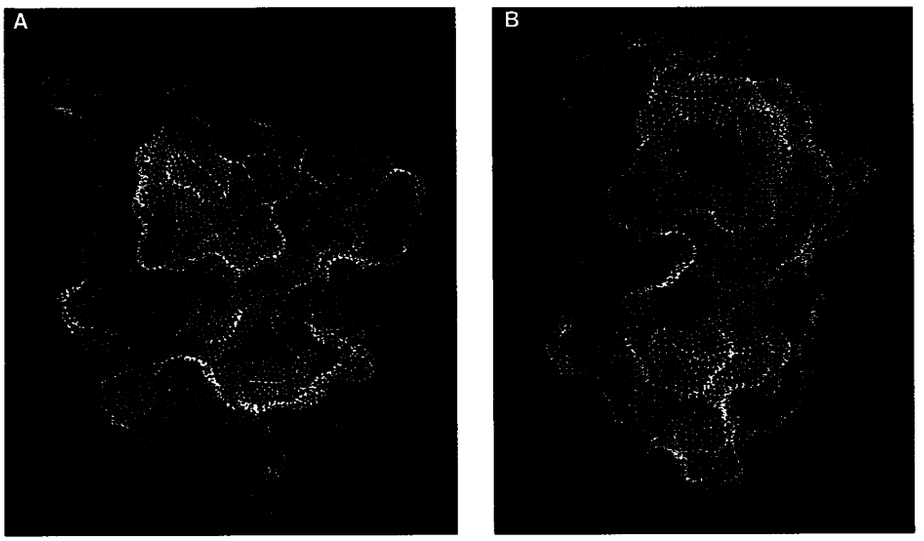

Fig. 4. Electrostatic potential at the surface of (**A**) the x-ray structure of poplar Pc and (**B**) one of the NMR structures of *S. obliquus* Pc. Note the distinctive shape of the acidic region of the poplar Pc. Surfaces were calculated with the algorithm of Connolly (*27*) with a 1.4 Å probe radius. The electrostatic potential was calculated by the method of Getzoff *et al.* (*28*) with a distance-dependent dielectric constant $\epsilon = \epsilon_0 r$, $\epsilon_0 = 4.0$ Å^{-1}. The surfaces are color-coded according to electrostatic potential: red, $V < -11$ kcal/mol; yellow, -11 kcal/mol $< V < -5$ kcal/mol; green, -5 kcal/mol $< V <$ +4 kcal/mol; blue, $V > +4$ kcal/mol.

reagents (*23*).

The acidic side chains of residues 42 to 44, 59, and 60 of higher plant plastocyanins define a highly distinctive negatively charged surface (Fig. 4A) that functions as a binding site for cationic electron transfer reagents (*4*) and cytochromes c and f (*5, 6*). The acidic residues at positions 42 to 44 are conserved in *S. obliquus* Pc and, together with Asp[53], Asp[58], and Glu[83], form a delocalized region of negative electrostatic potential. However, the deletion of residues 57 and 58 and the substitution of Glu[59] of poplar Pc by His (His[57] in the *S. obliquus* sequence) substantially alters both the shape and charge of the acidic patch in *S. obliquus* Pc (Fig. 4B). The most notable change is the elimination as a result of the deletions of a pronounced negative protrusion from the surface of the poplar Pc. Most of the acidic residues that form the negative patch are replaced by neutral or positive side chains in the Pc from the blue-green alga, *A. variabilis*, for which there is no observable binding of cationic electron transfer complexes (*26*). Thus it appears that during evolution from the blue-green algae through the green algae to higher plants, the acidic patch of Pc has become progressively a distinctive structural feature that probably plays an important role in recognition and binding of the physiological electron transfer partner, cytochrome f.

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Yeast HAP2 and HAP3: Transcriptional Activators in a Heteromeric Complex

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Transcription of the yeast *CYC1* gene (iso-1-cytochrome c) is regulated in part by the upstream activation site UAS2. Activity of UAS2 requires both the HAP2 and HAP3 activators, which bind to UAS2 in an interdependent manner. To distinguish whether these factors bound to UAS2 cooperatively or formed a complex in the absence of DNA, HAP2 and HAP3 were tagged by gene fusion to LexA and β-galactosidase, respectively, and purified through four chromatographic steps. The copurification of LexA-HAP2, HAP3 β-galactosidase, and UAS2 binding activity shows that HAP2 and HAP3 associate in the absence of DNA to form a multisubunit activation complex.

IN NUMEROUS BIOLOGICAL SYSTEMS, enzymes with catalytic functions have been found to exist as complexes bearing nonidentical subunits. For example, enzymes such as aspartate transcarbamoylase contain nonidentical subunits that separate their catalytic and regulatory functions (*1*). In macromolecular synthesis, enzymes that carry out catalytic functions have been found to exist as large complexes (DNA and RNA polymerases, splicosomes, and ribosomes). In contrast, transcriptional activators or repressors are usually not found as multisubunit complexes, but rather, as multimers (dimers or tetramers) with identical subunits (*2*). In prokaryotes, this oligomeric structure, which occurs, for example, in the

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lac repressor, enables the oligomer to bind to a DNA sequence that is rotationally symmetrical.

Transcription of the yeast *CYC1* gene encoding iso-1-cytochrome c is activated by two adjacent upstream activation sites, UAS1 and UAS2 (*3*). Activation of UAS2 is highly regulated by catabolite repression and is repressed about 30-fold by a shift of cells from lactate media to glucose (*3*). The activity of UAS2 depends on both the *HAP2* and *HAP3* genes (*3–5*). Mutations in *HAP2* or *HAP3* have identical phenotypes: the inability to activate UAS2 and a global defect in the expression of genes involved in respiratory metabolism. Wild-type UAS2 contains the sequence TGGTTGGT. A transition from G to A in UAS2 (termed UAS2-UP1) results in the conversion of this sequence to TGATTGGT, which matches that found in three other *HAP2,3*-regulated UASs, and the activity of UAS2 is increased tenfold in vivo (*3, 6*). Linker scanning analysis of UAS2 has confirmed that the consensus element is critical for activity of the site (*7*). This element is homologous to the CCAAT box of higher cells. The bases contacted in UAS2UP1 by HAP2-HAP3, as determined by methylation interference footprinting (*8*), are similar to those made by CCAAT box binding factors from higher cells (*9*).

Why are both *HAP2* and *HAP3* required to activate UAS2? We have begun a biochemical analysis of proteins that bind to UAS2 in vitro using crudely fractionated yeast extracts (*8*). By a gel-retardation assay, a UAS2-specific protein-DNA complex, complex C, is seen only in extracts of cells grown on nonfermentable carbon sources. The formation of complex C is greatly stimulated by the UAS2UP1 mutation. Strains containing bifunctional fusions of HAP3 β-galactosidase, HAP2 β-galactosidase, or LexA-HAP2 show altered mobility of complex C, indicating that complex C contains both the HAP2 and HAP3 proteins. A second complex, A, binds to sequences downstream from the consensus element (*7, 8*) and contains factors other than HAP2 or HAP3.

Neither HAP2 nor HAP3 binds to UAS2UP1 in the absence of the other HAP protein (*8*). This result suggests two alternative models for the interdependence of HAP2-HAP3 binding. In the first model (Fig. 1A), HAP2 and HAP3 do not associate in solution but bind cooperatively to adjacent sites at UAS2. Such a model of cooperative binding has been proposed from biochemical data for matα1 and a factor termed PRTF in activation of α-specific genes in yeast (*10*). In the second model (Fig. 1B), HAP2 and HAP3 form a multisubunit protein complex in the absence of DNA, and this complex binds to UAS2. This model has been proposed on the basis of genetic data for α2 and **a**1 in repression of haplo-specific genes in yeast (*11*). Experiments described below distinguish between these two alternative models for HAP2 and HAP3.

If HAP2 and HAP3 were part of a multisubunit transcriptional activation complex, we reasoned that the two proteins would copurify in the absence of UAS2 DNA. Our initial assay for copurification was the ability to bind UAS2UP1, since neither HAP2 nor HAP3 protein alone can detectably bind (*8*). To monitor recovery of HAP3 protein during purification, we used a strain containing a bifunctional HAP3 β-galactosidase fusion (*5*), which overproduced by a factor of 500 as compared to the wild-type level of HAP3. HAP2 was synthesized from the wild-type chromosomal gene and was probably much less abundant than HAP3 β-galactosidase (*4*). Thus, we expected that most of the HAP3 β-galactosidase in this strain would be inactive for DNA binding, because the supply of HAP2 would be limiting.

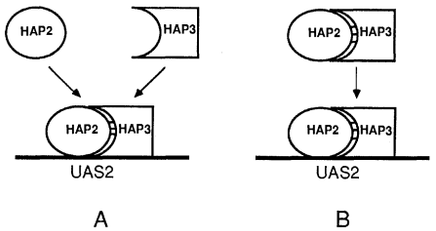

Fig. 1. Two alternative models for the interdependence of HAP2 and HAP3 for binding to UAS2: (**A**) HAP2 and HAP3 bind cooperatively; (**B**) HAP2 and HAP3 associate in the absence of DNA and bind as a complex to UAS2.

Extracts were prepared from lactate-grown cells and HAP3 β-galactosidase was purified as shown in Fig. 2. The gel-retardation assay was used to quantify the recovery of UAS2UP1 DNA binding activity (Fig. 2). The recovery of DNA binding activity from heparin-Sepharose, FPLC Mono Q, and Superose 6 gel filtration was closely parallel to the recovery of HAP3 β-galactosidase (Table 1). The molecular weight of the DNA binding species was ≥500,000 as determined by gel filtration (Fig. 2D). In contrast, most (99.6%) of the HAP3 β-galactosidase applied to a UAS2UP1 DNA affinity column did not bind and only about 30% of the applied DNA binding activity was recovered in this unbound fraction (Table 1). The bound HAP2-HAP3 β-galactosidase, 0.4% of the applied β-galactosidase activity, contained about 33% of the applied DNA binding activity (Table 1). Thus, the ratio of UAS2UP1 binding activity to β-galactosidase activity was about 250-fold greater in the fraction that bound to the DNA affinity column as compared to the flow through. We believe that this difference signifies that the material that binds to the affinity column contains HAP2, a point addressed below. The fact that extensive purification of HAP3 β-galactosidase did not result in a substantial loss of DNA binding activity suggests that HAP2 and HAP3 β-galactosidase have copurified and that the two proteins form a multisubunit complex in the absence of DNA.

To obtain stronger evidence that HAP2 and HAP3 copurified, we tagged HAP2 with the first 87 amino acids of *Escherichia coli* LexA by gene fusion (*4*). Antiserum directed against LexA now served as a means to assay for the presence of HAP2. The strain containing both LexA-HAP2 and HAP3 β-galactosidase displayed UAS2UP1 binding in the gel-retardation assay, indicating that the two fusion proteins can interact with one another.

HAP3 β-galactosidase from this strain was purified as above through the DNA affinity column followed by chromatogra-

Table 1. Purification of HAP2-HAP3 β-galactosidase. Purification was performed as described in text. Monomers HAP3 β-galactosidase were determined by measuring β-galactosidase activity according to (*17*) and were reproducible to within ±10%. Total UAS2UP1 DNA binding activity was measured by titration of the indicated fractions under the conditions of Fig. 2 and values are reproducible to within about 30% of the value given. Relative DNA binding specific activity is expressed as DNA binding activity per milligram of protein. The material applied to the Superose 6 column (load) was 40% of the material recovered from the DNA affinity column.

Step	Protein (mg)	Monomers HAP3 β-gal	UAS2UP1 DNA binding activity (% total)	Relative DNA binding specific activity
13–48% $(NH_4)_2SO_4$	600	7.8×10^{16}	100	1
Heparin	26	5.8×10^{16}	40	8.8
Mono Q	7.2	3×10^{16}	20	16
DNA affinity	0.03	1.2×10^{14}	6.6	1300
Superose 6 (load)	0.012	4.7×10^{13}	3.0	1300
Superose 6	0.003	2.2×10^{13}	1.3	2500

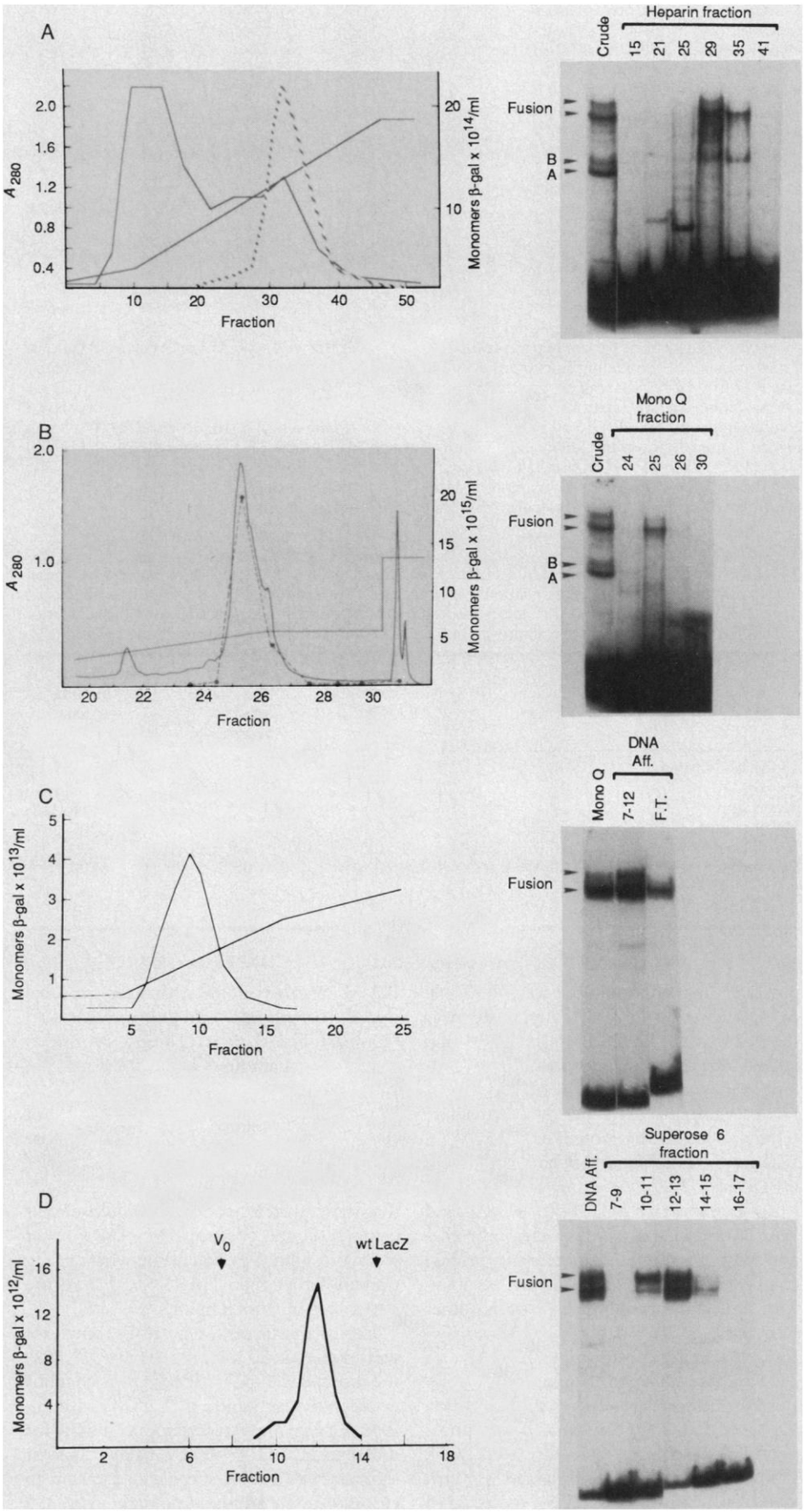

Fig. 2. Purification of HAP2-HAP3 β-galactosidase from yeast. Protein was purified (*15*) from lactate-grown protease-deficient strain BJ2168 (*16*) containing plasmid pSH151 (*5*) from which HAP3 β-galactosidase is synthesized under control of the *CYC1* promoter. (**A**) Heparin-Sepharose chromatography. The resuspended ammonium sulfate pellet (*15*) was applied to a 30-ml heparin-Sepharose column equilibrated in T buffer (*15*) + 40 m*M* KCl and eluted with a 120-ml linear gradient from 40 m*M* KCl to 0.5*M* KCl. Left side of (A) shows the elution profile: Diagonal line, the KCl gradient; solid line, the absorbance at 280 nm; and dashed line, the concentration of β-galactosidase monomers measured as described (*17*). Right side of (A) shows a DNA binding assay to UAS2UP1 DNA with the heparin column fractions numbered as on the left side. Crude sample is that applied to heparin. Fusion complex, complex A [(*8*) see text] and complex B (*8*) (probably a nonspecific UAS2 complex) are indicated. The binding assay was carried out as described (*8*). (**B**) FPLC Mono Q chromatography. The peak fractions of HAP3 β-galactosidase from (A) were pooled, concentrated, and equilibrated in T buffer with 10% glycerol. Protein was applied to a 1-ml Mono Q column and eluted with a linear salt gradient of T + 0*M* KCl to T + 0.4*M* KCl between fractions 14 and 26 (left side, stepped line); in fraction 30, the KCl concentration was raised to 1*M*. Solid line, absorbance at 280 n*M*; dashed line, β-galactosidase activity. DNA binding [right side of (B)] was assayed as in (A). (**C**). DNA affinity chromatography. Fraction 25 from the Mono Q column was applied to a 1-ml DNA Sepharose column (*18*). [Left side of (C)] Protein was applied in T + 0.1*M* KCl, 0.1% Brij 58, and salmon sperm DNA (4.5 μg/ml) and eluted with a linear gradient from 0.1*M* KCl to 1*M* KCl. [Right side of (C)] UAS2UP1 DNA binding activity of pooled fractions 7 to 12 (1.2×10^{11} β-galactosidase monomers per lane) and of the material that flowed through the column (F.T.) (3.8×10^{13} β-galactosidase monomers per lane) was measured in a tris buffer (*19*). Protein-DNA complexes was separated in 4% acrylamide, 10 m*M* tris (*p*H 8), and 1 m*M* EDTA gels at 4°C. (**D**) Superose 6 chromatography. The peak of DNA binding activity from the DNA affinity column was applied to a 25-ml Superose 6 FPLC column in T buffer with 10% glycerol, 0.3*M* KCl, and 0.1% Brij 58. Left side of (D) shows the column elution profile. Void volume of the column (V_o) and the position of wild-type β-galactosidase elution are indicated. UAS2UP1 DNA binding assay of fractions [right side of (D)] carried out as described in (C).

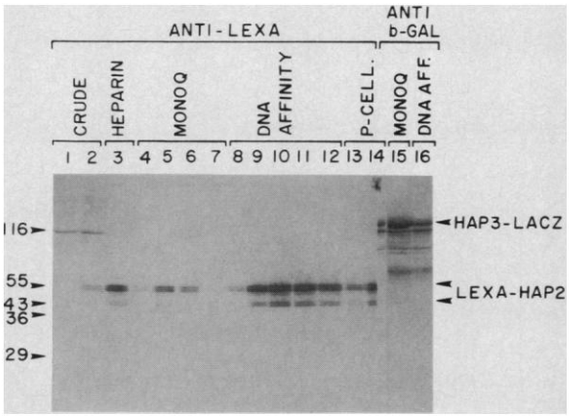

Fig. 3. Western blot of LexA-HAP2-HAP3 β-galactosidase. Western analysis was performed as described (*20*) by using LexA polyclonal antibody (*21*) (lanes 1 to 14), or a polyclonal antibody to β-galactosidase (lanes 14 to 16). Lane 1, crude 48% ammonium sulfate pellet from strain BJ2168 (*16*) with pSH151 (HAP3 β-galactosidase) but lacking LexA-HAP2. Lanes 2 to 16, protein derived from LexA-HAP2-HAP3 β-galactosidase strain BJ2168 with pSH151 and pJP300 (LexA-HAP2) (*9*). Monomers HAP3 β-galactosidase loaded per lane are given in parentheses. Lane 2, 48% ammonium sulfate pellet (1.3×10^{12}); lane 3, the peak of HAP2-HAP3 from heparin column (1.6×10^{13}). Lanes 4 to 7, Mono Q column fractions. Equal volumes of the leading fraction of β-galactosidase activity, lane 4 (4×10^{11}); the peak fraction, lane 5 (2.5×10^{12}); and trailing fractions, lane 6 (8×10^{11}); and lane 7 (3×10^{11}). Lanes 8 to 12 are DNA affinity column fractions. Equal volumes of the flow through, lane 8 (2×10^{13}); or pooled column fractions, lane 9 (2.5×10^{12}); lane 10 (3×10^{12}); lane 11 (2×10^{12}); lane 12 (10^{12}) were loaded. Lane 13, phosphocellulose column flow through (10^{12}); lane 14, phosphocellulose 0.3*M* KCl wash (10^{12}). Lane 14 was probed half with LexA antibody and half with an antibody to β-galactosidase as indicated. Lane 15, the peak fraction of Mono Q–purified protein (10^{13}); lane 16, pooled DNA affinity fractions (3×10^{12}). Molecular mass standards indicated in kilodaltons.

Table 2. Purification of LexA-HAP2-HAP3 β-galactosidase. Units defined as in Table 1. Material loaded onto the phosphocellulose column was eluted from the leading edge of the β-galactosidase peak from the DNA affinity column. The DNA binding specific activity of this material is roughly half that of pooled fractions eluted from the affinity column. F.T., material that flowed through the column.

Step	Protein (mg)	Monomers HAP3 β-gal	UAS2UP1 DNA binding activity (% total)	Relative DNA binding specific activity
13–48% $(NH_4)_2SO_4$	700	1.3×10^{17}	100	1
Heparin	45	2.9×10^{16}	50	7.8
Mono Q	9	1.2×10^{16}	21	16
DNA affinity	0.09	2×10^{14}	17	1200
P-Cellulose (load)	0.02	4.4×10^{13}	1.9	700
P-Cellulose (0.3*M* KCl)	0.0004	1.2×10^{13}	0.38	6800
P-Cellulose (FT)	0.0003	9.1×10^{12}	<0.04	<950

phy over a phosphocellulose column (Table 2). Quantitation of DNA binding activity showed that the HAP3 β-galactosidase that flowed through the phosphocellulose column had lost at least 90% of its original DNA binding activity per β-galactosidase activity, whereas the protein eluted at 0.3*M* KCl still retained its ability to bind UAS2-UP1 (Table 2).

To demonstrate that both HAP2 and HAP3 copurified in all of the above chromatography steps, we examined the protein composition by Western analysis, probing with polyclonal antibodies against either LexA or β-galactosidase. As shown in Fig. 3, LexA-HAP2 copurified with HAP3 β-galactosidase through heparin, Mono Q, and DNA affinity chromatography. Further, the protein that flowed through the phosphocellulose column and displayed weak DNA binding activity contained a much lower level of LexA-HAP2 per molecule of HAP3 β-galactosidase than the material eluted from phosphocellulose at 0.3*M* KCl (compare Fig. 3, lanes 13 and 14, which contain equal amounts of HAP3 β-galactosidase). Since the presence of LexA-HAP2 changed the chromatographic behavior of HAP3 β-galactosidase on phosphocellulose and since HAP2 and HAP3 copurified over four chromatographic steps, we conclude that these proteins comprise a multisubunit complex in the absence of DNA. Further work is required to determine whether any proteins other than HAP2 and HAP3 are also a part of this complex.

In prokaryotic cells, regulatory complexes with nonidentical subunits are rare. Rather, the host RNA polymerase itself contains nonidentical subunits, a structure that allows the enzyme to recognize promoters for different sets of genes by using different sigma factors that are specific for each set. In eukaryotes, the regulation of large gene sets by complexes of nonidentical subunits may afford subtle variation in control across the set. For example, it is possible that HAP2 and HAP3 associate with each other or with related proteins encoded by distinct regulatory loci. Such alternative forms of association would generate an array of transcriptional activation complexes from a simple set of proteins. Although combinatorial control has not yet been demonstrated in the HAP system, it clearly applied in the mating-type system in which α2 represses a-specific genes in α cells, but the proposed α2-a1 complex represses haplo-specific genes in diploid cells (*11*).

We imagine at least three functions that the HAP2-HAP3 complex must have. First, the complex must bind specifically to UAS2 and related sequences in the yeast genome. Second, it must activate transcription once bound at UAS2. Third, it must respond to a signal generated under inducing conditions in media with a nonfermentable carbon source. Experiments performed to date suggest that these three functions are not distributed cleanly between the two proteins. Both HAP2 and HAP3 are required for binding to UAS2UP1. Either HAP2 and HAP3 both make contacts with the DNA or one protein makes all the DNA contacts but must be held in the proper conformation for binding by the other. Further, the ability of the complex to activate transcription once bound at a UAS appears to require both proteins (*12*).

Will large gene families in eukaryotes generally be regulated by complexes with nonidentical subunits? A factor that binds to an upstream element of the chicken ovalbumen promoter appears to consist of two different subunits, one that binds to the site (COUP), and a second that stabilizes binding by reducing the rate of dissociation of COUP from the site (*13*). Further, the suggestion has been raised that the mammalian factor *fos* may interact with other nuclear proteins to generate DNA binding complexes (*14*). Quite possibly, regulatory complexes play important roles in eukaryotic organisms that range from yeast to humans.

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15. Cells (40 g) were disrupted with glass beads in 0.2*M* tris (*p*H 8), 0.4*M* ammonium sulfate, 10 m*M* $MgCl_2$, 1 m*M* EDTA, 10% glycerol, 1 m*M* dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF) (160 μg/ml), tosylamidophenylethyl chloromethyl ketone (TPCK) (50 μg/ml), and pepstatin A (1 μg/ml). The extract was centrifuged at 13,000*g* for 45 minutes. Ammonium sulfate was added for a final concentration of 48%, to the supernatant, which was then spun at 8000*g* for 20 minutes. The pellet was resuspended in T buffer [30 m*M* tris (*p*H 8), 2 m*M* EDTA, 20% glycerol, 1 m*M* DTT, PMSF (160 μg/ml), TPCK (25 μg/ml), and pepstatin A (1 μg/ml)] to a conductivity equal to that of T buffer + 40 m*M* KCl.
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GCCAGCTCATTTGGCGAGCGTTGATTGGTGGATCAA
CGAGTAAACCGCTCGCAACTAACCACCTAGTTCGGT
19. The buffer consisted of 4 m*M* tris (*p*H 8), 60 m*M* KCl, 2 m*M* $MgCl_2$, 4% glycerol, 1 m*M* DDT, bovine serum albumin (100 μg/ml), salmon sperm DNA (2.5 μg/ml), and 0.1% Brij 58 at 23°C.
20. M. Haldi and L. Guarente, in preparation.
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22. We thank B. Arcangioli, A. Brunelle, R. Schleif, and K. Pfeifer for advice on the gel-binding assay; E. Furfine, J. Greene, A. Ninfa, and L. Chodosh for advice on protein purification; and R. Brent for a gift of LexA antiserum. This work was supported by a Damon Runyon–Walter Winchell Cancer Fund Postdoctoral Fellowship to S.H. and a grant to L.G. from the National Institutes of Health (5RO1 GM3044-05).

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Interleukin-1 Immunoreactive Innervation of the Human Hypothalamus

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Interleukin-1 (IL-1) is a cytokine that mediates the acute phase reaction. Many of the actions of IL-1 involve direct effects on the central nervous system. However, IL-1 has not previously been identified as an intrinsic component within the brain, except in glial cells. An antiserum directed against human IL-1β was used to stain the human brain immunohistochemically for IL-1β–like immunoreactive neural elements. IL-1β–immunoreactive fibers were found innervating the key endocrine and autonomic cell groups that control the central components of the acute phase reaction. These results indicate that IL-1 may be an intrinsic neuromodulator in central nervous system pathways that mediate various metabolic functions of the acute phase reaction, including the body temperature changes that produce the febrile response.

INTERLEUKIN-1 (IL-1) IS A CYTOKINE that is elaborated by cells involved in host defense, such as macrophages (*1*). It mediates several components of the acute phase reaction, including the febrile response, secretion of adrenocorticotropic hormone (ACTH), and the synthesis of acute phase proteins. Plasma IL-1 is thought to enter the hypothalamus at the organum vasculosum of the lamina terminalis (OVLT), a circumventricular organ with no blood-brain barrier, located at the anteroventral tip of the third ventricle (*2*). IL-1 injection into the brain causes fever, secretion of adrenal corticosteroids, and synthesis of acute phase proteins by the liver (*3*). However, the site of action of IL-1 within the brain has not yet been determined.

A factor with IL-1–like activity has been isolated from the brain (*4*), but IL-1 has been demonstrated so far only in glial cell lines (*5*). We used immunohistochemistry to examine the cellular localization of IL-1 in the human brain. Our results indicate that IL-1 is contained in neural elements within the hypothalamus, where it may serve as a neuromodulator in the central component of the acute phase reaction.

Five normal human brains were collected at autopsy (*6*) and the basal forebrains were cut at 50 μm on a freezing microtome. Sections were stained immunohistochemically with an antiserum against human IL-1β (*7*). Control sections were prepared by omitting the primary antiserum or by using antiserum that had been adsorbed with recombinant human IL-1β (*8*). There was no fiber staining in this control material (Fig. 1, D and E). In addition, the IL-1β antiserum was adsorbed against a number of neuropeptides that have been identified in immunohistochemical studies of the hypothalamus (*8*), none of which reduced the intensity of staining. Sections through the hypothalamus were also stained with an antiserum to human IL-1α, which has approximately 30% amino acid identity with IL-1β (*8*). Only a few scattered fibers were stained by this antiserum, an indication that the staining with the IL-1β antiserum was specific.

IL-1β–like immunoreactive (ir) varicose fibers were seen in a characteristic distribution within the hypothalamus in each of the brains (Fig. 2). The densest accumulations of fibers were found in the periventricular regions that participate in anterior pituitary control. Immunoreactive fibers were found throughout the periventricular and arcuate nuclei of the hypothalamus, as well as in the parvocellular part of the paraventricular nucleus (Figs. 1C and 2). IL-1βir fibers could be traced into the infundibulum, including the region of the median eminence containing the hypophyseal portal vessels (Fig. 1B). In addition, IL-1βir innervation was seen in the magnocellular part of the paraventricular nucleus, and to a lesser extent in the supraoptic nucleus, among the cell bodies of neurons that secrete oxytocin and arginine vasopressin (AVP) from the posterior pituitary gland (Fig. 1A).

There was also IL-1βir innervation of hypothalamic structures that participate in autonomic control. In addition to the autonomic portions of the paraventricular nucleus, IL-1βir fibers were found in several other cell groups whose neurons directly innervate autonomic preganglionic nuclei (*9*), including the dorsomedial nucleus of the hypothalamus and the lateral hypothalamic area. IL-1βir fibers were also seen within the subfornical organ (Fig. 2E), the bed nucleus of the stria terminalis (Fig. 1G), the substantia innominata, the ventromedial nucleus of the hypothalamus (Fig. 1H), the posterior hypothalamic area (Fig. 1D), and the paraventricular nucleus of the thalamus, all structures thought to have a role in central autonomic control (*10*).

Our results indicate that the human hypo-

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