MOLECULAR AND CELLULAR BIOLOGY, June 1996, p. 2777–2786 Vol. 16, No. 6
0270-7306/96/$04.00+0

Participation of the Yeast Activator Abf1 in Meiosis-Specific Expression of the *HOP1* Gene

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Received 18 January 1996/Returned for modification 19 February 1996/Accepted 10 March 1996

The meiosis-specific gene *HOP1*, which encodes a component of the synaptonemal complex, is controlled through two regulatory elements, UAS_H and $URS1_H$. Sites similar to $URS1_H$ have been identified in the promoter region of virtually every early meiosis-specific gene, as well as in many promoters of nonmeiotic genes, and it has been shown that the proteins that bind to this site function to regulate meiotic and nonmeiotic transcription. Sites similar to the UAS_H site have been found in a number of meiotic and nonmeiotic genes as well. Since it has been shown that UAS_H functions as an activator site in vegetative haploid cells, it seemed likely that the factors binding to this site regulate both meiotic and nonmeiotic transcription. We purified the factor binding to the UAS_H element of the *HOP1* promoter. Sequence analysis identified the protein as Abf1 (autonomously replicating sequence-binding factor 1), a multifunctional protein involved in DNA replication, silencing, and transcriptional regulation. We show by mutational analysis of the UAS_H site, that positions outside of the proposed UAS_H consensus sequence (TNTGN[A/T]GT) are required for DNA binding in vitro and transcriptional activation in vivo. A new UAS_H consensus sequence derived from this mutational analysis closely matches a consensus Abf1 binding site. We also show that an Abf1 site from a nonmeiotic gene can replace the function of the UAS_H site in the *HOP1* promoter. Taken together, these results show that Abf1 functions to regulate meiotic gene expression.

Meiosis is a fundamental process in the life cycle of eukaryotes which occurs through a complex developmental pathway. It is apparent that many of the genes in this pathway are highly regulated in a coordinated manner and that failure to express these genes at the proper time and level in the meiotic pathway will often result in abnormal growth and development. Although relatively little is known about the process of meiosis in higher eukaryotes, the signals that control the entry into meiosis and the early steps in the regulatory pathway in the yeast *Saccharomyces cerevisiae* have been well studied (for a review, see reference 34).

In yeast cells, meiosis is initiated in diploid **a**/α cells grown under starvation conditions. These conditions trigger a cascade of regulatory events, leading to the induction of *IME1*, a gene coding for a central activator of meiosis (22, 23, 36, 48). Ime1 in turn stimulates the expression of early, middle, and late meiotic target genes through activation of *IME2*, which codes for a second inducer of meiosis, as well as through an *IME2*-independent pathway (35, 45, 55). In this cascade, *UME6*, which was originally isolated as a negative regulator of meiotic gene expression in mitotic cells, is also required for meiotic activation (2, 3, 40, 49, 50). Although there has been extensive analysis of the initiation of meiosis, comparatively little is known about the subsequent steps in the regulatory pathway. For example, many of the transcriptional factors that bind to the promoters of meiosis-specific genes have not been identified. This report examines the factor(s) that regulates an early meiosis-specific gene.

HOP1 encodes a meiosis-specific protein that is required for the formation of the synaptonemal complex during the first meiotic division (21). Hop1 is found along the entire length of the paired chromosomes, and therefore it has been proposed that it is a structural component of the synaptonemal complex rather than a regulatory factor. *hop1* mutants fail to form a synaptonemal complex and show reduced levels of meiotic recombination (20). These mutants are able to complete the process of meiosis and undergo sporulation but produce spores that are chromosomally imbalanced and therefore mostly inviable. The *hop1* mutations have no observable phenotype in mitotic cells, suggesting that the protein is required only during meiosis. Consistent with this finding, *HOP1* is not expressed in vegetative (mitotic) cells, but its transcription is strongly induced 3 to 4 h after yeast cells enter the meiotic pathway. Expression of *HOP1* is not detrimental in vegetative cells, but underexpression of the protein during sporulation prevents the completion of productive meioses (54). These results suggest that *HOP1* must be coordinately expressed with other components of the synaptonemal complex at the proper time and level in the meiotic pathway.

The specific regulation of *HOP1* expression during mitosis and meiosis is mediated by two upstream regulatory sequences, the activator site, UAS_H, and the repressor site, $URS1_H$ (54). During mitosis, *HOP1* expression is repressed by the function of the $URS1_H$ sequence. When the cells enter meiosis, the $URS1_H$ site no longer represses transcription and now acts as an activator site in conjunction with UAS_H. In the absence of a UAS_H site, activation by $URS1_H$ alone is insufficient to fully express *HOP1* to the required levels. This mutation has the same phenotype as a *hop1* null mutation (41, 54). Transcriptional activation by the UAS_H site does not appear to be meiosis specific, because in the absence of a $URS1_H$ site, UAS_H-dependent expression is observed in both mitotic and meiotic cells.

Sites similar to the UAS_H and $URS1_H$ elements have been found in the promoter regions of numerous meiotic and nonmeiotic genes (7, 34, 51, 54). In addition, sites similar in function to the UAS_H site, such as the T_4C and heat shock element

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sites, have been shown to act in conjunction with a URS1 site in the regulation of some meiosis-specific genes (2, 53). The URS1 site, which is bound by the Ume6 and Buf proteins, has been the most intensively studied among these regulatory sites. Ume6, also known as Car80, is a key regulator of meiotic and nitrogen metabolism genes, including *HOP1* (40, 50). Ume6 is required for repression of meiotic genes during vegetative growth and activation of these genes during sporulation (3, 50). The Buf proteins were purified as oligomeric factors that bind to a nonmeiotic URS1 site (30). Subsequent analysis has shown that Buf is the single-stranded DNA-binding replication factor Rpa of *S. cerevisiae* and that it binds to URS1 sites from meiotic and nonmeiotic promoters (1, 4, 15, 29, 47). The proteins that act at the UAS_H, T_4C, or heat shock element (in the context of meiosis) sites have not been identified, but a DNA-binding activity, called Ubf, that binds the UAS_H site has been observed (41).

We have focused our interest on the factor(s) that binds to the UAS_H site of the *HOP1* gene and the base pair specificity required for binding. We purified to homogeneity a protein that binds specifically to this site. Amino acid sequence analysis of the protein identified the UAS_H-binding factor as Abf1 (autonomously replicating sequence-binding factor 1). Furthermore, although the UAS_H site was previously defined by the consensus sequence (TNTGN[A/T]GT) that is derived from sequence alignments of sites found in many other promoters of meiotic and nonmeiotic genes (41, 54), this consensus does not seem to be required for transcriptional activation of *HOP1*. In fact, mutational analysis shows that the positions essential for transcriptional activation and DNA binding only partially overlap the proposed UAS_H consensus sequence but do correspond to an Abf1 binding site. Our results therefore define a role of the general transcriptional factor Abf1 in meiotic regulation.

MATERIALS AND METHODS

Yeast strains and media. Strains LNY2 (α *ura3 leu2*::*hisG trp1*::*hisG* lys2 *ho*::*LYS2*) and LNY3 (**a**/α *ura3*/*ura3 leu2*::*hisG*/*leu2*::*hisG trp1*::*hisG*/*trp1*::*hisG lys2*/*lys2 ho*::*LYS2*/*ho*::*LYS2*) are derivatives of SK1 and were provided by Lenore Neigeborn. The diploid strain YV16 (**a**/α *trp1*/*TRP1 ura3-52*/*ura3-x can1*/*CAN1 cyh2*/*CYH2 ade2-R8*/*ade2-1*) was used to assay the various *HOP1-lacZ* fusion constructs (54).

Sporulation medium (SPM) is composed of 3% potassium acetate and 0.02% raffinose. Yeast extract-peptone-dextrose (YEPD) and yeast extract-peptone-acetate (YEPAc) media consist of 2% Bacto Peptone, 1% yeast extract, and either 2% dextrose or 2% KAc, respectively. SD medium containing adenine consists of 0.1% adenine, 0.7% yeast nitrogen base without amino acids, and 2% glucose. SD medium lacking uracil consists of 0.1% uracil dropout powder, 0.7% yeast nitrogen base without amino acids, and 2% glucose.

Oligonucleotides. All oligonucleotides were synthesized in our laboratory on an Applied Biosystems 392-5 DNA synthesizer and, when appropriate, purified by C_{18} reverse-phase high-pressure liquid chromatography (HPLC). For electrophoretic mobility shift assays (EMSAs), oligonucleotides were end labeled with [γ-^{32}P]ATP by using polynucleotide kinase and purified by Nensorb columns (NEN) as instructed by the manufacturer. The oligonucleotides were made double stranded by being mixed with a threefold excess of the matching strand, incubated at 90°C for 20 min, and slowly cooled to 25°C overnight in a water bath.

The UAS_H site used to monitor the purification of the UAS_H-binding factor, cross-linking studies, and mutational analysis was the double-stranded 19-mer GATCCCG**TGTGAAGTG**ATA. This oligomer contains the minimal 13 bp of the UAS_H site required for transcriptional activation (54). The region corresponding to the proposed UAS_H consensus sequence is shown in boldface (41, 54). The top and bottom strands of this 19-mer (or 19-mers with mutations within the binding site) contain GATC sequences at their 5′ ends to facilitate cloning into the *Bgl*II site of plasmid pAV130. As a control for specific cross-linking, a mutant double-stranded site with the sequence GATCCGCTGTGAAGTGATA was used. For EMSAs, the double-stranded 26-bp oligonucleotide (TCGACTTAACCTG GGCGGCTAAATTC) containing the URS_H site was used as a nonspecific competitor (54). The double-stranded 30-bp oligonucleotide (GGAGATCTAC GATATATGTTT) containing the wild-type UAS_H site with its natural flanking sequences (positions −209 to −180 of the *HOP1* promoter) was used for affinity purification, and a double-stranded 30-mer oligonucleotide with an 8-bp mutation (GGAGATCTACGCAGATATCTATATATGTTT) was used as control. The oligonucleotides were biotinylated by the addition of biotin amidate (Applied Biosystems) as a final step of the synthesis and purified by C_{18} reverse-phase HPLC.

Construction and assays of *HOP1-lacZ* fusions containing UAS_H mutations. pAV130 contains a *HOP1-lacZ* fusion gene in which the UAS_H site in the *HOP1* promoter is disrupted with an 8-bp substitution (54). pCC83 is derived from pAV130 by inserting the 19-mer oligonucleotide containing the wild-type UAS_H site into the *Bgl*II site 10 bp upstream of the disrupted UAS_H site. The distance between the middle of the $URS1_H$ site and the middle of the disrupted UAS_H site is 26 bp. After insertion of a new UAS_H site into pAV130, the distance between the middle of the disrupted UAS_H site and the middle of the new UAS_H site is 21 bp. Therefore, the phase between the new UAS_H site and the $URS1_H$ site was not altered, but the distance was increased by two additional turns. To make *HOP1-lacZ* reporter vectors with mutant UAS_H sites, top and bottom strands of the 19-mer UAS_H site with the desired base pair substitution were synthesized, phosphorylated by T4 polynucleotide kinase, and cloned into the *Bgl*II site in pAV130. A *HOP1-lacZ* reporter vector containing the 13-bp Abf1 binding site from the *RPC40* gene (10) was constructed in the same manner. The resulting constructs were verified by DNA sequencing, and correct UAS_H mutant-containing plasmids were transformed into yeast strain YV16 as previously described (54).

HOP1-lacZ expression was assayed under meiotic conditions by selecting three independent YV16 transformants containing UAS_H mutant plasmids, growing the cells in SD lacking uracil to saturation, and diluting them 1:10 into YEPD medium for growth overnight. The cells were washed once with distilled water, diluted 1:10 in SPM, and incubated for 24 h. β-Galactosidase assays were performed as described previously (24). To assay β-galactosidase activity under mitotic conditions, three independent YV16 transformants containing UAS_H mutant plasmids were grown in SD lacking uracil to saturation and diluted 1:50 into SD lacking uracil for growth overnight.

Preparation of yeast cell extracts. Saturated cultures of strains YV16, LNY2, and LNY3 were inoculated at a 1:50 dilution into YEPD medium, grown for 12 h at 30°C, diluted 1:10 into YEPAc medium, and incubated overnight at 30°C. The cells were harvested by centrifugation at 4,000 rpm for 10 min, washed once with water, resuspended in the same volume of SPM, and incubated at 30°C. At various intervals, 200 ml of the culture was removed and centrifuged at 4,000 rpm for 10 min. The cell pellets were washed once with water, weighed, and suspended in an equal volume of extraction buffer containing 200 mM Tris-HCl (pH 8.0), 20 mM potassium acetate, 2 mM EDTA, 2 mM β-mercaptoethanol, 1 mM $MgCl_2$, 0.2% Triton X-100, and 10% (wt/vol) glycerol (1 g of cells per ml of extraction buffer). The cells were lysed with an equal volume of glass beads (0.5-mm diameter) in a Biospec bead beater by shaking for seven 1-min intervals and cooling on ice between periods. Glass beads and cell debris were removed by centrifugation at 12,000 rpm for 5 min in a microcentrifuge tube. The supernatants were cleared by centrifugation for 1 h at 12,000 rpm at 4°C and stored at −70°C. Protein concentration was determined by the method of Bradford (Bio-Rad protein assay), using bovine serum albumin (BSA) as a standard.

EMSA. Binding reactions for the UAS_H-binding factor were carried out in a mixture containing 10 mM Tris-HCl (pH 7.5), 40 mM NaCl, 4 mM $MgCl_2$, 6% (wt/vol) glycerol, 10 μg of sonicated salmon sperm DNA per ml, and ^{32}P-labeled oligonucleotide (10,000 cpm) in a total volume of 20 μl at room temperature for 20 min. Protein dilutions were made in 20 mM Tris-HCl (pH 8)–50 mM NaCl–1 mM EDTA, 1 mg of BSA per ml, 5 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride (PMSF). For supershift assays, 1 μl of various dilutions of rabbit anti-Abf1 antiserum (12) was added to the reaction mixture. Samples were analyzed on a 6% polyacrylamide gel (run in 0.5× Tris-borate-EDTA buffer for 60 min at 250 V). Gels were dried after electrophoresis, exposed to a phosphor screen, scanned, and quantitated on a model 425E Molecular Dynamics PhosphorImager.

Purification of UAS_H-binding factor. All steps were carried out at 4°C if not otherwise indicated. LNY2 cells (330 g) were harvested in late log phase, washed with distilled water, and suspended in 500 ml of lysis buffer (20 mM Tris-HCl [pH 8], 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM $MgCl_2$, 10% [wt/vol] glycerol, 2 mM benzamidine, 0.1 mM PMSF). An equal volume of glass beads (0.5-mm diameter) was added, and cells were disrupted by vigorous mixing as described above. Ammonium sulfate was added to the protein suspension to a final concentration of 10%. After stirring for 30 min, the suspension was centrifuged at 9,000 rpm for 20 min in a GSA rotor to remove cell debris and subsequently at 40,000 rpm for 90 min in a Ti40 rotor to sediment chromatin and membranes. Protein was precipitated with ammonium sulfate at 50% saturation by stirring for 1 h and centrifugation at 11,000 rpm for 45 min in a GSA rotor. The protein pellet was suspended in buffer A (50 mM Tris-HCl [pH 8], 0.1 mM EDTA, 1 mM dithiothreitol, 1 mM $MgCl_2$, 10% [wt/vol] glycerol, 2 mM benzamidine, 0.1 mM PMSF) containing 1 M ammonium sulfate to a final protein concentration of 35 mg/ml.

The solution was applied to a phenyl-Sepharose (Pharmacia) column (5 by 20 cm) equilibrated with buffer A containing 1 M ammonium sulfate. Protein was eluted with a 1.2-liter gradient of 1 to 0 M ammonium sulfate in buffer A. Active fractions were pooled and concentrated with ammonium sulfate at 80% saturation. The protein pellet was suspended in buffer A and desalted with a Sephadex G-25 (Pharmacia) column (2.6 by 25 cm). The desalted protein solution was

loaded onto a Q-Sepharose HR (Pharmacia) column (2.6 by 22 cm) and eluted with a 1-liter gradient of 0 to 400 mM NaCl in buffer A. Fractions containing activity were pooled, adjusted to 200 mM NaCl in buffer A, and applied to a 5-ml HiTrap-heparin (Pharmacia) column, equilibrated with buffer A without benzamidine and PMSF. Protein was eluted with a 150-ml gradient of 200 to 1 M NaCl. The pooled active fractions were concentrated at least fivefold by ultrafiltration in a Centriprep 30 (Amicon) as instructed by the manufacturer. Exchange into binding buffer was performed by desalting with Sephadex G-25 PD10 (Pharmacia) columns. Protein concentration at various purification steps was determined by the method of Bradford (Bio-Rad protein assay).

To prepare the oligonucleotide affinity beads, 10 mg of Dynabeads M-280 streptavidin (Dynal) was washed two times in 10 ml of TE (10 mM Tris-HCl [pH 8], 1 mM EDTA) and 1 M NaCl and resuspended in 1 ml of this buffer with 3,000 pmol of biotinylated, ^{32}P-labeled wild-type or mutant oligonucleotide. The mixture was rotated at room temperature for 1 h, and after magnetic separation with a magnetic particle concentrator (Dynal), the efficiency of coupling was measured by counting the remaining radioactivity in the supernatant. Beads were washed three times in TE with 1 M NaCl and stored in 1 ml of TE with 100 mM NaCl (TEN) at 4°C until further use. To prepare the beads for binding reactions, the beads were washed two times with 2 ml of TEN, two times with phosphate-buffered saline–0.1% BSA to block nonspecific binding sites, two times with binding buffer (10 mM Tris-HCl [pH 7.5], 40 mM NaCl, 4 mM $MgCl_2$, 6% glycerol) containing 1 M NaCl, and as a final step three times in binding buffer.

For identification of the UAS_H-binding factor, 50 μl of the concentrated heparin fractions was incubated with 20 μl of magnetic beads coupled with wild-type or mutant UAS_H-site for 40 min at room temperature. If prebatched with mutant UAS_H beads, the protein was incubated 10 min with mutant UAS_H beads and added after separation to wild-type UAS_H beads to incubate for an additional 30 min. After being washed three times with 50 μl of binding buffer, protein was eluted in 20 μl of binding buffer containing 1 M NaCl.

Cross-linking and SDS-PAGE. Protein fractions were incubated with ^{32}P-labeled oligonucleotide in assay buffer as described for EMSA and cross-linked by UV irradiation in an RPR100 photochemical reactor equipped with 16 253.9-nm tubes (Southern New England Ultraviolet, Hamden, Conn.) for 3.5 min at 22°C. Samples were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (12% gel) (27); gels were dried and exposed to a phosphor screen. To monitor the protein purification, gels were stained with either Coomassie blue or silver (Silver Stain Plus; Bio-Rad).

Molecular weight determination and amino acid sequencing. The native molecular weight of the UAS_H-binding factor was determined by gel filtration chromatography on a Superdex 200 HR (Pharmacia) column in 50 mM Tris-HCl (pH 8)–100 mM NaCl–0.1 mM EDTA–1 mM $MgCl_2$–1 mM dithiothreitol–10% (vol/vol) glycerol. Gel filtration standards, a mixture of molecular weight markers ranging in M_r from 1,350 to 670,000, were purchased from Bio-Rad. The amino acid sequence analysis of gel-purified UAS_H-binding factor (6.5 μg) was performed by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University.

RESULTS

UAS_H-binding activity is observed in different yeast extracts. We previously identified UAS_H (TGTGAAGTG) as a positive regulatory site in the *HOP1* promoter (54). A 19-mer oligonucleotide containing the UAS_H site was used in EMSAs to analyze yeast extracts for binding activity to this site. As shown in Fig. 1, an abundant, slowly migrating UAS_H-binding activity (arrow) is present in premeiotic cells (0 h of meiosis [lanes 2 and lanes 8 to 12]) and during different times of meiosis (2 to 24 h [lanes 3 to 7 and 13 to 17]). The UAS_H-binding activity in both nonmeiotic and meiotic extracts is competed for specifically by unlabeled UAS_H (lanes 8 to 10 and 13 to 15) but is not affected by the addition of the nonspecific competitor $URS1_H$ (lanes 11, 12, 16, and 17). At later times points during meiosis, faster-migrating, UAS_H-specific complexes are observed (lanes 3 to 7). These complexes probably represent proteolytic degradation products of the slowly migrating UAS_H-binding activity. *HOP1* expression is induced shortly after cells are shifted to SPM and decreases at later stages of meiosis (21, 54). It is possible that specific degradation of the UAS_H-binding activity is a mechanism to reduce *HOP1* expression. However, we never observe full degradation of the activity, and therefore it is likely that these faster-migrating complexes are due to nonspecific digestion by proteases present during sporulation (16). The slowly migrating UAS_H-binding activity is also detected in haploid cell extracts

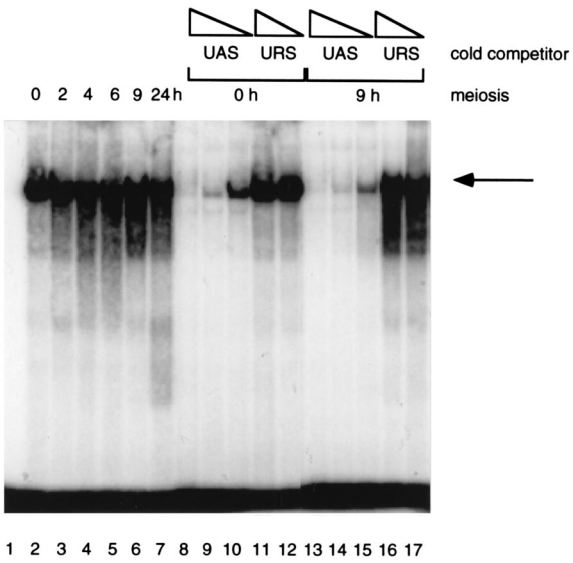

FIG. 1. UAS_H-binding activity in mitotic and meiotic extracts. The 19-mer UAS_H oligonucleotide was used to assay for UAS_H-binding activity (arrow) in an EMSA with yeast crude extracts as described in Materials and Methods. Lane 1, 19-mer UAS_H oligonucleotide without cell extract; lanes 2 to 7, UAS_H-binding activity during 0 to 24 h of meiosis; lanes 8 to 12, competition of UAS_H-binding activity of extracts from vegetative cells (0 h of meiosis) with unlabeled (cold) UAS_H site (molar excesses, 1,400-fold [lane 8], 350-fold [lane 9], and 70-fold [lane 10]) and unlabeled nonspecific $URS1_H$ site (molar excesses, 1,400 fold [lane 11] and 70-fold [lane 12]); lanes 13 to 17, competition of UAS_H-binding activity of extracts from cells at 9 h of meiosis with unlabeled UAS_H site (molar excesses, 1,400-fold [lane 13], 350-fold [lane 14], and 70-fold [lane 15]) and unlabeled nonspecific $URS1_H$ site (molar excesses, 1,400-fold [lane 16] and 70-fold [lane 17]).

(see Fig. 7, lanes 2 to 4) and is therefore independent of cell type or developmental stage. This observation is consistent with earlier in vivo results which show that a high level of *HOP1-lacZ* expression during mitosis in a mutant $URS1_H$ background is dependent on the UAS_H site (54). Taken together, these results indicate that a specific UAS_H-binding activity is present in mitotic and meiotic extracts and that this binding activity is not significantly altered when cells enter meiosis. The binding activity has a shift pattern that is very similar to one observed for binding to an UAS_H site, using different DNA probes and yeast strains (41).

Purification of the UAS_H-binding activity. Extracts from mitotic and meiotic cells produce the same slowly migrating complex of the UAS_H site (Fig. 1; see also Fig. 7) (41). To easily obtain large quantities of starting material for the isolation of the UAS_H-binding factor(s), we decided to purify the UAS_H-binding activity from vegetative cells. EMSAs were used to monitor the binding activity during the purification procedure. Cells were harvested in late log phase and centrifuged at high speed to remove cell debris and chromatin. An outline of the purification procedure is shown in Table 1. UAS_H-binding activity was completely bound to phenyl-Sepharose and eluted with a 1 to 0 M NaCl gradient. Most (95%) of the UAS_H-binding activity was present as the slowly migrating binding activity, as shown in Fig. 1. However, we also observed a ladder of faster-migrating complexes in fractions that eluted after the main activity, which were competed for specifically by the unlabeled UAS_H site. Figure 2 shows the elution profiles of the subsequent columns, Q-Sepharose (Fig. 2A) and heparin-Sepharose (Fig. 2B) along with the corresponding EMSAs of the UAS_H-binding activities. The EMSAs of the Q-Sepharose fractions show the separation of the UAS_H-binding activity in

TABLE 1. Purification of the UAS_H-binding factor

Fraction	Vol (ml)	Total protein (mg)	Total activity (U)[a]	Sp act (U/mg)	Yield (%)	Fold purification
50% $(NH_4)_2SO_4$	125	4,625	1.5×10^6	324	100[b]	1
Phenyl-Sepharose	100	760	8.1×10^5	1,065	54	3.3
Q-Sepharose	65	36	5.2×10^5	1.4×10^4	34.6	44
Heparin-Sepharose	36	0.4	2.9×10^5	7.2×10^5	19.2	2,222

[a] One unit of binding activity is defined as 25% of maximal binding, using the same quantity and preparation of labeled DNA.
[b] The yield of the UAS_H-binding factor after the 50% ammonium sulfate precipitation was used as reference for calculating the yield during the purification but is not identical with the initial amount of the UAS_H-binding factor in the crude yeast lysate.

the faster-migrating complexes and the slowly migrating complex. With the exception of a slowly migrating complex found in fractions 35 to 37, all of these binding activities are specifically competed for by the unlabeled UAS_H site and show similar DNA-binding specificities (data not shown). We continued the purification of the specific slowly migrating binding activity (fractions 44 to 46) by using a high-performance heparin resin (34-μm particle size) and achieved a 50-fold purification in this step after eluting the binding activity with 350 mM NaCl (Fig. 2B). At this point, 20% of the initial UAS_H-binding activity was recovered and was enriched more than 2,000-fold.

To identify the binding factor(s), we used an analytical DNA affinity purification step. A 30-bp oligonucleotide containing the wild-type UAS_H-binding site was coupled via biotin-streptavidin interaction to magnetic beads. As a control, we

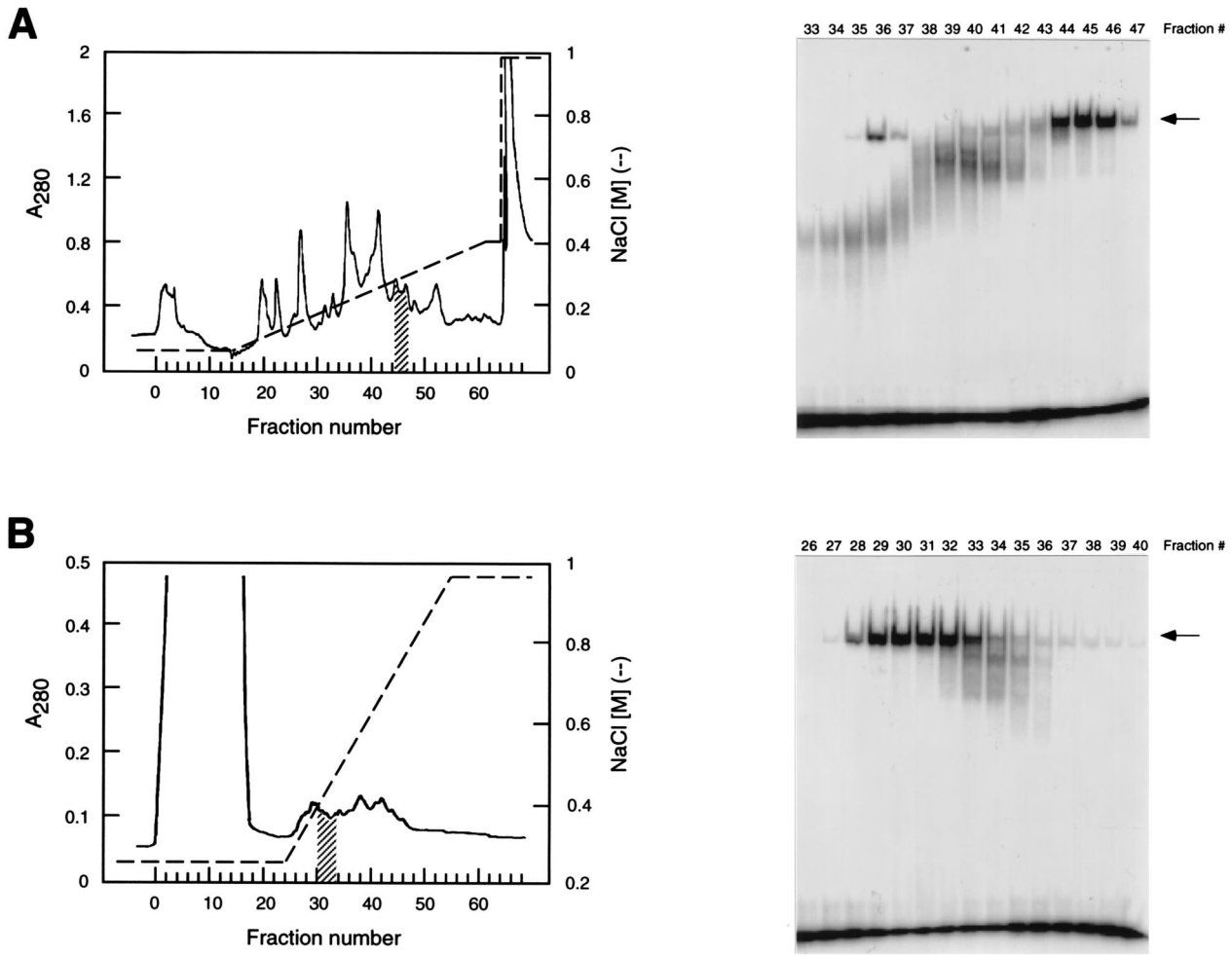

FIG. 2. Purification of UAS_H-binding activity. Elution profiles of Q-Sepharose (A) and heparin-Sepharose (B) with the corresponding EMSAs of UAS_H-binding activity. (A) UAS_H-binding activity eluted from the Q-Sepharose column (120 ml) at 200 to 320 mM NaCl and formed different complexes in the EMSAs: a fast-migrating complex (fractions 33 to 36), a ladder of complexes (fractions 37 to 42), and the original slowly migrating complex observed in crude lysates (fractions 43 to 47). The slowly migrating activity in fractions 35 and 36 is nonspecific, whereas all other complexes are specifically competed for by unlabeled UAS_H. (B) Q-Sepharose fractions 44 to 46 were loaded onto a 5-ml HiTrap-heparin column in the presence of 200 mM NaCl, and UAS_H-binding activity was eluted with 350 mM NaCl, migrating as the slowly migrating complex in EMSAs.

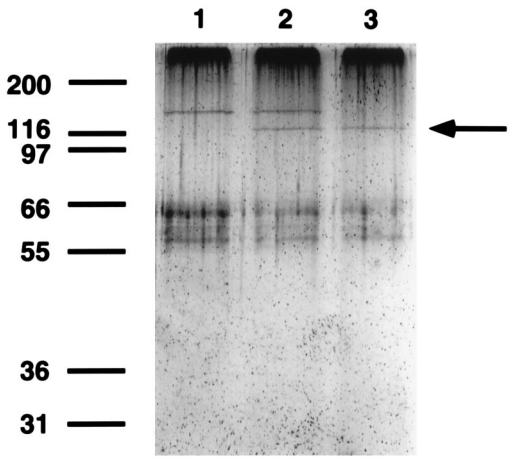

FIG. 3. Silver-stained SDS-polyacrylamide gel of UAS_H-binding activity eluted with 1 M NaCl from oligonucleotide affinity beads (Dynabeads M-280 streptavidin). Lane 1, a 140-kDa species is eluted from beads with a mutant UAS_H site; lane 2, in addition to the 140-kDa protein, a 120-kDa protein (arrow) is purified with beads containing the wild-type UAS_H site; lane 3, only the 120-kDa protein is eluted from beads with the wild-type UAS_H site if the protein sample was preincubated with beads containing the mutant UAS_H site. Positions of size markers are indicated in kilodaltons.

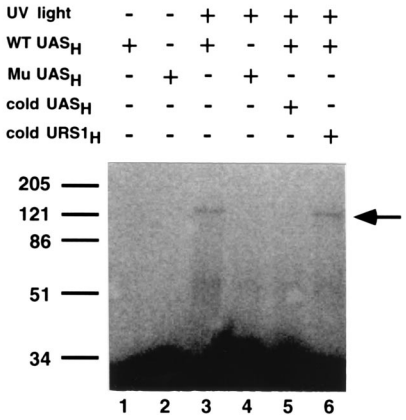

FIG. 4. UV cross-linking of UAS_H sites to protein (fraction 31) eluted from the heparin column. A 120-kDa band (arrow) was specifically cross-linked to the UAS_H site. Lane 1, wild-type (wt) UAS_H site incubated with protein but not irradiated with UV light; lane 2, mutant (Mu) UAS_H site incubated with protein but not irradiated with UV light; lane 3, protein cross-linked to the wild-type UAS_H site; lane 4, protein cross-linked to the mutant UAS_H site; lane 5, same as lane 3, but unlabeled (cold) wild-type UAS_H site was added at a 1,000-fold molar excess; lane 6, same as lane 3, but cold URS1 site as nonspecific competitor was added at a 1,000 fold molar excess. Positions of size markers are indicated in kilodaltons.

used beads coupled with a 30-bp oligonucleotide containing an 8-bp mutation in the UAS_H binding site. This substitution has been shown to completely destroy UAS_H function (54) and binding (data not shown). A sample of the heparin-enriched pool was incubated with either the wild-type or the mutant oligonucleotide beads. As an additional control, one protein sample was first incubated with the mutant oligonucleotide beads, and after magnetic separation, the supernatant was subsequently applied to wild-type affinity beads. The UAS_H-binding activity was almost completely removed from the supernatant of a protein fraction incubated with wild-type oligonucleotide beads, whereas no reduction of binding activity was observed in the supernatant incubated with mutant affinity beads (data not shown). The protein was then eluted from the beads with 1 M NaCl and analyzed on an SDS-gel. As shown in Fig. 3, a protein with a molecular mass of approximately 140 kDa was eluted from beads containing the mutant UAS_H binding site (lane 1), whereas an additional protein with 120 kDa was recovered from beads coupled with the wild-type oligonucleotide (lane 2, arrow). This result indicates that the 120-kDa protein binds specifically to the UAS_H binding site, while the 140-kDa protein binds nonspecifically either to the DNA or to the resin. This result is further supported by the finding that only the 120-kDa protein is eluted from wild-type affinity beads after preincubation with mutant oligonucleotide (lane 3). No other protein species could be detected in the eluents of the affinity beads on silver-stained gels. Both protein fractions eluted from the wild-type affinity beads (lane 2 and 3, respectively) bind the UAS_H oligonucleotide in the EMSA and produce a shift that is similar in mobility to the original complex (data not shown).

Identification of Abf1 as the 120-kDa UAS_H-binding factor. To further support the identification of a 120-kDa protein as the UAS_H-binding factor, we performed UV cross-linking experiments with ^{32}P-labeled oligonucleotides. As shown in Fig. 4, a 120-kDa protein was cross-linked to a wild-type UAS_H binding site (lane 3, arrow). This band was specifically competed for with an unlabeled UAS_H fragment (lane 5) but not with an unlabeled nonspecific competitor ($URS1_H$ [lane 6]). The 120-kDa cross-linked species was not observed when a mutant UAS_H binding site was used in the reaction (lane 4). In addition to the 120-kDa protein, a diffuse band (50 to 60 kDa) was observed after cross-linking to the wild-type UAS_H oligonucleotide (lane 3). However, this species could also be cross-linked to the mutant UAS_H site (lane 4) and is therefore not a UAS_H-binding protein. These data confirm that the 120-kDa protein isolated with the UAS_H-specific affinity beads is the UAS_H-binding factor.

We further characterized the UAS_H-binding factor by determining the apparent molecular weight by size exclusion chromatography (Superdex 200 HR) (data not shown). The UAS_H-binding activities of fractions from different purification steps (phenyl-Sepharose, Q-Sepharose, and heparin-Sepharose) eluted as a single peak with an M_r of 250,000, suggesting that the UAS_H-binding activity either is a dimer, associates with other proteins, or is an extended protein. In addition, we observed that under our assay conditions, the protein-DNA complex is fairly stable ($t_{1/2} \approx 15$ min) and has half-maximal binding in the range of 1 nM (data not shown).

Over 6 μg of the 120-kDa UAS_H-binding activity was gel purified for amino acid composition and sequence analysis. Amino acid sequence analysis of a peptide fragment derived from a digestion of the purified protein with trypsin revealed the sequence Gly-Leu-Asp-Asp-Glu-Ser-Gly-Pro-Thr-His-Gly-Asn-Asp-Ser-Gly-Asn-His-Arg. A search of the databases showed that this sequence matches exactly residues 202 to 219 of the transcription factor Abf1 (12, 43). Residue 201 of Abf1 is an arginine, and therefore cleavage of Abf1 with trypsin would have generated this peptide. The predicted molecular mass of this Abf1 fragment (1,865 Da) is the same as the molecular mass of the sequenced peptide fragment determined by mass spectrometry. The purified protein has the same electrophoretic mobility in SDS-PAGE as Abf1, which migrates between 120 and 140 kDa. Finally, the amino acid composition of the purified protein matches the composition deduced from the sequence of Abf1. These results indicate that the protein that we have purified is, indeed, Abf1.

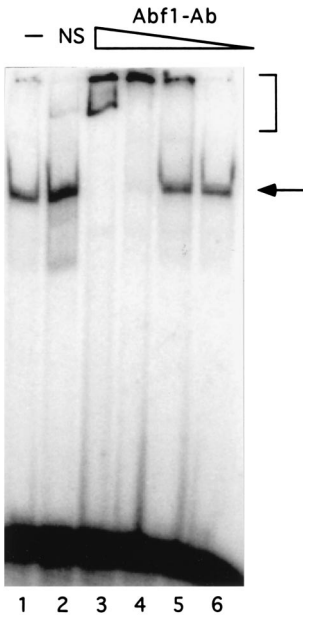

FIG. 5. Specific binding of Abf1 to UAS_H. EMSA using a protein fraction enriched after the purification step with heparin-Sepharose and incubation with no antiserum (lane 1), with nonspecific (NS) antiserum (raised against Rpa 70-kDa subunit) (lane 2), and with anti-Abf1 antiserum of various dilutions (lane 3, undiluted; lane 4, 1:10; lane 5, 1:100; lane 6, 1:1,000). Arrow, Abf1-DNA complex. The bracket indicates the supershift of the Abf1-DNA complex.

To further verify that the UAS_H-binding activity is Abf1, we performed EMSAs with antiserum raised against purified Abf1 protein (12). Addition of anti-Abf1 antiserum to the reaction mixture of purified protein with the UAS_H binding site led to complete transition of the original shift to a supershift (Fig. 5, lane 3 to 6). The supershift is not observed by the addition of nonspecific antiserum (lane 2). This finding confirms that the UAS_H-binding activity is the transcription factor Abf1. Furthermore, the faster-migrating, UAS_H-binding activity observed in fractions 33 to 36 in the Q-Sepharose eluent is supershifted with anti-Abf1 antibody as well (data not shown).

Sequence specificity of the UAS_H-binding activity. The UAS_H site was first defined through deletion analysis of the *HOP1* promoter (54). It was then shown that an 8-bp substitution of the site abolishes UAS_H function and that a 13-bp oligonucleotide that contains this site functions as an activator in heterologous promoters. Sequences similar to this site were found in the promoter regions of other meiotic and nonmeiotic genes, and a consensus sequence (TNTGN[A/T]CT) was proposed for this site (41, 54). Although these experiments showed that UAS_H is contained within a 13-bp site, there was little information about the base pair specificity for UAS_H function. To define the sequence requirements of UAS_H, we made a series of single base pair substitutions in the site and assayed the effects of the mutations on transcriptional activation in vivo and DNA binding in vitro.

Oligonucleotides containing the desired base pair substitutions were cloned into the promoter region of a *HOP1-lacZ* fusion vector, pAV130, and the constructs were assayed for β-galactosidase expression under sporulation conditions. The reporter vector, pAV130, contains an 8-bp mutation in the UAS_H site, which significantly reduces the level of meiotic activation compared with a construct that contains a wild-type *HOP1* promoter (pAV79 [Fig. 6A]) (54). Meiotic expression of the promoter is restored to nearly wild-type levels when a

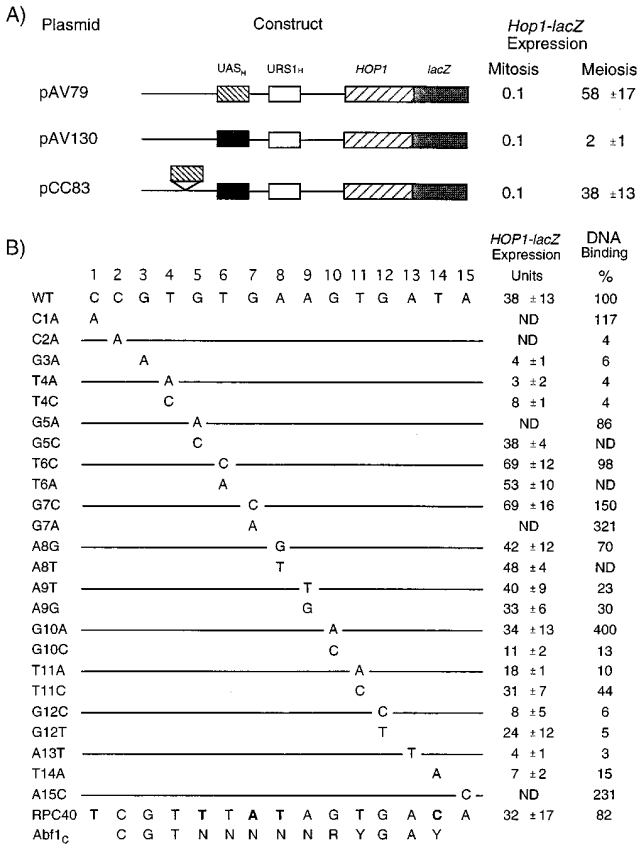

FIG. 6. Effects of substitutions in the UAS_H on DNA binding and *HOP1* expression. (A) Expression of *HOP1-lacZ* fusion during mitosis and meiosis of a wild-type promoter (pAV79), a UAS_H disrupted promoter (pAV130), and a reconstituted promoter containing a wild-type UAS_H site (pCC83). Each black box represents a UAS_H with an 8-bp substitution. Values are averages of the units from β-galactosidase assays of three independent transformants of YV16 under vegetative and sporulation conditions. (B) Effects of point mutations in the UAS_H site on *HOP1-lacZ* expression and DNA-binding activity. The first line shows the wild-type (wt) UAS_H sequence and activity. C1A to A15C are mutations for meiotic *HOP1-lacZ* expression from promoters containing UAS_H sites with substitutions at various positions. The next line (RPC40) shows an Abf1 site from the *RPC40* promoter that was cloned into pAV130 and assayed for meiotic expression of the *HOP1-lacZ* fusion. Boldface letters indicate positions in *RPC40* that are different from the *HOP1* site. The Abf1 consensus sequence ($Abf1_c$) is shown in the last line. β-Galactosidase values are averages for independent transformants of strain YV16 under sporulation conditions as described in Materials and Methods. Values for DNA binding represent binding activities of the purified UAS_H-binding factor to the various mutant sites. Numbers represent percentages relative to the wild-type site in an EMSA, quantitated with a PhosphorImager.

wild-type UAS_H site is cloned into the mutant promoter (pCC83). We have used this reconstitution system to measure the effects of substitutions in the UAS_H site (Fig. 6B). The results of the mutagenesis show that substitutions in base pairs on either end of the UAS_H site (positions 3 and 4 and positions 12 to 14) significantly reduce the level of meiotic activation of the *HOP1-lacZ* fusion. On the other hand, substitutions in the center of the site (positions 5 to 9) have relatively little effect on *HOP1-lacZ* expression.

We next tested the effects of these substitutions on DNA binding in vitro in order to corroborate our in vivo findings. Figure 7 shows an EMSA of some of the mutant sites with the purified UAS_H-binding factor. These results are also summarized in Fig. 6B. Substitutions which show large decreases in the level of activation in vivo, such as G3A and G12C, also

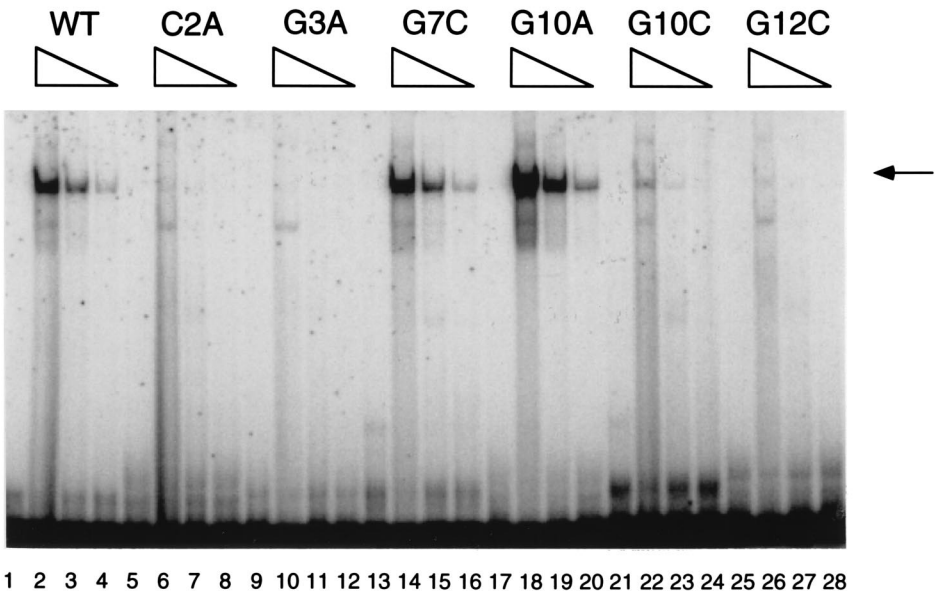

FIG. 7. UAS_H binding to mutant UAS_H sites. EMSAs of partially purified UAS_H-binding protein with the wild-type (WT) site and selected mutant sites are shown. Lanes 1, 5, 9, 13, 17, 21, and 25 contain labeled sites without UAS_H-binding protein; lanes 2 to 4, 6 to 8, 10 to 12, 14 to 16, 18 to 20, 22 to 24, and 26 to 28 contain UAS_H-binding protein that was serially diluted fourfold.

significantly reduce the level of DNA binding in vitro. Substitutions in the center of the site, such as G7C, which have little effect on activation in vivo, also have relatively little effect on DNA binding in vitro. There are a few discrepancies between in vitro binding and in vivo activation. For example, the G12T substitution results in a large decrease in binding affinity but only a slight decrease in transcriptional activity. However, it was shown that this deviation from the Abf1 consensus sequence represents a natural and functional Abf1 site in the *L2B* promoter of the ribosomal *L2* gene and that a substitution at this position in the *L2A* Abf1 site has only a small effect in vivo (9). Overall, our results show that the in vitro DNA affinity correlates with the in vivo data and suggest that the purified UAS_H-binding activity functions at this site in vivo. This analysis also demonstrates that the proposed UAS_H consensus site that is based on sequence similarity is not functionally conserved. We have therefore derived a new consensus sequence (5′-CGTNNNNNRYGAT-3′) for the UAS_H site that is based on the mutagenesis data. This site closely matches the consensus binding site for the Abf1 protein (5′-CGTNNNNNR YGAY-3′ [10, 13, 14]).

To obtain further proof that Abf1 functions at the *HOP1* promoter, we used the in vivo reconstitution system to replace the UAS_H site with an Abf1 site from a nonmeiotic gene. Our results show that the Abf1 site from the promoter of *RPC40*, a gene encoding a subunit shared by RNA polymerases A and C, can activate *HOP1* transcription at close to wild-type levels (Fig. 6B) (10). This nonmeiotic site remains repressed under mitotic conditions (data not shown). These results suggest that the factors that bind to the UAS_H site function at this nonmeiotic site as well. The analysis of the sequence requirements of the UAS_H site supports our observations from the biochemical analysis of the UAS_H-binding factor and suggests that Abf1 functions at the UAS_H site to regulate *HOP1* expression.

DISCUSSION

We have demonstrated the first purification of a transcription factor binding to an upstream activation sequence of a meiosis-specific gene. We have used conventional and affinity chromatography to purify a protein with an apparent molecular mass of 120 kDa, as determined by SDS-PAGE, and have shown that a 120-kDa protein can be specifically cross-linked to the UAS_H site. Amino acid sequence analysis of a peptide fragment generated from the purified protein revealed that the UAS_H-binding factor is the general transcription factor Abf1. This factor is an abundant, multifunctional site-specific DNA-binding protein which is essential for cell viability (43). Abf1 (also independently isolated as Baf1, Obf1, Taf, GfI, Suf, and Sbf-B) acts in replication by binding to sequences in origins of DNA replication (11, 31, 52). It also plays a role in silencing (6, 12, 28, 44) and in the transcriptional regulation of diverse sets of genes (5, 15, 18, 19, 26, 46). Our results demonstrate that Abf1 is also involved in the regulation of meiotic gene expression.

ABF1 encodes a 731-amino-acid protein with a predicted molecular mass of 81.7 kDa (12, 43). The protein, however, migrates as a double band and with an apparent molecular mass of 120 to 140 kDa in SDS-PAGE, an observation which was explained by its high asparagine and serine content and its low isoelectric point (10, 12, 17, 43). The UAS_H-binding factor, which we have shown is Abf1, migrates as a single band with a molecular mass of 120 kDa in SDS-PAGE. We have determined that Abf1 elutes with an apparent molecular weight of 250,000 in size exclusion chromatography. This result suggests that Abf1 may form a dimer in solution. Alternatively, if the Ser/Asp tracts are extended, the protein could be an elongated monomer. However, the large difference between the deduced monomer molecular mass of 81.7 kDa and the apparent molecular weight of 250,000 suggests that the high molecular weight represents a dimer rather than a rod-shaped monomer. Although we did not detect the presence of another factor after affinity purification, it is also possible that Abf1 is associated with other proteins, which would explain why the protein has a large apparent molecular weight.

In an EMSA, the UAS_H-binding activity produces a slowly migrating shift that is specific for the UAS_H site. In meiotic

extracts and during purification, we have also detected specific UAS_H-binding activities which show faster mobilities than the Abf1 shift (Fig. 1 and 2). DNA binding experiments with mutant UAS_H sites showed that these faster-migrating complexes are similar in sequence specificity to the slowly migrating complex, which contained only Abf1 (data not shown). In addition, these complexes were specifically supershifted with Abf1 antibody, indicating that Abf1 was a component of these shifts (data not shown). These faster-migrating UAS_H-binding complexes show strong similarity with the complexes produced after proteolytic digestion of Abf1 with trypsin or proteinase K (33). It therefore seems likely that these smaller, faster-migrating complexes represent degradation products of Abf1 rather than other binding factors. Similar faster-migrating shifts have been interpreted as breakdown products of Abf1 during starvation conditions or as a result of differential phosphorylation of Abf1 (19, 46). Although the faster-migrating complexes are most likely due to nonspecific digestion during meiosis and purification, it is possible that some Abf1 species represent different phosphorylation states. If phosphorylation does play a role in regulating Abf1 activity during meiosis, then it is tempting to speculate that after entering meiosis, Abf1 may be directly or indirectly modified by meiosis-specific kinases such as *IME2* or *MCK1* (37, 45).

Abf1 binds to the consensus sequence 5′-RTCRYNNNN NACG-3′, where R, Y, and N represent a purine, a pyrimidine, and any nucleotide, respectively (10, 13, 14). It was initially surprising that Abf1 binds to the UAS_H site in the *HOP1* promoter, because its recognition site did not show obvious similarity with the proposed UAS_H consensus sequence, TNT-GN(A/T)GT (41, 54). However, our mutational analysis demonstrated that an Abf1 binding site is indeed located in sequences overlapping on either side of UAS_H. We have shown that positions outside or close to the margins of the UAS_H consensus sequence are important for transcriptional activation of *HOP1* and for binding of the UAS_H-binding factor. A consensus site derived from the mutational analysis matches the consensus sequence of Abf1 in the reversed orientation (5′-CGTNNNNNRYGAY-3′). Our mutational analysis also conforms to the chemical modification data for the UAS_H-binding activity from crude yeast extracts (41) and for purified Abf1 (13, 17, 32). However, we also observe some discrepancies between in vitro binding and in vivo function; e.g., the mutation G12T significantly decreases binding but still mediates, although at a reduced level, transcriptional activation. Interestingly, it was shown that this deviation from the optimal Abf1 consensus sequence represents a natural Abf1 site in the *L2B* promoter of the ribosomal *L2* gene (9). The discrepancy between in vitro binding and transcriptional activation can be explained in several ways (10). Abf1 activity in vivo might be influenced by association of other proteins. It was also proposed that the relatively high DNA-binding affinity and abundance of Abf1 might allow a greater tolerance for point mutations in vivo (10). The effects of mutations in the UAS_H site are similar to the same substitutions in the Abf1 sites from nonmeiotic genes (10, 14, 18). It is of great significance that a nonmeiotic Abf1 site is able to substitute for UAS_H to mediate meiotic transcriptional activation. We have also found that there is no base pair specificity in the center of the UAS_H site. It therefore seems unlikely that other factors bind to the center of this site to regulate meiosis-specific activation.

Finally, while this work was being completed, it was found that anti-Abf1 antibody was capable of supershifting an EMSA complex derived from crude yeast extract and a promoter fragment of the meiosis-specific gene *SPR3* (38). Furthermore, it was noticed that Abf1 sites are present in other meiotic genes as well (38). We have searched the promoters of meiosis-specific genes for potential Abf1 sites. Six genes were found to have Abf1 sites which are potentially functional with respect to our mutational analysis and the analyses of others (Fig. 8) (10, 13, 14). Interestingly, we have found Abf1 sites in *RED1* and *ZIP1*, early genes which are expressed at the same time in the meiotic pathway as *HOP1* and are required for recombination. The other group of genes containing a potential Abf1 site are *SMK1*, *SPR3*, and *SGA*, middle and late genes which are involved in spore wall formation. It therefore seems likely that Abf1 is required to regulate genes at several different stages of meiosis. Although UAS_H sites have been identified in other meiosis-specific genes such as *DMC1*, *SPO16*, *SPO12*, *SPO11*, and *MER1* (34, 41, 54), by sequence comparison these sites do not appear to be Abf1 sites and therefore may not be important for transcriptional activation.

			ABF1		URS1
			2 3 4 5 6 7 8 9 10 11 12 13 14 CGTNNNNN$^{GT}_{AC}$GA$^{T}_{C}$		TCGGCGGCT
HOP1	→	-200	CGTGTGAAGTGAT	-173	TGGGCGGCT
RED1	→	-199	CGTTAAAAACGAT	-166	TCAGCGGCT
ZIP1	→	-241	CGTAAAAAATG**C**T	-22	TCGGCGGCT
SPR3	→	-324	CGTATTTAGTGAT		-
SMK1	←	-122	CGTCGCGCGCGAT		-
SGA	→	-231	CGTACAAGGTG**C**T		-
REC104	→	-114	CGTCAAAG**T**TGA**A**	-93	TTGGCGGCT
SPR1	←	-665	CGTCATTTATGA**A**		-

FIG. 8. Putative Abf1 binding sites in meiosis-specific genes. The promoter regions of meiosis-specific genes were searched for putative Abf1 binding sites by using the software tool MatInspector and choosing a matrix and core similarity of >0.8 (42). The first six genes listed have Abf1 sites which have a core similarity of >0.976 and are potentially functional with respect to our mutational analysis and the analyses of others (6, 10, 13, 14). The A13C deviation in *ZIP1* and *SGA* Abf1 sites is also present in the Abf1 site of ARS1, which is known to be functional (52). The T14A deviation found in *REC104* and *SPR1* Abf1 sites suggests that these sites may not be functional in vivo since a T14A substitution in the *HOP1* site destroys function. Deviations from the consensus Abf1 sequence are indicated by boldface letters. Numbers indicate the positions of the sites with respect to the start of the protein, and arrows indicate the orientations of the sites.

The *HOP1* promoter contains the two regulatory sites $URS1_H$ and UAS_H, which has now been shown to be the binding site for the Abf1. These sites have been found together in other meiotic and nonmeiotic promoters. For example, *CAR1*, *FOX3*, and *ARO3*, genes involved in nitrogen metabolism, β oxidation of fatty acids, and amino acid biosynthesis, respectively, all have the Abf1 and URS1 elements in common (15, 25, 26). This finding raises the question of whether Abf1 interacts with the transcription factors binding to the URS1 site. In the case of the *FOX3* gene, although Abf1 and URS1 sites are able to partially repress transcription independently of one another, they are both required in concert to mediate full glucose repression. In the context of the *HOP1* promoter, the Abf1 and $URS1_H$ sites appear to work synergistically to activate transcription during meiosis (54). This observation suggests that there may be interactions between the proteins or that they have a common target. An interaction of the factors might explain why the mutations like G12T in the UAS_H site have a large effect on DNA binding of purified Abf1 protein in vitro but have less effect on transcriptional activation in vivo. On the other hand, URS1 sites from the *HOP1*, *CAR1*, and *ARO3* promoters do not require the Abf1 site to fully repress

transcription (26, 51, 54). In addition, it has been shown that the URS1 sites from meiotic promoters can function on their own as meiosis-specific activator sites (2, 8). Fragments that contain both the *HOP1* UAS_H (Abf1) and $URS1_H$ sites migrate with the same mobility as a fragment containing the UAS_H site alone (41). This result indicates that the factors binding to the URS1 and Abf1 sites do not directly interact in vitro and suggests that they may work through a common target.

The participation of both Abf1 and Rpa in the regulation of *HOP1* suggests that there may be a close connection between DNA replication and the transcriptional regulation of meiosis-specific genes. Hop1 is a component of meiotic chromosomes and is required for the formation of the synaptonemal complex during the first meiotic division. It has been shown that precursors for the synaptonemal complex begin to appear approximately 4 h after the initiation of meiosis and about 1 h after the completion of DNA replication (39). General transcription factors like Abf1 and Rpa may function to coordinate DNA replication and the transcriptional activation of proteins, such as *HOP1*, that are required in the subsequent steps of meiosis.

ACKNOWLEDGMENTS

We thank Lenore Neigeborn, David Norris, Stephen Parent, and the members of our laboratory for critical reading of the manuscript. We thank Steven Brill for providing the Rpa antibodies, Leonore Neigeborn for yeast strains LNY2 and LNY3, and John Diffley and Bruce Stillman for the Abf1 antibodies. Amino acid sequence analysis was performed by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University.

This work was supported by a grant VM-67 from the American Cancer Society. V.G.-D. was supported by the Deutsche Forschungsgemeinschaft (Ga 549/1-1).

REFERENCES

1. **Alani, E., R. Thresher, J. D. Griffith, and R. D. Kolodner.** 1992. Characterization of DNA binding and strand-exchange stimulation properties of y-RPA, a yeast single-strand-DNA-binding protein. J. Mol. Biol. **227:**54–71.
2. **Bowdish, K., and A. Mitchell.** 1993. Bipartitite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**2172–2181.
3. **Bowdish, K., H. Yuan, and A. Mitchell.** 1995. Positive control of yeast meiotic genes by the negative regulator *UME6*. Mol. Cell. Biol. **15:**2955–2961.
4. **Brill, S., and B. Stillman.** 1991. Replication factor-A from *Saccharomyces cerevisiae* is encoded by three essential genes coordinately expressed at S phase. Genes Dev. **5:**1589–1600.
5. **Brindle, P. K., J. P. Holland, C. E. Willett, M. A. Innis, and M. J. Holland.** 1990. Multiple factors bind the upstream activation sites of the yeast enolase *ENO1* and *ENO2*: ABF1 protein, like repressor activator RAP1, binds *cis*-acting sequences which modulate repression or activation of transcription. Mol. Cell. Biol. **10:**4872–4885.
6. **Buchman, A. R., W. J. Kimmerly, J. Rine, and R. D. Kornberg.** 1988. Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**210–225.
7. **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.
8. **Chintamaneni, C., and A. K. Vershon.** Unpublished data.
9. **Della Seta, F., S.-A. Ciafré, C. Marck, B. Santoro, C. Presutti, A. Sentenac, and I. Bozzoni.** 1990. The ABF1 factor is the transcriptional activator of the L2 ribosomal protein genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2437–2441.
10. **Della Seta, F., I. Treich, J.-M. Buhler, and A. Sentenac.** 1990. ABF1 binding sites in yeast RNA polymerase genes. J. Biol. Chem. **265:**15168–15175.
11. **Diffley, J. F. X., and J. H. Cocker.** 1992. Protein-DNA-interactions at a yeast replication origin. Nature (London) **357:**169–172.
12. **Diffley, J. F. X., and B. Stillman.** 1989. Similarity between the transcriptional silencer proteins ABF1 and Rap1. Science **246:**1034–1038.
13. **Dorsman, J. C., M. M. Doorenbosch, C. T. C. Maurer, J. H. de Winde, W. H. Mager, R. J. Planta, and L. A. Grivell.** 1989. An ARS/silencer binding factor also activates two ribosomal protein genes in yeast. Nucleic Acids Res. **17:**4917–4923.
14. **Dorsman, J. C., W. C. van Heeswijk, and L. A. Grivell.** 1990. Yeast general transcription factor GF1: sequence requirements for binding to DNA and evolutionary conservation. Nucleic Acids Res. **18:**2769–2776.
15. **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.
16. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–287. *In* J. F. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Halfter, H., U. Müller, E.-L. Winnacker, and D. Gallwitz.** 1989. Isolation and DNA-binding characteristics of a protein involved in transcription activation of two divergently transcribed, essential yeast genes. EMBO J. **8:**3029–3037.
18. **Hamil, K. G., H. G. Nam, and H. M. Fried.** 1988. Constitutive transcription of yeast ribosomal protein gene *TCM1* is promoted by uncommon *cis*- and *trans*-acting elements. Mol. Cell. Biol. **8:**4328–4341.
19. **Holland, J. P., P. K. Brindle, and M. J. Holland.** 1990. Sequences within an upstream activation site in the enolase gene *ENO2* modulate repression of *ENO2* expression in strains carrying a null mutation in the positive regulatory gene *GCR1*. Mol. Cell. Biol. **10:**4863–4871.
20. **Hollingsworth, N. M., and B. Byers.** 1989. *HOP1*: a yeast meiotic pairing gene. Genetics **121:**445–462.
21. **Hollingsworth, N. M., L. Goetsch, and B. Byers.** 1990. The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61:**73–84.
22. **Kao, G., J. Shah, and M. J. Clancy.** 1990. An RME1-independent pathway for sporulation control in *Saccharomyces cerevisiae*. Genetics **126:**823–835.
23. **Kassir, Y., D. Granot, and G. Simchen.** 1988. *IME1*, a positive regulator of meiosis in *Saccharomyces cerevisiae*. Cell **52:**853–862.
24. **Keleher, C. A., C. Goutte, and A. D. Johnson.** 1988. The yeast cell-type-specific repressor $\alpha 2$ acts cooperatively with a non-cell-type specific protein. Cell **53:**927–936.
25. **Kovari, L. Z., and T. G. Cooper.** 1991. Participation of ABF-1 protein in expression of the *Saccharomyces cerevisiae CAR1* gene. J. Bacteriol. **173:**6332–6338.
26. **Künzler, M., C. Springer, and G. H. Braus.** 1995. Activation and repression of the yeast *ARO3* gene by global transcription factors. Mol. Microbiol. **15:**167–178.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
28. **Loo, S., P. Laurenson, M. Foss, A. Dillin, and J. Rine.** 1995. Roles of *ABF1*, *NPL3*, and *YCL54* in silencing in *Saccharomyces cerevisiae*. Genetics **141:**889–902.
29. **Luche, R., W. Smart, T. Marion, M. Tillman, R. 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.
30. **Luche, R. M., W. Smart, and T. G. Cooper.** 1992. Purification of a heteromeric protein binding to the URS1 transcriptional repression site in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**7412–7416.
31. **Marahrens, Y., and B. Stillman.** 1992. A yeast chromosomal origin of DNA replication defined by multiple functional elements. Science **255:**817–823.
32. **McBroom, L. D. B., and P. D. Sadowski.** 1994. Contacts of the ABF1 protein of Saccharomyces cerevisiae with a DNA binding site at *MAT*a. J. Biol. Chem. **269:**16455–16460.
33. **McBroom, L. D. B., and P. D. Sadowski.** 1994. DNA bending by *Saccharomyces cerevisiae* ABF1 and its proteolytic fragments. J. Biol. Chem. **269:**16461–16468.
34. **Mitchell, A. P.** 1994. Control of meiotic gene expression in *Saccharomyces cerevisiae*. Microbiol. Rev. **58:**56–70.
35. **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.
36. **Mitchell, A. P., and I. Herskowitz.** 1986. Activation of meiosis and sporulation in yeast by repression of the *RME1* product in yeast. Nature (London) **319:**738–742.
37. **Neigeborn, L., and A. P. Mitchell.** 1991. The yeast MCK1 gene encodes a protein kinase homolog that activates early meiotic gene expression. Genes Dev. **5:**533–548.
38. **Ozsarac, N., M. Bhattacharyya, I. W. Dawes, and M. J. Clancy.** 1995. The *SPR3* gene encodes a sporulation-specific homologue of the yeast *CDC*/*10*/*11*/*12* family of bud neck microfilaments and is regulated by ABF1. Gene **164:**157–162.
39. **Padmore, R., L. Cao, and N. Kleckner.** 1991. Temporal comparison of recombination and synaptonemal complex formation during meiosis in *S. cerevisiae*. Cell **66:**1239–1256.
40. **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.
41. **Prinz, S., F. Klein, H. Auer, D. Schweizer, and M. Primig.** 1995. A DNA

binding factor (UBF) interacts with a positive regulatory element in the promoters of genes expressed during meiosis and vegetative growth in yeast. Nucleic Acids Res. **23:**3449–3456.

42. **Quandt, K., K. Frech, H. Karas, E. Wingender, and T. Werner.** 1995. MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Res. **23:**4878–4884.
43. **Rhode, P. R., K. S. Sweder, K. F. Oegema, and J. L. Campbell.** 1989. The gene encoding ARS-binding factor I is essential for the viability of yeast. Genes Dev. **3:**1926–1939.
44. **Shore, D., D. J. Stillman, A. H. Brand, and K. A. Nasmyth.** 1987. Identification of silencer binding proteins from yeast: possible roles in SIR control and DNA replication. EMBO J. **6:**461–467.
45. **Sia, R. A. L., and A. P. Mitchell.** 1995. Stimulation of later functions of the yeast meiotic protein kinase Ime2p by the IDS2 gene product. Mol. Cell. Biol. **15:**5279–5287.
46. **Silve, S., P. R. Rhode, B. Coll, J. Campbell, and R. O. Poyton.** 1992. ABF1 is a phosphoprotein and plays a role in carbon source control of *COX6* transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**4197–4208.
47. **Singh, K. K., and L. Samson.** 1995. Replication protein A binds to regulatory elements in yeast DNA repair and DNA metabolism genes. Proc. Natl. Acad. Sci. USA **92:**4907–4911.
48. **Smith, H. E., and A. P. Mitchell.** 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**2142–2152.
49. **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.
50. **Strich, R., R. Surosky, C. Steber, E. B. Dubois, F. Messenguy, and R. Esposito.** 1994. *UME6* is a key regulator of nitrogen repression and meiotic development. Genes Dev. **8:**796–810.
51. **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.
52. **Sweder, K. S., P. R. Rhode, and J. L. Campbell.** 1988. Purification and characterization of proteins that bind to yeast *ARS*s. J. Biol. Chem. **263:** 17270–17277.
53. **Szent-Gyorgyi, C.** 1995. A bipartite operator interacts with a heat shock element to mediate early meiotic induction of *Saccharomyces cerevisiae HSP82*. Mol. Cell. Biol. **15:**6754–6769.
54. **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.
55. **Yoshida, M., H. Kawaguchi, Y. Sakata, K. Kominami, M. Hirano, H. Shima, R. Akada, and I. Yamashita.** 1990. Initiation of meiosis and sporulation in *Saccharomyces cerevisiae* requires a novel protein kinase homologue. Mol. Gen. Genet. **221:**176–186.