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A DNA binding factor (UBF) interacts with a positive regulatory element in the promoters of genes expressed during meiosis and vegetative growth in yeast

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ABSTRACT

We have studied the bipartite regulatory element UAS_H/URS1 in the promoter of *HOP1*, whose product is required for synapsis and correct pairing of homologous chromosomes during the first meiotic division. *HOP1* is transcriptionally repressed by the URS1 motif during vegetative growth and induced during meiotic prophase by the UAS_H motif in cooperation with the bifunctional URS1 site, which is required for full induction of *HOP1*. While URS1 is bound *in vitro* by the Buf and Ume6 repressor proteins, we demonstrate for the first time by electrophoretic mobility shift assays and interference footprinting that the UAS_H site interacts *in vitro* with a novel factor called UBF (UAS_H binding factor) which is present in haploid and diploid cycling, as well as sporulating cells. Point mutations in the *HOP1* UAS_H motif abolish UBF-dependent DNA binding activity *in vitro* and meiotic *HOP1* gene expression *in vivo*. Furthermore, we show that UBF binds *in vitro* to UAS_H-like sequences in the promoter regions of several meiosis-specific and non-specific genes and propose that UBF mediates gene expression through its interaction with the UAS_H motif in both cycling and sporulating cells.

INTRODUCTION

Early meiosis-specific genes in yeast provide a good model system for three essential problems in transcriptional regulation: cell type specificity, developmental stage specificity and precise timing of gene expression while undergoing a differentiation program. MATa/α cells of the budding yeast *Saccharomyces cerevisiae* commit themselves in late G1 (*start*) to another round of cell division or, upon starvation, undergo two meiotic divisions to differentiate into haploid spores. Entry into meiosis is controlled by the mating type locus (MAT) and triggered by nitrogen starvation and lack of a fermentable carbon source. Induction of meiosis activates *IME1*, which activates *IME2* through a negative feedback loop. Subsequently sets of early, middle and late meiotic genes are consecutively activated and repressed following a tightly regulated pattern (1–4, for a review see 5).

To date two positive elements are known that mediate early meiotic gene expression in cooperaton with URS1, a bifunctional modulator sequence: the T_4C site, originally discovered as a regulatory element in the *IME2* promoter (TTTTCNNCG; 6), and the UAS_H motif in the *HOP1* promoter (upstream activator site of *HOP1*, TGTGAAGTG; 7). This motif appeares to mediate partial gene activation during both vegetative growth and sporulation (7). Hop1 is associated with precursors (axial elements) of the synaptonemal complex (SC), a multicomponent structure formed in the course of chromosome synapsis during prophase of the first meiotic division (8,9). Many genes essential for early meiotic events show similar expression profiles to *HOP1* and their promoters often contain either a T_4C site or a UAS_H motif or both (10–25, for a review see 5; see also Fig. 5). In genes expressed exclusively in meiosis these positive regulatory sites are invariably linked to the URS1 motif ($T^G/_CGGCGG-^G/_CT$). This sequence represses genes required for metabolic functions during vegetative growth and is also involved in transcriptional control during meiosis (for details see 5,26). Two URS1 motifs from the *CAR1* and *SPO13* promoters are specifically recognized *in vitro* by the heterotrimeric factor Buf (also called *RF-A*; 27–29) and the Ume6 protein (30). In the context of meiotic promoters the URS1 motif is believed to be involved in their repression during mitotic growth (for a review see 5). In the cases of *HOP1*, *IME2* and *SPO13* this sequence also mediates transcripitional activation of a *lacZ* fusion reporter gene during meiosis, most likely through its interaction with the Ime1-dependent activator form of Ume6 (6,7,13,31). There is a considerable overlap in trancriptional activation patterns of genes during mitotic growth and meiotic differentiation, as a number of them are expressed at different levels in mitotic and sporulating cells, like *IME1*, *SPO12*, *MCK1* and *SPO7* (1,18,32,33). *GAL10* and *RAD2* have meiotic expression profiles similar to that of

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HOP1. While the former is dispensable for transition through meiosis, the latter is required for spore formation in a *rad18* mutant backround (34–36). *UME6* is constitutively transcribed in both dividing and sporulating cells (30). It is not known how these genes are regulated during vegetative growth and spore differentiation.

In this paper we present a detailed study of a novel DNA binding factor called UBF that interacts with the UAS_H motif *in vitro*. We show that point mutations within this site abolish UBF-dependent DNA binding activity and inactivate *HOP1* gene expression *in vivo*. These results indicate an essential role for the UAS_H motif in the chromosome in gene activation and show that the URS1 site alone cannot mediate *HOP1* expression during sporulation. UAS_H-like sequences are located within the upstream regions of a number of potential target genes other than *HOP1* and we find three of them to be specifically bound by UBF present in vegetative and sporulating cells *in vitro*. The presence of a UAS_H motif in the *IME1* promoter and several other genes expressed in dividing cells suggests a function for UBF as a general transactivator. This is fully consistent with the observation that several meiotic genes whose promoters contain UAS_H sites are mitotically derepressed in a *ume6* mutant and the previously described UAS_H-mediated *lacZ* reporter gene activation during vegetative growth when the adjacent Ume6 target site was mutated (7,30).

MATERIALS AND METHODS

Oligonucleotides

The following oligonucleotides were used as EMSA probes (5′→3′ sequences are shown, relevant binding sites are underlined, base changes are in bold):
UAS-URS, tcgacGGAGATCTACGTGTGAAGTGATATATGTTTTTAACCTGGGCGGCTAAATTGTACc;
UASm1, tcgacGGAGATCTACGTGTGAA**TC**TATATATGTTTTTAACCTGGGCGGCTAAATTGTACc;
UASm2, tcgacGGAGATCT**CCTT**GTGAA**TC**TATATATGTTTTTAACCTGGGCGGCTAAATTGTACc;
UAS-URSm, tcgacGGAGATCTACGTGTGAAGTGATATATGTTTTTAACC**TCTCGAGCT**AAATTG
TACc; UAS, tcgacGGAGATCTACGTGTGAAGTGATATATGTTTTTc;
URS, tcgacTTTAACCTGGGCGGCTAAATTGTc;
UASZ, tcgacTTTCTTTGAGATTCGGAAGTAAAATACCATAc;
UASS, tcgacTTATTTTCTCTCTGTGTAGTGACAATTTTCAGCc;
UASI, gatccGCATAAAATTGTGATGTTGATTAAAACg;
2×UAS, gatccGATCTACGTGTGAAGTGATATATGATCTACGTGTGAAGTGATATATg;
nsp, tcgacGATCAAGTAACAGCAGGTGCAAAATAAAGTg.

Plasmids

To complement the *hop1* phenotype of our promoter mutant the strain was transformed with pA62-3 (15). Plasmid pSP1 was constructed by ligating a 2.5 kb *Eco*RI–*Sac*I *HOP1* fragment isolated from pA62-3 into a *Eco*RI/*Sac*I-digested pUC19A vector. The *HOP1* disruption plasmid was cloned by ligation of a *Bgl*II–*Bam*HI fragment from pIC19R *URA3*, whose *Sac*I site was blunted with Klenow enzyme, into pSP1 linearized with *Bgl*II/*Bam*HI. pSP1 was used as a template to PCR amplify a fragment containing five point mutations in the *HOP1* UAS_H motif: primer A, 5′-GTAAAGGGAGATCTCCTTGTGAATCTATATATG-3′; primer B, 5′-TATGATCATAGGAAACTGCAGTCAATTTCTTTC-3′. Primer A contains five mismatches, introducing the UASm2 sequence and a diagnostic *Hin*fI restriction site. The PCR fragment was digested with *Bgl*II and *Pst*I and ligated into *Bgl*II/*Pst*I-linearized pSP1. A *Hin*dIII–*Bam*HI fragment from pSP3 containing the PCR fragment was subcloned into Bluescript pKS (Stratagene) for sequence analysis.

Yeast strains

Chromosomal integrations and disruptions followed the one-step gene replacement procedure (38). The strain harboring the mutated *HOP1* promoter (UASm2) was constructed in two steps. First, *HOP1* and its promoter were replaced by *URA3* using the *Eco*RI–*Sac*I fragment of pSP2. Subsequently Yep13 and the *Eco*RI–*Sac*I fragment of pSP3 were co-transformed to integrate *HOP1* and the mutated promoter. Leu^+ transformants were replica plated onto FOA-containing medium to identify $ura3^-$ clones. Strain SP2 was transformed with Yep-*HO* to obtain a diploid strain homozygous for the UASm2 mutation. Integrations were confirmed by Southern blot analysis of genomic DNA digested with *Eco*RI and *Sac*I using *HOP1* as the probe.

Yeast cell extracts

Yeast extracts were prepared from logarithmically growing or sporulating cells according to Pfeifer *et al.* (39). Sporulation was induced as follows. Cultures were incubated overnight in YEPD medium and re-inoculated into PSP-medium (1% potassium acetate, 2% peptone, 1% yeast extract). Cultures containing 2×10^7 cells/ml were resuspended in 0.5 vol. SPO-medium (2% potassium acetate) and incubated at 30°C. Protein concentrations were determined using the BioRad Bradford protein assay. Extract aliquots were stored at –80°C.

DNA binding assays

Oligonucleotide probes were end-labeled with Klenow DNA polymerase and two ^{32}P-labeled radioactive nucleotides. EMSAs were performed as described in Primig *et al.* (40), except that the samples were loaded onto 5 or 4% gels in 0.25× TBE. Poly(dI·dC) (0.2 μg/μg protein extract) (Pharmacia) was added as a non-specific competitor prior to addition of labeled probe. Non-labeled oligonucleotides were added to the extracts and incubated for 10 min prior to addition of labeled probe in specific competition assays.

Northern analysis

RNA isolation was as described in Cross and Tinkelenberg (41). The RNA was loaded onto a 1% agarose gel containing formaldehyde. Hybridization was in Church buffer (0.5 M sodium phosphate, pH 7.2, 7% SDS). pGB430 was digested with *Eco*RV and pNH-41 with *Bam*HI and *Hin*dIII to yield probes specific for *SPO11* and *HOP1*, respectively. rRNA was visualized by staining the filter with methylene blue (2%) for 10 min at room temperature.

DEPC interference assays

DEPC assays were carried out as described in Sturm *et al.* (42), with some modifications. Water (10 µl) containting 1 µg single-stranded oligonucleotide was boiled for 5 min and placed on ice for 10 min. Modification buffer (190 µl) (50 mM sodium cacodylate, pH 7.2, 1 mM EDTA) and 10 µl DEPC were added and incubated at 37°C for 20 min. The reaction was terminated by adding 50 µl stop buffer (1.5 M sodium acetate, 1 M β-mercaptoethanol) and 750 µl ethanol. The modified oligonucleotide (100 ng) was then end-labeled with PNK and annealed to its complementary strand. The resulting probe was purified on a 5% acrylamide:bisacrylamide 20:1 gel and subsequently incubated with 20 µg of a 40% ammonium sulfate fraction of a crude yeast cell extract in a preparative EMSA. The free and retarded probes were excised, eluted overnight (0.5 M ammonium acetate, 1 mM EDTA), ethanol precipitated and resuspended in a 1:10 dilution of piperidine. The DNA was cleaved for 30 min at 90°C, ethanol precipitated, resuspended in loading buffer (80% formamide, 1 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol) and resolved on a 10% sequencing gel.

SC preparation

SC preparation was carried out as described in Klein *et al.* (43).

RESULTS

A factor present in haploid and diploid cells binds to the UAS_H motif in the *HOP1* promoter

It was previously demonstrated that the UAS_H/URS1 element located within a 207 bp sequence upstream of the first ATG of *HOP1* is sufficient for the onset of meiosis-specific transcription (7,8). The results of electrophoretic mobility shift assays (EMSAs) using different probes that contain the UAS_H/URS1 element and the individual sites are summarized in Figure 1. We find a prominent binding activity (referred to as UBF) present in dividing MATa and MATa/α cell types (see Table 1; Fig. 1B, lane 2, and C, lanes 14 and 15) that is specific for the UAS_H motif, as demonstrated by its insensitivity to mutations in the URS1 motif (lanes 17 and 18). Furthermore, while UBF binding can be specifically competed by the non-labeled probe UAS-URS, an oligonucleotide covering only the URS1 motif (URS; compare lanes 3 and 4 with lanes 5–7) cannot. These results are confirmed by an EMSA using a probe covering only the UAS_H motif: UBF binds and is competed with unlabeled probe (UAS) and a probe covering both motifs (UAS-URS), but not by a non-specific oligonucleotide with an unrelated sequence (nsp, lanes 9–12). To determine whether induction of meiosis would affect the UBF binding activity we performed an EMSA using extracts prepared from starved haploid and sporulating diploid cells. Figure 1D, lanes 19–25, indicates that the electrophoretic mobility of UBF-dependent binding signals on probes covering the UAS_H and the URS1 motifs (UAS-URS) or just UAS_H (UAS) are not affected by starvation. We interpret the slight retardation of the binding signal in lanes 20 and 23 to be the consequence of a moderate increase in binding activity present in sporulating diploid cells, but we do not as yet understand its significance. Experiments with an excess of probe yielded the same result (data not shown). We did not observe any significant Buf- or Ume6-like binding signals to our UAS_H/URS1 probe, irrespective of whether we used the synthetic oligonucleotides poly(dI·dC)/poly(dA·dT) or salmon sperm DNA as non-specific competitor (data not shown). These data suggest that the DNA binding activity of UBF is neither cell type-specifically nor developmental stage-specifically regulated and does not depend upon cooperation with Ume6/Buf proteins at a level detectable by EMSAs.

Table 1. Strain list

Strain	Genotype	Reference
SK1a	MATa, *ho::LYS2, lys2, leu::hisG, his4X, ura3*	44
AP1	MATa/α	8
SP1	MATa, *ho::LYS2, lys2, leu::hisG, his4X, ura3, hop1::URA3*	This work
SP2	MATa, *ho::LYS2, lys2, leu::hisG, his4X, ura3, HOP1*-UASm2	This work
SP3	MATa/α, *ho::LYS2, lys2, leu::hisG, his4X, ura3, HOP1*-UASm2	This work

Modified bases within or close to the UAS_H motif interfere with UBF binding *in vitro*

To determine the footprint patterns of UBF from dividing cells versus sporulating cells we performed carbethoxy interference experiments using a UAS-URS probe. The results are shown in Figure 2A. The interference patterns of UBF from dividing MATa and MATa/α and sporulating MATa/α cells are similar and the interfering bases are clustered in the 5′ and 3′ regions of the UAS_H motif, respectively, and extend beyond its previously defined consensus sequence (see Figure 2B, lanes 3, 4, 6, 7, 10, 11, 13 and 14). This indicates that UBF factors from dividing and sporulating cells share similar DNA binding specificities. Our data do not rule out the possibility that a factor of small molecular weight which does not interact with DNA directly is present in different stages or cell types.

We subsequently assayed the effect of point mutations in the UAS_H motif on UBF binding activity. The UBF binding signal from dividing haploid (MATa) and diploid (MATa/α) cells is greatly reduced by three base changes in the 3′ footprint (Fig. 3; UASm1, lanes 8 and 9) and completely abolished by five mutations covering both interference signal clusters (UASm2, lanes 11 and 12). Two point mutations in the 5′ interference signal corresponding to those shown in UASm2 (leaving the 3′ sequence triplet intact) also strongly reduced the binding activity (data not shown). These results were supported by a competition experiment. A 100-fold molar excess of unlabeled wild-type probe containing the UAS_H and URS1 motifs (UAS-URS) and a probe containing a mutated URS1 motif (UAS-URSm) can compete UBF binding, while a probe with an intact URS1 site and five point mutations in the UAS_H motif cannot (UASm2, lanes 3–5). A set of experiments using protein extracts from sporulating diploid cells yielded the same results (data not shown).

Point mutations in the UAS_H motif that abolish UBF binding deregulate *HOP1* gene expression *in vivo*

To assess the *in vivo* effect of point mutations which abolish UBF DNA binding we introduced five base changes corresponding to UASm2 into the *HOP1* promoter of an otherwise wild-type

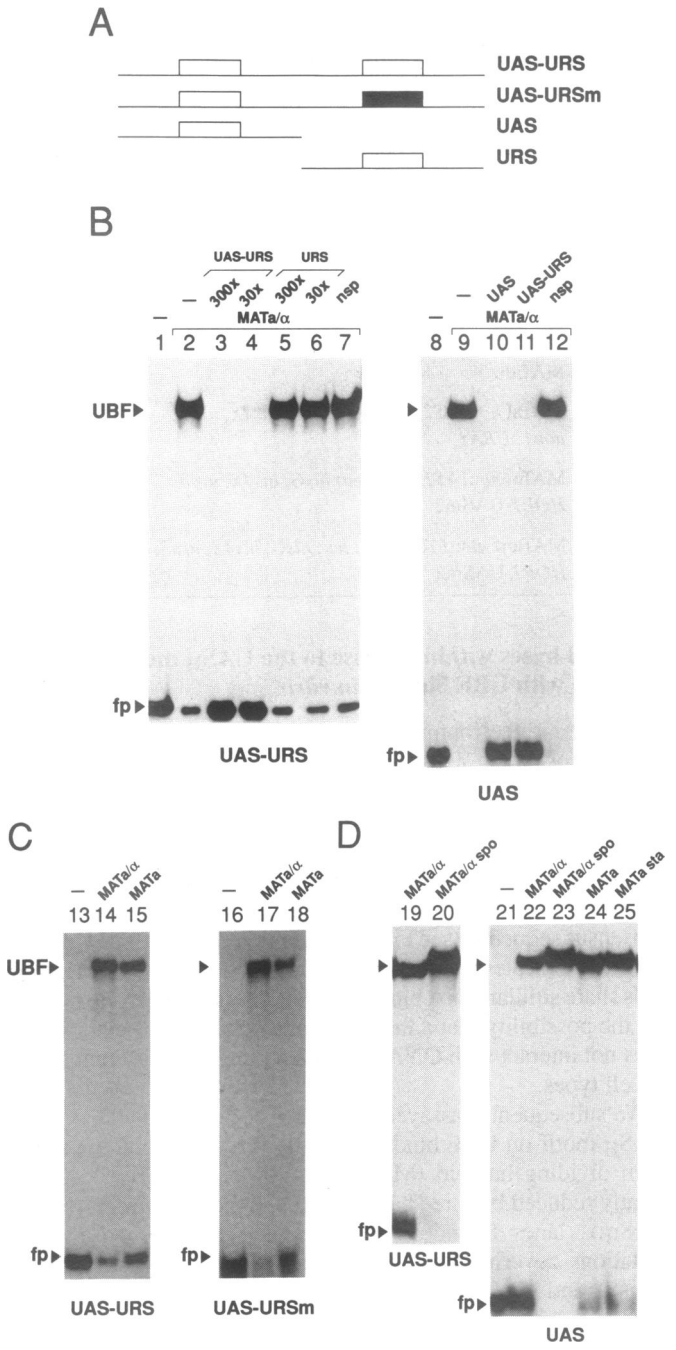

Figure 1. UBF is present in dividing haploid and diploid cells, as well as in sporulating diploid cells, and binds to the UAS_H site in the *HOP1* promoter. (**A**) Oligonucleotide probes used in the EMSA. UAS-URS covers both consensus sites in the *HOP1* promoter. UAS and URS1 are probes containing either UAS_H or URS1 sites and UAS-URSm is a probe with a mutated URS1 site. (**B**) UBF from MATa/α cells binds to UAS_H. (**C**) UBF from MATa cells binds to UAS_H. (**D**) UBF from dividing and sporulating MATa/α and from dividing and starved MATa cells binds to UAS_H. Protein aliquots (10 μg) of extract from cycling diploid cells (MATa/α) were added to 1 ng labeled probe in each assay. Competition with unlabeled probes was at 30-fold molar excess in lanes 4 and 6, at 100-fold molar excess in lanes 10, 11 and 12 and at 300-fold molar excess in lanes 3, 5 and 7. Protein extracts prepared from sporulating diploid cells were added to lanes 20 and 23 (MATa/α spo). The extracts added to lanes 15, 18 and 24 were prepared from dividing haploid cells (MATa), the extract in lane 25 was from starved haploid cells (MATa sta). No extract was added to lanes 1, 8, 13, 16 and 21. UBF, binding activity; fp, free probe; nsp, oligonucleotide with an unrelated DNA sequence used as a non-labeled competitor. The radioactive probes used are indicated at the bottom of the figure.

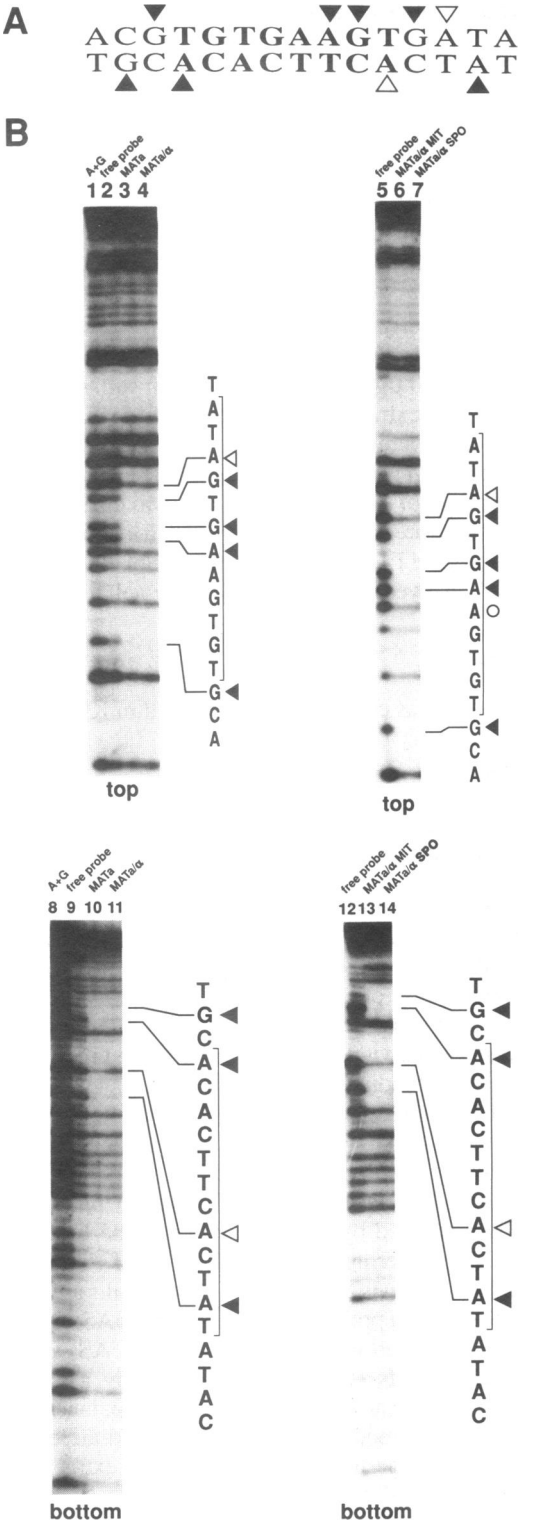

Figure 2. *In vitro* carbethoxylation interference analysis. (**A**) Summary of the interference pattern. Black and white arrowheads mark strongly and weakly interfering bases. (**B**) Comparison of the cleavage patterns of partially modified probes in lanes 1 and 8 (A+G) with the free probes in lanes 2, 5, 9 and 12 and the probes bound by UBF from dividing haploid and diploid (lanes 3, 4, 6, 10, 11 and 13) and sporulating diploid cells (lanes 7 and 14), with the top and bottom strands labeled as indicated. MATa/α spo are sporulating and MATa/α MIT are mitotic cells. The relevant DNA sequences are given; strong and weak interference signals are marked by black and white arrowheads. A non-reproducible interference signal on the top strand is marked with a circle.

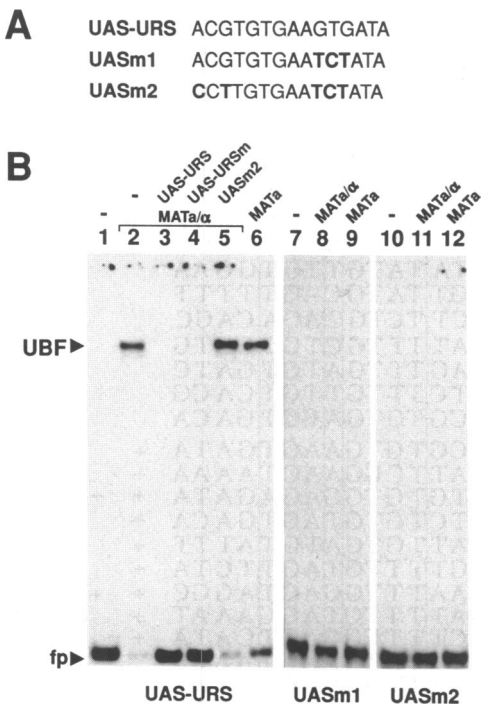

Figure 3. Point mutations in UAS_H abolish UBF-dependent DNA binding *in vitro*. (**A**) Wild-type (UAS-URS) and mutant (UASm1 and UASm2) probes. Only the relevant sequences are shown. (**B**) Aliquots of protein extract (5 μg) from dividing diploid cells (MATa/α in lanes 2 to 5, 8 and 11) and haploid cells (MATa in lanes 6, 9 and 12) were added to 0.5 ng labeled wild-type or mutant probes as indicated. Non-labeled oligonucleotides were added at a 100-fold molar excess to lanes 3, 4 and 5 as indicated. No extracts were in lanes 1, 7 and 10. UBF, binding activity; fp, free probe. The radioactive probes used are indicated at the bottom of the figure.

SK1-derived strain. The constructs used are shown in Figure 4A. The levels of *HOP1* mRNA mediated by the wild-type and mutated UAS_H motifs were determined by Northern analysis (Fig. 4B). RNA samples prepared from cycling and sporulating diploid wild-type cells (lanes 1–6) and two independently diploidized mutant strains (lanes 7–18) were compared at different time points. This experiment shows that induction of *HOP1* expression in sporulating diploid cells is UAS_H dependent and that the URS1 site alone cannot activate *HOP1* to the level required for detection by Northern analysis. Re-hybridization of the membrane with a *SPO11*-specific probe confirms that the *HOP1* UAS_H mutant strains induce transcription of an early meiotic gene whose UAS_H motif is intact (Fig. 4B). Analysis of 10 tetrads from the mutant strain UAS_Hm2-1 (SP2 in Table 1) produced only non-viable spores; this phenotype was completely restored upon transformation with a vector expressing wild-type *HOP1* (data not shown), indicating that the UAS_H mutations caused a *hop1* phenotype. This interpretation is supported by a detailed cytological analysis of a UAS_H mutant strain. Spread nuclei were double stained with Hop1 and Zip1 antibodies (*ZIP1* encodes a structural component of the SC). Zip1-positive nuclei were scored as definitely meiotic and their capacity to form SCs was investigated in a wild-type SK1 and an isogenic *HOP1* promoter mutant strain. About 95–99% of the wild-type Zip-1 positive nuclei showed extensive SC formation (Fig. 4C, panel a); in 1–5% of the cases we observed partially unsynapsed axial elements (SC precursors, panel b). Inactivation of the *HOP1* UAS_H motif results in the complete absence of SC formation in 95–99% of the meiotic nuclei (panel c), with about 1–5% of the cases showing partial SC and axial element formation (panel d). Furthermore, we observe frequent formation of so called poly-complexes (PC): this structure is thought to be typical of perturbed SC formation, as it consists of mispackaged SC subunits, probably axial elements (panel c). We conclude from this experiment that an inactive UAS_H motif yields a phenotype virtually identical to that observed in an isogenic *hop1* null strain, where synapsis of axial elements, but not their formation, is completely abolished (see Fig. 4C; described in detail in 9).

The UAS_H consensus site is present in the promoters of meiotic genes and other genes activated during mitosis and meiosis

If the UAS_H motif was a regulatory element playing a role during both vegetative growth and spore differentiation one would predict it to be present in the promoters of genes expressed in dividing and sporulating cells. We defined the UAS_H motif as $TNTGN^A/_TGT$ and searched a number of meiotic genes and mitotic genes that are also expressed during sporulation. We did not include the most 5′ and 3′ interfering bases in the consensus sequence, because they might not be directly contacted by UBF, but rather destabilize the complex due to non-specific steric hindrance. Indeed, these bases appear to be non-conserved. Our findings are summarized in Figure 5. Figure 5A shows the UAS_H in the *HOP1* promoter and indicates the bases which strongly or weakly interfere with UBF binding *in vitro*. Figure 5B shows a list of sequences that match the UAS_H consensus to variable degrees. The first group covers genes expressed in cycling and sporulating cells (with the exception of *BUF1* and *BUF2*, whose meiotic expression pattern is as yet unknown). Intriguingly, genes like *RAD2* and *GAL10* show precisely the same profile of activation during early sporulation as *HOP1* and promoter deletion studies are consistent with a role of the UAS_H motif in their regulation (34,36 and references therein). The second group comprises meiosis-specific genes. We also found UAS_H motifs in the promoter regions of *SPO12*, which is activated in mid to late meiosis, and *UME6*, indicating that the role of this positive element might not be limited to the expression of early meiotic genes. The UAS_H positions are variable and in some cases far upstream by yeast standards, but *IME1* is an example of a yeast gene with a large regulatory region (discussed in 37). We believe that the UAS_H motif in the *IME1* promoter is biologically relevant because it is located in a 250 bp element that is known to be required for regulation of *IME1* expression (see Discussion).

UBF-like factors bind *in vitro* to promoter elements of the *IME1*, *SPO11* and *ZIP1* genes

One prediction of our results is that UAS_H motifs from genes other than *HOP1* should be bound by a UBF-like activity *in vitro*. To test this idea we performed EMSAs using labeled probes covering UAS_H sequences from the *SPO11*, *ZIP1* and *IME1* promoters. The binding activities from cycling diploid cells which we observe using these probes are weaker than the UBF-dependent complex binding to the *HOP1* probe, but share the same specificity because they are competed by a *HOP1* probe with either one (see Fig. 6, compare lanes 6, 10 and 15) or two UAS_H sites (tested for *IME1*, lane 17) and not by a mutated *HOP1*

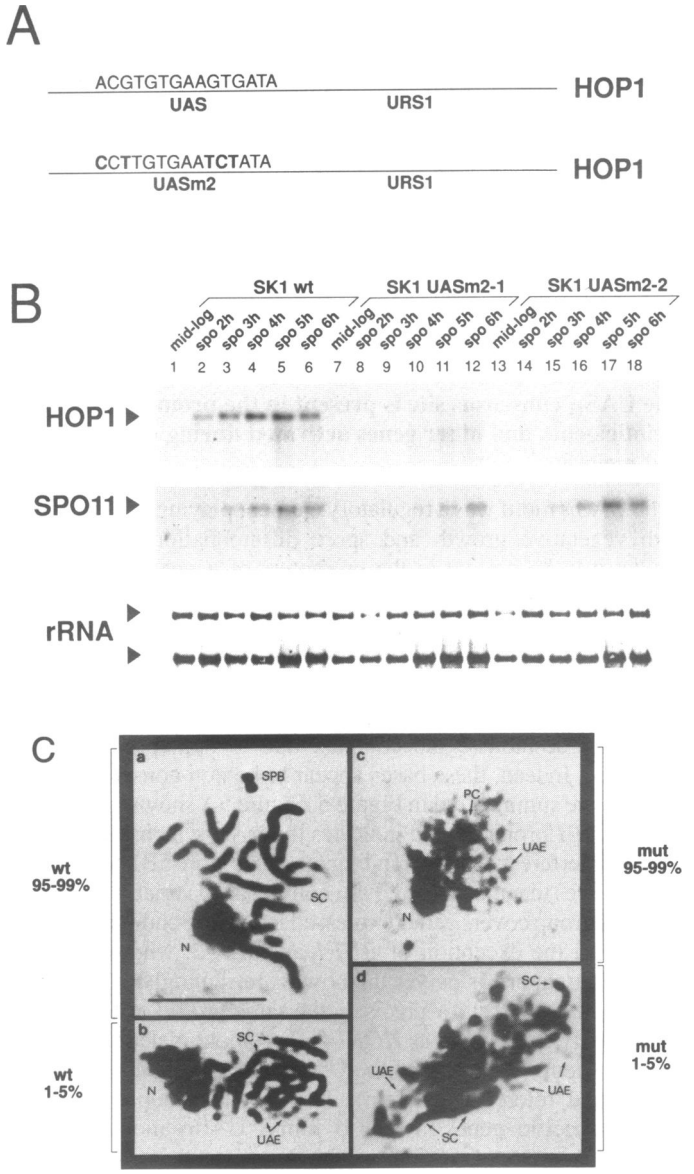

Figure 4. Point mutations that impair UBF-dependent DNA binding activity *in vitro* abolish UAS_H-mediated *HOP1* gene activation during early meiosis *in vivo*. (**A**) Wild-type (upper) and mutant (lower) versions of the *HOP1* promoter constructs. (**B**) The result of a Northern analysis comparing wild-type and mutated *HOP1* UAS_H sites. Total RNA samples (15 µg) prepared from cycling and sporulating diploid cells at different time points as indicated were hybridized with *HOP1*- and *SPO11*-specific probes as shown. (**C**) Silver stained light micrographs of meiotic yeast nuclei were produced using a cooled CCD camera (CH250 Photometrics). The images were processed using Photoshop software. (a) Wild-type pachytene nucleus with paired chromosomes (bivalents); (b) early wild-type nucleus with partially synapsed axial elements; (c) mutant pachytene nucleus showing only unsynapsed axial elements and poly-complexes; (d) mutant pachytene nucleus showing partially synapsed axial elements. N, nucleolus; UAE, unsynapsed axial element; SC, synaptonemal complex; SPB, spindle pole body; PC, poly-complex. The bar is 5 µm.

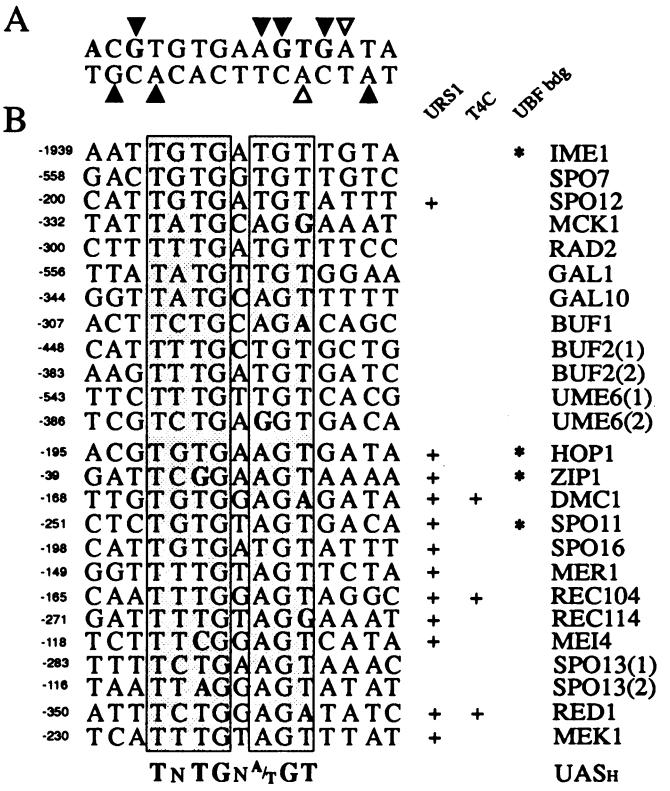

Figure 5. Alignment of UAS_H motifs from meiotic and mitotic genes. (**A**) Interference pattern on the *HOP1* UAS_H. Bases crucial for UBF binding *in vitro* and gene activation *in vivo* are given in bold. (**B**) Summary of the UAS_H motifs and their positions in the promoters of relevant genes (the position of the first T is given relative to the ATG). URS1 elements (+), T4C sites (+) and UBF-dependent *in vitro* DNA binding activities (*) on the respective sites are indicated. The UAS_H consensus is shown at the bottom of the list.

probe or a non-specific oligonucleotide (lanes 7, 8, 11, 16 and 18). Furthermore, they are also present in sporulating diploid and dividing haploid cells (data not shown). The relevance of faster migrating complexes on the *SPO11* and *IME1* probes which are less efficiently competed by the *HOP1* UAS_H probe is unclear (white arrowheads, lanes 4–8 and 13–18). These results suggest that UBF present in MATa and MATa/α cells binds *in vitro*, with variable affinities, to its target sites in the promoters of *HOP1*, *ZIP1*, *SPO11* and *IME1*.

DISCUSSION

This study provides the first insight into the function of the UAS_H and URS1 motifs in the *HOP1* promoter under physiological conditions. We show that: (i) the UAS_H motif is absolutely essential for sporulation-specific gene expression *in vivo*. Furthermore, evidence is presented that: (ii) the URS1 motif does not mediate meiotic *HOP1* gene expression on its own; (iii) the UAS_H motif interacts with the novel DNA binding factor UBF *in vitro*; (iv) there is a direct correlation between the ability of the UAS_H site to bind UBF *in vitro* and to transcriptionally activate *HOP1 in vivo*; (v) several functionally important bases in the UAS_H motif are highly conserved in a large number of UAS_H-like motifs located in the promoters of potential target genes; (vi) three different UAS_H sites from genes other than *HOP1*, expressed in cycling and sporulating cells, are bound *in vitro* by UBF; (vii) UBF DNA binding activity is detectable in all cell types, it is not developmental stage-specifically regulated and does not depend on protein interaction with the Ume6/Buf factors.

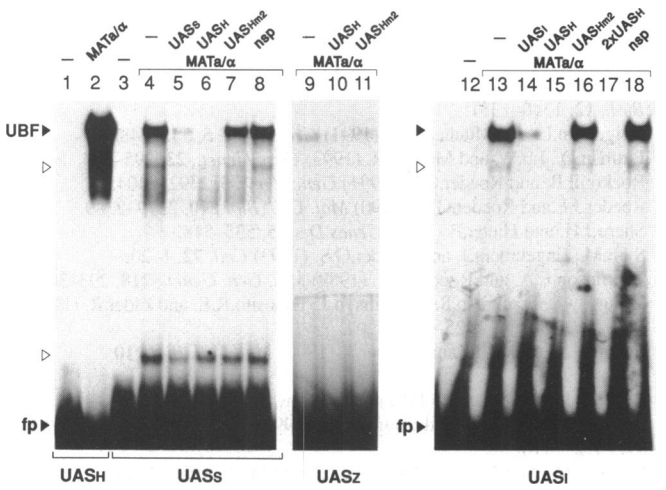

Figure 6. UBF-dependent DNA binding activities to the UAS_H sites in the promoters of three different genes. Aliquots of protein extract (10 μg) from cycling diploid cells were incubated with 1 ng labeled *HOP1* probe (UASH, lane 2), *SPO11* probe (UASS, lanes 4–8), *ZIP1* probe (UASZ, lanes 9–11) and *IME1* probe (UASI, lanes 12–18). Non-labeled oligonucleotides were added at a 20-fold molar excess to lanes 5–8, 10–11 and 13–18 as indicated. No extract was added to lanes 1, 3 and 12. No specific competitor was added to lanes 2, 4, 9 and 13. The major binding activity (UBF), fast migrating complexes (open triangle) and the free probes (fp) are indicated. The different probes used are shown at the bottom of the panels.

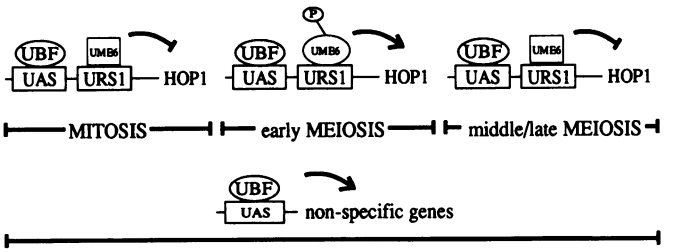

Figure 7. Model for the role of UBF during vegetative growth and sporulation. In cycling cells Ume6 inhibits UBF. During early sporulation Ume6 is modified and converted to a co-activator which mediates *HOP1* expression in cooperation with UBF. During middle and late meiosis Ume6 might be re-modified to become a repressor and thus inactivate *HOP1*. In the absence of a URS1 motif (as in *UME6*) UBF could act as a non-specific constitutive activator, possibly in cooperation with as yet unknown regulator sites.

The UAS_H and URS1 motifs, UBF and their function

Our data extend a previous study that defined the UAS_H site as a transcriptional activator of a *lacZ–HOP1* reporter gene construct (7). We find the UAS_H motif to be absolutely essential for meiosis-specific *HOP1* gene activation and the URS1 site to be insufficient to mediate even low level gene expression. However, our data do not rule out the possibility that the URS1 motif is required for full *HOP1* gene expression during early meiosis. Analysis of the UBF binding site revealed a number of bases clustered in the 5′ and 3′ regions of the UAS_H motif that are critical for its interaction with UBF *in vitro*. We found some of them to be essential for UAS_H-mediated gene activation *in vivo* as well (see Fig. 4). Our DNA sequence search yielded a number of matches in the promoters of a variety of meiotic genes that were previously thought not to contain any UAS_H-like sequences, notably *SPO13* and *ZIP1*. The former contains two significant matches to UAS_H motifs, one of which is immediately upstream of a URS1 site shown to be required for meiosis-specific *SPO13* gene expression in the context of a 60 bp promoter fragment (13; see Fig. 5). The latter contains a UAS_H site in its upstream region bound by UBF *in vitro*, suggesting a possible activator function in meiotic *ZIP1* expression (23). Further support is lent to the notion that the UAS_H motif might be a positive element widely used in meiotic promoters by our finding that UBF binds to its target site in the *SPO11* promoter. The differences in the relative affinities of UBF for its binding sites can be explained by target sequence diversity. The UAS_H motifs of *ZIP1* and *HOP1* differ at positions 2 and 3 and, indeed, the *ZIP1* probe is bound much more weakly. UAS_H sites from *SPO11* and *IME1* differ only at positions 5 or 6, respectively, and are bound with higher affinities as compared with *HOP1* (see Figs 5 and 6). Thus deviation from the proposed consensus sequence could be correlated with a reduction in UBF binding activity and hence with transcriptional activation *in vivo*. Such a phenomenon was also suggested for strong or weak matches to the T_4C site (6).

A simple interpretation of our results is that UAS_H mediates gene activation through interaction with UBF in all cell types both during vegetative growth and (in MATa/α cells) during sporulation (see the model in Fig. 7). In meiosis-specific promoters this positive effect is overcome by the Buf/Ume6 repressors, while the absence of URS1 motifs in non-specific promoters permits gene expression in both cycling and sporulating cells (see Figs 5 and 7). During early meiosis the Buf/Ume6 repressor might be phosphorylated through an *IME1*-dependent mechanism and thus act as a co-activator for UBF to contribute to full expression of early meiotic genes. Subsequent dephosphorylation of Buf/Ume6 might then lead to rerepression of early meiotic genes during later stages of spore development (see also the discussion in 30). We found no evidence for cooperative binding of UBF and Buf/Ume6 to their DNA binding sites in the *HOP1* promoter *in vitro*. Since both sites are clearly required for gene regulation *in vivo*, their activities (i) might not involve physical contact of protein complexes as a prerequisite for DNA binding and (ii) might be mediated by factors that do not interact with each other under non-physiological conditions.

In summary, we suggest that *HOP1* gene regulation is exerted by the combined activities of UAS_H and URS1 motifs, whereby the UAS_H site is essential for non-specific transcriptional activation, while URS1 provides the timing of mitotic repression, early meiotic activation and, most likely, subsequent inactivation during later stages of meiosis. Such a model predicts continuous basal level transcription of *HOP1* in the absence of Ume6. It was shown by Bowdish *et al.* (31) that this is indeed the case. While *HOP1* remains repressed in a mutant that fails to convert Ume6 to a co-activator during early meiosis (*ime1*) and a mutant that contains a constitutive repressor allele of Ume6 (*rim16-12*), it is expressed at a basal level in cycling cells and until late sporulation in a *ume6* deletion mutant (see Fig. 2 in 31); we note that *SPO13* and *IME1* also appear to be derepressed in vegetatively growing and late sporulating cells when Ume6 is absent (Fig. 2).

Our data provide a detailed characterization of a *cis*-acting regulatory sequence in the *HOP1* promoter and its specific binding activity that can play a role in dividing as well as in

differentiating eukaryotic cells. Experiments to further characterize UBF at the molecular level are currently under way.

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