The HAP2 subunit of yeast CCAAT transcriptional activator contains adjacent domains for subunit association and DNA recognition: model for the HAP2/3/4 complex

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Budding yeast HAP2 is required in concert with HAP3 and HAP4 to form a heterotrimeric CCAAT-binding transcriptional activation complex at the UAS2 element of *CYC1*. Functional homologs of HAP2 and HAP3 have been conserved in HeLa cells where HAP2 activity corresponds to a chromatographic fraction designated CP1B. Here, we describe deletion and codon insertion mutagenesis of the *Saccharomyces cerevisiae* HAP2 subunit. DNA binding was assessed both in vitro and in vivo whereas subunit association and transcriptional activation were analyzed in vivo by using a bifunctional lexA-HAP2 fusion. The results indicate that the 265-amino-acid HAP2 protein contains an "essential core" of 65 amino acids, which is entirely sufficient for assembly and DNA binding of the HAP2/3/4 complex. The essential core can be divided further into a region of 44 amino acids, which is sufficient for subunit association, and a region of 21 amino acids, which is required specifically for DNA recognition. The remainder of the HAP2 protein is dispensable. The roles of HAP3 and HAP4 in the heterotrimeric complex are also assessed by using lexA fusions and a fusion of the GAL4 acidic activation domain to HAP2, which partially bypasses a deletion of *HAP4* but not *HAP3*. These data indicate that subunits HAP2 and HAP3 are primarily responsible for site-specific DNA binding by the complex, whereas the HAP4 subunit provides the primary transcriptional activation domain. A model for the function of this regulated transcriptional activation complex is presented.

[Key Words: Yeast transcription; CCAAT element; HAP2/3/4; heteromeric complex; *Saccharomyces cerevisiae*]

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Transcriptional activators affect gene expression by first binding to specific DNA sequences near genes and then promoting the initiation of transcription by RNA polymerase II via the various basic transcription factors. Analysis of several eukaryotic transcriptional activators has revealed that they commonly possess a modular structure consisting of separate and independent DNA-binding and transcriptional activating regions (Brent and Ptashne 1985). Site-specific DNA binding has been shown to occur through various common DNA-binding motifs such as the helix-turn-helix (for review, see Pabo and Sauer 1984; Brennan and Matthews 1989), zinc finger (for review, see Klug and Rhodes 1987; Berg 1989) leucine zipper (Landschulz et al. 1988), and helix-loop-helix (Murre et al. 1989a,b) structures. Less is known about the specific structure of transcriptional activating regions, except that such sequences are often enriched in acidic amino acids (Ma and Ptashne 1987), glutamines (Courey and Tjian 1988), or prolines (Mermod et al. 1989).

Most transcriptional activators possess both DNA-

binding and transcriptional activating functions in a single polypeptide. Recently, however, complex transcriptional activators consisting of two or three different protein subunits have been recognized and characterized. For example, the genetically defined HAP2 and HAP3 (Olesen et al. 1987) and, later, HAP4 (Forsburg and Guarente 1989a) proteins have been shown to bind as a complex heterotrimeric structure to the sequence CCAAT located in the UAS2UP1 element of *Saccharomyces cerevisiae* *CYC1*. Mutations in any one of these subunits abolish transcriptional activation from UAS2. The HAP2/3/4 heterotrimer also activates transcription through CCAAT "boxes" located upstream of *HEM1* (Keng and Guarente 1987), as well as *COX4* and *CYT1* (Schneider 1989). All of these genes encode components of the mitochondrial electron transport chain. As a consequence, *hap2*, *hap3*, and *hap4* mutant yeast strains fail to express such genes adequately, resulting in an inability to grow on nonfermentable carbon sources where oxidative phosphorylation is essential for energy production. The primary induction of UAS2 (CCAAC) of

CYC1, or other genes regulated by the complex, occurs when cells are shifted from a fermentable carbon source such as glucose to medium containing a nonfermentable carbon source, such as lactate. The precise mechanism by which this regulation occurs is not fully understood, although changes in the amount of HAP2/3/4 CCAAT-binding activity clearly play a major role (Olesen and Guarente 1987). An additional form of UAS2 (CCAAC) regulation is the modulation of HAP2/3/4 activity by heme. In heme-deficient cells, HAP2/3/4 activity from UAS2 is tenfold lower than in cell synthesizing heme. This form of regulation is thought to make the CCAAT-responsive respiratory genes sensitive to the availability of environmental oxygen, which is required in the biosynthesis of heme (for review, see Forsburg and Guarente 1989b).

Remarkably, the HAP2/3/4 CCAAT-binding complex appears to have been conserved over the millions of years of evolutionary divergence between yeast and man. Chromatographic fractionation of human cells has identified a similar multicomponent CCAAT-binding complex (Chodosh et al. 1988a). Cooperative binding between the human and yeast subunits has demonstrated that the HAP2 human homolog is contained in a fraction designated CP1B, whereas the human homolog of HAP3 occurs in fraction CP1A (Chodosh et al. 1988b). These studies revealed that both protein-protein interactions between the subunits and protein-DNA interactions allowing site-specific binding have been conserved between yeast and man by the subunits of this complex transcriptional activator. More recently, the heterodimeric structure of mammalian Fos-Jun has been analyzed in detail (for review, see Curran and Fianza 1988), and MyoD1/E12 and other helix-loop-helix protein heterodimers have also been described (Murre et al. 1989b).

None of the subunits of the HAP2/3/4 complex possesses any homology to known DNA-binding motifs; therefore, the subunits and protein domains involved are not apparent. Subsequent to binding to the site specifically, the HAP2/3/4 complex must promote transcription of the downstream gene—presumably by contacting the transcriptional machinery. Interestingly, two of the subunits possess features found in known transcriptional activating regions. HAP2 contains a polyglutamine stretch similar to the transcriptional activating sequences of mammalian SP1 (Courey and Tjian 1988) and also found in numerous *Drosophila* developmental genes (Wharton et al. 1985), whereas HAP4 contains an essential highly acidic carboxy-terminal region that is interchangeable in function with the acidic transcriptional activation domain of GAL4 (Forsburg and Guarente 1989a). In addition to DNA binding and transcriptional activation functions, each subunit of the HAP2/3/4 heterotrimer must contain surfaces that allow association with the other subunits of the complex, “heterodimerization domains.” Indeed, the available evidence suggests that the HAP2, HAP3, and HAP4 subunits are associated together in solution and do not need to be bound to DNA to form the requisite protein-

protein interactions for assembly of the complex (Hahn and Guarente 1988, and this paper). Here, we describe experiments that begin a molecular dissection of the HAP2/3/4 complex and the HAP2 subunit, in particular.

Results

The HAP2 protein contains an essential core of 65 amino acids

Mapping sequences in HAP2 required for DNA binding by HAP2/3/4 The HAP2 protein is 265 amino acids long and possesses two interesting features in its sequence. One is a repeat of 14 glutamines in a row, extending from amino acid 120 to amino acid 133. The other is a highly basic (27% lysine or arginine) amino acid content in the carboxy-terminal 100 amino acids. A similarly charged region of GCN4 has been shown to possess sequence-specific DNA-binding activity (Hope and Struhl 1986), and comparable basic protein domains appear to be involved in DNA recognition by the Fos-Jun family of proteins, as well as MyoD1 and related proteins (see Kouzarides and Ziff 1989). To determine the minimal region of HAP2 required for sequence-specific DNA binding, a series of amino-terminal and carboxy-terminal deletions of the HAP2 protein were synthesized by in vitro transcription of deleted HAP2 templates with SP6 RNA polymerase. The resulting RNA was translated in vitro in a rabbit reticulocyte lysate as described in Materials and methods. Because HAP2 binds to the UAS2UP1 CCAAT box as a complex with HAP3 (Olesen et al. 1987) and HAP4 (Forsburg and Guarente 1989a), regions of the HAP2 protein required for DNA binding may be involved in either subunit association or direct contact with the DNA double helix or both protein-protein and protein-DNA interactions.

Full-length HAP2 protein was generated from the template pSP64:HAP2, which contains the SP6 promoter cloned upstream of the 265 amino acid HAP2 open reading frame (ORF). Gel shift DNA-binding assays performed with in vitro-translated unlabeled HAP2 protein and radiolabeled UAS2 probes revealed the presence of a UAS2UP1 DNA-binding activity (Fig. 1, lane 4), which was absent with a UAS2 probe containing a non-consensus CCAAC sequence (lane 3). Binding of HAP2 in the absence of exogenously supplied HAP3 and HAP4 is presumably due to the presence of the evolutionarily conserved CP1A activity in the mammalian reticulocyte lysate. The endogenous CA1A/CP1B activity was evident as a faint band in the mock translation control (lane 2). THE CP1A-like activity must be limiting for binding when HAP2 is synthesized in vitro, because adding purified CP1A from HeLa cells to HAP2 reticulocyte translation resulted in a large increase in CCAAT DNA-binding activity (cf. lanes 4 and 6). In the presence of an adequate amount of competitor nucleic acid and supplemental HeLa CP1A, binding of the in vitro-synthesized HAP2 protein was readily evident without interference from the CP1 CCAAT-binding activity endogenous to the rabbit reticulocyte lysate (cf. lanes 5 and 6). Further-

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Figure 1. Detection of DNA-binding activity from in vitro-synthesized HAP2. Radiolabeled UAS2 and UAS2UP1 probes were utilized as indicated in gel mobility-shift DNA-binding assays with nonradiolabeled mock or HAP2 in vitro translation products prepared as described in Materials and methods. Lanes 1–4 contain 0.5 μ g single-stranded (salmon sperm) DNA and 0.5 μ g of poly[d(I-C)/d(I-C)] competitor nucleic acid. The HAP2 CCAAT-binding activity detected in lane 4 reflects the presence of a CP1A-like activity endogenous to the rabbit reticulocyte lysate. Detection of the HAP2/3/4 CCAAT DNA-binding activity was facilitated by the addition of more competitor nucleic acid [3 μ g of single-stranded DNA and 3 μ g of poly[d(I-C)/d(I-C)] and either HeLa CP1A fraction (lanes 5 and 6), or a yeast HAP3/HAP4-containing extract (lanes 7 and 8).

more, the in vitro-synthesized HAP2 was also capable of interacting with HAP3 and HAP4 supplied from a crude yeast extract to form the HAP2/3/4 CCAAT-binding species (cf. lanes 7 and 8). We conclude that this in vitro assay accurately reflects the DNA-binding properties of yeast HAP2.

A series of amino-terminally deleted HAP2 proteins was generated by construction of in-frame deletions of HAP2 by use of either the wild-type (amino acid 1) or a downstream (amino acid 43) ATG initiation codon. Synthesis of the expected size protein was confirmed by translation of a small amount of the RNA in the presence of [35 S] methionine and molecular weight analysis of the translation products on an SDS-PAGE gel, as described in Materials and methods (results not shown). The remaining RNA was translated in the presence of unlabeled methionine, and the resulting unlabeled HAP2 protein was examined for site-specific DNA binding in the gel retardation assay. Binding of the amino-terminal deletion proteins to UAS2UP1 is shown in Figure 2A, and results are summarized in Figure 2B. Deletions extending to amino acid 154 (lanes 1–7) did not disrupt DNA binding, although they increased the mobility of the complex, as would be expected from the decrease in molecular weight of the HAP2 component. Deleting further to amino acid 169 resulted in loss of CCAAT-binding activity (lane 8). Thus, the first 153 amino acids of HAP2, including the polyglutamine stretch, are not required for site-specific DNA binding.

Carboxy-terminal deletions of HAP2 were generated by truncation of pSP64 : HAP2 template at a series of restriction sites in the ORF. As shown in Figure 2A and summarized in Figure 2B, deletions up to amino acid 218 were still able to bind site specifically (lanes 9–12). Truncating further to amino acid 197 obliterated binding (lane 13). Furthermore, to confirm that there was no redundancy in DNA-binding function of the amino- and carboxy-dispensable sequences, truncations of Δ N154 were generated (lanes 18–24). The 65-amino-acid region extending from 154 to 218 was capable of forming a CCAAT-binding complex (Δ N154 Δ C218, lane 22), demonstrating that the entire DNA-binding domain of HAP2 is contained within this region. Furthermore, representative HAP2 deletions were also examined for cooperative CCAAT-binding activity with yeast HAP2/HAP4 (assays not shown). The results, summarized in Figure 2B, indicate that the region extending from amino acid 154 to amino acid 218 is adequate for the formation of a CCAAT-binding complex with either mammalian CP1A or yeast HAP3/HAP4. We conclude that these residues constitute a core of the HAP2 protein, which makes the necessary protein–DNA and/or protein–protein interactions sufficient to assembly HAP2 into a heterotrimeric CCAAT-binding complex.

Codon insertion mutations in the DNA-binding core domain obliterate HAP2 function in vivo As a first step toward determining the regions of HAP2 required for its in vivo function, we introduced a total of 20 codon insertion mutations throughout the HAP2 protein (Barany 1985). Net insertions from 1 to 4 amino acids and a single net deletion of 3 amino acids were constructed primarily by introduction of linkers at specific restriction sites such that the reading frame of the HAP2 protein was maintained (see Materials and methods).

The amino acid sequence changes made are summarized in Figure 3A. The mutations are named according to the amino acid residue(s) and codon position(s) eliminated by the change, followed by a slash and the amino acids inserted at that site. The ability of the resulting HAP2 mutant proteins to support growth on lactate and to activate a UAS2–*lacZ* fusion is summarized in Figure 3B. Only two of the mutations—13 (Y168/SRD) and 15 (G209/AGSR)—affected complementation of a *hap2* null mutation. Both of these codon insertions occur in the HAP2 DNA-binding core domain defined above.

Interestingly, mutant 14 (K180–184/DP), which occurs between these two mutations and is in the center of the DNA-binding domain, had no apparent phenotype. Mutants 4, 6, 7, 8, and 11–20 fully complemented the *hap2* mutant. We conclude that in vivo function of the HAP2 protein is quite insensitive to codon insertion mutations occurring outside of the core region required for site-specific DNA binding.

Deletion mutations reveal that the core domain is sufficient for HAP2 function in vivo To determine whether the regions of HAP2 not required for site-specific DNA binding and not affected by codon insertion mutagenesis are essential to the functioning of the protein in vivo, we

Figure 2. Amino- and carboxy-terminal deletions define the minimal DNA-binding domain of HAP2. (A) The indicated HAP2 deletion was synthesized in vitro and used in a gel retardation DNA-binding assay with a radiolabeled UAS2UP1 (CCAAT) probe, and HeLa CP1A fraction in the presence of 3 μ g single-stranded DNA and 3 μ g of poly[d(I-C)/d(I-C)] competitor. (B) Summary of amino- and carboxy-terminal deletion analysis of HAP2. The 265-amino-acid HAP2 ORF (WT HAP2) is represented schematically with the polyglutamine stretch (stippled) and the positively charged carboxyl terminus (hatched) indicated. The structure of each of the HAP2 deletions is indicated below. The number of each deletion construct indicates the first HAP2 codon present in the case of amino-terminal deletions and the last HAP2 codon present in the case of carboxy-terminal deletions. Amino-terminal deletion constructs utilize the native HAP2 initiation codon and first 16 amino acids except Δ N43, which starts with codon 43, and Δ N83, which starts with codons 43 and 44. Codons added by linker sequences to amino-terminal deletion constructs are described in Materials and methods. (+) Detection of a Δ HAP2-containing CCAAT-binding activity in the presence of HeLa CP1A (left column) or yeast HAP2/HAP4 (right column). (-) No CCAAT-binding detected; (nd) binding of the given construct in the presence of yeast HAP2/HAP4 was not determined.

constructed carboxy- and amino-terminal deletions of the HAP2 proteins as fusions to the bacterial repressor *lexA* (see Materials and methods). As reported earlier (Pinkham et al. 1987), the *lexA*-HAP2 fusion protein is capable of activating transcription from a yeast promoter containing the *lexA* operator but no yeast upstream promoter elements. This fusion protein also fully complements a *hap2* null mutation for growth on lactate. The *lexA*-HAP2 fusion is thus bifunctional in that it binds site specifically at both the *lexA* operator and at the UAS2UP1 CCAAT box as a complex with the HAP3 and HAP4 subunits. Figure 4A summarizes the phenotypes

observed for amino-terminal deletions of HAP2 fused to *lexA*. In this section, we discuss complementation of the *hap2* null mutant for growth on lactate by these mutant *lexA*-HAP2 constructs. Deletions extending to amino acid 154 (mutants *lx* Δ N45-*lx* Δ N154) did not affect complementation. As expected, further deletions that impinge on the DNA-binding domain of HAP2 (mutants *lx* Δ N169-*lx* Δ N255) abolished complementation.

Figure 4B shows the results of carboxy-terminal deletion mutagenesis of the *lexA*-HAP2 fusion. Deletions extending to the carboxyl end of the DNA-binding domain (amino acid 218, mutant *lx* Δ C218) did not detect-

Figure 3. Codon insertion mutagenesis of HAP2. (A) Summary of the 20 codon insertion mutations constructed as described in Materials and methods. The sequence changes made are indicated by the HAP2 amino acids and codon numbers destroyed, followed by a slash and the amino acids inserted at that site by linker sequence and/or Klenow fill-in. The net addition (or deletion) of codons is also indicated. (B) Summary of the position, size, and phenotype of the 20 codon insertion mutations. The structure of HAP2 is represented schematically, and the positions of the polyglutamine tract (Q14, stippled) and carboxy-terminal basic region (hatched) are indicated. The minimal region of HAP2 required for assembly and CCAAT DNA binding of the HAP2/3/4 complex (amino acids 154–218) is indicated below. The net insertion of codons is proportional to the size of the base of the inverted triangle. Complementation of a *hap2* null mutant for growth on lactate is indicated by plus (+) for wild-type levels of complementation and minus (–) for no detectable complementation.

ably affect lactate growth. Further deletions that impinge on the DNA-binding domain (mutant Δ C208) abolished complementation. The presence of the bacterial DNA-binding protein *lexA* in the HAP2 deletion constructs is unlikely to have affected these observations because deletions of HAP2 fused to the bacterial enzyme β -galactosidase gave similar results (data not shown).

To demonstrate that there is no redundancy in function of the first 154 and last 47 amino acids, mutants with both regions deleted were constructed. Figure 4C shows that residues 154–218 expressed as a fusion to *lexA* were entirely sufficient to support growth on lactate (mutant Δ N154 Δ C218). We conclude that residues 154–218 constitute an essential core of the HAP2 protein. This essential core provides all the protein structure necessary for assembly of HAP2 into the HAP2/3/4 heterotrimer, site-specific DNA binding of the resulting complex, and subsequent transcriptional activation of the promoter-bound complex as judged by *in vivo* complementation for growth on lactate.

The essential core is divisible into subunit association and DNA recognition domains

An assay for subunit interaction in the HAP2/3/4 complex The ability of a *lexA*–HAP2 fusion to activate at the *lexA* site is an assay for assembly of the HAP complex, because HAP4 must be incorporated into the active complex. As suggested earlier (Forsburg and Guarente 1989a) and shown below, HAP4 contains an acidic domain that activates transcription. To dissect sequences in the HAP2 essential core required for interaction with other subunits (HAP3 and/or HAP4) from sequences that may be involved in site-specific DNA interactions in the intact complex, we therefore examined in greater detail the properties of a *lexA*–HAP2 fusion protein that contains HAP2 codons 16–265 fused to the DNA-binding domain of *lexA* (codons 1–87). Figure 5 shows that the *lexA*–HAP2 fusion bound at the *lexA* operator activated transcription, as assayed by a *CYC1*–*lacZ* fusion, in the same carbon source and heme-dependent manner that occurs at UAS2. The levels of β -galactosidase were derepressed 3-fold in galactose and 13-fold in lactate over levels observed in glucose. Similarly, low heme conditions repressed the levels by 11-fold. These regulatory effects at the *lexA* site paralleled those observed at UAS2UP1 with wild-type HAP2 or with the bifunctional *lexA*–HAP2 (not shown). To demonstrate that the effects at the *lexA* operator were specific, a *lexA*–GAL4 fusion was also checked and was neither strongly affected by intracellular heme levels nor carbon source (Fig. 5). In contrast to the behavior of *lexA*–HAP2, the activity of *lexA*–GAL4 actually decreased in lactate. This reduction in activity is likely due to a decrease in expression of the *lexA*–GAL4 fusion from the ADH1 promoter, which is reportedly repressed by growth on nonfermentable carbon sources (Denis et al. 1983).

The critical test of the *lexA*–HAP2/HAP3/HAP4 interaction assay is that activity at the *lexA* operator should depend on HAP4 and, possibly, HAP3. The roles of the other two subunits of the HAP2/3/4 transcriptional activator were investigated by examining the function of *lexA*–HAP2 in strains mutant for either HAP3 or HAP4. Although both HAP3 and HAP4 are required for binding to UAS2, activation by *lexA*–HAP2 at the *lexA* operator is independent of the ability to bind UAS2. The effect of removing either HAP3 or HAP4 thus directly reflects the role of the protein in transcriptional activation. As shown in Figure 5, a *hap3* null mutation resulted in a twofold decrease in transcriptional activation in glucose and an eightfold decrease in galactose. Levels of activation in lactate could not be tested because of the inability of the *hap3* mutant strain to grow on a nonfermentable carbon source. Nonetheless, the decrease in expression in glucose and lack of derepression in galactose strongly suggests that the *lexA*–HAP2 fusion is dependent on the HAP3 subunit for the formation of a strong, regulatable transcriptional activation complex at the *lexA* operator. Similarly, the activity of *lexA*–HAP2 in a *hap4-2* mutant background failed to show galactose-derepressible transcriptional activation at the *lexA* operator. Although the effect was

CCAAT transcriptional activation complex

A	Name (Plasmid)	Structure	Complementation (lactate growth)	@UAS2UP1		@lexAoperator (WT)			@lexAoperator (hap3 ⁻)			HAP2/3/4 ASSEMBLY
				Glucose	Lactate	Glucose	Galactose	Lactate	Glucose	Galactose	Lactate	
	lxHAP2 (pJP300)		++	113	420	6.1	16	78	3.0	2.0	ng	+
	lxΔN45 (pJO301)		++	90	370	5.3	nd	92	nd	nd	ng	+
	lxΔN83 (pJO302)		++	86	275	3.3	nd	78	nd	nd	ng	+
	lxΔN92 (pJO303)		++	55	205	2.8	7.3	40	1.0	0.5	ng	+
	lxΔN107 (pJO304)		++	46	141	1.4	3.5	27	0.8	0.4	ng	+
	lxΔN141 (pJO305)		++	41	116	1.0	4.3	16	0.5	0.4	ng	+
	lxΔN154 (pJO306)		++	35	176	1.5	3.6	21	0.6	0.4	ng	+
	lxΔN169 (pJO307)		-	1.1	ng	0.3	0.2	0.2	0.3	0.2	ng	-
	lxΔN185 (pJO308)		-	1.1	ng	0.3	0.2	0.2	0.3	0.2	ng	-
	lxΔN230 (pJO309)		-	1.1	ng	0.3	0.2	0.2	0.3	0.2	ng	-
	lxΔN255 (pJO310)		-	1.1	ng	0.3	0.2	0.2	0.3	0.2	ng	-

B	Name (Plasmid)	Structure	Complementation (lactate growth)	@UAS2UP1		@lexAoperator (WT)			@lexAoperator (hap3 ⁻)			HAP2/3/4 ASSEMBLY
				Glucose	Lactate	Glucose	Galactose	Lactate	Glucose	Galactose	Lactate	
	lxHAP2 (pJP300)		++	113	420	6.1	16	78	3.0	2.0	ng	+
	lxΔC258 (pJO311)		++	98	328	8.0	nd	90	nd	nd	ng	+
	lxΔC230 (pJO312)		++	68	169	12	nd	173	nd	nd	ng	+
	lxΔC218 (pJO313)		++	49	189	8.4	15	95	4.0	1.0	ng	+
	lxΔC208 (pJO314)		-	1.2	ng	35	69	147	7.4	3.8	ng	+
	lxΔC197 (pJO315)		-	1.0	ng	23	44	142	15	2.9	ng	+
	lxΔC178 (pJO316)		-	1.0	ng	42	18	9.0	24	20	ng	-
	lxΔC167 (pJO317)		-	1.3	ng	7.1	3.4	5.0	7.3	4.7	ng	-
	lxΔC153 (pJO318)		-	1.1	ng	3.1	2.0	1.6	3.5	1.8	ng	-
	lxΔC139 (pJO319)		-	1.1	ng	2.2	1.4	0.7	2.5	1.3	ng	-
	lxΔC91 (pJO320)		-	1.1	ng	2.0	nd	0.7	nd	nd	ng	-
	lxΔC45 (pJO321)		-	1.2	ng	0.3	nd	0.1	nd	nd	ng	-

Figure 4. (Continued on next page.)

C	Name (Plasmid)	Structure	Complementation (lactate growth)	@UAS2UP1		@lexAoperator (WT)			@lexAoperator (hap3 ⁻)			HAP2/3/4 ASSEMBLY
				Glucose	Lactate	Glucose	Galactose	Lactate	Glucose	Galactose	Lactate	
	lxHAP2 (pJP300)		++	113	420	6.1	16	78	3.0	2.0	ng	+
	lxΔN141ΔC218 (pJO322)		++	50	532	3.5	10	28	0.6	0.6	ng	+
	lxΔN154ΔC218 (pJO323)		++	58	494	1.2	3.0	16	0.3	0.2	ng	+
	lxΔN154ΔC197 (pJO324)		-	0.9	ng	3.1	7.6	24	0.6	0.5	ng	+
	lxΔN154ΔC178 (pJO325)		-	0.7	ng	0.4	0.5	0.2	0.3	0.3	ng	-
	lxcI153 (pJO327)		++	80	450	7.6	32	85	2.0	3.3	ng	+
	lxcI168 (pJO328)		-	1.0	ng	1.5	0.8	0.9	1.0	0.7	ng	-
	lxcI180-184 (pJO329)		++	39	306	4.7	7.8	13	2.5	2.6	ng	+
	lxcI209 (pJO330)		-	0.7	ng	12	32	49	3.6	2.9	ng	+
	lxcI218 (pJO331)		++	71	429	9.0	nd	73	nd	nd	ng	+

Figure 4. Analysis of transcriptional activation and HAP3/HAP4 subunit assembly by *lexA*- Δ HAP2. The structure of the original *lexA*-HAP2 fusion (lxHAP2) and deletion derivatives thereof are shown schematically with the positions of the polyglutamine tract (Q14, stippled) and carboxy-terminal basic region (hatched) indicated. Numbers represent the first HAP2 codon present in amino-terminal deletions and the last HAP2 codon present in carboxy-terminal deletions. Codons introduced during the construction by added linker sequences are summarized in Materials and methods in the case of deletion constructs and in Fig. 3A in the case of codon insertion constructs. Complementation of a *hap2* null mutation is indicated for growth on lactate by the indicated *lexA*- Δ HAP2 fusion: (++) wild-type complementation; (-) no complementation detected. The data represent the average of at least four independent determinations of units of β -galactosidase from UAS2UP1 (pLGD265UP1) in *hap2* null strain (first two columns of numbers) or from the *lexA* operator (PRB1155) in a wild-type (WT) yeast strain (next three columns) or in a *hap3* null strain (last three columns of numbers) and the indicated carbon source. The final column indicates whether the given *lexA*- Δ HAP2 fusion is assembling with HAP3/HAP4 and is based on the difference between the wild-type and *hap3* null strains for levels of activation in glucose and galactose at the *lexA* operator. Constructs that require the presence of HAP3 for significant levels of activation are judged to be assembling into a *lexA*- Δ HAP2/3/4 complex at the *lexA* operator, whereas those that do not (e.g., lx Δ C178 and lx Δ C167) are judged to be activating transcription at the *lexA* operator independently of the other subunits. (+ and -) The given *lexA*- Δ HAP3 construct assembles with HAP3/HAP4 or does not, respectively. (A) Amino-terminal deletions of *lexA*-HAP2; (B) carboxy-terminal deletions of *lexA*-HAP2; (C) Double (amino- and carboxy-terminal) deletions of *lexA*-HAP2, and codon insertion *lexA*-HAP2 constructs.

less marked than in the *hap3* background, this is likely due to the use of an ethylmethane sulfonate (EMS)-derived point mutant (*hap4-2*) that is somewhat leaky, rather than a null mutant as was used for the *hap3* experiment. Control experiments with the *lexA*-GAL4 fusion showed that neither *hap3* nor *hap4* mutant backgrounds nonspecifically affected transcriptional activation through the *lexA* operator (Fig. 5, and results not shown). We conclude that the HAP2 subunit is capable of assembling in solution with HAP3 and HAP4, and that the activity of *lexA*-HAP2 at the *lexA* site is a measure of this assembly. HAP2 in the absence of HAP3 or HAP4 behaves as a weak constitutive transcriptional activator. These observations, which imply the formation

of a stable HAP2/3/4 heterotrimer in solution, are consistent with the observation that all three subunits copurify through four chromatographic steps in the absence of DNA (Hahn and Guarente 1988).

The HAP2 essential core contains two domains—one sufficient for subunit association and the other required specifically for CCAAT DNA recognition To map the regions of HAP2 required for association with the HAP3 and HAP4 subunits, amino- and carboxy-terminal deletions of *lexA*-HAP2 were examined for HAP3-dependent activation at UAS2UP1 and at the *lexA* operator. As summarized in Figure 4A, amino-terminal deletions extending to amino acid 154 still responded to carbon

Activator	Strain	Promoter	Glucose	Galactose	Lactate	low HEME	high HEME
1) HAP2	WT		95	229	607	58	350
2) <i>lexA</i> -HAP2	WT		6	16	78	2.4	27
3) <i>lexA</i> -HAP2	<i>hap3</i> ⁻		3	2	ng		
4) <i>lexA</i> -HAP2	<i>hap4</i> ⁻		5	5	ng		
5) <i>lexA</i> -GAL4	WT		260	185	80	162	152
6) <i>lexA</i> -GAL4	<i>hap3</i> ⁻		200	240	ng		

Figure 5. Transcriptional activation by HAP2/3/4 at UAS2UP1, and by *lexA*-HAP2/3/4 and *lexA*-GAL4 at the *lexA* operator. Numbers indicate the average of a least four independent determinations of units of β -galactosidase activity detected from the indicated *lacZ* promoter fusion under the indicated carbon source and heme conditions. The putative configuration of activator proteins in each yeast strain is also indicated. For the sake of simplicity, a single *lexA*-fusion protein is shown binding to the *lexA* operator (the *lexA* repressor actually binds its operator as a dimer), and a single copy of each HAP subunit in the complex is also assumed. HAP2 (black oval), HAP3 (open circle), and HAP4 (stippled form) are shown assembled and bound at the UAS2UP1 CCAAT box (line 1) or the *lexA* operator via the *lexA*-HAP2 fusion (line 2). The complex is shown incompletely assembled in either a *hap3* null strain (line 3) or a *hap4* null strain (line 4) (see Discussion). Activation by *lexA*-GAL4 fusion at the *lexA* operator (lines 5 and 6) is also summarized. This fusion is unaffected by the absence of HAP3 (line 6) or HAP4 (not shown).

source by derepressing strongly in lactate and were HAP3-dependent. Although overall levels of activation decreased as sequences were deleted, no effect was observed on the degree of carbon source regulation or HAP3 dependence. Interestingly, no effect was observed from deletion of the polyglutamine tract (cf. *lxΔN107* with *lxΔN141*). Further deletions (*lxΔN169*) impinging on the essential core of HAP2 resulted in the complete loss of activation at UAS2UP1 and the *lexA* operator in all carbon sources, with or without HAP3. We conclude that, although the amino terminus of HAP2 may play a minor role in augmenting transcription of the heterotrimeric complex, it is not involved in assembly of HAP2

with the HAP3 and HAP4 subunits into a regulatable transcriptional activating complex.

The results of carboxy-terminal mutagenesis of *lexA*-HAP2 are summarized in Figure 4B. Deletions extending to amino acid 218 (*lxΔC218*) did not significantly alter gross levels of activation, carbon source regulation, or HAP3 dependence at UAS2UP1 or the *lexA* operator. The region extending from amino acid 218 to 265 thus appears entirely dispensable for HAP2 function.

Further carboxy-terminal deletions (*lxΔC208* and *lxΔC197*) extending into the essential core of HAP2 are critical in that they indicate that HAP2 has distinct regions dedicated to UAS2 binding and subunit interac-

tion. These deletions abolished complementation and activation at UAS2 but did not affect carbon source regulation or HAP3 dependence at the *lexA* operator. We conclude that this region (amino acid 197–218) is essential for site-specific DNA binding but is not required for assembly with the HAP3 and HAP4 subunits to form a regulatable transcriptional activation complex. Further carboxy-terminal deletions (*lxΔC178* and *lxΔC167*) obliterated carbon source regulation and HAP3 dependence at the *lexA* operator. These mutants showed low constitutive levels of activation at the *lexA* site, consistent with the proposed function of the amino terminus of HAP2 as a transcription-augmenting domain.

Combined amino- and carboxy-terminal deletions of *lexA*–HAP2 were also examined for their activity at the *lexA* operator. As summarized in Figure 4C, two essential core-encompassing fusions, *lxΔN141ΔC218* and *lxΔN154ΔC218*, showed normal carbon source regulation and HAP3 dependence at both UAS2UP1 and the *lexA* operator. Furthermore, deletion *lxΔN154ΔC197*, which removes the region required for site-specific DNA binding, functioned normally at the *lexA* operator but not at UAS2, whereas deletion of additional sequences extending into the putative subunit recognition domain eliminated this activity (*lxΔN154ΔC178*). We conclude that amino acids 154–197 of HAP2 are both necessary and sufficient for association with HAP3 and HAP4.

To examine further the specificity of the proposed subunit association and DNA-contacting domains of the essential core, we constructed *lexA* fusion derivatives of several of the codon insertion mutants described above (Fig. 4C). Codon insertion mutants 13 (Y168/SRD) and 15 (G209/AGSR) occur in the subunit association and DNA-contacting domains, respectively. As would be expected for a mutant that specifically disrupts CCAAT binding, *lxc209* was inactive at UAS2 but showed normal carbon source regulation and HAP3 dependence at the *lexA* operator. In contrast, mutant *lxc168* was neither carbon source-regulated nor HAP3 dependent at the *lexA* operator, as would be expected for a mutant that disrupts assembly with the HAP3 and HAP4 subunits. Mutant *lxc180–184* (K180–K184/DP), which fully complements for growth on lactate, showed slightly compromised levels of activation at the *lexA* operator but was nonetheless HAP3-dependent, consistent with a slight impairment in the ability to assemble with HAP3 and HAP4 in solution. Other codon insertion mutations examined did not affect the behavior of *lexA*–HAP2.

We conclude that amino acids 154–197 are involved specifically in subunit association and amino acids 198–218 are involved specifically in DNA recognition. Amino acids 180–184 appear relatively dispensable for assembly with HAP3 and HAP4. These results are summarized in Figure 6, which presents a four-domain model for the structure of HAP2.

Roles of HAP3 and HAP4

It was critical to validate the view that HAP4 contains

the principal activation domain for the HAP2/3/4 complex. Accordingly, we constructed a bifunctional *lexA*–HAP4 fusion, containing the DNA-binding domain of the *lexA* repressor and codons 10–554 of HAP4. In contrast to the behavior of the *lexA*–HAP2 fusion at the *lexA* operator, *lexA*–HAP4 fusion produced from the ADH1 promoter was extremely active in promoting transcription in all carbon sources and under both heme-limiting and heme-sufficient conditions (Fig. 7A). Furthermore, levels of transcriptional activation actually increased slightly in the absence of HAP2 and/or HAP3. These data show that HAP4 encodes a strong transcriptional activation domain.

Is the sole role of HAP4 to bring the activation domain to the complex? Previous work suggested that HAP4 was required for sequence-specific DNA binding, because a gel shift complex from *hap4* mutant cells was not observed (Forsburg and Guarente 1989a). However, we do not believe that this binding assay is sensitive. We therefore employed a genetic scheme to examine whether HAP4 was absolutely required for UAS2-specific DNA binding in the complex. If the only role of the HAP4 subunit is to provide a transcriptional activation domain to the HAP2/3/4 heterotrimer, then we reasoned that the need for HAP4 could be bypassed by merely fusing an acidic transcriptional activation domain to one of the other two subunits.

As shown in Figure 7B, fusing the 129-amino-acid GAL4 acidic tail to the carboxyl terminus of HAP2 results in a partial bypass of the need for HAP4. The HAP2–GAL4 acid blob fusion supported partial growth on lactate in *hap4* null background. Thus, HAP2 and HAP3 alone will bind to the CCAAT sites in the yeast genome. The suboptimal level of complementation may be because HAP4 has a quantitative effect on binding of the HAP complex to its sites. Alternatively, it may be due to repression of the GAL4 activation domain by GAL80, by instability of the HAP2–GAL4 fusion protein, or an unfavorable effect of the acidic activation domain on site-specific DNA binding by HAP2.

Notably, the HAP2–GAL4 acid blob fusion did not eliminate the need for HAP3. This finding indicates that the role of HAP3 in the complex is not merely to serve as a connector between HAP2 and HAP4. Rather, the HAP3 subunit must be essential for site-specific DNA binding. Thus, the subunits of the HAP2/3/4 hetero-

Figure 6. Four-domain model for the structure of HAP2. The essential core of HAP2 (amino acids 154–218) is shown encompassing both subunit association (crosshatched) and DNA recognition (hatched) domains. The gap occurring in the subunit association domain is meant to represent the nonessential sequences (amino acids 180–184) occurring in this region (see codon insertion mutagenesis). The two nonessential domains—the amino-terminal transcription-augmenting and the carboxy-terminal-dispensable tail region—are also indicated.

A	Activator	Strain		Glucose	Galactose	Lactate	low	high
							HEME	HEME
1)	lexA-HAP4	WT		143	108	298	88	136
2)	lexA-HAP4	hap2-, hap3-		383	265	ng	90	204
3)	lexA-GAL4	WT		212	316	100	165	255
4)	lexA-GAL4	hap2-, hap3-		227	178	ng	127	221

growth on lactate

B	Δ hap2	Δ hap3	Δ hap4
+++	—	—
+++	—	+

Figure 7. Transcriptional activation by *lexA*-HAP4 and *lexA*-GAL4 at the *lexA* operator. (A) Numbers indicate the average of at least four independent determinations of units of β -galactosidase activity detected from the *lexA* operator-*lacZ* promoter fusion under the indicated carbon source and heme conditions. The putative configuration of activator proteins in each yeast strain is also indicated. For the sake of simplicity, a single *lexA*-fusion protein is shown binding to

the *lexA* operator (the *lexA* repressor actually binds its operator as a dimer), and a single copy of each HAP subunit in the complex is also assumed. HAP2 (black oval), HAP3 (open circle) and HAP4 (stippled form) are shown. Essentially identical results were obtained for *lexA*-HAP4 in either the *hap2/hap3* null strain (line 2) or individual *hap2* null and *hap3* null strains (data not shown). (B) A HAP2-GAL4 acidic domain fusion partially bypasses the need for HAP4. The structures of HAP2 and the HAP2-GAL4 fusion containing the acidic carboxy-terminus of GAL4 are shown schematically. Complementation for growth on lactate is shown in each of the three *hap* null strains. (+++) Fully wild-type levels of lactate growth; (+) slow but significant growth on lactate. (—) No detectable complementation of the lactate growth defect phenotype.

trimer can be crudely divided into DNA binding (HAP2 and HAP3) and transcriptional activation (HAP4) functions. These results are summarized in Figure 8, which presents a functional model for the HAP2/3/4 complex.

Discussion

In most cases of well-examined eukaryotic transcriptional activators, a single polypeptide has been shown to encode small discrete domains for both DNA binding and transcriptional activation. Increasingly, complex transcriptional activators consisting of two or more different polypeptides have been recognized and characterized. Currently, this class of regulatory proteins includes

yeast HAP2/3/4 and its apparent mammalian homolog CP1 (Olesen et al. 1987; Chodosh et al. 1988a,b), leucine zipper-containing proteins such as mammalian fos/jun and other AP-1-related complexes (for review, see Curran and Franza 1988), as well as the activating transcription factor (ATF) family of transcription factors (Hai et al. 1989). MyoD and other proteins containing the helix-loop-helix dimerization/DNA-binding motif (Murre et al. 1989a) have also been shown to form DNA-binding heterodimers (Murre et al. 1989b). The discovery of an ever-increasing number of complex transcriptional regulators involved in the control of gene expression in higher cells may have profound implications for understanding metazoan gene regulation through combina-

torial control mechanisms. A thorough understanding of the structure and function of this class of regulators not only requires a knowledge of the subunit protein domains involved in DNA binding and transcriptional activation but also the protein sequences involved in heteromeric protein–protein interactions. In this study, we begin a functional dissection of the HAP2/3/4 CCAAT-binding complex by investigating the general roles of the HAP2, HAP3, and HAP4 subunits in transcriptional activation and DNA binding. In addition, we begin a molecular dissection of the complex by localizing a region of HAP2 responsible for interaction with HAP3 and possibly HAP4, and a region of HAP2 specifically involved in DNA recognition.

Structure of HAP2

Using deletion mutagenesis and an *in vitro* DNA-binding assay, we demonstrated that amino acids 154–218 of HAP2 provide all of the necessary information for assembly of HAP2 into a CCAAT-binding complex. This region of HAP2 was necessary and sufficient for cooperative binding with both *S. cerevisiae* HAP3/HAP4 and with mammalian CP1A, the functional HeLa homolog of these yeast activities (Chodosh et al. 1988b). Deletions of HAP2 had identical effects on cooperative

Figure 8. Proposed structure of the HAP2/3/4 CCAAT-binding complex and division of site-specific DNA binding and transcriptional activation functions between the subunits. The model hypothetically assumes a single copy of each of the three subunits in the heterotrimeric complex. The detailed structure of HAP2 is shown, with its essential core (ovals) consisting of subunit association (crosshatched oval touching HAP3) and DNA recognition (hatched oval touching the DNA double helix) domains. The putative transcription-augmenting amino terminus is represented by a random wavy line connected to the subunit association domain via the polyglutamine tract (Q14). The carboxy-terminal dispensable tail is also indicated. HAP3 and HAP4 subunits are depicted as uncharacterized shapes. HAP3 is shown contacting the DNA, HAP2, and HAP4. In contrast, HAP4 is presumed not to contact the DNA and is shown making protein–protein contacts with HAP3 and, possibly, HAP2. The HAP4 acidic transcriptional activation domain is represented by the negative charges at the top of that subunit.

binding with yeast HAP3/HAP4 and HeLa CP1A, thus providing further evidence that the yeast and mammalian CCAAT-binding protein complexes have been conserved extensively in both structure and function throughout the period of evolutionary divergence between yeast and man. In other work (J. Olesen and L. Guarente, unpubl.), we present evolutionary evidence that the HAP2 core is a highly constrained protein structure that has changed in amino acid sequence very little since the divergence of budding yeast, fission yeast, and metazoan eukaryotic cell lineages. By use of codon insertion and deletion mutagenesis of HAP2, we have demonstrated that this core region of HAP2 is entirely sufficient for functional complementation *in vivo*. HAP2 residues 154–218 thus constitute an essential core domain of this protein.

The observation of carbon source-regulated, heme-dependent, and HAP3/HAP4-dependent activation by *lexA*–HAP2 tethered to a yeast promoter via the *lexA* operator provides an *in vivo* assay for the association of HAP2 with the other subunits in the complex. Using this assay, we localized a small region of the HAP2 protein that is both necessary and sufficient for association with HAP3 and HAP4. The subunit association domain is a subset of the essential core of HAP2, which encodes both subunit association and DNA-recognition activities. HAP2 residues 154–197 span the subunit association domain because they are sufficient for the assembly of the HAP2/3/4 complex in solution at the *lexA* operator. A carboxy-terminal deletion to residue 178 destroyed subunit association with HAP3/HAP4 (see Fig. 4B, *lxΔC178*, and Fig. 4C, *lxΔN154ΔC178*), whereas a mutation substituting codons 180–184 with two other codons resulted in only a slight impairment of subunit association as measured at the *lexA* operator. This suggests that residues 180–184 are somewhat dispensable and that either the precise spacing between residues 154 and 178 and 185 and 197 is also not critical for the function of the subunit association domain, or that the latter region is also dispensable and the minimal subunit association domain extends only to amino acid 179.

The remainder of the HAP2 essential core (amino acids 197–218) is clearly critical for site-specific DNA binding because it is required for activity at the UAS2UP1 CCAAT box. The clear implication from these results is that site-specific DNA binding is determined by these HAP2 residues, along with the other subunits. The DNA-binding domain of HAP2 thus constitutes the entire essential core structure, including the subunit association domain. This arrangement is similar to that occurring in the DNA-binding domain of FOS (Neuberg et al. 1989), Jun, and related leucine zipper and helix–loop–helix proteins. Indeed, the HAP2 subunit association and DNA-recognition domains apparently constitute novel, previously undescribed structures for heteromeric protein/protein interaction and site-specific DNA binding because they show no homology to previously described DNA-binding motifs, the leucine zipper (Landschulz et al. 1988), helix–loop–helix (Murre et al. 1989a), helix–turn–helix (Brennan and Matthews

1989), homeo domain (Scott et al. 1989), or zinc finger (Klug and Rhodes 1987).

Whereas residues 219–265 comprise a dispensable tail region of HAP2, the amino-terminal 154 residues appear to have subtle effects on transcriptional activation of the assembled complex assayed at the *lexA* operator. Gross levels of activation, but not the degree of carbon source regulation, at UAS2UP1 and the *lexA* operator diminished as sequences 16–154 were deleted (*lexΔN45–lexΔN154*, Fig. 4A). Significantly, however, deletion of the polyglutamine motif had no effect on transcriptional activation at the *lexA* operator or UAS2. The amino terminus of HAP2 on its own functioned as a weak constitutive transcriptional activator at the *lexA* operator (see Fig. 4B, *lexΔC178–lexΔC139*). In contrast, the dispensable tail region had no such activity (*lexΔN169–lexΔN255*, Fig. 4A). The calculated net charge of this putative transcriptional augmenting domain is -10 which, although not as densely acidic as previously identified activating regions (Ma and Ptashne 1987a), is consistent with a minor role in transcriptional activation of the assembled HAP2/3/4 complex.

Division of functions among the subunits

In addition to mapping subunit association and DNA-recognition domains of HAP2, we presented evidence for a division of function between the subunits of the heterotrimer. The nature of the HAP2/3/4 complex requires that all three subunits encode surfaces for protein–protein interaction between the subunits that allow assembly of the complex. In contrast, each of the remaining functions of a transcriptional activator, that is, site-specific DNA binding and transcriptional activation, need not be specified by all of the subunits of the complex. Indeed, the experiments presented here suggest that HAP2 and HAP3 appear to provide the specificity for CCAAT DNA binding, whereas HAP4 appears to provide the primary transcriptional activation surface to the HAP2/3/4 complex (Fig. 8). The evidence justifying this division of function among the subunits is as follows.

HAP4 encodes the principle transcriptional activation domain of the complex It has been demonstrated previously that HAP4 contains an essential and highly acidic protein domain at its carboxyl terminus (Forsburg and Guarente 1989a). This domain can be exchanged with the acidic activation domain of GAL4 without impairing the function of the CCAAT activation complex, suggesting that the HAP4 acidic sequence plays the same role in transcriptional activation as the acidic sequence of GAL4. We sought independent confirmation that the HAP4 subunit provides the principle transcriptional activation domain for the entire complex by assaying each of the subunits independently for its activation potential at the *lexA* operator. In the absence of HAP4 neither *lexA*–HAP2 nor *lexA*–HAP3 (not shown) is capable of activating transcription at high levels through the *lexA* operator. This suggests that strong ac-

tivation surfaces do not appear on either HAP2 or HAP3. In contrast to *lexA*–HAP2 and *lexA*–HAP3 fusions, *lexA*–HAP4 conferred very strong carbon source and heme-independent transcriptional activation on the *lexA* operator-driven promoter. Furthermore, the HAP2 and HAP3 subunits were not required for transcriptional activation by *lexA*–HAP4.

A different approach also indicated that HAP4 brings the activation domain to the complex. Fusion of the acidic activation domain of GAL4 to the carboxyl end of HAP2 partially bypassed a *hap4* deletion mutation. This result shows that HAP4 is not absolutely required for DNA site recognition by the complex.

HAP2 and HAP3 encode the CCAAT DNA-binding specificity The HAP2–GAL4 fusion that partially bypassed the need for HAP4 clearly did not bypass the need for HAP3. Because HAP3 is not needed for the nuclear localization of HAP2 (Pinkham et al. 1987), this result strongly suggests that HAP3 is required for HAP2 to recognize the CCAAT box sequence. Furthermore, a protein fusion of HAP2 to HAP4, while fully complementing both *hap2* and *hap4* null mutations, does not bypass the need for HAP3, as might be expected if HAP3 functioned only to link the two subunits together. (J.T. Olesen and L. Guarente, unpubl.). An additional argument for a role of HAP3 in site-specific DNA binding is the UAS2 element itself is bipartite and asymmetric (Forsburg and Guarente 1988). Binding to the asymmetric UAS2 might involve an asymmetric arrangement of dissimilar subunits (i.e., HAP2 and HAP3) of the complex.

Regulation by the HAP2/3/4 complex

The static model for the HAP2/3/4 complex does not explain how carbon source and heme regulation of UAS2 occurs. In the case of another complex transcriptional activator, mammalian Fos–Jun heterodimer, transcriptional regulation through the Fos–Jun-binding site (TRE) occurs in response to serum stimulation via changes in the amount of Fos protein synthesized.

The data presented here, in combination with existing evidence, suggests that transcriptional regulation through the UAS2UP1 CCAAT box by HAP2/3/4 occurs via a similar modulation of the amount of assembled heteromeric complex. In this case, it is the HAP4 subunit that appears to play the rate-limiting role served by Fos in the Fos–Jun complex. The evidence for this is as follows. First, measurable DNA-binding activity by HAP2/3/4 requires the presence of all three subunits of the complex and is strongly induced by carbon source (Olesen et al. 1987; Forsburg and Guarente 1989a). Furthermore, both the HAP3 and HAP4 subunits are required for induction by *lexA*–HAP2 at the *lexA* operator. Together, these facts suggest that induction of the HAP2/3/4 DNA-binding activity occurs by assembly of the HAP2/3/4 complex in solution. Second, evidence that the HAP4 subunit is synthesized in limiting amounts under repressing conditions comes from anal-

ysis of *HAP4* gene expression (Forsburg and Guarente 1989a). Northern analysis reveals that levels of *HAP4* mRNA increase fivefold in a shift from glucose to lactate and similar effects are observed for β -galactosidase levels from a *HAP4-lacZ* fusion under control of the *HAP4* promoter. Future analysis of the *HAP4* promoter may reveal a control element, analogous to the *c-fos* SRE, which is responsive to carbon source growth conditions.

It is interesting to note that for both the Fos–Jun (Lech et al. 1988) and the proposed *HAP2/3/4* regulation scheme, the limiting subunit possesses a potent transcriptional activation domain. Similar strong transcriptional activators have been shown to have an adverse effect on RNA polymerase II-dependent mRNA synthesis when present in high concentrations (for review, see Ptashne 1988). Limiting the synthesis of the most potent transcriptional activating subunit in a heteromeric complex avoids this problem while, at the same time, providing a straightforward mechanism for controlling activation from the heteromeric complex.

Further analysis of the structure and function of the *HAP2/3/4* complex will provide important insights into the nature of the subunit association and DNA-recognition motifs utilized by this heteromeric transcriptional regulator. In addition, analysis of the *HAP2/3/4* regulatory network will aid our understanding of the elaborate combinatorial control mechanisms affecting eukaryotic gene regulation.

Materials and methods

Strains and media

The *S. cerevisiae* strain BWG 1-7a (*MAT α leu2-3, 112 his4-519 ade1-100 ura3-52*; Guarente and Mason 1983) or an isogenic derivative was used in all experiments described. *HAP2* complementation was assessed by using strain JO1-1a, which contains a chromosomal deletion of the *HAP2* gene obtained by the two-step gene transplacement technique (Rothstein 1983). The *hap3-1* and *hap3 : HIS4* null strain SHY40 have been described (Hahn et al. 1988). The strain JO2-1 is an isogenic *hap2, hap3 : HIS4* double null strain derived from JO1-1a by the disruption of the *HAP3* gene, as described previously (Hahn et al. 1988). The *hap4-2* and *hap4 : LEU2* null strain SLF401 have also been described previously (Forsburg and Guarente 1989a). The *hem1* null strain MH1-4c (Haldi and Guarente 1989) was used in the heme induction experiments.

Rich media and synthetic minimal media were supplemented with 2% glucose, 2% galactose, or 2% lactate, as described by Sherman et al. (1986). Low heme synthetic media contained 2% galactose and 0.1 μ g/ml δ -ala heme biosynthetic precursor, whereas high heme synthetic media contained 2% galactose and 50 μ g/ml of δ -ala.

Plasmids

Vectors pRB1155 (Brent and Ptashne 1985), pJP300 (Pinkham et al. 1987), and pLG265 [UAS2(CCAAC)–*lacZ*; Guarente et al. 1984] and pLG265UP1(CCAAT)–*lacZ*; Guarente et al. 1984] have been described.

β -Galactosidase assays

The appropriate isogenic yeast strain was transformed with the indicated *lacZ* reporter (2 μ , *URA3*⁺) and *lexA* fusion effector plasmids (2 μ , *LEU2*⁺) by sequential transformation, using the lithium acetate protocol (Sherman et al. 1986). Transformants were grown selectively in minimal media (salts plus glucose/uracil/leucine) and assayed for β -galactosidase activity as described previously (Guarente and Mason 1983). Values reported were normalized to a control and represent the average of not less than four independent determinations.

Codon insertion mutagenesis

DNA isolation and manipulation techniques were, in general, as described in Sambrook et al. (1989). *HAP2* codon insertion mutants were constructed by use of vector pSP64 : *HAP2*, which contains a 1.4-kb *MnII*–*EcoRI* fragment encompassing the entire *HAP2* ORF, as well as 22 bp 5' and 600 bp 3' cloned into the *SmaI*–*EcoRI* backbone of pSP64 (Promega Biotech). pSP64 : *HAP2* was linearized by total or partial digestion with the following enzymes for the indicated codon insertion construct: *BglII* (1), *StyI* (2), *MboII* (3), *HinfI* (4), *StyI* (5), *AccI* (6 and 7), *PstI* (8), *Fnu4HI* (9 and 10), *RsaI* (11), *NlaIV* (12), *ScaI* (13), *AluI* (14), *MboII* (15), *DdeI* (16), *NheI* (17), *SphI* (18), *PvuII* (19), and *PstI* (20). Full-length linearized vectors were gel-purified by standard techniques in the case of constructs utilizing partial digests before further manipulation to assure cutting at only one site in the plasmid. Linearized ends were rendered blunt with Klenow fragment (Boehringer Mannheim), resulting in a net insertion at 5' ends (1 bp, mutants 9 and 10; 2 bp, mutants 6 and 7; 3 bp, mutants 4 and 16; 4 bp, mutants 1, 2, 5, and 17) and a net deletion at 3' ends (1 bp, mutants 3 and 15; 4 bp, mutants 8, 18, and 20). Mutant 7 was constructed by chewing back the 2-base 5' *AccI* site overhang with mung bean nuclease. Adjacent *AluI* sites resulted in a net deletion of 17 bp in construct 14. The remaining codon insertion mutants were at blunt sites and did not remove *HAP2* sequence. Standard phosphorylated linkers (New England Biolabs, NEB) were ligated to the blunted ends, and the plasmid was recircularized with T7 DNA ligase. Linker size was adjusted to retain the reading frame of *HAP2* across the insertion site as follows: *SmaI* 6-mer (mutants 11, 12, 13, 14, and 19), *BamHI* 8-mer (mutants, 1, 2, 5, 7, 9, 10, and 17), *BamHI* 10-mer (mutants 3, 6, 8, 15, 18, and 20), or no linker (mutants 4 and 16). The resulting pSP64 : *HAP2* codon insertion constructs were linearized with the linker site enzyme, religated, and retransformed to ensure that only a single linker remained at the insertion site. The sequence changes resulting from these manipulations are listed in Figure 3. These codon insertion mutants were reconstructed from the 1.35-kb *BglII*–*EcoRI* *HAP2* mutant fragment and the *BglII*–*EcoRI* backbone of the ars-cen yeast vector pJP103 (Pinkham and Guarente 1985) for analysis of *hap2* complementation. The sequences of the reconstructed mutants were confirmed by restriction analysis, and, in the case of all codon insertion mutants yielding a *hap2* phenotype and a majority of those yielding a *HAP2*⁺ phenotype, by M13 DNA sequencing using the Sanger method and commercial reagents (Sequensase).

HAP2 DNA-binding analysis

Analysis of in vitro DNA binding by *HAP2* has performed as follows. The vector pSP64 : *HAP2* (described above) was linearized with *EcoRI*, and synthetic *HAP2* RNA was prepared with SP6 RNA polymerase and reagents supplied by Promega Biotech under the conditions recommended for the production of

large amounts of uncapped and unlabeled RNA. Control RNA was prepared from linearized control pSP64 templates supplied by the manufacturer. The resulting RNA was extracted twice with phenol and ethanol precipitated with 2 M Na acetate. The RNA was translated *in vitro* by use of a commercially available rabbit reticulocyte lysate (Promega Biotech) under the reaction conditions recommended for the preparation of large amounts of unlabeled protein. The mock translation reactions were prepared by use of the control RNA described above. The use of control RNA translation products or translations in the absence of exogenous synthetic RNA produced the same results as with this mock translation. Small amounts of these reactions were translated in the presence of [³⁵S]methionine (Amersham), and the resulting radiolabeled proteins were analyzed for size in an SDS-PAGE gel basically as described in Pfeifer et al. (1989). DNA binding was assessed either in the absence of HAP2/HAP3 activities, or in the presence of 10 µg of HeLa fraction AB containing the CP1A activity (Chodosh et al. 1988b) or yeast HAP2/HAP3 activities supplied from 30 µg of crude whole-cell extract of yeast strain JO1-1a (*Δhap2*) prepared as described previously (Olesen et al. 1987). Preparation of UAS2 and UAS2UP1 probes, as well as binding conditions and native polyacrylamide gels for the resolution of protein–DNA complexes, were as described previously (Olesen et al. 1987; Chodosh et al. 1988b).

Amino- and carboxy-terminal deletions of HAP2 were generated by using the above protocol on deleted derivatives of the pSP64 : HAP2 template prepared as follows. ΔN43 is a *Bgl*III–*Bam*HI deletion of pSP64 : HAP2, which makes HAP2 codon 43 the first ATG downstream of the SP6 promoter. ΔN83 is a *Sty*I–*Sty*I in-frame deletion of ΔN43 that deletes amino acids 45–82 and leaves codons 43 and 44 (M,P) at the amino terminus of the ORF. ΔN92 is a *Bgl*III–*Bam*HI deletion of codon insertion 7, which leaves the first 16 amino acids of HAP2 plus a P codon fused to codon 92. ΔN107 was obtained by ligation of blunted *Bgl*III and *Spe*I sites of pSP64 : HAP2, which leaves the first 16 amino acids plus a single codon (P) fused to codon 107. ΔN141 is a blunted *Bgl*III–*Sma*I deletion of codon insertion 11, which leaves the first 16 amino acids plus two codons (R,D) fused to HAP2 codon 107. ΔN154 is a *Bgl*III–*Bam*HI deletion of a derivative of codon insertion 12, which contains a *Bam*HI 8-mer linker at the introduced *Sma*I site (see above). ΔN154 thus encodes the first 16 amino acids of HAP2 plus two codons (P,G) fused to residue 154. ΔN169 is a *Bgl*III (blunted)–*Sma*I deletion of 14, which leaves the first 16 amino acids plus two codons (R,D) fused to HAP2 codon 169. Most of the carboxy-terminal deletion mutants were generated from pSP64 : HAP2 truncated at a restriction site internal to the HAP2 ORF as follows: ΔC258 (*Pvu*II), ΔC230 (*Nhe*I), ΔN154ΔC218 (*Dde*I), ΔC167 (*Sca*I), ΔC110 (*Pst*I). ΔC197 was created by Klenow fill-in of a *Hin*FI site in pSP64 : HAP2, which creates an ochre stop codon after residue 197. Double deletions were created by truncation of ΔN154 as follows: ΔC258 (*Pvu*II), ΔC252 (*Sph*I, ΔN154 ΔC230 (*Nhe*I), ΔC218 (*Dde*I), ΔN154ΔC208 (*Mbo*II), and ΔN154ΔC197 (*Hin*FI). Constructs were checked for the synthesis of the expected size protein as described above, and the sequence of all templates testing negative in the DNA-binding assay as well as most templates testing positive was confirmed by M13 sequencing.

lexA–HAP2 deletion constructions

pJP300 has been described (Pinkham et al. 1987). Deletions of HAP2 fused to the first 87 codons of *lexA* (plasmids pJO301–pJO331) were constructed via the intermediate pSP64 : *lexA*–HAP2, which contains the 1.7-kb *Hind*III–*Hind*III *lexA*–HAP2 fragment from pJP300 cloned into the *Hind*III site of pSP64RI⁺.

pSP64RI⁺ was derived from pSP64 by restriction with *Eco*RI, Klenow fill-in, and religation to destroy the *Eco*RI site. Amino-terminal deletions were reconstructed by ligation of the appropriate size *Bam*HI linker (NEB) onto Klenow-blunted ends of pSP64 : HAP2 linearized with the indicated restriction enzyme as described below, for the given construct: *Sty*I + *Bam*HI 12-mer (ΔN45), *Sty*I + *Bam*HI 12-mer (ΔN83), *Acc*I [blunted with mung bean nuclease (NEB)] + *Bam*HI 8-mer (ΔN92), *Spe*I + *Bam*HI 10-mer (ΔN107), *Sca*I + *Bam*HI 10-mer (ΔN169), *Alu*I + *Bam*HI 8-mer (ΔN185), *Nhe*I + *Bam*HI 10-mer (ΔN230), and *Sph*I + *Bam*HI 10-mer (ΔN255). ΔN141 and ΔN154 were constructed from codon insertion mutants 11 and 12 by adding a *Bam*HI 10-mer and a *Bam*HI 8-mer at the *Sma*I insertion site, respectively. The resulting *Bam*HI–*Eco*RIΔHAP2 restriction fragment was reconstructed as a *lexA* fusion by ligation into the *Bgl*III–*Eco*RI (partial) backbone of pSP64 : *lexA*–HAP2. The *lexA*–ΔHAP2 fusion was released from this intermediate construct on a *Hind*III–*Hind*III fragment and inserted into the *Hind*III site of pAAH5 (Ammerer 1983) for expression in yeast. Insert orientation was screened to ensure transcription of the *lexA*–ΔHAP2 construct by the ADHI promoter. Codons introduced at the *lexA*/ΔHAP2 fusion junction by added linker sequences are as follows: (P,R) added in ΔN45 and ΔN83; (P) added in ΔN92, ΔN185, and ΔN230; (P,A) added in ΔN107 and ΔN255; (P,G,D) added in ΔN141; (P,G) added in ΔN154; and (P,D) added in ΔN169.

Carboxy-terminal deletions of *lexA*–HAP2 were constructed in a similar manner by the addition of an *Xba*I 14-mer linker (NEB, 1062), which encodes a TAG nonsense codon in all three reading frames, at appropriate Klenow-blunted restriction sites of pSP64 : HAP2 as follows: *Pvu*II (ΔC258), *Nhe*I (ΔC230), *Dde*I (ΔC218), *Mbo*II (ΔC208), *Alu*I (ΔC178), *Sca*I (ΔC167), *Nla*IV (ΔC153), *Rsa*I (ΔC139), *Acc*I (ΔC91), and *Sty*I (ΔC45). These plasmids were also cut and blunted at the downstream *Nde*I site so that all HAP2-coding sequence downstream of the carboxy-terminal deletion endpoint was removed, except in the case of ΔC197, which was constructed by Klenow fill-in of a *Hin*FI site, as described above for ΔC197. Reconstruction of these carboxy-terminal deletions as *lexA* fusions was described above except that a *Bgl*III–*Eco*RI fragment encompassing the *lexA*–HAP2ΔC was used. Codons introduced at the carboxyl terminus of these *lexA*–HAP2ΔC constructs by the addition of the *Xba*I stop codon linker are as follows: (A,S,L,D) added in ΔC258, ΔC208, and ΔC178; (S) added in ΔC230; (D) added in ΔC218; (S,S,L,D) added in ΔC167 and ΔC139; and (L,V) added in ΔC153, ΔC91, and ΔC45.

Codon insertion mutations of *lexA*–HAP2 were constructed from the appropriate codon insertion pSP64 : HAP2 derivatives described above. Double deletions (amino- and carboxy-terminal) of *lexA*–HAP2 were derivatives of ΔN141 and ΔN154 obtained by adding the *Xba*I 14-mer stop codon linker at the same restriction sites as described for the corresponding single carboxy-terminal deletion constructs except in the case of ΔN154ΔC197, which was obtained from ΔN154 by Klenow fill-in of a *Hin*FI site and addition of the *Xba*I 14-mer linker. Codons introduced by added linker sequences at the *lexA*/ΔHAP2 fusion and carboxyl terminus of double deletion constructs are as described above for the corresponding single deletion mutations except ΔN154ΔC197 in which the amino acid sequence (S,S,L,D) is added at the carboxyl terminus.

Construction of *lexA*–HAP4

Fusion of *lexA* codons 1–87 to HAP4 codons 10–554 was

achieved by ligating a *Bam*HI 10-mer linker (NEB) to a *Stu*I digest of pSLF406 (Forsburg and Guarente 1989a). Following digestion with *Sac*I, blunting with Klenow, and restriction with *Bam*HI, a 2-kb *Bam*HI–*Sac*I fragment carrying all but the first 9 codons of the *HAP4* ORF was isolated and reconstructed into the *Bgl*II–*Eco*RI backbone of intermediate construct pSP64 : *lexA*–*HAP2*, as described above for the carboxy-terminal deletions of *lexA*–*HAP2*. The *lexA*–*HAP4* fusion was released on a *Hind*III–*Hind*III fragment and reconstructed into yeast vector pAAH5 as described above for the *lexA*–*HAP2* deletion constructs. The codon (P) is added at the *lexA*–*HAP4* fusion junction by linker sequence.

Construction of *HAP2*–*GAL4* acidic domain fusion

Fusion of the carboxy-terminal 129 amino acids of *GAL4*–*HAP2* codon 258 was achieved as follows. A *Pvu*II–*Pvu*II fragment, carrying *GAL4* codons 753–881, was isolated from plasmid pRB1027 (Brent and Ptashne 1985). *Bam*HI 12-mer linkers were ligated onto the ends and restricted with *Bam*HI. The resulting fragment was cloned into the *Bam*HI site at the *HAP2*/*lacZ* fusion junction of pJP258 (Pinkham et al. 1987). Introduced linker sequence creates the codons (A,R,S,A) between *HAP2* amino acid 258 and *GAL4* amino acid 753. Single inserts in each orientation were obtained, and these constructs were screened for complementation in *hap2*, *hap3*, and *hap4* null strains (described above). Only constructs in which the *GAL4* acidic activation domain was fused to *HAP2* resulted in a partial complementation in the *hap4* null strain.

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References

- Ammerer, G. 1983. Expression of genes in yeast using the *ADC1* promoter. *Methods Enzymol.* **101**: 192–201.
- Barany, F. 1985. Two-codon insertion mutagenesis of plasmid genes by using single-stranded hexameric oligonucleotides. *Proc. Natl. Acad. Sci.* **82**: 4202–4206.
- Berg, J.M. 1989. DNA binding specificity of steroid receptors. *Cell* **57**: 1065–1068.
- Brennan, R.G. and B.W. Matthews. 1989. The helix-turn-helix DNA binding motif. *J. Biol. Chem.* **264**: 1903–1906.
- Brent, R. and M. Ptashne. 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. *Cell* **43**: 729–736.
- Chodosh, L., A. Baldwin, R. Carthew, and P. Sharp. 1988a. Human CCAAT-binding protein have heterologous subunits. *Cell* **53**: 11–24.
- Chodosh, L., J. Olesen, S. Hahn, A. Baldwin, L. Guarente, and P. Sharp. 1988b. A yeast and a human CCAAT-binding protein have heterologous subunits that are functionally interchangeable. *Cell* **53**: 25–35.
- Courey, A.J. and R. Tjian. 1988. Analysis of SP1 in vivo reveals multiple transcriptional domains, including a novel glutamine rich activation motif. *Cell* **55**: 887–898.
- Curran, T. and B.R. Franza. 1988. Fos and jun: The AP-1 connection. *Cell* **55**: 395–397.
- Denis, C.L., J. Ferguson, and E.T. Young. 1983. mRNA levels for the fermentative alcohol dehydrogenase of *Saccharomyces cerevisiae* decrease upon growth on a nonfermentable carbon source. *J. Biol. Chem.* **258**: 1165–1171.
- Forsburg, S.L. and L. Guarente. 1988. Mutational analysis of upstream activation sequence 2 of the *CYC1* gene of *Saccharomyces cerevisiae*: A *HAP2*–*HAP3*-responsive site. *Mol. Cell Biol.* **8**: 647–654.
- . 1989a. Identification and characterization of *HAP4*: A third component of the CCAAT-bound *HAP2*/*HAP3* heteromer. *Genes Dev.* **3**: 1166–1178.
- . 1989b. Communication between mitochondria and the nucleus in regulation of cytochrome genes in the yeast *Saccharomyces cerevisiae*. *Annu. Rev. Cell Biol.* **5**: 153–180.
- Guarente, L. and T. Mason. 1983. Heme regulates transcription of the *CYC1* gene of *S. cerevisiae* via an upstream activation site. *Cell* **32**: 1279–1286.
- Guarente, L., B. Lalonde, P. Gifford, and E. Alani. 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene of *S. cerevisiae*. *Cell* **36**: 503–511.
- Hahn, S. and L. Guarente. 1988. Yeast *HAP2* and *HAP3*: Transcriptional activators in a heteromeric complex. *Science* **240**: 317–321.
- Hahn, S., J. Pinkham, R. Wei, R. Miller, and L. Guarente. 1988. The *HAP3* regulatory locus of *Saccharomyces cerevisiae* encodes divergent overlapping transcripts. *Mol. Cell Biol.* **8**: 655–663.
- Hai, T., F. Liu, W.J. Coukos, and M.R. Green. 1989. Transcription factor ATF cDNA clones: An extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes Dev.* **3**: 2083–2090.
- Haldi, M. and L. Guarente. 1989. N-terminal deletions of a mitochondrial signal sequence in yeast. *J. Biol. Chem.* **264**: 17107–17112.
- Hope, I.A. and K. Struhl. 1986. Functional dissection of eukaryotic transcriptional activator protein, GCN4 of yeast. *Cell* **46**: 885–894.
- Keng, T. and L. Guarente. 1987. Constitutive expression of the yeast *HEM1* gene is actually a composite of activation and repression. *Proc. Natl. Acad. Sci.* **84**: 9113–9117.
- Klug, A. and D. Rhodes. 1987. Zinc fingers: A novel protein motif for nucleic acid recognition. *Trends Biochem. Sci.* **12**: 464–469.
- Kouzarides, T. and E. Ziff. 1989. Leucine zippers of *fos*, *jun* and GCN4 dictate dimerization specificity and thereby control DNA binding. *Nature* **340**: 568–571.
- Landschulz, W.H., P.F. Johnson, and S.L. McKnight. 1988. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. *Science* **240**: 1759–1764.
- Lassar, A.B., J.N. Buskin, D. Lockshon, R.L. Davis, S. Apone, S.K. Hauschka, and H. Weintraub. 1989. MyoD is a sequence-specific DNA binding protein requiring a region of *myc* homology to bind to the muscle creatine kinase enhancer. *Cell* **58**: 823–831.
- Lech, K., K. Anderson, and R. Brent. 1988. DNA-bound Fos proteins activate transcription in yeast. *Cell* **52**: 179–184.
- Leszczynski, J.F. and G.D. Rose. 1986. Loops in globular proteins: A novel class of protein secondary structure. *Science* **234**: 849–855.
- Ma, J. and M. Ptashne. 1987a. Deletion analysis of *GAL4* defines two transcriptional activating sequences. *Cell* **48**: 847–853.
- Mermod, N., E.A. O'Neill, T.J. Kelly, and R. Tjian. 1989. The

- proline-rich transcriptional activator of CTF/NF-1 is distinct from the replication and DNA binding domain. *Cell* **58**: 741–753.
- Murre, C., P.S. McCaw, and D. Baltimore. 1989a. A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell* **56**: 777–783.
- Murre, C., P.S. McCaw, H. Vaessin, M. Caudy, L.Y. Jan, Y.N. Jan, C.V. Cabrera, J.N. Buskin, S.E. Hauschka, A.B. Lassar, H. Weintraub, and D. Baltimore. 1989b. Interactions between heterologous helix-loop-helix proteins generate complexes that bind specifically to a common DNA sequence. *Cell* **58**: 537–544.
- Neuberg, M., M. Schuermann, J.B. Hunter, and R. Muller. 1989. Two functionally different regions in Fos are required for the sequence-specific DNA interaction of the Fos/Jun protein complex. *Nature* **338**: 589–590.
- Olesen, J.T., S. Hahn, and L. Guarente. 1987. Yeast HAP2 and HAP3 activators both bind to the *CYC1* upstream activation site UAS2 in an interdependent manner. *Cell* **51**: 953–961.
- Pabo, C.O. and R.T. Sauer. 1984. Protein DNA recognition. *Annu. Rev. Biochem.* **53**: 293–321.
- Pfeifer, K., K.S. Kim, S. Kogan, and L. Guarente. 1989. Functional dissection and sequence of yeast transcriptional activator HAP1. *Cell* **56**: 291–301.
- Pinkham, J.L. and L. Guarente. 1985. Cloning and molecular analysis of the *HAP2* locus: A global regulator of respiratory genes in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **5**: 3410–3416.
- Pinkham, J.L., J.T. Olesen, and L. Guarente. 1987. Sequence and nuclear localization of the *Saccharomyces cerevisiae* HAP2 protein, a transcriptional activator. *Mol. Cell Biol.* **7**: 578–585.
- Ptashne, M. 1988. How eukaryotic transcriptional activators work. *Nature* **335**: 683–689.
- Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101**: 202–245.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Schneider, J.C. 1989. "Coordinate control of cytochrome genes." Ph.D. thesis. M.I.T., Cambridge, MA.
- Scott, M.P., J.W. Tamkun, and G.W. Hartzell III. 1989. The structure and function of the homeodomain. *Biochim. Biophys. Acta* **989**: 25–48.
- Sherman, R., G.R. Fink, and J.B. Hicks. 1986. In *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Wharton, K.A., B. Yedvobnick, V.G. Finnerty, and S. Avtavanis-Tsakonas. 1985. *opa*: A novel family of transcribed repeats shared by the notch locus and other developmentally regulated loci in *D. melanogaster*. *Cell* **40**: 55–62.
- Wiederrecht, G., D. Seto, and C.S. Parker. 1988. Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. *Cell* **54**: 841–853.

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