The Saccharomyces cerevisiae genome contains a predicted gene, YPR008w, homologous to the gene encoding the copper-activated transcription factor Ace1. The product of the YPR008w gene, designated Haa1, regulates the transcription of a set of yeast genes, many of which encode membrane proteins. Two main target genes of Haa1 are the multidrug resistance gene YGR138c and the YOR2 homolog to the plasma membrane Hsp30. Haa1 is localized to the nucleus. Haa1-induced expression of YGR138c and YOR2 appears to be direct. Induction of HAA1 using a GAL1/HAA1 fusion gene resulted in rapid galactose-induced expression of both HAA1 and target genes. Although Haa1 has a sequence very similar to the Cu-activated DNA binding domain of Ace1, expression of Haa1 target genes was found to be independent of the copper status of cells. Haa1 does not exhibit metalloregulation in cells incubated with a range of transition metal salts. Haa1 does not exhibit any cross-talk with Ace1. Overexpression of Haa1 does not compensate for cells lacking a functional Ace1. The lack of metalloregulation of Haa1 despite the strong sequence similarity to the copper regulatory domain of Ace1 is discussed.

The yeast Saccharomyces cerevisiae appears to have undergone an ancient genome duplication event resulting in extensive gene redundancy (1). Gene duplication confers evolutionary diversity and protection in that redundant gene products may ensure against loss of essential functions (2). Nearly 16% of the yeast proteome consists of protein pairs (3). Some of these paralogs are functionally redundant, such as the Hsp82 and 83 chaperones (97% identical) and the identical L1 ribosomal proteins Rpl1A and B (4). Other protein pairs are differentially localized. Two yeast citrate synthases, 81% identical in amino acid sequence, are localized to distinct cellular compartments (5). Some protein pairs are expected to have distinct functions for physiological challenges not encountered in laboratory conditions (1). Whereas specific metabolic challenges may require one particular member of a protein pair, other growth conditions may permit either member to be functional. A number of transcriptional activators exist as protein pairs. Paralogsous transcription factors often exhibit differential regulation or have distinct functions. Swi5 and Ace2 have highly homologous Zn finger DNA binding domains and bind to the same DNA promoter sequences in vitro but transactivate different genes due to context effects and negative regulators (6). Likewise, Yap1 and Yap2 have overlapping but distinct biological functions (7). The paralogous bZIP Yap1/Yap2 pair and the binuclear Zn cluster Pdr1/Pdr3 pair are regulated differentially. Pdr1 and Pdr3 modulate the expression of two ABC type multidrug resistance genes (7–9).

The Ace1 transcription factor has a robust homolog in YPR008w (35% sequence identity). Ace1 activates expression of three genes in a copper-dependent manner (10–14). The three genes encode two metallothioneins, Cup1 and Crs5, and the Sod1 superoxide dismutase. Each of these molecules is capable of buffering cytosolic copper ion levels thereby preventing Cu-induced cytotoxicity (15–17). Thus, Cu-induced expression of these molecules is a protective response. Targeted disruption of ACE1 results in a copper-sensitive cellular phenotype (15). Ace1 is a 225-residue polypeptide with an N-terminal DNA binding domain and C-terminal transactivation domain. Molecular dissection studies on Ace1 and the orthologous Amt1 in Candida glabrata indicate that Cu(I) activation involves Cu-induced DNA binding in the N-terminal segment (10, 18). This DNA binding segment of Ace1 and Amt1 consists of two subdomains, a 40-residue module stabilized by one Zn(II) ion and a 70-residue copper regulatory module (19, 20). The copper regulatory domain (CuRD) binds four Cu(I) ions through eight cysteinyl thiolates forming a polymetallic cluster (20). Based on Ace1 orthologs in other species, a consensus sequence of CX3CX14CXX10–27CXX6CXC is predicted to exit within the CuRD. The only site of significant variability within the CuRD is the segment separating the two halves, which contain four cysteine each. The domain is predicted to consist of a two-lobe structure with the tetracopper center sandwiched between them. DNA binding by Ace1 requires both the Zn and copper regulatory domains for major groove and minor groove DNA interactions (21).

YPR008w, designated here as Haa1, is homologous to both the N-terminal Zn module and copper regulatory domain of Ace1 (Fig. 1). The predicted Zn(II) ligands and their spacing are identical in the two proteins. The spacing of the Cys residues in the CuRD fits the consensus sequence shown above except in the variable spacer separating four Cys segments. Sequences in
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| Zn Domain Ligands | 40 |
|-------------------|----|
| Ace1: MVLINQYVA C28C IGKRAAQ C37H TQDLMRKKCFP88 | |
| Haa1: MVLINQYVA C28C IGKRVVT C37H TQDLMXKCFP88 | |

| Tetracopper Domain Ligands | 80 |
|-----------------------------|----|
| Ace1: 7T CUC6 KEIKTVFQPGC C6C AS–AR–REAUSK–RE–6C | |
| Haa1: 7T CD6C KQKLXNANFES C6C GLEEEKLVAKVKEKAR | |

![Image](https://example.com/image.png)

**FIG. 1.** Sequence comparison of the N-terminal segment of Haa1 and the DNA binding domains of Ace1. The DNA binding domains of Ace1 include the N-terminal Zn module and adjacent copper regulatory domain.

The domain important for DNA binding in Ace1 is conserved in Haa1. One significant difference between Ace1 and Haa1 is that the residue corresponding to the last Cys residue in the Ace1 CuRD is a Tyr in Haa1 (residue 101). Mutation of that Cys residue in the Ace1 CuRD (C90S) abolishes function, but does not impair polycopper cluster formation (19, 22).

The Ace1 ortholog Amt1 from *C. glabrata* resembles Ace1 only in the DNA binding domain (23). No homology is evident in the transactivation domain. Likewise, Haa1 lacks any similarity to Ace1 downstream of the DNA binding domain. The lack of homology in the transactivation domain is also evident in the Yap1/Yap2 pair and Aft1/Atf2 transcriptional activator pair.

The similarity of Haa1 and Ace1 in the copper regulatory domain suggests that Haa1 may be a copper metalloregulatory factor. Microarray experiments were carried out to determine whether Haa1 is a transcription factor and to identify any potential target genes. Haa1 was found to be a transcriptional activator of a set of genes encoding membrane stress proteins. Curiously, the function of Haa1 is unaffected by the copper status of cells.

**MATERIALS AND METHODS**

**Strains and Culture Conditions**—The strains used in this study include CM66/Δaa1 (MATa, gcn4–101, ura3–52, ino1–13, FREL/HIS3::LEU2, Δaa1::URA3), CM666 (MATa, gcn4–101, ura3–52, ino1–13, FREL/HIS3::LEU2), Sub62Δaa1ΔMATa, lys2–801, leu2–3, ura3–52, his3Δ00, trp1–1, HAA1::HIS3), CM3260 (MATa, trp1–63, leu2–3,112, gcn4–101, his3Δ00, ura3–52, BY4741 (MATa, his3Δ1, leu2Δ0, met1530, ura3Δ0, BY4741Δaa1 (MATa, his3Δ1, leu2Δ0, met1530, ura3Δ0), BY4741Δaa1 (MATa, his3Δ1, leu2Δ0, met1530, ura3Δ0, ace1::kanMX), and BY4742Δae1 (MATa, his3Δ1, leu2Δ0, lys2Δ0, ace1::kanMX)). Cells were propagated either in yeast extract-peptone-dextrose, synthetic complete medium plus dextrose, or selective medium plus raffinose. RNA transformations were performed using a lithium acetate procedure.

**Vectors**—To construct the HAA1/GFP fusion gene, the sequence coding for HAA1 was amplified by polymerase chain reaction using Pfu polymerase (Stratagene). The 5′ oligonucleotide used contained a BanHI site and the 3′ oligonucleotide removed the stop codon and added a CaaI site. The product generated was then subcloned into pYEF2-GFP as a BanHI/CaaI fragment resulting in plasmid pGAL1/ HAA1/GFP. Expression of GFP in the pYEF2-GFP vector is under the control of the GAL1 promoter (24). To construct the HAA1VP16 and ACE1/VP16 fusion genes, the sequence coding for the VP16 activation domain was polymerase chain reaction-amplified creating 5′ BanHI/ CaaI sites and a 3′ stop codon and EcoRI site. This product was then subcloned into pYEF2 as a BanHI/EcoRI fragment. Sequences encoding the DNA binding domains of Haa1 (codons 1–124) and Ace1 (codons 1–123) were polymerase chain reaction-amplified with 5′ BanHI and 3′ CaaI sites and ligated into the previously constructed pYEF2- VP16AD as BanHI/CaaI fragments yielding in-frame fusion of HAA1/ VP16 and ACE1/VP16. Both genes were under the control of the GAL1 promoter. Sequences were verified of each construct. The mutant form of Haa1 creating a Y101C substitution was engineered in pYEF2 HAA1/VP16 using site-directed mutagenesis.

**RNA Isolation and Microarray Analysis**—Total RNA was isolated from cells cultured in complete synthetic medium to an A600nm of 0.6 by the hot acid phenol method. Quantitation of RNA was carried out by UV spectroscopy. mRNA was isolated from total RNA using the PolyATtract® mRNA Isolation System IV kit from Promega following manufacturer’s instructions. Cy3-dUTP or Cy5-dUTP (Amersham Pharmacia Biotech) was incorporated during reverse transcription of the polyadenylated RNA. Fluorescently labeled products were recovered and hybridized to microarrays, washed, and scanned as described previously (25).

**mRNA Quantitation by S1 Nuclease Analysis**—Total RNA isolated from mid-logarithmic cells was hybridized with a 32p-labeled, single-stranded DNA oligonucleotide and digested with S1 nuclease. The samples were electrophoresed through an 8% polyacrylamide, 5 μ urea gel and data quantified with a Bio-Rad FX Imager and Quantity One software (Bio-Rad Inc.). S1 probes used included 60–66 nucleotides of the 5′ open reading frame sequences of candidate genes and 40 nucleotides of the calmodulin (CDM1) 5′ open reading frame.

**Immunofluorescence**—Haa1-GFPpYEF2, GFPpYEF2, and pYEF2 in BY4741Δaa1 were grown in synthetic medium lacking uracil with 2% raffinose at 30 °C to A600nm of 0.5. Expression of the GFP fusion was induced by addition of galactose to a final concentration of 2% for a 3-h period. Expression was then repressed by addition of glucose to 2% and cells were incubated an additional 2 h. Cells were then bound onto polylysine-coated slides and washed twice with phosphate-buffered saline buffer. The bound cells were overlaid with a mounting solution (phosphate-buffered saline, 50% glycerol, and 50 ng/ml DAPI) and sealed with a coverslip. The slides were stored at 4 °C in the dark for 4–18 h before viewing by fluorescence microscopy. Microscopy was performed using a Nikon Diaphot microscope (Nikon) equipped with a Micromax cooled CCD camera (Roper Scientific). DAPI and GFP fluorescence were detected using a multiband dichroic emission filter combination (Omega Optical; part number FX57 and individual excitation filters (400DF15 for DAPI and 485DF15 for GFP).

**RESULTS**

To identify potential target genes of Haa1, we compared wild-type and Δhaa1 cells for differential expression of genes. DNA microarray experiments were used to quantify differential expression in cells cultured in the presence of 100 μM CuSO4 or 100 μM bathocuprine sulfonate (BCS), a Cu(1) chelator, for 30 or 60 min. The addition of 100 μM CuSO4 to cultures activates Ace1-dependent expression of CUP1 and CRS1 (13, 14). In contrast, the addition of 100 μM BCS to cultures lowers the availability of copper ions resulting in the activation of Mac1 (26, 27). Transcriptional activation by Mac1 is specifically inhibited in copper-replete cells (26–28). In multiple microarray experiments with independently grown cultures, more than 10 genes were elevated in their expression in wild-type cells with respect to Δhaa1 cells (Table I). The observed differential expression of these genes was independent of the copper status of the yeast. Several of the differentially

**TABLE I**

Differential expression of genes in a pairwise comparison of HAA1 and ΔHAA1 cells

| Gene                  | Cy3/Cy5 ratio |
|-----------------------|--------------|
| YP157w                | 11 ± 3       |
| YGR138c               | 10 ± 2       |
| YPR02                 | 8 ± 2        |
| PHMS                  | 5 ± 2        |
| YGP1                  | 4 ± 0.4      |
| YLR297w               | 5 ± 0.5      |
| YIR035w               | 4 ± 0.4      |
| YPR156c               | 3 ± 0.1      |
| YPI122c               | 3 ± 0.1      |
| YER130c               | 2 ± 0.6      |

Wild-type (CM66) and Δhaa1 (CM66Δhaa1) cells cultured in complete synthetic medium were incubated in 100 μM bathocuprine sulfonate or 100 μM CuSO4 for 30 or 60 min prior to cell harvest. mRNA isolated from the four cultures was used for each microarray experiment. Since expression of the following genes was independent of the copper status of the cells, a mean ratio of Cy3/Cy5 fluorescence is shown of four independent experiments.
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**Fig. 2. S1 analysis of Haa1 target genes in wild-type and Δhaa1 mutant cells.** Cells were grown in synthetic complete medium to \(A_{600\,\text{nm}}\) 0.6 and harvested. RNA was extracted and 40 \(\mu\)g of RNA was hybridized with specific single-stranded oligonucleotides for S1 analysis as described in “Materials and Methods.” Calmodulin (CMD1) was used as a loading control.

The Haa1-dependent expression of YGR138c and the other genes listed in Table I was confirmed by quantitation of mRNA using the S1 nuclease assay (Fig. 2). Expression of YGR138c, YPR157w, and YRO2 was markedly attenuated in Δhaa1 cells, whereas expression of PHMS and YGP1 was weakly attenuated in Δhaa1 cells.

To confirm that the differential expression of these genes correlated with the HAA1 genotype, two experiments were performed. First, wild-type and Δhaa1 cells were crossed to create a diploid strain and subsequent tetrad dissection was performed on sporulated cells. The resulting haploid cells were then analyzed by S1 analysis. Expression of YGR138c and YRO2 correlated with the HAA1 genotype. A representative S1 blot of one tetrad is shown in Fig. 3. Secondly, a GAL1/HAA1 fusion gene was constructed to enable galactose-inducible expression of HAA1. The episomal fusion gene containing the entire HAA1 open reading frame was transformed into Δhaa1 cells. Cells pregrown in raffinose to mid-log phase were induced with galactose, and cells were harvested at varying times after the addition of galactose. Within 15 min of the addition of galactose, HAA1 expression was prominent (Fig. 4). Expression of YGR138c paralleled HAA1 expression. YGR138c expression was visible at 15 min of galactose addition, but increased markedly at 30 min. No further increase was observed in either HAA1 or YGR138c expression in cells cultured for longer times in galactose. Expression of YPR157w, YRO2, PHMS and YGP1 was also prominent after 30 min of HAA1 induction. Cells transformed with a control vector showed no galactose-induced expression of these genes. The rapid induction of all these genes following Haa1 induction suggests that their activation by Haa1 is likely to be direct.

If Haa1 is a transcriptional activator, we would expect it to be found in the nucleus under conditions in which its target genes are activated. A vector containing the GAL1/HAA1/GFP fusion gene was transformed into Δhaa1 cells to assess Haa1/GFP localization. The fusion protein was functional in that YGR138c was induced in galactose cultures. Cells pregrown in raffinose were treated with galactose for 3 h and incubated an additional 2 h after the addition of glucose to inhibit GAL1 expression. Fluorescence microscopy revealed clear nuclear staining of the Haa1/GFP fusion (Fig. 5). The Haa1/GFP fluorescence was similar to DAPI fluorescence in nuclei. Cells expressing GFP not fused to Haa1 showed only diffuse cellular fluorescence.

The homology between Haa1 and Ace1 in the Ace1 Cu-responsive DNA binding domain suggests that Haa1 may be copper-regulated in its function, although no evidence of metalloregulation was observed in the initial microarray experiments. Two additional experiments verified that expression of Haa1 target genes is independent of the copper status of cells. First, wild-type cultures incubated with 100 \(\mu\)M CuSO₄ for 30 min to 2 h failed to show any change in YGR138c expression (data not shown). Furthermore, the addition of other metal salts at 100 \(\mu\)M, including Zn(II), Fe(II), and Cd(II), failed to alter YGR138c expression levels. Second, Δhaa1 cells transformed with the GAL1/HAA1/GFP fusion gene were preincubated under conditions resulting in either copper deficiency with the use of BCR or copper loading. Thus, transformants were cultured in medium containing either 100 \(\mu\)M bathocuproine sulfonate or 10 \(\mu\)M CuSO₄ prior to a 30-min induction with galactose. Expression of YGR138c was equivalent in the Cu-deficient and Cu-supplemented cells containing the GAL1/HAA1/GFP fusion gene (Fig. 6). CTR1 expression was monitored as a control to assess the efficiency of BCR in generating Cu-deficient cells. CTR1 expression is maximally induced in Cu-deficient cells (27). These experiments are consistent with a lack of metalloregulation by Haa1.

To determine whether the N-terminal segment homologous to Ace1 represented the minimal DNA binding domain, fusion genes were constructed in which the Cu-responsive DNA bind-
**Fig. 5. Immunofluorescence of Δhha1 cells transformed with pGAL1/HAA1/GFP or a control pGAL1/GFP vector.** Cells were grown in synthetic medium lacking uracil with 2% raffinose at 30 °C to A_{600nm} of 0.5. Expression of the HAA1/GFP fusion was induced by addition of galactose to a final concentration of 2% for a 3-h period. Expression was then repressed by addition of glucose to 2%, and cells were incubated an additional 2 h. Cells were then bound onto poly-lysine-coated slides and prepared for immunofluorescence as described in “Materials and Methods.” Panels A, D, and G show GFP fluorescence of cells containing pGAL1/HAA1/GFP (panel A), pGAL1/GFP (panel D), or control vector (panel G), respectively. Panels B, E, and H show the DAPI fluorescence, and panels C, F, and I show the Nomarski cell image. The bar shown represents 5 μm.

**Fig. 6. Haa1-dependent expression of YGR138c is independent of the copper status of cells.** Cells (Δhha1) were transformed with either pGAL1/HAA1/GFP or a control vector. Cells were pregrown in low copper medium (Bio101) containing 2% raffinose. These were then diluted to A_{600nm} 0.1 in medium containing either 10 μM CuSO_{4} or 100 μM bathocuproine sulfonate, and cells were grown to an A_{600nm} of 0.5. Galactose was added to 2%, and the cells were harvested 30 min later. RNA was extracted for S1 analysis probing for expression of YGR138c or CTR1 with CMD1 included as a loading control.

**Fig. 7. Expression of YGR138c by a Haa1/VP16 fusion.** Cells (Δhha1) containing a HAA1/VP16 fusion gene or a mutant HAA1/VP16 fusion encoding a Y101C substitution were cultured in the presence of 100 μM CuSO_{4} or 100 μM bathocuproine sulfonate prior to cell harvest and subsequent S1 analysis.

Cells lacking a functional Haa1 are viable and have no detectable growth defects with different carbon sources or show any heat sensitivity. Δhha1 cells do not exhibit any enhanced sensitivity to copper depletion or copper toxicity (data not shown). Cells lacking a functional Ace1 are copper-sensitive in that they fail to propagate in medium containing 0.1 mM CuSO_{4}. The disruption of HAA1 in an ace1 background did not augment or inhibit the known Cu sensitivity of ace1 cells (data not shown). Furthermore, the Cu-sensitive phenotype of ace1 cells was not suppressed by transformation of the cells with the episomal GAL1/HAA1/VP16 fusion gene, although suppression was complete by transformation with an episomal GAL1/ACE1/VP16 fusion gene (Fig. 8).

**DISCUSSION**

Haa1 is shown to be a transcriptional regulator of a series of novel yeast genes, many of which are predicted to encode membrane proteins. Consistent with a role as a transcriptional activator, Haa1 is localized within the nucleus. Induction of HAA1 using a GAL1/HAA1 fusion gene resulted in the accumulation of HAA1 mRNA within 15 min after addition of the galactose to the culture. Haa1 target genes were rapidly induced suggesting that Haa1 is a primary activator of these genes.

The gene most responsive to a Haa1 is YGR138c. This gene encodes one of 28 putative multidrug resistance permeases in the major facilitator superfamily (MFS) in *S. cerevisiae* (29). MFS permeases differ from ABC multidrug resistance permeases in that they are energized by a proton-motive force rather than ATP hydrolysis. YGR138c and YPR156c belong to a group of 11 other putative multidrug resistance genes predicted to have 12 transmembrane helices (30). Haa1 as shown in these microarray studies alters none of the other multidrug resistance genes in this group in their expression. Multidrug permeases may be localized to different membranes; for example, the Tpo1 polyamine permease is localized in vacuolar membranes (31). Only a few of the 13 yeast MFS molecules comprising the cluster containing YGR138c have known substrates. These include amiloride, cycloheximide, fluconazole, quinidine, polyamines, and organic acids (31–33). It is significant that Haa1 is the major regulator of two MFS genes in this group, YGR138c and YPR156c, consistent with the suggestion that Haa1 is a regulator of resistance to one or more unidentified compounds. YGR138c exhibits marked similarity to a benzylmethylthioate resistance protein in *Candida albicans* (30), although haa1 cells show no obvious enhanced sensitivity to methotrexate or cycloheximide. Most of the Haa1 target genes have not yet been characterized. Many are predicted to be membrane proteins. One such example is YRO2 that encodes a protein resembling two plasma membrane proteins, Hsp30 (Yro1) and Mrh1. Ygp1 is predicted to be a glycoprotein synthesized in response to nutrient limitation.

The sequence similarity of Haa1 to the Cu-activated DNA
binding domain of Ace1 suggested that Haa1 would be a copper-metalloregulated transcriptional activator (Fig. 1). Expression of Haa1 target genes was found to be independent of the copper status of cells. Expression of YGR138c was not altered in cells cultured in medium varying in Cu(II) or other transition metal ion concentrations. In fact, expression of YGR138c was unaffected by culturing cells in Cu-deficient medium. These results suggest that DNA binding by Haa1 is copper-independent. This is in contrast to Ace1 in which DNA binding requires formation of a tetra-copper thiolate cluster in one domain of the DNA binding segment (34). Copper metalloregulation of YGR138c is not observed even in the Y101C mutant Haa1 that restores all eight essential Cu regulatory domain cysteines in the Ace1 copper regulatory domain. The lack of copper metalloregulation in Haa1 is not related to its transactivation domain. Fusion of the heterologous transactivator VP16 to the Ace1 and Haa1 minimal DNA binding domains retained Cu-induction of CUP1 expression by Ace1, but no Cu modulation of YGR138c expression was observed. Thus, Haa1 is not a metalloregulatory protein. It is curious that Ace1 is Cu-regulated, whereas Haa1 is not considering that they both share a conserved Cu regulatory domain and are nuclear factors. It is possible that Cu-metalloregulation of Ace1 is dependent on specific Cu(I) presentation to Ace1 through a metallochaperone. Despite attempts to identify a nuclear copper metallochaperone, no genes have been identified for this role.

The lack of metalloregulation in Haa1 does not imply that a Cu-thiolate cluster does not stabilize the CuRD in Haa1. The folded conformation of the CuRD in Ace1 is dependent on the presence of the tetra-copper cluster. In the absence of bound Cu(I), the domain is unstructured. The marked similarity between the CuRD in Ace1 and the corresponding segment in Haa1 may suggest that a polycopper thiolate cluster likewise stabilizes the DNA-binding conformer of Haa1. A key difference may be that the cluster in Ace1 may undergo facile dissociation, thereby showing metalloregulation. Future studies will address whether Haa1 contains a stable polycopper cluster required for DNA binding. The presence of a polycopper cluster in Haa1 may permit other stress conditions such as oxidative stress to destabilize Haa1 through cluster disruption.

The sequence similarities between Haa1 and Ace1 may imply related DNA binding sites for the two proteins. Ace1 makes major groove contacts at two ends of its DNA promoter elements and minor groove contacts in the middle A/T-rich region. The Zn domain contacts DNA in one major groove region and the minor groove. Haa1 is expected to have related contacts for the Zn domain. The residues in the copper regulatory domain of Ace1 responsible for DNA binding have not been mapped, so it is not possible to predict whether this domain in Haa1 will contact a similar DNA sequence to that of Ace1. No obvious Ace1-like promoter sites are apparent in YGR138c. Mapping studies of the Haa1 binding sites in YGR138c are in progress.

The Ace1/Haa1 paralogs do not resemble other paralogous transcription factors that exhibit partial overlapping functions. Swi5 and Ace2 share a similar DNA binding structural motif and show cross-regulation when overexpressed (6). Overexpression of Haa1 does not restore CUP1 expression in ace1 cells. This is somewhat surprising given the strong sequence similarity in the segments of Ace1 that appear responsible for major groove DNA binding. The lack of suppression of ace1 cells by overexpression of HAA1 is consistent with an absence of cross-talk between Ace1 and Haa1. The two factors obviously have distinct functions.

Haa1 was found to interact with Man5 in a genomic two-hybrid experiment (35). Msn5 is required for the export of the Pho4 transcription factor from the nucleus under phosphate repression conditions (36). Thus, one intriguing possibility is that Haa1 is a functional transcription factor only under a set of conditions, including the standard growth conditions we examined, and may be exported from the nucleus under other growth conditions.

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