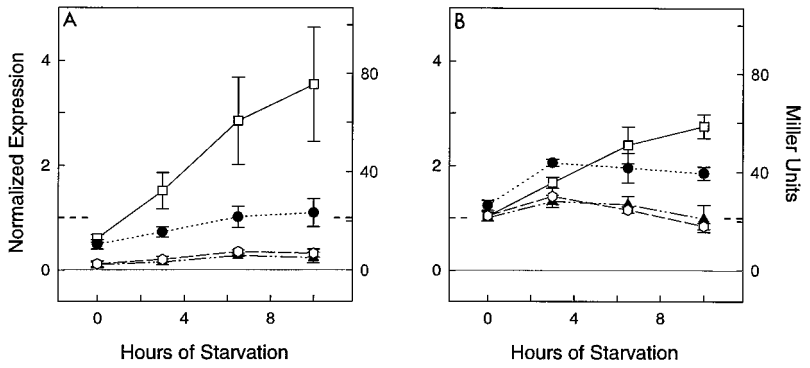

FIG. 8. *IME2* plays a major role in starvation-dependent induction. Haploid α cells of the indicated genetic backgrounds were starved in 2% acetate sporulation medium. Symbols: ○, WT; □, pRS P_{ACT1}-*IME1* (a multicopy plasmid that constitutively expresses an *ACT1* promoter-*IME1* fusion gene [69]); ▲, *ime2*Δ (an *ime2-2*::*LEU2* chromosomal disruptant [48]); ●, P_{ACT1}-*IME1 ime2*Δ. (A) Expression of an *HSP82-lacZ* reporter carrying an intact URS1-ARE element; (B) expression of an *HSP82-lacZ* reporter carrying a random replacement of the URS-ARE motif (Ø UAB). All constructs incorporate 5′ *HSP82* DNA from −188 to +1. To differentiate between activation and repression, data are normalized to the vegetative expression level of the operatorless reporter in WT cells (Ø UAB, 0 h of starvation; the reference activity level is shown by horizontal dotted lines).

that the activating URS1 variants and Ume6p apparently modulate the same machinery to mediate vegetative activation. It suggests that the antagonism of Ume6p function is inherent to early meiotic induction, as might be expected of a protein essential for the mitotic repression of all tested early meiotic genes. The equivalent functionality of the repressing and activating URS1 elements at the height of meiotic expression furthermore suggests that these motifs may bind similar protein complexes during vegetative growth despite supporting very different levels of vegetative transcription (see Discussion). This view is consistent with the observation that the random replacement mutation, which cannot bind a repressing or activating form of the URS1 factor, supports a lower level of early meiotic expression (Fig. 7A and C).

***IME1* expression antagonizes URS1-mediated vegetative repression.** To investigate how the major regulatory factor that controls entry into the meiotic pathway might interact with operator sequences, a multicopy plasmid that constitutively overexpressed *IME1* was transformed into cells carrying integrated minimal *HSP82-lacZ* reporters. In haploid cells grown in acetate, a construct bearing a replacement of the entire URS1-ARE operator displayed the same expression in the presence or absence of ectopic Ime1p (Fig. 8B, 0 h). For constructs bearing an intact URS1 version of the operator, overexpressing *IME1* increases transcription sixfold above the normal repressed level to about half that seen in the absence of the entire URS1-ARE motif (Fig. 8A, 0 h). This partial-derepression phenotype suggested that only a single component of the bipartite operator might be the target of *IME1* action. Therefore, constructs containing specific replacements of either URS1 or the ARE were evaluated in *IME1*-overexpressing strains. In wild-type cells, reporters bearing lesions in either component transcribed at 70% of the level seen for the extended URS-ARE lesion (Fig. 9A). When *IME1* was overexpressed, transcription of the ARE mutation recovered to that of the extended lesion, whereas transcription of the URS1 mutation declined slightly. This pattern indicates that the URS1 element is the specific target of *IME1* antagonism.

The loss of specific interactions between URS1 and an activation element may enable secondary modes of induction. To test whether meiotic specificity is a simple combinatorial property of known meiotic repression and activation elements, HSE 1 was replaced by the *IME2* T_4C activation element (Fig. 9B). No induction during sporulation was observed for T_4C constructs that did or did not include the consensus URS1-ARE motif (data not shown). Both T_4C constructs supported the same high level of vegetative activity, about 30 times that of *HSP82* with URS1 (Fig. 9B, rows 5 to 8). In an *ime1*Δ background, vegetative expression of the T_4C constructs decreased about twofold, but it changed little for the HSE 1 counterparts. Interestingly, for cells grown in glucose, vegetative expression was virtually absent for T_4C constructs but only slightly decreased for those with HSE 1 (rows 9 to 12). The loss of T_4C-mediated transcription may reflect glucose repression, because addition of small amounts of glucose to acetate-grown cells severely reduced vegetative activity (data not shown).

The pattern of expression mediated by T_4C here differs radically from that of the composite T_4C-URS1 UAS in *IME2*; in this case, *IME1*-dependent expression increases 100-fold over extremely low vegetative levels (7). However, when an isolated T_4C element is used as the UAS in a *CYC1-lacZ* reporter, it behaves much as it does in *HSP82* (8). A simple explanation that integrates all of the foregoing observations asserts that high T_4C-dependent constitutive expression corresponds to a derepressed state. The repression element cannot function because the ARE cannot substitute for a similar unidentified *IME2* ancillary element or because the mutual context of URS1 and T_4C is altered in terms of spacing, orientation, or distance from promoter. As is the case for HSE 1 (see below), *IME1* can only minimally stimulate T_4C directly. The *IME1*-dependent induction reported for the native T_4C-URS1 module (7) may thus entail, via an altered interaction between factors bound to URS1 and T_4C, the unmasking of latent induction machinery bound to T_4C, which in this case is glucose repressible.

***IME2* plays a major role in starvation-dependent induction.** When haploid cells that overexpressed *IME1* were starved in acetate sporulation medium, an additional time-dependent increase in β-galactosidase activity occurred in the reporter fusion containing the URS1-ARE motif (Fig. 8A). A lesser increase with similar kinetics was noted for the reporter in which random sequence had replaced the bipartite operator (Fig. 8B), consistent with the absence of the previously described URS1-HSE activation mechanism. If the chromosomal *IME2* gene was disrupted and *IME1* was overexpressed, a very small increase in transcription occurred in the URS1-ARE reporter; in the operatorless construct, expression increased modestly for the first 3 h and thereafter declined slowly. For both reporters, *IME2* disruption alone led to no induction. It is clear that *IME2* function is required for efficient induction under

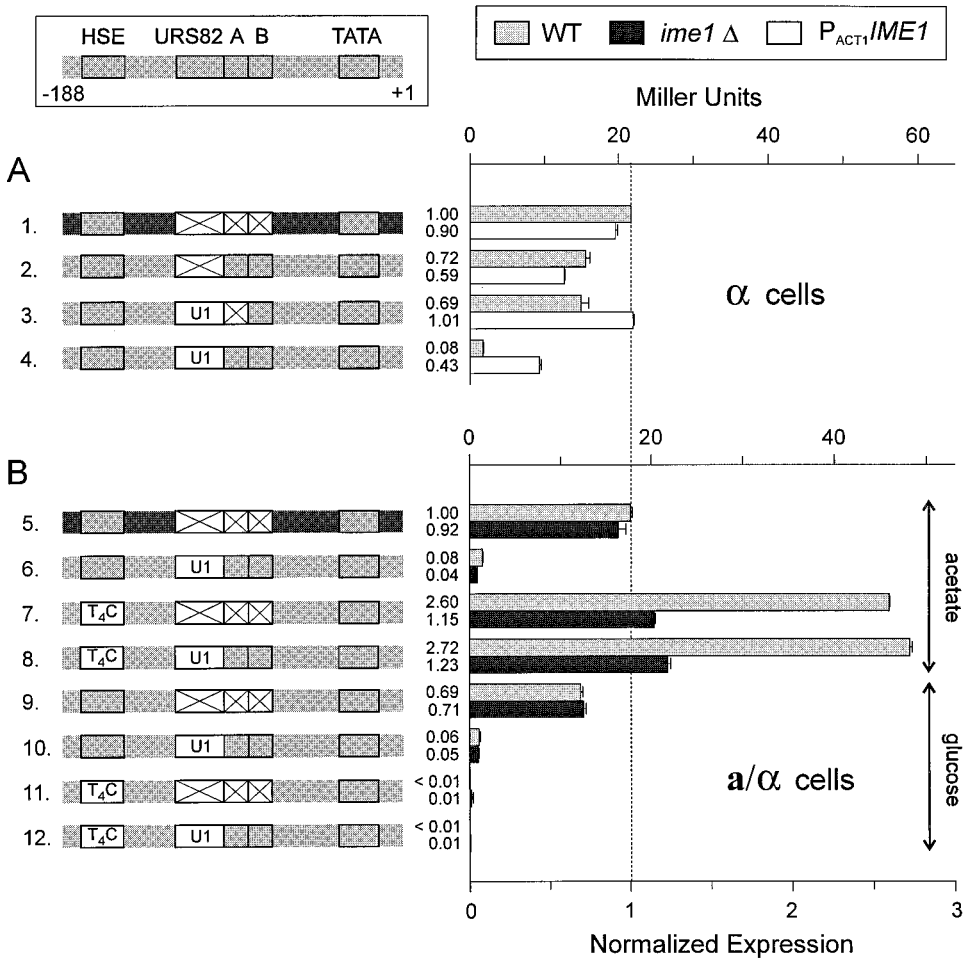

FIG. 9. (A) Overexpressed *IME1* specifically antagonizes the function of the URS1 component of the URS1-ARE motif. β-Galactosidase activity was assayed in acetate-grown WT or P_{ACT1}-*IME1* (69) α haploid cells. To better examine URS1 function, data are normalized to the expression of an operatorless *HSP82* construct (row 1, dark construct). (B) An *IME2* activation element does not support URS1-dependent repression and activation when substituted for the *HSP82* HSE. Constructs containing the *IME2* T_4C activation element (7) or HSE 1 were integrated into P_{ACT1}-*IME1* and *ime1-12* (62) **a**/α diploid cells. Expression was measured in cells grown in synthetic acetate or glucose medium as indicated. The operatorless integrant in acetate-grown WT cells was used to normalize β-galactosidase activity (row 5, dark construct).

starvation conditions and that to a considerable degree, *IME2* can stimulate transcription in the absence of the bipartite repression element, especially later in the response.

Interestingly, *IME1-IME2*-dependent ectopic induction of *HSP82* also occurs in acetate-grown haploid cells subsequently starved in 2% glucose but not in cells starved in water (data not shown). Starvation of haploids in the presence of a carbon source allows the eventually suicidal progression of the cell cycle, whereas starvation in water leads to a protective cell cycle arrest (16, 17). This finding suggests that in addition to responding to limiting nutrients, ectopically expressed *IME1-IME2* pathway components also monitor cell cycle progression.

DISCUSSION

Absence of positive early meiotic determinants. A primary aim of this study was to identify *cis*-acting DNA elements that regulate the induction of *HSP82* transcription during sporulation. Surprisingly, *HSP82* was found to be a member of the so-called early meiotic genes. *HSP82* has two features in common with all members of this family: its meiotic induction is under control of the *IME1-IME2* transcriptional cascade, and it possesses an URS1 element. However, *HSP82* also differs from members in two respects: the translated product clearly has functions other than in meiosis or sporulation (6, 55), and it is the only currently identified gene that is highly transcribed prior to meiotic induction.

HSP82 shares an unusual trait with two other early meiotic genes whose regulatory regions have been dissected, *SPO13* and *HOP1*, in that no single sequence capable of regulating meiotic transcription in a heterologous reporter has been identified. In all three cases, proper regulation requires less than 200 bp of immediate upstream sequence; each includes a nonspecific activation element that lies 20 to 50 bp 5′ to a functional URS1 element. Whereas the positive elements in *SPO13* (undescribed) and *HOP1* (UAS_H) are poorly characterized, HSE 1 and other yeast HSEs have been thoroughly studied. An isolated HSE can nonspecifically support meiotic induction, but there must be other meiotic regulatory mechanisms in vivo because many heat shock genes are not induced during sporulation. Therefore, attention is focused on the element in common, URS1. The widespread occurrence of URS1 in nonmeiotic genes indicates a general function for this element but

does not preclude an obligate supporting role in conferring meiotic specificity.

Two modes of meiotic activation. The absence of a positively acting meiotic determinant suggests a mechanism in which specificity is conferred through the regulated relief of URS1-mediated repression. Although the means by which early meiotic transcriptional regulators interact with URS1 to initiate relief is still obscure, the data presented here suggest that full meiotic activation of *HSP82* actually involves two distinct mechanisms: (i) the relief of URS1-mediated repression, followed by URS1 and the HSE acting jointly to stimulate meiotic expression; and (ii) the *IME2* kinase directly or indirectly stimulating an HSE-bound factor. URS1-dependent activation contributes about a third of full meiotic expression, and *IME2*-dependent activation contributes about two-thirds.

The relative proportion of meiotic expression that is *IME2* dependent appears to be larger for *HSP82* than for *SPO13* and *HOP1* (48, 63), whereas the meiotic expression driven by the upstream T_4C-URS1 UAS is completely independent of *IME2* (7). These variations further affirm that *IME2*-mediated activation is mechanistically distinct from URS1-mediated activation. *IME2* has been shown to weakly stimulate meiotic transcription of *IME2* that is mediated by sequences separate from the T_4C-URS1 UAS (7). However, the presence of URS1 in all early meiotic genes suggests that URS1-mediated repression may have to be lifted prior to any *IME2*-mediated activation. The existence of a relatively strong *IME2*-dependent mode of activation only for genes in which the UAS resides near the promoter suggests that factors proximal to the basal transcriptional machinery may be specifically involved in this type of activation, consistent with the behavior of *HSP82* reported here.

The vegetative activation conferred by *ume6*Δ or rare point mutations in URS1 may utilize the same transcriptional machinery involved in URS1-mediated activation during early meiosis. Vegetative and meiotic modes of activation require the collaboration of URS1 and the HSE. The absolute increases in expression are similar for vegetative and meiotic activation, and in each mode URS1 supports a somewhat greater increase than URS82. Time course experiments provide evidence that the two activation modes are directly linked by showing that the vegetative activation state is encompassed by the meiotic activation pathway. The URS1-associated mechanisms by which vegetative expression can be artificially upregulated may thus provide insight into URS1-mediated meiotic activation.

The role of the bipartite repression element. Although it is known that sequence context is sometimes important for URS1 repression function in nonmeiotic genes (42), URS1 is in isolation a general repression element, and therefore specific meiotic function must be conferred by other sequences. A major finding of these experiments is that an ancillary element, the ARE, is required for efficient URS1-dependent repression. This is the first identification of an obligate accessory element in an early meiotic gene; it has been recently reported that an URS1 and an ABF1 element jointly mediate the glucose repression of a metabolic gene, *FOX3* (13). The ARE is operationally distinct from the core URS in that the ARE motif is at least to a limited extent physically separable from URS1 and in that it facilitates only repression in all tested environments, in contrast to the variable nature of URS1. The extended homology between the URS1-ARE region in *HSP82* and corresponding sequences in *SPO13* suggests that an ARE-bound factor(s) might specifically interact with early meiotic regulators. However, ectopic overexpression of *IME1* in vegetatively growing cells partially antagonizes repression mediated by the URS1 component rather than the ARE. It is surprising that the nonspecific URS1 is the target for a presumed early step in meiotic activation, but this is a limited stimulus under conditions quite different from those that initiate sporulation. It is possible that additional stimuli mediated by other meiotic regulators or a modified Ime1p target the ARE. Sequence analysis of 14 early meiotic genes (47) identifies 10 that contain good matches (6 of 7 bp or better) to the ARE within 50 bp of URS1 (Fig. 10). In eight cases, including *HSP82*, *HOP1*, and *SPO13* (a second match), the ARE motif is downstream of URS1. It is currently unknown whether any of the candidate ARE motifs have a function corresponding to that of the *HSP82* element. Note that the URS1 motif as compiled from early meiotic genes extends the published URS1 consensus (42) to 10 bp, with pronounced variability confined to a single position.

	ARE			URS		
HSP82	TGAGAAT	-	-185	TAaCCGCtCA*	-	-195
SPO13	TGAGAAA	+	-104	TAGCCGCCGA*	+	-97
	TGAGAcT	-	-78			
HOP1	aGAGAAA	+	-133	TAGCCGCCCA*	-	-173
IME2	gGAGAAA	-	-580	TAGCCGCCGt*	-	-552
				TtGCCGCCGA*	-	-457
DMC1	TGgGAAA	-	-183	TAGCCGCCCA	+	-138
ZIP1	aGAGAAT	+	-5	TAGCCGCCGA	-	-22
	TGAGAtT	+	-45			
RED1	TGAaAAA	-	-664	TAGtCGCCAA	-	-691
	TGAGcAA	-	-179	TAGCCGCtGA	-	-166
	TGcGAAA	-	-144			
SPO16	cGAGAAA	+	-76	TAGCCGCCCA	-	-90
REC114	aGAGAAT	+	-109	TAGCCGCCCA	-	-90
REC104	TcAGAAA	+	-48	TAGCCGCCAA	-	-93
MER1	TGAGAAA	+	-199	TAGCCGCCGA	+	-112
MEK1	TGAaAAA	-	-284	TAGCCGCCAt	-	-150
				TAGCCGCCGA	-	-136
MEI4	TGtGAAA	+	-43	TAGCCGCCCA	-	-98
SPO11	TGtGAAT	-	+163	TAGCCGCCAA	-	+253
CONSENSUS	TGAGAAA/T			TAGCCGCCCA/G/A		$URS1_E$
				AGCCGCCGA		URS1

FIG. 10. Relationship of ARE and URS motifs in early meiotic genes. To facilitate comparison, orientations of motifs (sense [+] and antisense [−]) are relative to the published URS1 consensus sequence (42). Positions of the motifs represent the 5′-most base pairs relative to the 5′-3′ gene orientation and are numbered with respect to the translation start. Lowercase letters indicate mismatches to the derived consensus sequences. Asterisks denote URS1 elements that reside within UAS or promoter regions demonstrated to support early meiotic induction.

The mechanism of URS1 function. It is striking that the URS1-bound factor can be converted from a repressor to an activator of vegetative transcription by two experimental means, the deletion of *UME6* or the introduction of certain point mutations into URS1. URS1 is operationally distinct from classical activation elements in *S. cerevisiae* because it requires an adjacent UAS (HSE) in order to function. Despite uncertainty as to the nature of the factor(s) that binds to URS1, there are several lines of evidence that suggest that much of the functional repressor complex remains bound to URS1 when it functions as an activator. First, in a gel shift experiment using crude WT yeast cell extract, a 26-bp *SPO13* oligonucleotide that spans URS1 and 5 bp of the ARE homology produces six shifted complexes (67). In an extract prepared from a *ume6* disruptant, the two most slowly migrating complexes were replaced by two faster complexes, consistent with

Ume6p directly or indirectly interacting with a multisubunit complex that remains bound to the oligonucleotide. Second, a preparation purified by virtue of its affinity to the prototypal *CAR1* URS1 element was found to be almost exclusively replication factor A (RPA), a heterotrimeric complex implicated in early events of DNA replication in *S. cerevisiae* and mammals (40). Interestingly, this complex binds the activating G→C URS1 variant described earlier as well as it binds to the prototypal URS1 (30). Furthermore, the activating sequence within a classical *CAR1* UAS is an RPA-binding motif related to this activating URS1 variant (30), suggesting that in vivo URS1-bound complexes that repress or activate may modulate the same regulatory machinery. Third, as reported in this study, the G→C (and also the T→C) URS1 variant is epistatic to *ume6*Δ, suggesting that a functionally equivalent activator complex binds to URS1 in the presence of these *cis* and *trans* lesions. Last, the observation here that the most efficient repressor becomes the most efficient activator when *UME6* is deleted suggests that the affinities of both forms of complex for their binding sites are related and thus perhaps reflect the retention of crucial protein-DNA contacts. Since activating point mutations of the prototypal URS1 elicit the same phenotype as the intact URS1 in a *ume6*Δ background, it might be thought that the point mutations achieve their functional effect simply by eliminating a DNA-binding site for Ume6p (or an *UME6*-dependent protein) (67). However, among the many point mutations assayed, those that strongly activate are rare (30, 42), suggesting that activation may instead result from an alternate conformation of bound complex that is stabilized by an altered binding site. There is indirect evidence that yeast RPA may exist in vivo in two forms that have different physical properties and different affinities for single-stranded DNA and double-stranded DNA (30).

A recent study of the T_4C-URS1 UAS of *IME2* has led to the suggestion that *IME1* activates meiotic genes by converting URS1-bound Ume6p from a negative regulator to a positive regulator, thereby establishing URS1 as an independently acting UAS (8). The *IME2* analysis and the present study are in agreement that URS1 is functionally converted from an element that mediates repression to one that mediates activation, but the mechanism proposed for *IME2* induction is difficult to reconcile with these *HSP82* experiments. It is unknown whether *UME6* is a positive regulator of other meiotic genes, but levels of *SPO13*, *SPO11*, and *SPO16* expression seen in *ume6*Δ vegetative SK1 cells approach the levels seen in WT meiotic SK1 cells (67). It is likely that the high *ume6*-dependent vegetative expression of these early meiotic genes corresponds to the *ume6*-triggered URS1-dependent vegetative activation seen in *HSP82*. Such a correspondence strengthens the argument that *UME6* plays no positive role in URS1-mediated meiotic activation of these genes.

A role for HSF. Although the experiments reported here make no attempt to directly identify the factor(s) that binds HSE 1 during early sporulation, it is likely to be HSF because (i) no other HSF-like proteins in *S. cerevisiae* that bind HSEs have been identified (65, 79); (ii) HSEs from nonmeiotic genes can substitute for HSE 1 during sporulation, making improbable the existence of HSF-like proteins that recognize only a subset of HSEs; (iii) variant HSEs support similar relative levels of induction during early meiosis and heat shock; (iv) yeast HSF binds HSE 1 constitutively (19, 46, 72), and haploids or *ime1* diploids retain vegetative levels of transcription under sporulation conditions; and (v) early meiotic induction occurs at a time when there is only minimal synthesis of new sporulation-specific proteins. Induction can be driven ectopically in haploids by first expressing *IME1* and *IME2* and subsequently starving the cells. This finding is consistent with the idea that later starvation-dependent steps in the transcriptional activation of *HSP82* may involve only modifications of extant proteins.

Yeast HSF contains two spatially and functionally separable activation domains (3, 11, 26, 50, 64). A broad N-terminal region contains a stress-inducible activator (it does not support constitutive expression) that cannot be resolved from the DNA-binding site, whereas a C-terminal domain can function independently as a strong constitutive activator. Under conditions of steady-state growth, a small region near the N terminus can operationally mask the C-terminal activator in inverse proportion to environmental temperature, thereby eliciting a sustained heat-dependent vegetative response (11, 64). In heat-shocked cells, only the N-terminal activator can independently support a transient increase in expression; the presence of the C-terminal domain simply increases the overall level of expression without altering the magnitude of the induction.

The pattern of *HSP82* regulation is consistent with this understanding of HSF structure and function. The activating URS1 point variants mediate sustained HSF-dependent vegetative activation that is distinct from heat shock, suggesting that the URS1-bound complex may directly or indirectly unmask the HSF C-terminal domain. However, this model remains speculative pending experiments using engineered forms of HSF. An HSE found in *CUP1* mediates a sustained induction upon glucose starvation that is dependent on *SNF1* and *SNF4*, known regulators of glucose starvation-induced genes (73). In *CUP1*, the carboxyl domain of HSF is critical to the sustained starvation response and also supports transient *SNF*-independent heat shock induction (73). The regulated use by two pathways of the HSF carboxyl domain in *CUP1* hints that this domain may contact other regulatory components, but DNA-binding sites for such factors have not been identified. Secondary regulatory elements in *SSA1*, *SSA3*, and *HSP26* that may depend at least in part on an HSE for their function have been identified or can be inferred, but little is known about their mode of action (5, 53, 71, 81).

Mechanism of *IME2*-mediated induction. It is a paradox that *IME1-IME2* expression plus starvation is able to induce *HSP82* transcription in the absence of any *cis*-acting element specific to early meiotic genes. Although URS1 supports about a third of increased meiotic transcription, it appears as if *IME2* transmits a starvation signal that independently stimulates other components of the *HSP82* transcriptional machinery. These experiments do not distinguish whether starvation increases the transcription or translation of *IME2* or, alternatively, activates or recruits existing Ime2p. Evidence presented here suggests that a factor binding the HSE could be directly activated by *IME2*. Phosphorylation of yeast HSF in vivo is important for the downregulation of transcription following heat shock (23). It remains to be tested whether in response to starvation *IME2* can directly or indirectly modify HSF.

It also remains possible that *IME2* can efficiently activate only a complex composed of HSF and URS1 factors; namely, components of the URS1 complex also may be able to function in *trans* when an URS1 binding site is absent. Precedent for this type of scenario is found in the TATA-binding protein's ability to function with promoters that lack a cognate binding site (68). In such a model, the function of URS1 would be to tether general transcriptional machinery adjacent to regulatory factors that impart specificity and adjacent to UAS-bound activators that drive transcription. By maintaining contact with the activation domains of UAS activators, an URS1-bound complex could also effectively screen these enhancer proteins from inappropriate interactions with other general or specific

transcriptional components. In the case of most early meiotic genes, which are toggled from an off state to an on state, a *cis*-binding URS1 complex may well provide the only meiotically regulated means to mask and unmask the activation domains of activator proteins that otherwise support significant vegetative expression. This model is consistent with the vegetative transcription seen for early meiotic genes in an *ume6* background (67), in which the URS1 complex can no longer function as a repressor. It is perhaps noteworthy that *UME6* and *SRB10* (*UME5*), a *CDC28*-like kinase component of the RNA polymerase II holoenzyme (38), were each isolated in screens for the relief of vegetative repression of *SPO13* (66, 67). Both gene products have roles in repression and activation (this study; 31, 76). This parallel suggests that URS1 could tether or otherwise interact with the polymerase II holoenzyme.

Although there is no direct evidence that *IME2* and the rat mak1 kinase genes are functional homologs, one (*HSP84*) of the two members of the murine *HSP90* genes is specifically expressed in male meiotic germ cells (36), suggesting that the relationship of these meiotic regulatory kinases to these target promoters may indeed be phylogenetically conserved. Meiotic induction of rodent *HSP84* may furthermore involve HSF because HSF2 accumulates in sperm cells and is able to constitutively bind to HSEs of testis-specific *HSP70* genes (57). A developmental role for HSF has also been proposed for *Drosophila melanogaster* (78).

The observation made here that a nonspecific starvation signal plus cell cycle progression may be required for *IME2*-mediated regulation suggests that the expression and/or activity of this kinase depends on general cellular parameters that coexist only after an initial decision has been made to enter the meiotic cell cycle. The ability of the T_4C element to be independently repressed by glucose provides a transcriptional mechanism that enables *IME2* to directly respond to the nutrient environment after URS1-mediated repression has been relieved. Under appropriate conditions, the *IME1*-dependent T_4C-URS1-mediated induction of *IME2* may thus be reversed, and cells may be directed to return to mitotic growth.

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REFERENCES

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
2. **Becker, D. M., and L. Guarente.** 1991. High-efficiency transformation of yeast by electroporation. Methods Enzymol. **194:**182–187.
3. **Bonner, J. J., S. Heyward, and D. L. Fackenthal.** 1992. Temperature-dependent regulation of a heterologous transcriptional activation domain fused to yeast heat shock transcription factor. Mol. Cell Biol. **12:**1021–1030.
4. **Boorstein, W. R., and E. A. Craig.** 1990. Structure and regulation of the *SSA4* HSP70 gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **265:**18912–18921.
5. **Boorstein, W. R., and E. A. Craig.** 1990. Regulation of a yeast HSP70 gene by a cAMP responsive transcriptional control element. EMBO J. **9:**2543–2553.
6. **Borkovich, K. A., F. W. Farrelly, D. B. Finkelstein, J. Taulien, and S. Lindquist.** 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol. Cell. Biol. **9:**3919–3930.
7. **Bowdish, K. S., and A. P. Mitchell.** 1993. Bipartite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**2172–2181.
8. **Bowdish, K. S., H. E. Yuan, and A. P. Mitchell.** 1995. Positive control of yeast meiotic genes by the negative regulator *UME6*. Mol. Cell. Biol. **15:**2955–2961.
9. **Brunt, S. A., and J. C. Silver.** 1991. Molecular cloning and characterization of two distinct hsp85 sequences from the steroid responsive fungus Achlya ambisexualis. Curr. Genet. **19:**383–388.
10. **Buckingham, L. E., H. T. Wang, R. T. Elder, R. M. McCarroll, M. R. Slater, and R. E. Esposito.** 1990. Nucleotide sequence and promoter analysis of *SPO13*, a meiosis-specific gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:**9406–9410.
11. **Chen, Y., N. Barlev, O. Westergaard, and B. Jakobsen.** 1993. Identification of the C-terminal activator domain in yeast heat shock factor: independent control of transient and sustained transcriptional activity. EMBO J. **12:**5007–5018.
12. **Ding, D., S. M. Parkhurst, S. R. Halsell, and H. D. Lipshitz.** 1993. Dynamic *Hsp83* RNA localization during *Drosophila* oogenesis and embryogenesis. Mol. Cell. Biol. **13:**3773–3781.
13. **Einerhand, A. W. C., W. Kos, W. C. Smart, A. J. Kal, H. F. Tabak, and T. G. Cooper.** 1995. The upstream region of the *FOX3* gene encoding peroxisomal 3-oxoacyl-coenzyme A thiolase in *Saccharomyces cerevisiae* contains ABF1- and replication protein A-binding sites that participate in its regulation by glucose repression. Mol. Cell. Biol. **15:**3405–3414.
14. **Farrelly, F. W., and D. B. Finkelstein.** 1984. Complete sequence of the heat-shock-inducible *HSP90* gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **259:**5745–5751.
15. **Grandin, N., and S. I. Reed.** 1993. Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol. Cell. Biol. **13:**2113–2125.
16. **Granot, D., and M. Snyder.** 1991. Glucose induces cAMP-independent growth-related changes in stationary-phase cells of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88:**5724–5728.
17. **Granot, D., and M. Snyder.** 1993. Carbon source induces growth of stationary phase yeast cells, independent of carbon source metabolism. Yeast **9:**465–479.
18. **Gross, D. S., C. C. Adams, S. Lee, and B. Stentz.** 1993. A critical role for heat shock transcription factor in establishing a nucleosome-free region over the TATA-initiation site of the yeast *HSP82* heat shock gene. EMBO J. **12:**3931–3945.
19. **Gross, D. S., K. E. English, K. W. Collins, and S. W. Lee.** 1990. Genomic footprinting of the yeast *HSP82* promoter reveals marked distortion of the DNA helix and constitutive occupancy of heat shock and TATA elements. J. Mol. Biol. **216:**611–631.
20. **Guarente, L., and M. Ptashne.** 1981. Fusion of *Escherichia coli lacZ* to the cytochrome c gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **78:**2199–2203.
21. **Herschlag, D., and F. B. Johnson.** 1993. Synergism in transcriptional activation: a kinetic view. Genes Dev. **7:**173–179.
22. **Hoffman, T., and B. Hovemann.** 1991. Heat-shock proteins, Hsp84 and Hsp86, of mice and men: two related genes encode formerly identified tumour-specific transplantation antigens. Gene **74:**491–501.
23. **Hoj, A., and B. K. Jakobsen.** 1994. A short element required for turning off heat shock transcription factor: evidence that phosphorylation enhances deactivation. EMBO J. **13:**2617–2624.
24. **Honigberg, S. M., C. Conicella, and R. E. Esposito.** 1992. Commitment to meiosis in *Saccharomyces cerevisiae*: involvement of the *SPO14* gene. Genetics **130:**703–716.
25. **Honigberg, S. M., and R. E. Esposito.** 1994. Reversal of cell determination in yeast meiosis: postcommitment arrest allows return to mitotic growth. Proc. Natl. Acad. Sci. USA **91:**6559–6563.
26. **Jakobsen, B. K., and H. R. Pelham.** 1991. A conserved heptapeptide restrains the activity of the yeast heat shock transcription factor. EMBO J. **10:**369–375.
27. **Jinno, A., K. Tanaka, H. Matsushime, T. Haneji, and M. Shibuya.** 1993. Testis-specific Mak protein kinase is expressed specifically in the meiotic phase in spermatogenesis and is associated with a 210-kilodalton cellular phosphoprotein. Mol. Cell. Biol. **13:**4146–4156.
28. **Kawaguchi, H., M. Yoshida, and I. Yamashita.** 1992. Nutritional regulation of meiosis-specific gene expression in *Saccharomyces cerevisiae*. Biosci. Biotechnol. Biochem. **56:**289–297.
29. **Kobayashi, N., and K. McEntee.** 1993. Identification of *cis* and *trans* components of a novel heat shock stress regulatory pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**248–256.
30. **Kovari, L. Z., I. Kovari, and T. G. Cooper.** 1993. Participation of RAP1 protein in expression of the *Saccharomyces cerevisiae* arginase (*CAR1*) gene. J. Bacteriol. **175:**941–951.
31. **Kuchin, S., P. Yeghiayan, and M. Carlson.** 1995. Cyclin-dependent protein kinase and cyclin homologs *SSN3* and *SSN8* contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA **92:**4006–4010.
32. **Kurtz, S., J. Rossi, L. Petko, and S. Lindquist.** 1986. An ancient developmental induction: heat-shock proteins induced in sporulation and oogenesis. Science **231:**1154–1157.
33. **Landt, O., H. P. Grunert, and U. Hahn.** 1990. A general method for rapid site-directed mutagenesis using the polymerase chain reaction. Gene **96:**125–128.
34. **Lee, M. S., and W. T. Garrard.** 1991. Transcription-induced nucleosome

'splitting': an underlying structure for DNase I sensitive chromatin. EMBO J. **10:**607–615.
35. **Lee, M. S., and W. T. Garrard.** 1992. Uncoupling gene activity from chromatin structure: promoter mutations can inactivate transcription of the yeast *HSP82* gene without eliminating nucleosome-free regions. Proc. Natl. Acad. Sci. USA **89:**9166–9170.
36. **Lee, S.** 1990. Expression of *HSP86* in male germ cells. Mol. Cell. Biol. **10:** 3239–3242.
37. **Li, W., and F. Sherman.** 1991. Two types of TATA elements for the *CYC1* gene of the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**666–675.
38. **Liao, S., J. Zhang, D. A. Jeffery, A. J. Koleske, C. M. Thompson, D. M. Chao, M. Viljoen, J. J. van Vuuren, and R. A. Young.** 1995. A kinase-cyclin pair in the RNA polymerase II holoenzyme. Nature (London) **374:**193–196.
39. **Lopes, J. M., K. L. Schulze, J. W. Yates, J. P. Hirsch, and S. A. Henry.** 1993. The *INO1* promoter of *Saccharomyces cerevisiae* includes an upstream repressor sequence (URS1) common to a diverse set of yeast genes. J. Bacteriol. **175:**4235–4238.
40. **Luche, R. M., W. C. Smart, and T. G. Cooper.** 1992. Purification of the heteromeric protein binding to the URS1 transcriptional repression site in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**7412–7416. (Erratum, **89:**11107.)
41. **Luche, R. M., W. C. Smart, T. Marion, M. Tillman, R. A. Sumrada, and T. G. Cooper.** 1993. *Saccharomyces cerevisiae* BUF protein binds to sequences participating in DNA replication in addition to those mediating transcriptional repression (URS1) and activation. Mol. Cell. Biol. **13:**5749–5761.
42. **Luche, R. M., R. Sumrada, and T. G. Cooper.** 1990. A *cis*-acting element present in multiple genes serves as a repressor protein binding site for the yeast *CAR1* gene. Mol. Cell. Biol. **10:**3884–3895.
43. **Malone, R. E.** 1990. Dual regulation of meiosis in yeast. Cell **61:**375–378.
44. **Mandel, S., K. Robzyk, and Y. Kassir.** 1994. *IME1* gene encodes a transcription factor which is required to induce meiosis in *Saccharomyces cerevisiae*. Dev. Genet. **15:**139–147.
45. **Matsushime, H., A. Jinno, N. Takagi, and M. Shibuya.** 1990. A novel mammalian protein kinase gene (*mak*) is highly expressed in testicular germ cells at and after meiosis. Mol. Cell. Biol. **10:**2261–2268.
46. **McDaniel, D., A. J. Caplan, M. S. Lee, C. C. Adams, B. R. Fishel, D. S. Gross, and W. T. Garrard.** 1989. Basal-level expression of the yeast *HSP82* gene requires a heat shock regulatory element. Mol. Cell. Biol. **9:**4789–4798.
47. **Mitchell, A. P.** 1994. Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. **58:**56–70.
48. **Mitchell, A. P., S. E. Driscoll, and H. E. Smith.** 1990. Positive control of sporulation-specific genes by the *IME1* and *IME2* products in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2104–2110.
49. **Moehle, C. M., and A. G. Hinnebusch.** 1991. Association of RAP1 binding sites with stringent control of ribosomal protein gene transcription in *Saccharomyces cerevisiae*. Mol. Cell Biol. **11:**2723–2735.
50. **Nieto-Sotelo, J., G. Wiederrecht, A. Okuda, and C. S. Parker.** 1990. The yeast heat shock transcription factor contains a transcriptional activation domain whose activity is repressed under nonshock conditions. Cell **62:**807–817.
51. **Ochman, H., A. S. Gerber, and D. L. Hartl.** 1988. Genetic applications of an inverse polymerase chain reaction. Genetics **120:**621–623.
52. **Park, H. D., R. M. Luche, and T. G. Cooper.** 1992. The yeast *UME6* gene product is required for transcriptional repression mediated by the *CAR1* URS1 repressor binding site. Nucleic. Acids Res. **20:**1909–1915.
53. **Park, H. O., and E. A. Craig.** 1991. Transcriptional regulation of a yeast HSP70 gene by heat shock factor and an upstream repression site-binding factor. Genes Dev. **5:**1299–1308.
54. **Perisic, O., H. Xiao, and J. T. Lis.** 1989. Stable binding of Drosophila heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. Cell **59:**797–806.
55. **Picard, D., B. Khursheed, M. J. Garabedian, M. G. Fortin, S. Lindquist, and K. R. Yamamoto.** 1990. Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature (London) **348:**166–168.
56. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
57. **Sarge, K. D., O. K. Park-Sarge, J. D. Kirby, K. E. Mayo, and R. I. Morimoto.** 1994. Expression of heat shock factor 2 in mouse testis: potential role as a regulator of heat-shock protein gene expression during spermatogenesis. Biol. Reprod. **50:**1334–1343.
58. **Sherman, A., M. Shefer, S. Sagee, and Y. Kassir.** 1993. Post-transcriptional regulation of *IME1* determines initiation of meiosis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **237:**375–384.
59. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
60. **Slater, M. R., and E. A. Craig.** 1987. Transcriptional regulation of an hsp70 heat shock gene in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**1906–1916.
61. **Smith, H. E., S. E. Driscoll, R. A. L. Sia, H. E. Yuan, and A. P. Mitchell.** 1993. Genetic evidence for transcriptional activation by the yeast *IME1* gene product. Genetics **133:**775–784.
62. **Smith, H. E. and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**2142–2152.
63. **Smith, H. E., S. S. Su, L. Neigeborn, S. E. Driscoll, and A. P. Mitchell.** 1990. Role of *IME1* expression in regulation of meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**6103–6113.
64. **Sorger, P. K.** 1990. Yeast heat shock factor contains separable transient and sustained response transcriptional activators. Cell **62:**793–805.
65. **Sorger, P. K., and H. R. Pelham.** 1988. Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell **54:**855–864.
66. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86:**10018–10022.
67. **Strich, R., R. T. Surosky, C. Steber, E. Dubois, F. Messenguy, and R. E. Esposito.** 1994. *UME6* is a key regulator of nitrogen repression and meiotic development. Genes Dev. **8:**796–810.
68. **Struhl, K.** 1994. Duality of TBP, the universal transcription factor. Science **263:**1103–1104.
69. **Su, S. S., and A. P. Mitchell.** 1993. Identification of functionally related genes that stimulate early meiotic gene expression in yeast. Genetics **133:**67–77.
70. **Sumrada, R. A., and T. G. Cooper.** 1987. Ubiquitous upstream repression sequences control activation of the inducible arginase gene in yeast. Proc. Natl. Acad. Sci. USA **84:**3997–4001.
71. **Susek, R. E., and S. Lindquist.** 1990. Transcriptional derepression of the *Saccharomyces cerevisiae HSP26* gene during heat shock. Mol. Cell. Biol. **10:**6362–6373.
71a. **Szent-Gyorgyi, C.** Unpublished data.
72. **Szent-Gyorgyi, C., D. B. Finkelstein, and W. T. Garrard.** 1987. Sharp boundaries demarcate the chromatin structure of a yeast heat-shock gene. J. Mol. Biol. **193:**71–80.
73. **Tamal, K. T., X. Liu, P. Silar, T. Sosinowski, and D. J. Thiele.** 1994. Heat shock transcription factor activates yeast metallothionein gene expression in response to heat and glucose starvation via distinct signalling pathways. Mol. Cell. Biol. **14:**8155–8165.
74. **Treger, J. M., K. A. Heichman, and K. McEntee.** 1988. Expression of the yeast *UB14* gene increases in response to DNA-damaging agents and in meiosis. Mol. Cell. Biol. **8:**1132–1136.
75. **Vershon, A. K., N. M. Hollingsworth, and A. D. Johnson.** 1992. Meiotic induction of the yeast *HOP1* gene is controlled by positive and negative regulatory sites. Mol. Cell. Biol. **12:**3706–3714.
76. **Wahi, M., and A. D. Johnson.** 1995. Identification of genes required for $\alpha 2$ repression in *Saccharomyces cerevisiae*. Genetics **140:**79–90.
77. **Werner-Washburne, M., J. Becker, J. Kosic-Smithers, and E. A. Craig.** 1989. Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. J. Bacteriol. **171:**2680–2688.
78. **Westwood, J. T., J. Clos, and C. Wu.** 1991. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. Nature (London) **353:** 822–827.
79. **Wiederrecht, G., D. Seto, and C. S. Parker.** 1988. Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. Cell **54:**841–853.
80. **Wu, C.** 1980. The 5′ ends of *Drosophila* heat shock genes in chromatin are hypersensitive to DNase I. Nature (London) **286:**854–860.
81. **Young, M. R., and E. A. Craig.** 1993. *Saccharomyces cerevisiae* HSP70 heat shock elements are functionally distinct. Mol. Cell. Biol. **13:**5637–5646.