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Genetics

Constitutive expression of the yeast *HEM1* gene is actually a composite of activation and repression

(opposing forms of regulation/*HAP2–HAP3* global activation system)

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ABSTRACT We show that *HEM1* (encoding 5-aminolevulinate synthase) expression, while constitutive under all steady-state growth conditions tested, is activated by the *HAP2–HAP3* global activation system that controls expression of apocytochromes. This finding creates a paradox because apocytochrome activation by *HAP2–HAP3* is highly regulated, subject to induction by heme, and subject to further derepression by a shift from glucose medium to one containing a nonfermentable carbon source. We clarify this issue by showing that *HEM1* is subject to two additional layers of control that mask regulatory changes. First is a second activation system acting at a site close to the *HAP2–HAP3* target sequence that keeps *HEM1* turned on under conditions of heme deficiency. Second is a regulated negative control site downstream of the upstream activation site that counteracts derepression in medium containing a nonfermentable carbon source. Thus, transcription of the constitutive gene is actually a composite of opposing regulatory sites. This complex regulatory arrangement may exist to allow *HEM1* to be coordinated transiently with apocytochromes for transition to respiratory growth. Conversely, it may reflect the alteration of *HEM1* from a regulated to a constitutive gene over evolution.

Regulated genes typically encode products that are required when cells grow under specific physiological conditions, or, in multicellular organisms, that are expressed in particular cell types. Constitutive genes encode products required in the maintenance of basic cellular processes or architecture. In some cases, the product of a single gene may be related to more than one function, encompassing both regulated and constitutive processes.

One example of such a gene is the *HEM1* locus of *Saccharomyces cerevisiae* encoding 5-aminolevulinate synthase. This enzyme catalyzes the first step of the heme biosynthetic pathway (1–3). The pathway also provides precursors for the synthesis of vitamin B_{12} precursors and siroheme (reviewed in ref. 4). Heme is involved in the maturation of cytochromes and other hemoproteins, while siroheme is the prosthetic group of sulfite reductase, which is involved in sulfur assimilation and biosynthesis of cysteine and methionine. Cytochromes are expressed at low levels in the absence of respiration (5). Thus, the need for heme is greatest when cells are grown in an aerobic environment, especially when utilizing a nonfermentable carbon source (6). The need for siroheme and B_{12} precursors is essentially constitutive when these compounds are not supplied in the medium.

In yeast, the regulation of cytochrome synthesis by oxygen and carbon source has been extensively studied. Transcription of the *CYC1* gene encoding the iso-1-cytochrome *c* is induced about 100-fold by oxygen and an additional 10-fold by shifting from glucose medium to one containing a nonfermentable carbon source. Heme itself is the effector that mediates induction by oxygen. In the absence of oxygen, enzymes late in the heme biosynthetic pathway, which are oxygenases, do not function, and heme is not made (4, 7).

Regulation of *CYC1* is mediated by tandem upstream activation sites 1 and 2 (UAS1 and UAS2) (8). UAS1 is activated by the *HAP1* gene product, which binds to the site in a heme-dependent manner *in vitro* (9). UAS2 activity requires both the *HAP2* and *HAP3*, gene products. The *HAP2–HAP3* system appears to regulate respiratory genes globally, since mutations in either locus render cells unable to grow on nonfermentable carbon sources. Transcription of at least one other respiratory gene, *COX4*, encoding subunit 4 of cytochrome oxidase, has been shown to require *HAP2* and *HAP3* gene products (C. Schneider and L.G., unpublished data). The activity of both UAS2 and the *COX4* UAS is regulated by heme and carbon source.

Under conditions in which cells are shifted from an anaerobic to an aerobic environment or from a medium containing glucose to one containing a nonfermentable carbon source, it might be advantageous for cells to induce the synthesis of apocytochromes and heme coordinately. As the gene encoding the first step in heme biosynthesis, *HEM1* would be a presumed target of such regulation. Alternatively, the requirement for the *HEM1* gene product in the synthesis of B_{12} precursors and siroheme is constant. How does a cell balance these opposing needs?

In this report we show that, indeed, *HEM1* expression is fairly constitutive under all steady-state conditions tested. However, paradoxically, *HEM1* expression is regulated by the *HAP2–HAP3* system. The steady-state regulation by oxygen and carbon source, typical of *HAP2–HAP3* control at UAS2 and *COX4*, is masked in the case of *HEM1* by additional layers of control. The molecular mechanisms of these additional forms of regulation are inferred from an analysis of elements that act in cis to control *HEM1* expression. Thus, the constitutive expression of *HEM1* is actually a composite of multiple control systems.

MATERIALS AND METHODS

Strains. *S. cerevisiae* strain BWG1-7a (*MAT***a** *leu2-3 leu2-112 his4-519 ade1-100 ura3-52*) was the wild-type strain used in this study. LGW32 (*hap1-1*), LGW1 (*hap2-1*), and JP40-1 (*hap3-1*) are isogenic derivatives of BWG1-7a. Strain TP7H$^-$L$^+$, derived from BWG1-7a by insertion of *LEU2* at *HEM1*, was obtained from Toni Prezant (Massachusetts Institute of Technology, Cambridge). LG40 is a *hem1* mutant of BWG 9A-1 (8), a strain related to 1-7A.

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Abbreviation: UAS, upstream activation site.

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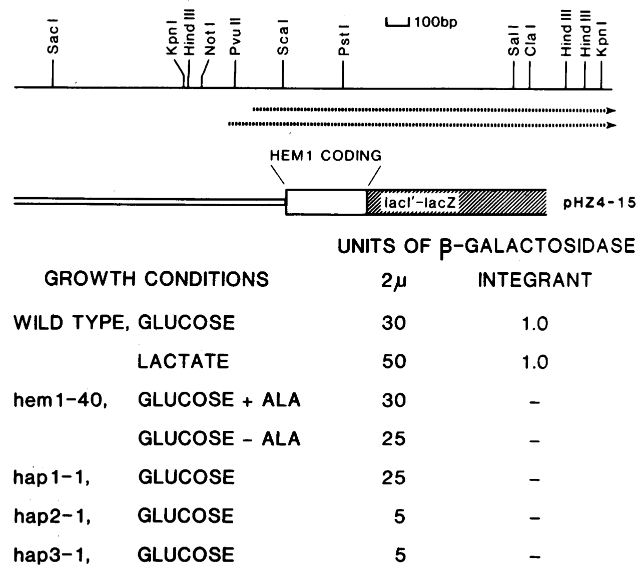


GROWTH CONDITIONS		UNITS OF β-GALACTOSIDASE 2μ	INTEGRANT
WILD TYPE,	GLUCOSE	30	1.0
	LACTATE	50	1.0
hem1-40,	GLUCOSE + ALA	30	-
	GLUCOSE - ALA	25	-
hap1-1,	GLUCOSE	25	-
hap2-1,	GLUCOSE	5	-
hap3-1,	GLUCOSE	5	-

FIG. 1. Full-level expression of *HEM1-lacZ* is dependent on *HAP2* and *HAP3*. A restriction map of the *HEM1* coding region is diagramed above together with the positions of the two RNA initiation sites. The *HEM1* coding and 5′ noncoding region present on pHZ4-15 are represented by open bars. The wild-type strain used was BWG1-7a. The *hem1* strain used was either LG40 or TP7H$^-$L$^+$. Bars indicate values not determined. ALA, 5-aminolevulinate.

Media and Assays. Cells were grown in synthetic medium containing yeast nitrogen base without amino acids to an OD_{600} of about 1.0 for RNA extractions or β-galactosidase assays as described (10). 5-Aminolevulinate was added at 0.5 μg/ml (low heme) or 50 μg/ml (high heme). Cells were transformed by the lithium acetate method (11). β-Galactosidase assays were performed as described (12).

RNA Isolation and Mapping of 5′ Ends of HEM1 mRNA. Total RNA was isolated by glass-bead disruption and phenol extraction (13). The 5′ ends of HEM1 mRNA were mapped by primer extension of an oligonucleotide primer complementary to the *lacI′* sequence approximately 67 nucleotides (pHZ328) or 55 nucleotides (pHZB7) 3′ to the start of translation; 5 ng of ^{32}P-end-labeled primer was hybridized to 20 or 50 μg of total yeast RNA for primer extension (14).

DNA Sequencing and Manipulations. The DNA sequence of the *HEM1* upstream region was determined by the chain-termination method as modified by Biggin *et al.* using M13 phages mp18 and mp19 to generate single-stranded templates (15).

5′ deletion derivatives were derived from the parent plasmid pHZ4-15, which contains 2 kilobases (kb) of *HEM1* upstream DNA and 112 codons of *HEM1* fused to *lacZ* (3). pTK1448, pTK1427, pTK1392, pTK1367, and pTK1223 were constructed by standard recombinant DNA techniques and were identical to pHZ4-15 except that they now contain only 448, 427, 392, 367, or 223 base pairs (bp) upstream of the *HEM1* translational start site, respectively (16).

The *HEM1-CYC1* hybrid promoter fusions all contain a *CYC1-lacZ* fusion and 247 bp of *CYC1* upstream DNA. These plasmids are missing the sequences that constitute the *CYC1* UAS but have all the "TATA" and RNA transcription initiation sites (6). All *HEM1* sequences are inserted in the same orientation with respect to the TATA and initiation elements, as they are upstream of *HEM1*. pTK1011 was constructed by converting the unique *Not* I site at −367 into a *Xho* I site with the insertion of *Xho* I octamer linkers and ligating the *Kpn* I–*Xho* I fragment (−448 to −367) to a *Kpn* I–*Xho* I backbone provided by pLGΔ265Kpn. pTK1012 was constructed in a similar manner by converting the *Xmn* I site at −310 into a *Xho* I site and contains the sequences from −448 to −310 upstream of the *HEM1* coding region.

The *CYC1*-UAS2-*HEM1* hybrid promoter fusion, pTK-1050, was constructed by ligating the *Not* I (filled-in)–*Sac* I fragment from pTK1012, extending from −367 through part of *lacZ* to the *Xho* I (filled-in)–*Sac* I backbone from pLGΔ229UP1. This plasmid contains a *CYC1-lacZ* fusion driven by UAS2UP1, a derivative of UAS2 containing a G→A transition increasing the activity of the site about 10-fold (8). The resulting plasmid contains the *Not* I–*Xmn* I fragment (−367 to −310) from the 5′ noncoding region of *HEM1* inserted between UAS2UP1 of *CYC1* and the *CYC1* TATA and initiation sequences.

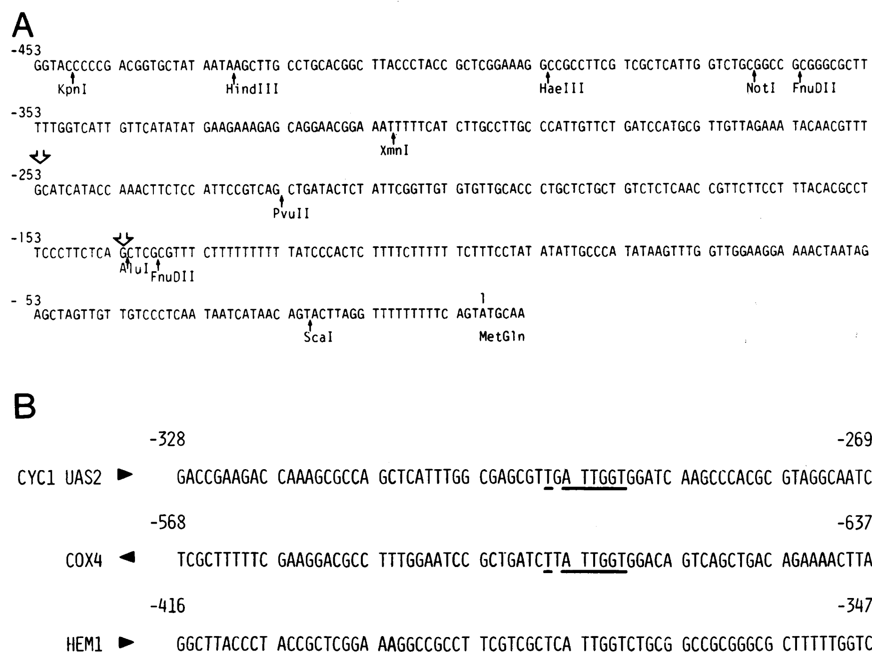


FIG. 2. (*A*) DNA sequence of the 5′ noncoding region of *HEM1*. The 5′ ends of the RNAs are marked by open arrows. (*B*) DNA sequence homologies between the *HEM1* regulatory region and those of *CYC1* UAS2UP1 and *COX4*. The homologies are underlined.

RESULTS

Regulation of *HEM1*. To examine the regulation of the *HEM1* gene of *S. cerevisiae*, we used a fusion to the *lacZ* gene of *Escherichia coli* that has been described (3). The fusion contains 112 codons of *HEM1* fused to *lacZ* and is preceded by 2 kb of yeast DNA upstream of the *HEM1* gene. We studied this fusion either as a part of an autonomous plasmid bearing the 2-μm origin of replication or integrated into the *HEM1* locus of the yeast chromosome.

The levels of β-galactosidase activity expressed from the plasmid, pHZ4-15, were measured in synthetic glucose or lactate media. There was at most a 2-fold increase in the level of β-galactosidase activity in the nonfermentable carbon source (Fig. 1). The same results were obtained using strains in which the *HEM1-lacZ* fusion had been integrated at the *HEM1* locus. Integration occurred by a single crossover between a plasmid bearing the fusion and genomic DNA, generating an intact *HEM1* locus and the fused gene.

The role of heme in the expression of *HEM1* was next investigated. pHZ4-15 was introduced into an isogenic *hem1* strain. There was no change in the level of β-galactosidase activity observed in the presence or absence of the heme supplement 5-aminolevulinate, indicating that the *HEM1* expression is not heme-dependent and that heme does not play a negative regulatory role in *HEM1* expression (Fig. 1). Further, supplying the amino acids cysteine and methionine in the form of Casamino acids did not alter the level of expression (not shown).

Gene Products Required in trans for *HEM1* Expression. We next examined whether the *HAP1*, *HAP2*, or *HAP3* loci, shown to encode positive activators of transcription of cytochromes, played a role in *HEM1* expression. The levels of *HEM1-lacZ* expression were found to be reduced by a factor of 5–6 by mutations in *HAP2* (*hap2-1*) (8) or *HAP3* (*hap3-1*) (S. Hahn, personal communication) (Fig. 1). In contrast, the *hap1-1* mutation had no effect on expression. Thus, *HEM1* appears to be a member of the group of respiratory functions regulated by the *HAP2–HAP3* global activation system. However, unlike *CYC1* and *COX4*, expression of *HEM1*, as shown above, is not regulated by the carbon source or heme levels, raising the possibility that the *HAP2–HAP3* effect on heme expression is indirect. Analysis of UAS2 and the *COX4* UAS revealed a consensus sequence, TNATTGGT (N can be any nucleotide), that is required for *HAP2–HAP3* responsiveness (S. Forsburg, C. Schneider, and L.G., unpublished data). The sequence of DNA upstream of *HEM1*, shown in Fig. 2*A*, was compared to UAS2 and the *COX4* UAS (Fig. 2*B*). The only homology that could be observed in all three sequences was the consensus TNATTGGT at approximately −379 in *HEM1*. The importance of this sequence has been underscored by the G→A mutation in UAS2 (to form UAS2UP1), which converts TNGTTGGT to TNATTGGT, and which increases the activity of the site 10-fold (8). This sequence homology between *HEM1* and other genes regulated by *HAP2–HAP3* strengthens the notion that *HAP2–HAP3* is directly involved in the activation of *HEM1* expression. *In vitro* binding experiments using partially purified HAP3–β-galactosidase fusion protein (S. Hahn, J. Olesen, and L.G., unpublished data) show that the protein binds to these *HEM1* sequences (J. Olesen, S. Hahn, and L.G., unpublished data).

Defining the Functional *HEM1* UAS. To determine whether the sequence mentioned above was functional and to uncover other possible sites mediating *HEM1* control, we undertook a mutational analysis of the *HEM1* upstream activation region. We first determined the *HEM1* transcription initiation sites by primer extension, using a primer that is specific for *HEM1-lacZ* (see the legend to Fig. 3). Fig. 3 *B* and *C* show that there are two major clusters of transcription initiation

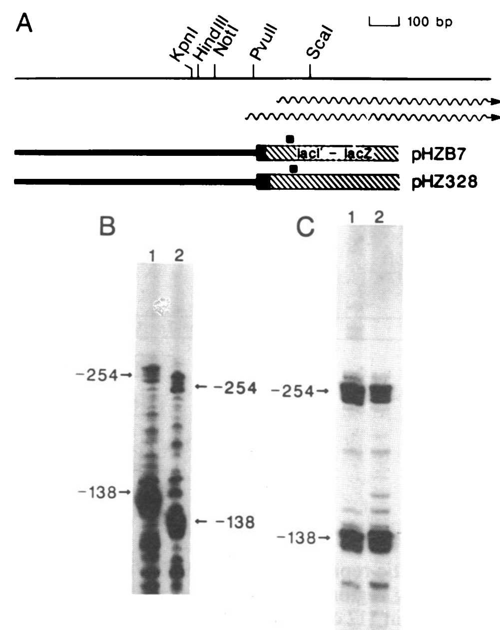
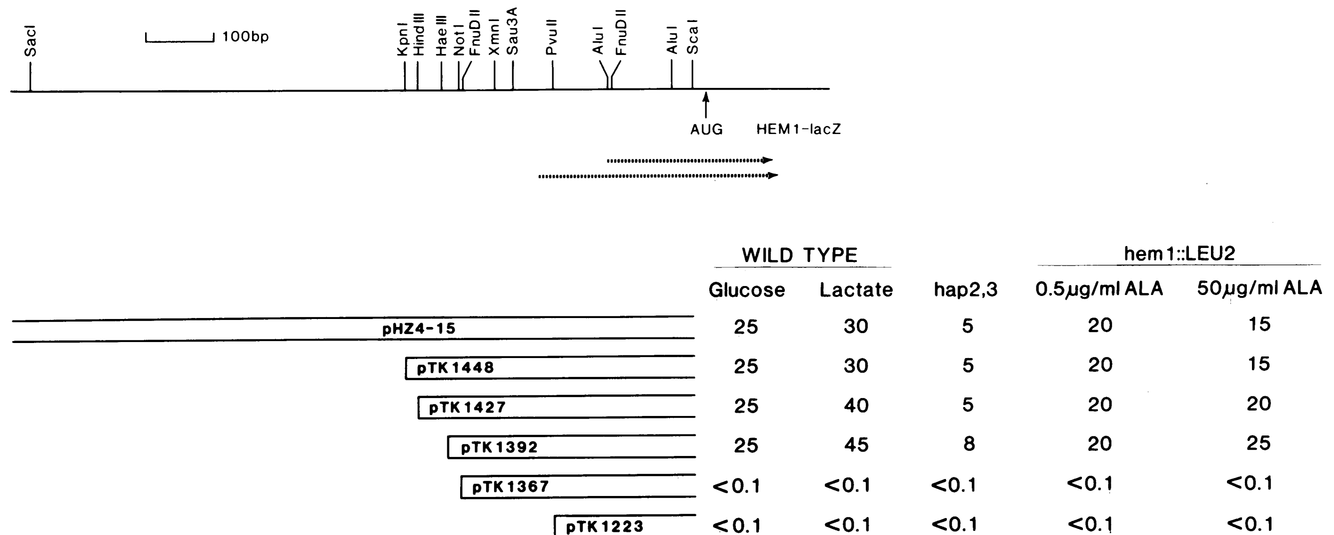


FIG. 3. Mapping of *HEM1* transcription initiation sites. RNA was extracted from glucose-grown BWG1-7a cells containing (*i*) plasmid pHZB7, a multicopy plasmid containing 2 kb of yeast DNA upstream of *HEM1* and five codons of HEM1 fused to *lacI'-lacZ*, or (*ii*) plasmid pHZ328, a similar plasmid with nine codons of *HEM1* fused to *lacI'-lacZ* (3, 13). The 5' ends of the mRNAs were determined by primer extension of an end-labeled oligonucleotide complementary to the lacI' sequence in the fusions. (*A*) Map of part of the *HEM1* coding region. The two *HEM1-lacZ* fusions used in these studies are drawn. Black bars represent the region of *HEM1* coding and 5' noncoding region present on the plasmids. The black squares over the bars represent the oligonucleotide and are positioned above the region of complementary sequence. (*B*) The reactions include 50 μg of RNA isolated from BWG1-7a containing pHZ328 (lane 1) or pHZB7 (lane 2). (*C*) Primer extensions using 20 μg (lane 1) or 50 μg (lane 2) of RNA isolated from BWG1-7a containing pHZ328. The reactions were sized against a sequencing ladder. The numbers indicate positions relative to the translational start.

sites, mapping about 143 and 253 nucleotides upstream of the translation initiation codon. Identical results were obtained for two different *HEM1-lacZ* fusions bearing different extents of *HEM1* coding sequences.

Next, the 5' boundary of the UAS of *HEM1* was defined by making deletions of sequences upstream of the *HEM1-lacZ* fusion on plasmid pHZ4-15. Deletion of all sequences upstream of the *Hae* III site at 392 nucleotides upstream of the coding region (pTK1392) did not result in any changes in the levels of β-galactosidase activity (see Fig. 4). These deletion mutants also responded in the same manner as their parent plasmid pHZ4-15 to *hap2-1* and *hap3-1* mutations. In addition, like the parent plasmid, the expression of β-galactosidase activity from the deletion plasmids was unaffected by the levels of heme precursor. However, deletions of sequences upstream of the *Not* I site at −367 (pTK1367) resulted in a decrease of >2 orders of magnitude in β-galactosidase activity (Fig. 4). These results place the 5' boundary of the UAS of *HEM1* to between −392 and −367.

Activation of a Heterologous Gene by *HEM1* Upstream Segments. To define the 3' boundary of the *HEM1* UAS, we chose to place segments of *HEM1* DNA at a site upstream of the *CYC1* TATA box–mRNA initiation region of pLGΔ-178 (Fig. 5). This site resides 247 nucleotides upstream of the translational start of a *CYC1-lacZ* fusion and has been used to identify and characterize numerous yeast regulatory sequences for UAS activity. These sequences will activate transcription initiation at the *CYC1* initiation sites in a



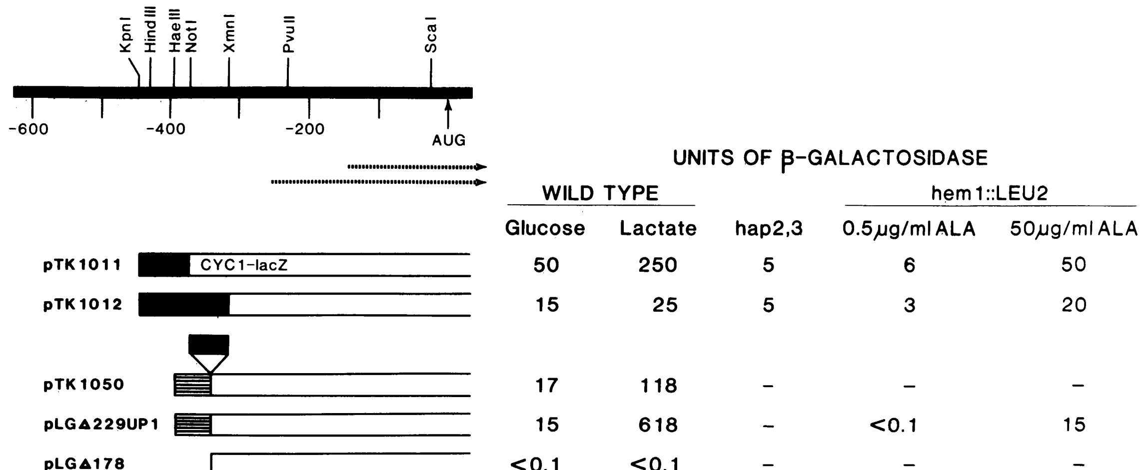
	WILD TYPE			hem1::LEU2	
	Glucose	Lactate	hap2,3	0.5μg/ml ALA	50μg/ml ALA
pHZ4-15	25	30	5	20	15
pTK1448	25	30	5	20	15
pTK1427	25	40	5	20	20
pTK1392	25	45	8	20	25
pTK1367	<0.1	<0.1	<0.1	<0.1	<0.1
pTK1223	<0.1	<0.1	<0.1	<0.1	<0.1

FIG. 4. Schematic representations of *HEM1-lacZ* fusions constructed and the levels of β-galactosidase activity observed. A restriction map of the 5′ noncoding region of *HEM1* is represented together with the positions of the RNA initiation sites. All plasmids were derived from pHZ4-15 and contain 112 codons of *HEM1* fused to *lacZ*. DNA sequences present on the plasmids are represented by open bars. The wild-type strain used was BWG1-7a. The *hem1*::*LEU2* strain used was TP7H$^-$L$^+$.

manner subject to regulation encoded by the UAS. This approach allows us to determine whether the isolated *HEM1* UAS has all of the regulatory properties of the intact promoter.

Thus, the *HEM1* fragments from −448 to −367 (pTK1011) and −448 to −310 (pTK1012) were inserted into pLGΔ-178 and found to activate *CYC1-lacZ* expression in a *HAP2–HAP3*-stimulated manner. However, two differences were noted in the expression driven by the *HEM1* segment when compared to that driven by UAS2UP1 (Fig. 5). First, the *HEM1* segment retained partial activity in the absence of heme, and, second, it retained partial activity in the absence of *HAP2* or *HAP3* gene products. This residual activity in *hap2* or *hap3* mutants was also observed and was somewhat greater in the intact *HEM1* promoter (Fig. 1). We infer, from these results, that the *HEM1* UAS contains sequences that can be activated by a system other than *HAP2–HAP3*. This system could maintain expression of *HEM1* in the absence of heme.

A Negative Site Rendering *HEM1* Constitutive with Respect to the Carbon Source. Although pTK1011 and pTK1012 were similar in their responses to heme deficiency or mutations in *HAP2* or *HAP3*, they differed strikingly in their ability to be derepressed by shift to media containing a nonfermentable carbon source. While pTK1011 was derepressed about 5-fold by such a shift, similar to derepression observed for UAS2, pTK1012 did not derepress significantly (Fig. 5). This result raised the possibility that the additional sequences in pTK-1012 (between −367 and −310) contained a negative site that was active in preventing derepression. To test this possibility, a DNA segment encompassing DNA sequences between −366 and −310 was inserted between UAS2UP1 and a *CYC1-lacZ* fusion (Fig. 5). Although the presence of these sequences had no effect on expression in glucose, it inhibited derepression of UAS2UP1 in lactate medium by a factor of 5. Insertion of random DNA fragments of similar lengths between the UAS and TATA sequences of *CYC1* has been shown to have minimal effects on expression and derepres-



	WILD TYPE			hem1::LEU2	
	Glucose	Lactate	hap2,3	0.5μg/ml ALA	50μg/ml ALA
pTK1011	50	250	5	6	50
pTK1012	15	25	5	3	20
pTK1050	17	118	–	–	–
pLGΔ229UP1	15	618	–	<0.1	15
pLGΔ178	<0.1	<0.1	–	–	–

FIG. 5. β-Galactosidase activities measured from *CYC1-HEM1* hybrid promoter fusion plasmids. A map of the upstream region of *HEM1* is represented together with the translational start and transcription initiation sites. The plasmids constructed were introduced into wild-type strain BWG1-7a, *hap2-1* or *hap3-1* mutant strain, or *hem1*::*LEU2* strain TP7H$^-$L$^+$. The filled bars represent *HEM1* DNA sequences present in the hybrid promoter fusions. The *CYC1-lacZ* fusion is represented by an open bar. Horizontally striped regions represent the UAS2UP1 of *CYC1*.

sion of the gene (17). Thus, we conclude that *HEM1* contains a site just downstream from the UAS that prevents derepression in medium containing a nonfermentable carbon source.

DISCUSSION

The surprising finding reported here is that the constitutive *HEM1* gene is actually regulated by a pair of oppositely regulated sites, one positive and the other negative. The positive UAS site, located between −392 and −368 is activated by the *HAP2–HAP3* system known to activate expression of several genes encoding apocytochromes. However, *HEM1* expression, as previously noted (2, 18), does not display derepression by nonfermentable carbon sources typical of other genes regulated by *HAP2–HAP3* (ref. 8; C. Schneider and L.G., unpublished data). The inability of *HEM1* to derepress cannot be due to the TATA box–mRNA initiation region because a high level of galactose-inducible expression of a *HEM1-lacZ* fusion can be obtained by the positioning of the UAS of *GAL1-10* immediately upstream (3). Rather, derepression is prevented by a negative element that lies between −366 and −310. When this segment was placed downstream of the UAS2 of the *CYC1* gene in pTK1050 (Fig. 5), it reduced derepression of that site by a factor of 5. When positioned downstream of the *HEM1* UAS in pTK1012, the fragment prevented derepression, while exerting a modest reduction in activity in glucose medium. Thus, we conclude that the −366 to −310 fragment is a negative control site that mediates repression, which is greatest when cells grow in medium containing a nonfermentable carbon source. Why this repression site is not completely effective when adjacent to UAS2 is not clear but may be related to the higher derepressed activity of that site as compared with the *HEM1* UAS.

Further, also unlike other genes activated by *HAP2–HAP3*, *HEM1* is transcribed under conditions of heme deficiency. Transcription of *HEM1*, under heme-sufficient or heme-deficient conditions, requires the −392 to −368 UAS. Like other sites that respond to *HAP2–HAP3*, this segment contains the sequence TNATTGGT. A hybrid promoter containing the *HEM1* UAS and *CYC1* TATA region, while able to respond to *HAP2-3*, maintained a substantial basal level of expression under conditions of heme deficiency or in strains mutant in *HAP2* or *HAP3*. Thus, we believe that this UAS contains sequences that respond to an activation system that is independent of *HAP2–HAP3* and that functions in the absence of heme. Whether the sequences recognized by this system are identical to or overlap the *HAP2–HAP3* responsive element cannot be deduced as yet. We do not know why the residual activity under heme-deficient conditions is higher for the intact *HEM1* promoter (Fig. 1) than for the hybrid construct (Fig. 5). We presume that *HEM1* sequences downstream of −367, missing in the hybrid construct, aid the UAS region in the maintenance of this residual activity.

Why does the *HEM1* promoter contain both a UAS and an opposing negative site? As detailed in the Introduction, the position of the *HEM1* gene product in biosynthesis dictates that its expression remain relatively constitutive under all growth conditions. The increased rate of heme synthesis under derepressed conditions is probably due to a lifting of feedback inhibition of 5-aminolevulinate synthase by heme when the latter is bound by apocytochromes (19). However, this increase in 5-aminolevulinate synthase activity may not be sufficient when cells are making the transition to medium containing a nonfermentable carbon source. In this period, cells may coordinate the derepression of apocytochromes and heme synthesis by the *HAP2–HAP3* system. Consistent with this idea, Mahler and Lin have found that the expression of the *HEM1* product is transiently induced when cells are shifted from medium containing glucose to one containing a nonfermentable carbon source (18). Positive and negative sites have also been reported for the yeast *CYC7* promoter (20). In this case, the role of the negative site does not seem to be regulatory but rather to reduce overall levels of *CYC7* transcription under repressed or derepressed conditions.

It is possible that a variety of eukaryotic genes whose products are synthesized constitutively under steady-state conditions will be regulated by a composite set of control systems. One such example described here involves both a UAS and a site of negative control that are regulated inversely. In such a case, regulation characteristic of the activation system acting at the UAS will be masked. Thus, it may be difficult to discern what genes fall under the control of a common activation system without a genetic or biochemical handle on the activator itself.

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1. Bard, M. & Ingolia, T. D. (1984) *Gene* **28**, 195–199.
2. Urban-Grimal, D., Robes, V. & Labbe-Bois, R. (1984) *Curr. Genet.* **8**, 327–331.
3. Keng, T., Alani, E. & Guarente, L. (1986) *Mol. Cell. Biol.* **6**, 355–364.
4. Tait, G. H. (1978) in *Heme and Hemoproteins*, eds. DeMatteis, F. & Aldridge, W. N. (Springer, New York), pp. 1–48.
5. Sherman, F. & Stewart, J. (1981) *Annu. Rev. Genet.* **5**, 257–296.
6. Guarente, L. & Mason, T. (1983) *Cell* **32**, 1279–1286.
7. Mattoon, J. R., Lancashire, W. E., Sanders, H. K., Carvajal, E., Malamud, D., Brag, G. R. C. & Panek, A. D. (1979) in *Biochemical and Clinical Aspects of Oxygen*, ed. Caughey, W. J. (Academic, New York), pp. 421–435.
8. Guarente, L., Lalonde, B., Gifford, P. & Alani, E. (1984) *Cell* **36**, 503–511.
9. Pfeifer, K., Arcangioli, B. & Guarente, L. (1987) *Cell*, in press.
10. Sherman, F., Fink, G. R. & Hicks, J. B. (1983) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
11. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153**, 163–168.
12. Guarente, L. (1983) *Methods Enzymol.* **101**, 181–191.
13. Teem, J. & Rosbash, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4403–4407.
14. Hahn, S., Hoar, E. & Guarente, L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8562–8566.
15. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3963–3965.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Guarente, L. & Hoar, E. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7860–7864.
18. Mahler, H. R. & Lin, C. C. (1974) *Biochem. Biophys. Res. Commun.* **61**, 963–970.
19. Malamud, D. R., Dadrao, J. R. B., Borralho, L. M., Arrese, M., Panek, A. D. & Mattoon, J. R. (1983) *Braz. J. Med. Biol. Res.* **16**, 203–213.
20. Wright, C. & Zitomer, R. (1984) *Mol. Cell. Biol.* **4**, 2023–2030.