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Cloning and Molecular Analysis of the *HAP2* Locus: a Global Regulator of Respiratory Genes in *Saccharomyces cerevisiae*

JENNIFER L. PINKHAM *and* LEONARD GUARENTE*

Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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We report here the cloning of the *HAP2* gene, a locus required for the expression of many cytochromes and respiratory functions in *Saccharomyces cerevisiae*. The cloned sequences were found to direct integration of a marked vector to the chromosomal *HAP2* locus, and derivatives of these sequences were shown to yield chromosomal disruptions with a Hap2$^-$ phenotype. The gene maps 18 centimorgans centromere proximal to *ade5* on the left arm of chromosome VII, distinguishing it from any other previously characterized nuclear petite locus. The *HAP2* locus encodes a 1.3-kilobase transcript which is present at extremely low levels and which is derepressed in cells grown in media containing nonfermentable carbon sources. Levels of *HAP2* mRNA are not reduced in strains bearing a mutation at the *HAP3* locus, which is also required for expression of respiratory functions. Models outlining possible interactions of the products of the *HAP2* and *HAP3* genes are presented.

Regulation of eucaryotic transcription can depend on sequences up to hundreds of nucleotides removed from the region of transcription initiation. Such sequences include enhancers and proximal regulatory sites in mammalian systems and upstream activation sites (UASs) in *Saccharomyces cerevisiae*. Enhancers can confer hormone inducibility (6), viral host range (18), or tissue-specific expression (2, 9) on the genes with which they are associated, whereas UASs mediate regulation by specific physiological signals. It is presumed that enhancers and UASs interact with specific DNA-binding proteins which recognize the sites and can activate transcription. In *S. cerevisiae* such *trans*-acting, UAS-specific proteins as well as *trans*-acting negative regulators have been identified genetically. For example, the *GCN4* gene is believed to encode a positive regulator of the genes derepressed by amino acid starvation (15, 29). Likewise, the *GAL4* gene product activates transcription of the *GAL1*, *GAL7*, and *GAL10* genes in response to galactose (28). The product of the mating type gene *MATα2* is a negative regulator of **a**-specific genes (14). The demonstration that *MATα2* (14) and *GAL4* (28) are localized to the nucleus provides further evidence that they are direct regulators. Confirmation that both proteins are direct regulators has been obtained recently by the finding that they bind specifically to DNA sequences near the genes that they control (3, 10, 17).

Transcription of the yeast *CYC1* gene, encoding iso-1-cytochrome *c*, is activated by two tandem UASs, UAS1 and UAS2, each of which can function independently to activate transcription in response to heme and carbon source (12, 13). In the absence of heme, or under anaerobic growth conditions, both sites are inactive. Under heme-sufficient conditions in glucose media, UAS1 activity is 10-fold greater than that of UAS2. Under derepressed conditions in lactate media UAS1 is derepressed 10-fold, whereas UAS2 is derepressed 100-fold. Thus, in glucose most of *CYC1* transcription is driven from UAS1, and in lactate transcription is driven equally from both UASs.

Although UAS1 and UAS2 bear homologous regions, *trans*-acting regulatory mutations discriminate between the two sites (12). Mutations in a locus *HAP1* abolish UAS1 activity without affecting UAS2, whereas mutations in either *HAP2* or *HAP3* (Pinkham and Guarente, manuscript in preparation) exert the converse effect. The *hap2-1* and *hap3-1* mutations are pleiotropic, resulting in a reduction in levels of many cytochromes and in an inability to grow on nonfermentable carbon sources. Thus, the *HAP2-HAP3* system may comprise a global activation system for yeast genes whose products are involved in respiration. The HAP2 and HAP3 products may form a complex which directly activates transcription by binding to UAS2 and to the UASs of other genes. Alternatively one gene may control the synthesis or activity of the product of the other, which itself is the direct activator. It is also possible that neither HAP product directly activates transcription. To begin to investigate these possibilities, we report the cloning of *HAP2* and the use of the clone to characterize the locus physically and genetically.

MATERIALS AND METHODS

Strains and general genetic methods. Tables 1 and 2 list the *S. cerevisiae* strains and plasmids used. Standard yeast genetic procedures were followed (27). Growth on nonfermentable carbon sources was scored on complex or synthetic minimal medium with 2% lactate added. Yeast transformations were performed by the lithium acetate method (16). *Escherichia coli* YMC9 (*ΔlacU169 hsdR hsdM*$^+$) transformations were done by the calcium chloride procedure (19), and transformants were grown in LB or M9 medium (21) containing ampicillin.

DNA isolation and techniques. Restriction digests and other enzyme reactions were performed as recommended by the suppliers. DNA fragments were isolated for cloning from acrylamide gels by crushing and soaking (11). Plasmid DNA for most procedures was prepared on cesium chloride-ethidium bromide equilibrium gradients, and small-scale DNA isolation from *E. coli* was accomplished by rapid boiling lysis (19). Plasmid and genomic DNA was isolated from yeast by the method of Sherman et al. (27).

β-Galactosidase assays. Cells were grown in minimal medium of nitrogen base without amino acids and ammonium

* Corresponding author.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source or reference
BWG2-9A-1	α *ade6 his4-519 ura3-52*	(13)
BWG1-7A	**a** *ade1-100 his4-519 leu2-3,2-112 ura3-52*	(13)
LGW1	**a** *ade1-100 his4-519 leu2-3,2-112 ura3-52 hap2-1*	(12)
TM2	*hem1 ura3-52*	(13)
CSH 86L	α *spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	G. Fink
CSH 88L	α *spo11 ura3 his2 leu1 lys1 met4 pet8*	G. Fink
CSH 90L	α *spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	G. Fink
F341	α *lys5 gal2*	G. Fink
GS320	**a** *cyh2 ade2 ura3 can^{r}1 leu1 rme1*	G. Fink
1614A	**a** *ade5*	G. Fink
444-1C	α *met13-25 gln1-37*	(21)
JP27	**a** *ade1-100 his4-519 leu2-3,2-112 HAP2*::pJP161	This work
JP50	**a** *ade1-100 his4-519 ura3-52 LEU2::CYC1-lacZ*	This work
JP1-1C	α *ade1-100 ade6 his4-519 ura3-52 leu2-3,2-112 hap2-1*	This work
JP3-3B	**a** *his4-519 ura3-52 LEU2::CYC1-lacZ hap2-1*	This work
JP4-5C	**a** *his4-519 ura3-52 LEU2::CYC1-lacZ hap3-1*	This work
JP4-8C	α *ade1-100 his4-519 ura3-52 LEU2::CYC1-lacZ*	This work
JP41	α *ade1-100 his4-519 HAP2'::URA3 LEU2::CYC1-lacZ*	This work
JP8-2B	**a** *his4-519 leu2-3,2-112 ura3-52 hap2-1*	This work
JP9-1C	α *ade1-100 his4-519 leu2-3,2-112 trp5 HAP2*::pJP161	This work
JP13-12D	α *lys5 hap2-1*	This work

sulfate before assays. The medium was supplemented with 2% carbon source and 40 μg of the required amino acids per ml. β-Galactosidase activity was determined in a liquid assay with cells permeabilized with chloroform and sodium dodecyl sulfate and on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside plates as described previously (11).

Construction of *HAP2'::URA3*. The bulk of the *URA3* gene encoded on YCp50 was removed from pJP103 by cutting the plasmid with *Sma*I and *Eco*RV and reclosing the backbone such that a 2.3-kilobase (kb) segment of DNA containing pBR322 sequences and all but about 0.1 kb of the *URA3* gene was deleted. The resulting plasmid, pJP109, was the recipient of the 1.1-kb *Hin*dIII fragment bearing the *URA3* gene, whose ends had been rendered flush, ligated into the unique *Pvu*II site within the *HAP2* complementing insert. The plasmid bearing the disruption, pJP110, had a 3.2-kb *Eco*RI fragment containing the disrupted *HAP2* gene, which was used for the transplacement (26) of the wild-type *HAP2* gene in strains containing the UAS2 *CYC1-lacZ* fusion (see Fig. 3).

TABLE 2. Plasmids[a]

Plasmid	Genetic markers
pJP100	*LEU2, CYC1-lacZ*
pJP112	*URA3, HAP2*, 2μm
pJP161	*URA3, HAP2*, 2μm
pJPA4	*URA3, HAP2, CEN4 ARS1*
pJPC2	*URA3, HAP2, CEN4 ARS1*
pJP102	*URA3, HAP2*, 2μm
pJP103	*URA3, HAP2, CEN4 ARS1*
pJP109	*HAP2, CEN4 ARS1*
pJP110	*HAP2'::URA3, CEN4 ARS1*

[a] All plasmids bear the *bla* gene and the colE1 origin of replication from pBR322.

Synthesis of riboprobes. The 0.5-kb *Pvu*II-*Eco*RI fragment (see Fig. 2) was gel isolated and ligated into *Sma*I-*Eco*RI-digested pSP64 and pSP65 vectors from Promega Biotec, Inc. These constructs were the templates for the A and A' riboprobes in Fig. 4. Likewise, the 0.8-kb *Bgl*II-*Pvu*II fragment cloned into *Sma*I-*Bam*HI-digested vectors was the source of the B and B' riboprobes. A 3.5-kb *Eco*RI-*Bam*HI fragment from pRB149 containing the actin gene (24) was cloned into pSP65 restricted with *Eco*RI and *Bam*HI to provide antisense actin RNA. The resulting plasmids were linearized to serve as templates for the SP6 RNA polymerase. Radioactively labeled RNA was synthesized and prepared as described previously (20).

RNA isolation and Northern blots. Total cellular RNA was isolated by glass bead lysis of cells grown in minimal medium to an optical density at 600 nm of 0.5 to 1.0 as described previously (13). Yeast strains with UAS2 *CYC1-lacZ* integrated at *LEU2* were used where possible so that β-galactosidase activities of each culture could be assayed. The cellular RNA (10 to 40 μg) was size fractionated on 1% agarose–37% formaldehyde–20 mM 3-(*N*-morpholine)propanesulfonic acid (pH 7.0)–5 mM sodium acetate–1 mM EDTA gels and transferred to nitrocellulose as described previously (13, 31). Hybridization of the riboprobe was done at 55°C in 50% formamide–50 mM sodium phosphate (pH 6.5)–0.8 M NaCl–1 mM EDTA–0.1% sodium dodecyl sulfate–0.05% bovine serum albumin–0.05% Ficoll–0.05% polyvinylpyrrolidone.

Southern blots. Genomic DNA was restricted, size fractionated in 1% agarose–90 mM Tris borate (pH 8.3)–25 mM EDTA gels and transferred to nitrocellulose (29) in 5× SSC (1× SSC is 0.15 M NaCl–0.015 M sodium citrate). Hybridization with riboprobe was done at 42°C in 50% formamide and the buffer described for the hybridization of Northern blots.

RESULTS

Isolation of *HAP2* clones. *Hap2*$^-$ mutants possess two phenotypes, a reduction of UAS2-directed transcription and poor growth in lactate (nuclear petite). Plasmids which complemented the latter phenotypes were isolated from yeast libraries constructed in the high-copy vector YEp24 (5) and the single-copy vector YCp50 (J. Thomas and M. Rose, personal communication). DNA was isolated from candidates in which the Ura$^+$ and lactate-positive phenotypes cosegregated, and this DNA was introduced into *E. coli* by selecting ampicillin-resistant bacterial transformants. Four unique clones were isolated which complemented both the lactate-negative phenotype of strain JP3-3B and restored activation of UAS2 as measured in a *CYC1-lacZ* fusion integrated at the *LEU2* locus (Fig. 1) upon retransformation.

The physical maps of these four clones are shown in Fig. 2. All four clones bear overlapping inserts. Three of the four clones, two high copy (pJP112, pJP161) and one single copy (pJPC2), fully complement the lactate-negative phenotype and activate UAS2 to wild-type levels, whereas one single-copy clone (pJPA4) only partially complements the lactate-negative phenotype and activates UAS2 to only 25% of

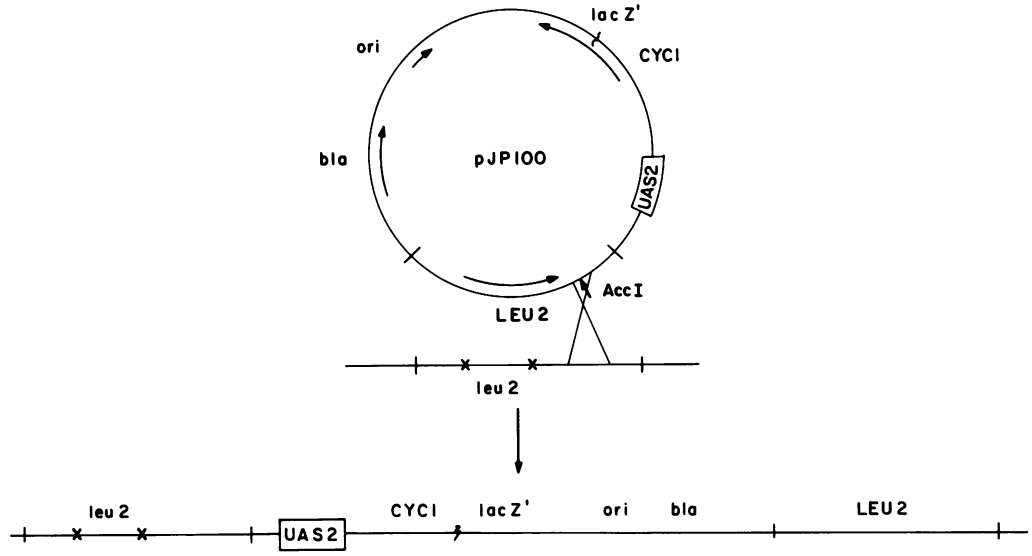

FIG. 1. Derivation of the chromosomally integrated UAS2 *CYC1-lacZ* fusion. pJP100 contains the *bla* and *LEU2* genes of YIp32 (1, 4) joined to a UAS2 (UP1)-driven *CYC1-lacZ* fusion (12). This plasmid was partially digested with *Acc*I such that one-fourth of the linear molecules would be restricted at the *Acc*I site 0.1 kb downstream of the *LEU2* coding region (1). Strain BWG1-7A was transformed to leucine prototrophy with this linear DNA. Stable leucine prototrophs resulted from homologous recombination of the plasmid *LEU2* gene and the genomic *leu2-3,2-112* allele and possessed regulated β-galactosidase activity. Such a transformant was JP50, and it was the parent strain of JP4-8C, JP41, JP4-5C, and JP3-3B.

wild-type levels (see below, Delineation of the *HAP2* gene). We note that plasmids bearing *HAP2* in multicopy do not give rise to elevated UAS2 activity in glucose-repressed or in lactate-derepressed conditions.

***HAP2* clone directs integration at the *HAP2* locus.** To determine whether the cloned gene was the wild-type allele of the gene defined by the *hap2-1* mutant, we targeted integration of the pJP161 plasmid into the yeast chromosome. pJP161 was restricted within the insert DNA by a partial *Pvu*II digest, and BWG1-7A was transformed to

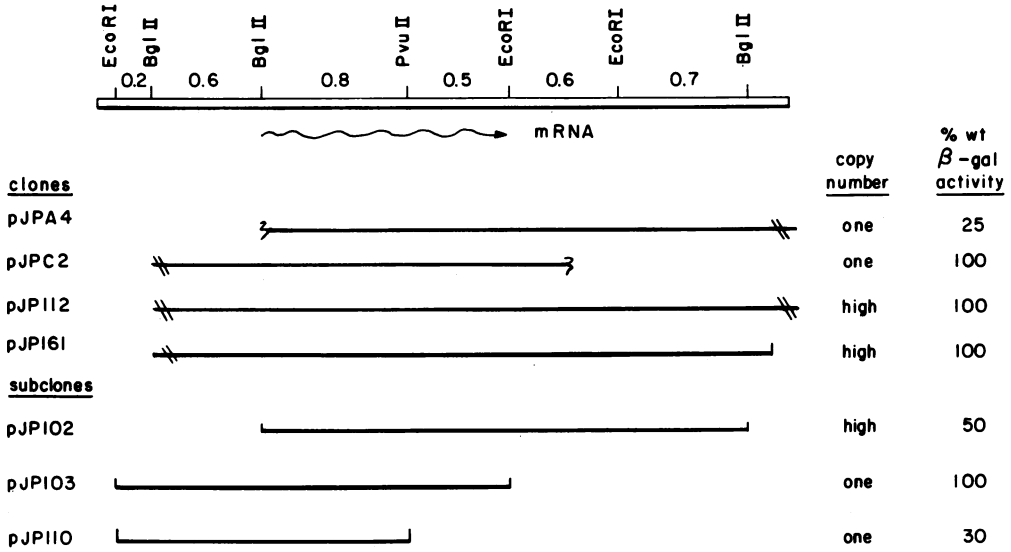

FIG. 2. Restriction map of the *HAP2* gene region, the structure of genomic clones and subclones, and the quantitation of *hap2-1* complementation. The open bar represents the DNA in the *HAP2* region depicted with sizes of the restriction fragments in kilobases. The wavy line represents the approximate position of the *HAP2* mRNA, and the arrow indicates the 3′ end of the mRNA. The solid bars show the cloned yeast DNA; the junctions with vector DNA are depicted by the vertical wavy lines; the double slash signifies that the cloned DNA includes several kilobases beyond the *HAP2*-complementing region. pJPA4 has a left junction at or near the *Bgl*II site (see the text) an is 9 kb in size; pJPC2 has a right junction within the 0.6-kb *Eco*RI fragment and is 8 kb; pJP112 is 8 kb; pJP161 possesses a fortuitous *Bam*HI site at its right junction, about 0.1 kb beyond the *Bgl*II site. Subclones pJP102 and pJP103 are described in the text, and pJP110 depicts the fragment of *HAP2* which yields partial activity in the *HAP2′::URA3* transplacement (Fig. 3). The copy number is stated as one when the DNA has been cloned into a YCp50 backbone and high when it is cloned into YEp24. Percent wild-type β-galactosidase activity was assayed in glucose-grown transformants of the *hap2-1* strain, JP3-3B, which untransformed has 5% of wild-type activity.

TABLE 3. Genetic mapping of *hap2-1*[a]

Cross	Gene pair	PD	NPD	TT	Map distance (cM)[b]
GS320 × JP41	*leu1* × *HAP2'::URA3*	3	3	14	
CSH90L × JP27	*trp5* × *HAP2*::pJP161	4	2	18	
GS320 × JP41	*cyh2* × *HAP2'::URA3*	3	5	12	
444-1D × LGW1	*met13* × *hap2-1*	7	3	22	62
F341 × JP8-2B, 1614A × JP13-2D	*lys5* × *hap2-1*	14	4	22	58
1614A × JP13-2D	*ade5* × *hap2-1*	19	1	2	18
1614A × JP13-2D	*lys5* × *ade5*	5	2	16	62[c]

[a] Abbreviations: PD, parental ditype; NPD, nonparental ditype; TT, tetratype.
[b] Map distances in centimorgans (cM) were calculated with the formula of Perkins (25).
[c] Published distance, 76 centimorgans (22).

uracil prototrophy with the linear DNA. Integration of the linear DNA into the yeast chromosome was ascertained by the mitotic stability of the Ura$^+$ phenotype and by 2:2 segregation of the Ura$^+$ phenotype when the integrant strain JP27 was crossed with *ura3-52* tester strains. A *MAT*α derivative of the integrant strain JP27, JP9-1C, was crossed with a *hap2-1* strain, LGW1, the diploids were sporulated, and tetrads were dissected. The *hap2-1* phenotype (poor growth on lactate) and the Ura$^+$ phenotype were found to segregate in opposition in nine of nine tetrads.

Chromosomal location of *HAP2*. The chromosome on which the *HAP2* locus resided was determined by the 2 μm mapping procedure (7, 8). JP27, which carries pJP161 integrated at the *HAP2* locus, was crossed with three multiply marked tester strains. Ura$^-$ segregants were identified by replica plating; 1 to 5% of the Ura$^-$ segregants from the diploids heterozygous for *trp5* were also Trp$^-$. From this result we inferred that *HAP2* resided on the left arm of chromosome VII (23). Accordingly, strains bearing either *hap2-1* or a *URA3* marker integrated at the *HAP2* locus, JP27 or JP41 (described below), were crossed with several strains marked at various locations along the left arm of chromosome VII (Table 3). Tetrad analysis of these sporulated diploids indicated that the *HAP2* locus lay 18 centimorgans centromere proximal to *ade5* (see Table 3). This location indicates that the *hap2-1* mutation is not allelic with any previously characterized nuclear mutations with petite phenotypes.

Delineation of the *HAP2* gene. A comparison of the four original clones suggests that the *HAP2*-coding sequence lies within a 2.1-kb *Eco*RI fragment. Indeed a subclone, pJP103, bearing this restriction fragment inserted into the *Eco*RI site of YCp50 fully complemented the *hap2-1* mutation, in both the ability to grow on a nonfermentable carbon source and the ability to activate UAS2.

Alterations in either end of the *Eco*RI fragment were found to partially reduce *HAP2* complementing activity. The partially complementing, single-copy clone, pJPA4, was truncated at a *Bgl*II site 0.8 kb into the *Eco*RI fragment (Fig. 2). A subclone, pJP102, bearing the 2.7-kb *Bgl*II fragment inserted into the *Bam*HI site of YEp24, fully complemented the lactate-negative phenotype, but only restored UAS2 activity to 50% of wild-type levels. Thus we inferred that a portion of either the promoter or the 3′ region of *HAP2* lies on the 0.8-kb *Eco*RI-*Bgl*II fragment.

Next, the genomic *HAP2* gene was disrupted by the insertion of the *URA3* gene into the *Pvu*II site 0.8 kb to the right of the *Bgl*II site described above (Fig. 3). The structure of the integrant was confirmed by Southern blot analysis of genomic DNA. The strain bearing this disruption, JP41, possessed a lactate-negative phenotype less severe than that caused by the *hap2-1* mutation and was partially deficient in activation of UAS2 (Fig. 2). The lactate-negative phenotype was complemented by mating with an *HAP2*$^+$ strain, but not by mating with a *hap2-1* strain, showing that JP41 was Hap2$^-$. However, since partial activity remained in the strain bearing the disruption, we concluded that sequences required for optimal *HAP2* expression must lie to the right of the *Pvu*II site in Fig. 2.

To determine the direction of *HAP2* transcription probes were constructed bearing the 0.8-kb *Bgl*II-*Pvu*II fragment or the 0.5-kb *Pvu*II-*Eco*RI fragment adjacent to the SP6 promoter in both orientations. Radioactively labeled RNA was synthesized from each construct and was hybridized to cellular RNA which had been size fractionated on formaldehyde-agarose gels and transferred to nitrocellulose paper. The riboprobes synthesized from the 0.5-kb *Pvu*II-*Eco*RI fragment, A and A′ (Fig. 4), hybridized to 1.3- and 1.9-kb transcripts, respectively. However, only the 1.3-kb transcript was detected by the 0.8-kb *Bgl*II-*Pvu*II-B riboprobe (Fig. 4). The other strand of the *Bgl*II-*Pvu*II fragment did not

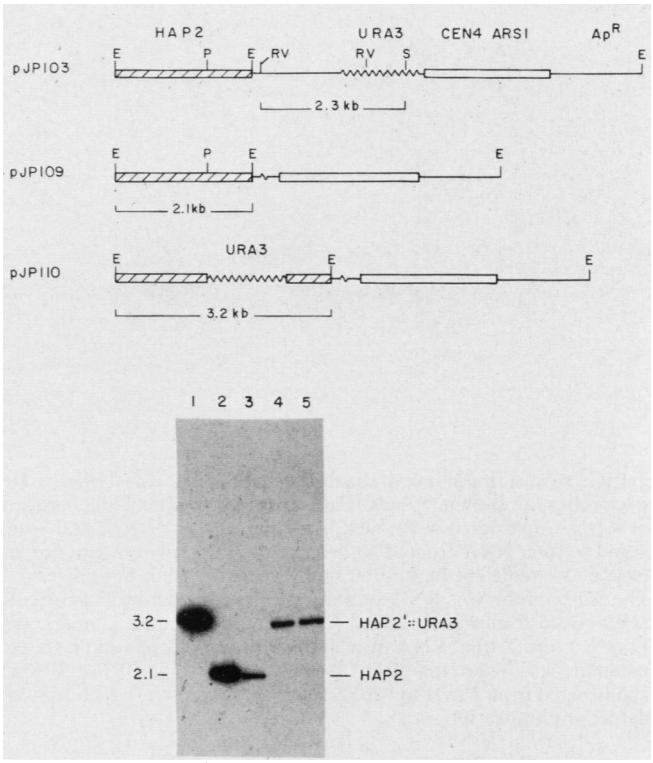

FIG. 3. Transplacement of *HAP2* with *HAP2'::URA3*. The construction of pJP103, pJP109, and pJP110 is described in Materials and Methods. The hatched box denotes the 2.1-kb *Eco*RI fragment containing the *HAP2* gene. The solid line indicates pBR322 DNA. The wavy line represents the *URA3* gene, and the open bar indicates the *CEN4 ARS1* DNA. Restriction sites: E, *Eco*RI; P, *Pvu*II; RV, *Eco*RV; S, *Sma*I. The lower panel shows a Southern blot of genomic DNA isolated from strains with and without the transplacement, restricted with *Eco*RI, and probed with riboprobe A (*HAP2* antisense RNA; Fig. 4). Lanes: 1, pJP110 DNA control; 2, pJP109 DNA control; 3, genomic DNA from *HAP2*$^+$ strain JP4-8C; 4 and 5, genomic DNA from two independent *HAP2'::URA3* derivative strains, JP41 and JP42, respectively.

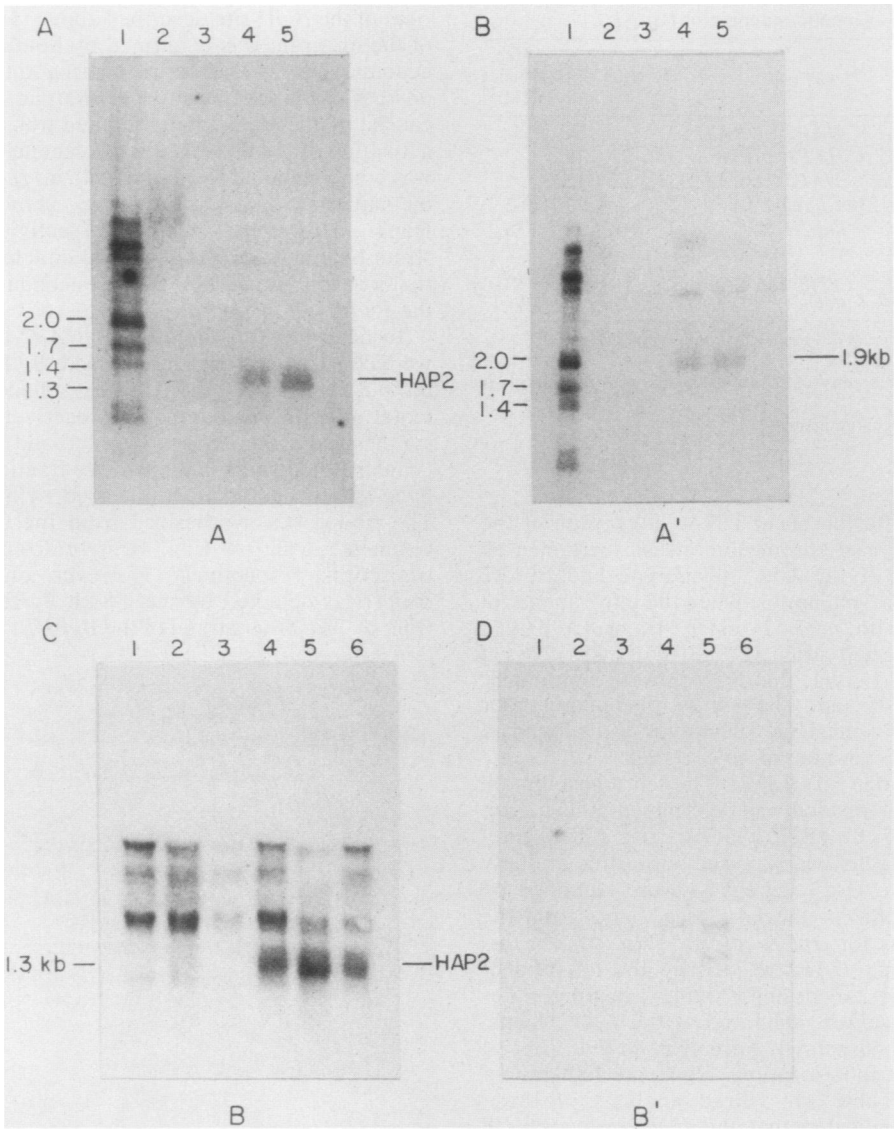

FIG. 4. Determination of the direction of *HAP2* transcription. Northern blots of 20 μg of total RNA per lane hybridized with four different riboprobes are shown. Panels A and B display identical blots probed with riboprobes A and A′, respectively. Lanes: 1, *Hin*dIII-*Eco*RI digest of λ DNA, hybridized with nick-translated (19) λ DNA; 2 and 3, total RNA from JP50 grown in glucose and in lactate media, respectively; 4 and 5, total RNA from JP50 bearing pJP161 grown in glucose and lactate media, respectively. Hybridization is detectable only in RNA isolated from pJP161-bearing strains. The A riboprobe hybridizes to a 1.3-kb transcript, and the A′ riboprobe hybridizes to a 1.9-kb transcript. The A riboprobe was RNA synthesized from the 0.5-kb *Pvu*II-*Eco*RI fragment in the direction *Eco*RI toward *Pvu*II, and the A′ riboprobe is synthesized from *Pvu*II toward *Eco*RI (Fig. 2). Panels C and D display identical blots hybridized with riboprobes B and B′, respectively. Lanes: 1 and 2, total RNA from JP50 grown in glucose and lactate media, respectively; 3, total RNA from JP4-5C (*hap3-1* strain) in glucose medium; 4, 5, and 6, total RNA from strains bearing pJP161, JP50 glucose, JP50 lactate, and JP4-5C glucose, respectively. The B riboprobe, synthesized from *Pvu*II toward *Bgl*II, hybridizes to a 1.3-kb transcript detectable only in pJP161-bearing strains. The B′ riboprobe does not detect any transcript.

hybridize to any RNA species. Thus the 1.3-kb RNA transcribed from left to right in Fig. 4 must be the *HAP2* transcript. It is not known whether the 1.9-kb transcript located downstream of the *HAP2* locus and transcribed from the opposite strand overlaps the 3′ end of the *HAP2* mRNA. If we infer from the complementation data that the *HAP2* transcript starts to the right of the *Bgl*II site, 0.8 kb from the *Eco*RI site, then the boundaries of the transcript must be closely defined by the 1.3-kb *Bgl*II-*Eco*RI fragment.

Transcriptional regulation of the *HAP2* gene. We wished to determine whether levels of *HAP2* transcription were influenced by mutations at the *HAP3* locus or by the physiological signals affecting UAS2 activity. Northern blot analysis of the *HAP2* transcript gave several important results (Fig. 5). First, the levels of *HAP2* message encoded by the chromosomal locus were extremely low, roughly 0.1% of actin mRNA levels. Second, levels of the *HAP2* message were regulated by carbon source, but not by the availability of heme or by mutations in *HAP3*. Densitometry of autoradiograms indicated that levels of the *HAP2* message are about fivefold higher under derepressed conditions of growth in lactate medium than in glucose medium. Third, pJP161,

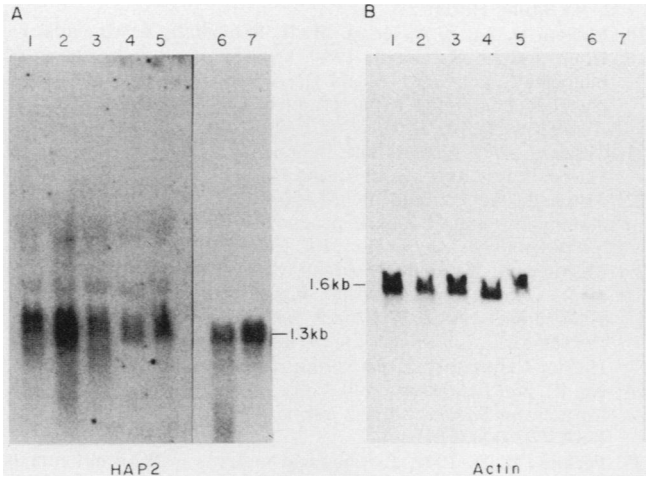

FIG. 5. Regulation of *HAP2* transcription. Panels A and B display the same Northern blot hybridized with the *HAP2* A riboprobe and actin riboprobe, respectively. Lanes 1 through 5 contain 40 μg of total RNA, lanes 6 and 7 contain 10 μg of total RNA. Lanes: 1, JP50, glucose medium; 2, JP50, lactate medium; 3, JP4-5C, glucose medium; lane 4, TM2, heme-sufficient medium (13); 5, TM2, medium lacking heme (13); 6, JP50 bearing pJP161, glucose medium; 7, JP50 bearing pJP161, lactate medium. The *HAP2* riboprobe detects the 1.3-kb *HAP2* transcript, and densitometry of the autoradiogram indicates a fivefold derepression of *HAP2* mRNA in cells grown in lactate medium compared with cells grown in glucose medium. However, in cells bearing pJP161 only a twofold derepression is apparent. An estimation of the relative amounts of *HAP2* and actin mRNAs was done by densitometry of the autoradiograms. The radioactively labeled *HAP2* and actin riboprobes were synthesized at the same specific activity and hybridized under identical conditions separately to the same nitrocellulose filter. The actin probe was 3.5 kb, and the *HAP2* probe was 0.5 kb. In panel A, the filter with lanes 1 through 5 was exposed for 15 days; in panel B, the filter was exposed for 30 min. Exposures were done at −70°C with intensifying screens, and we estimate that the *HAP2* mRNA is a fraction of a percent of actin mRNA levels.

which does not derepress UAS2 in glucose-grown cells, nevertheless gives rise to elevated levels of *HAP2* mRNA under these conditions.

DISCUSSION

In this report we have described the isolation of a gene which complements the *hap2-1* mutation. Four unique clones with overlapping sequences were isolated. Evidence that the cloned sequences correspond to the *HAP2* gene is threefold. First, cloned sequences complement in single copy. Second, the cloned sequences direct plasmid integration to the *HAP2* locus in the yeast genome. Third, a disruption in the genomic sequence corresponding to the clone results in an Hap2$^-$ phenotype. The map position of the *HAP2* locus in the yeast genome, 18 centimorgans centromere proximal to *ade5* on the left arm of chromosome VII, indicates that this gene is different from the nine previously mapped nuclear petite loci (23).

Physical analysis indicates that the *HAP2* locus encodes a 1.3-kb transcript. Deletion of a restriction fragment close to the start of the mRNA partially inactivates the gene, possibly because it removes a UAS required for expression. Further, truncating the *HAP2* gene at a site roughly 0.8 kb from the start of transcription leaves significant *HAP2* activity intact. Recent experiments with *lacZ* fusions indicate that the *HAP2*-coding sequence extends past this restriction site (unpublished data). Thus, it appears that the carboxyl terminus of the HAP2 protein is not strictly required for its activity.

Analysis of *HAP2* transcription has yielded several interesting findings. First, the steady-state level of *HAP2* mRNA is very low, a fraction of a percent of actin mRNA levels. Second, levels of RNA are fivefold higher in cells grown in a nonfermentable carbon source than in cells grown with glucose as the carbon source. These two findings are consistent with the notion that *HAP2* is the direct regulator of transcription of *CYC1* and other cytochromes. Analogously, the *GCN4* gene, thought to be the direct regulator of genes controlled by general amino acid control, is itself regulated by general control (14, 30). Third, *HAP2* transcription is not reduced in *hap3*$^-$ strains. Further, by using gene fusions it has been determined that *HAP2* translation is not affected by mutations in *HAP3* (unpublished data). These findings rule out the possibility that *HAP3* is required for expression of *HAP2*. We also think it unlikely that *HAP2* is simply required for expression of *HAP3*, since in that instance it might be expected that high-copy clones of *HAP3* could complement an *hap2* lesion. As indicated above, all complementing clones isolated contained the *HAP2* locus. Thus we favor the hypothesis that the products of the *HAP2* and *HAP3* genes act as a complex to activate UAS2 and the UASs of other genes encoding products involved in respiration. One prediction of this model is that the products of both *HAP2* and *HAP3* are nuclear proteins and will bind to UAS2 and UASs of other cytochrome genes. Recent experiments indicate that the *HAP2* product is indeed nuclear (unpublished data). According to this view, derepression of UAS2 and other genes involved in respiration requires a simultaneous increase in the synthesis of the *HAP2* and *HAP3* gene products. We imagine that heme could serve as a cofactor required for the activity but not the synthesis of the HAP2 and HAP3 products.

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