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Positive and Negative Transcriptional Control by Heme of Genes Encoding 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase in *Saccharomyces cerevisiae*

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Responses of the yeast genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase, *HMG1* and *HMG2*, to in vivo changes in heme concentrations were investigated. Expression of the genes was determined by direct measurement of the mRNA transcribed from each gene, by direct assay of the enzyme activity encoded by each gene, and by measurement of the expression of *lacZ* fusions to the control regions of each gene. These studies indicated that expression of *HMG1* was stimulated by heme, whereas expression of *HMG2* was repressed by heme. The effect of heme on *HMG1* expression was mediated by the *HAP1* transcriptional regulator and was independent of *HAP2*. Thus, the genes encoding the 3-hydroxy-3-methylglutaryl coenzyme A reductase isozymes join a growing list of gene pairs that are regulated by heme in opposite ways.

In the yeast *Saccharomyces cerevisiae*, expression of a number of genes is regulated by the level of oxygen in the environment. Many of the genes whose expression is regulated by oxygen encode proteins involved in cellular respiration, such as the cytochromes (15, 21, 50, 55). Other oxygen-regulated genes include *PUT1*, which encodes a component of the oxygen-dependent proline utilization pathway (54) as well as several genes of unknown function (29). Oxygen increases the expression of some genes and decreases the expression of others (29, 51). In several cases, the effects of oxygen on gene expression are mediated by heme, a molecule whose synthesis requires molecular oxygen (33). Three genes, *HAP1* (*CYP1*), *HAP2*, and *HAP3*, encode transcription factors that serve to activate the expression of a number of oxygen-regulated genes (39–41). The ability of *HAP1* to activate transcription is heme dependent (15, 17, 53). The *REO1* and *ROX1* genes encode proteins that act to repress the expression of genes in aerobically grown cells (28, 30, 50). Thus, expression of oxygen-regulated genes is controlled by a set of oxygen-sensitive transcription factors.

In addition to respiration, another cellular function that occurs only in aerobically growing cells is the biosynthesis of sterols. Certain steps in the sterol biosynthetic pathway require heme and molecular oxygen (13, 19). In many organisms, sterol biosynthesis is controlled in part by regulating the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the rate-limiting step in sterol biosynthesis (reviewed in reference 8). HMG-CoA reductase activity has been reported to be higher in aerobically grown cells than in anaerobically grown cells (7, 43). Studies with the yeast *S. cerevisiae* found HMG-CoA reductase activity to be lower in heme-deficient cells than in heme-sufficient cells, suggesting that heme acts as a positive regulator of HMG-CoA reductase (27). Since heme can act as a signal of oxygen levels in yeast cells, heme levels may mediate the effect of oxygen on the expression of the HMG-CoA reductase genes.

Yeast cells contain two structural genes for HMG-CoA reductase, *HMG1* and *HMG2* (6). Yeast genes that occur in pairs often have different patterns of regulation. In this study, the effect of heme levels on expression of the two isozymes of HMG-CoA reductase was evaluated by using *lacZ* gene fusions to *HMG1* and *HMG2*. The regulation of the intact HMG-CoA reductase genes was also monitored by mRNA analysis and HMG-CoA reductase assays. The results indicated that heme levels mediated transcriptional control of *HMG1* and *HMG2* and that heme stimulated expression of *HMG1* and decreased expression of *HMG2*.

MATERIALS AND METHODS

Materials. [α-^{32}P]dCTP (800 Ci/mmol), 3-hydroxy-3-methyl[3-^{14}C]glutaryl ([^{14}C]HMG)-CoA (52 mCi/mmol), and the random priming kit for radiolabeling DNA probes were from Amersham Corp. (Arlington Heights, Ill.). Nitrocellulose and Nytran filters were from Schleicher & Schuell, Inc. (Keene, N.H.) Hemin type I (bovine) and δ-aminolevulinic acid (δ-ALA) were from Sigma Chemical Co. (St. Louis, Mo.).

Strains. The yeast strains used are described in Table 1. Plasmid pTP*hem1*::*LEU2* (42) was used to introduce the *hem1*::*LEU2* disruption mutation into JRY438 by one-step gene disruption methods (46). The predicted structure of the disrupted *HEM1* locus in the resulting strain, JRY1236, was confirmed after disruption by a gel transfer hybridization experiment.

Strains carrying the *hem1*::*LEU2* disruption were used to study the role of heme in the expression of the *HMG1* and *HMG2* genes. The *HEM1* gene encodes the enzyme δ-aminolevulinate synthase, and the *hem1*::*LEU2* disruption blocks the synthesis of the heme precursor δ-ALA. Supplementation of media with either a low (0.5 μg/ml) or a high (50 μg/ml) concentration of δ-ALA allowed growth of *hem1* strains. However, cells grown in low δ-ALA were heme deficient, whereas cells grown in high δ-ALA were heme sufficient (42).

The effect of the *hem1*::*LEU2* mutation on *HMG1* and *HMG2* expression was determined by using the *HMG1-lacZ* and *HMG2-lacZ* gene fusions. The *hem1*::*LEU2* mutation was introduced into strains carrying these *lacZ* fusions as

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TABLE 1. Strains used

Strain[a]	Genotype	*lacZ* fusion[b]
JRY438	α *his4-519 leu2-3,112 ura3-52*	None
JRY527	**a** *his3Δ200 lys2-801 ade2-101 met ura3-52*	None
JRY528	α *his3Δ200 lys2-801 ade2-101 tyr1 ura3-52*	None
JRY986	α *his3Δ200 lys2-801 ade2-101 met tyr1 ura3-52 hmg2::HIS3*	None
JRY1159	**a** *his3Δ200 lys2-801 ade2-101 met ura3-52 hmg1::LYS2*	None
JRY1160	**a** *his3Δ200 lys2-801 ade2-101 met ura3-52 hmg2::HIS3*	None
JRY1236	α *his4-519 leu2-3,112 ura3-52 hem1::LEU2*	None
JRY1314	JRY527	pJR436
JRY1443	JRY527	*HMG1-lacZ*
JRY1751	JRY527	*HMG1-lacZ*
JRY1775	JRY527	*HMG2-lacZ*
JRY2041	**a** *his3Δ200* and/or *his4-519 ade2-101 met ura3-52 leu2-3,112*	*HMG1-lacZ*
JRY2042	**a** *his3Δ200* and/or *his4-519 lys2-801 ura3-52 hem1::LEU2*	*HMG1-lacZ*
JRY2060	α *his4-519 leu2-3,112 ura3-52*	*HMG2-lacZ*
JRY2063	JRY2060 with *hem1::LEU2*	*HMG2-lacZ*
JRY2076	**a** *ade1-100 his4-519 ura3-52 leu2-3,112*	*HMG1-lacZ*
JRY2077	**a** *ade1-100 his4-519 ura3-52 leu2-3,112*	*HMG2-lacZ*
JRY2079	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap1::LEU2*	*HMG1-lacZ*
JRY2080	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap1::LEU2*	*HMG2-lacZ*
JRY2082	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap2::LEU2*	*HMG1-lacZ*
JRY2083	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap2::LEU2*	*HMG2-lacZ*
JRY2090	α *lys2-801 met ade2-101 ura3-52*	None
JRY2091	α *his3Δ200* and/or *his4-519 ade2-101 met ura3-52 leu2 hem1::LEU2*	None
JRY2092	**a** *his3Δ200* and/or *his4-519 lys2-801 ura3-52 leu2 hem1::LEU2*	None
JRY2093	**a** *his3Δ200* and/or *his4-519 ura3-52*	None
JRY2098	α *his3Δ200 ade2-101 met ura3-52 hmg1::LYS2 hem1::LEU2*	None
JRY2100	α *his3Δ200 ade2-101 met ura3-52 leu2 hmg1::LYS2*	None
JRY2101	α *his3Δ200 ade2-101 met ura3-52 hmg1::LYS2 hem1::LEU2*	None
JRY2102	**a** *his3Δ200 ade2-101 met ura3-52 leu2 hmg1::LYS2*	None
JRY2103	**a** *his3Δ200 ade2-101 met ura3-52 hmg1::LYS2 hem1::LEU2*	None
JRY2108	α *lys2-801 met ura3-52 hmg2::HIS3 hem1::LEU2*	None
JRY2110	**a** *lys2-801 met ura3-52 hmg2::HIS3*	None
JRY2111	α *lys2-801 ade2-101 met ura3-52 hmg2::HIS3 hem1::LEU2*	None
JRY2112	α *lys2-801 met ura3-52 hmg2::HIS3 hem1::LEU2*	None
JRY2113	**a** *lys2-801 ade2-101 met ura3-52 hmg2::HIS3*	None
BWG1-7a	**a** *ade1-100 his4-519 ura3-52 leu2-3,112*	None
BWG1-7a	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap1::LEU2*	None
BWG1-7a	**a** *ade1-100 his4-519 ura3-52 leu2-3,112 hap2::LEU2*	None

[a] Plasmid pTL*hem1::LEU2*, used to construct JRY1236, was obtained from T. Prezant and L. Guarente. The isogenic BWG1-7a series was obtained from L. Guarente.

[b] Unless otherwise noted, *HMG1-lacZ* fusions were integrated at *HMG1* by using plasmid pJR502. All *HMG2-lacZ* fusions were on the multicopy plasmid pJR550.

follows. For the *HMG1-lacZ* fusion, a diploid heterozygous for the *HMG1-lacZ* fusion and for the *hem1::LEU2* disruption (JRY1443 × JRY1236) was sporulated. Expression of *HMG1-lacZ* was characterized in six *HEM1* and six *hem1* segregants from this diploid. JRY2041 (*HEM1*) and JRY2042 (*hem1*) were segregants from one tetrad of this cross, and expression of *HMG1-lacZ* in these strains was representative of expression in the initial group of 12 segregants. For the *HMG2-lacZ* fusion, isogenic *HEM1* (JRY438) and *hem1::LEU2* (JRY1236) strains were transformed with the *HMG2-lacZ* fusion plasmid pJR550 to generate the strains JRY2060 (*HEM1*) and JRY2063 (*hem1::LEU2*). Additional *HEM1* and *hem1* strains carrying *HMG2-lacZ* were isolated as segregants from a diploid made by crossing JRY1775, which carried the *HMG2-lacZ* fusion, with JRY1236. Expression of *HMG2-lacZ* was characterized in six tetrads from this cross as well as in JRY2060 and JRY2063. Expression of *HMG2-lacZ* in JRY2060 and JRY2063 was representative of expression in the other *HEM1* and *hem1* strains studied.

Media and genetic methods. Yeast minimal medium (YM) containing 2% glucose and sporulation media were prepared as described previously (3). Amino acids and base supplements were added, when needed, at 30 μg/ml. Solid media contained 2% agar. Standard genetic manipulations were performed as described previously (35). Yeast cells were transformed by the method of Hinnen et al. (20) except that spheroplasts were prepared with lyticase, a gift from the laboratory of R. Schekman. A 5-mg/ml stock solution of δ-ALA was made in water and used in media at a concentration of 50 μg/ml (high δ-ALA) or 0.5 μg/ml (low δ-ALA). A 3.75-mg/ml stock solution of hemin type I, an oxidized form of heme, was made in 50% ethanol–20 mM sodium hydroxide and used at a final concentration of 13 μg/ml. Cells were grown aerobically at 30°C with vigorous shaking in tubes or flasks containing a volume of medium less than one-fourth the total container volume.

Plasmid constructions. For construction of pJR436, a single-copy plasmid carrying an *HMG1-lacZ* fusion at the first ATG of the *HMG1* open reading frame, the 0.25-kilobase-pair (kbp) *Hin*dIII-*Bam*HI fragment of plasmid pAB120 (obtained from K. Zaret and F. Sherman) that carries the *CYC1* transcription terminator (TERM; described in reference 41) (Fig. 1) was inserted into the *Hin*dIII-*Bam*HI sites of the yeast centromere vector YCp50 (38) to generate plasmid pJR422. Next, the 0.95-kbp *Hin*dIII-*Sph*I fragment

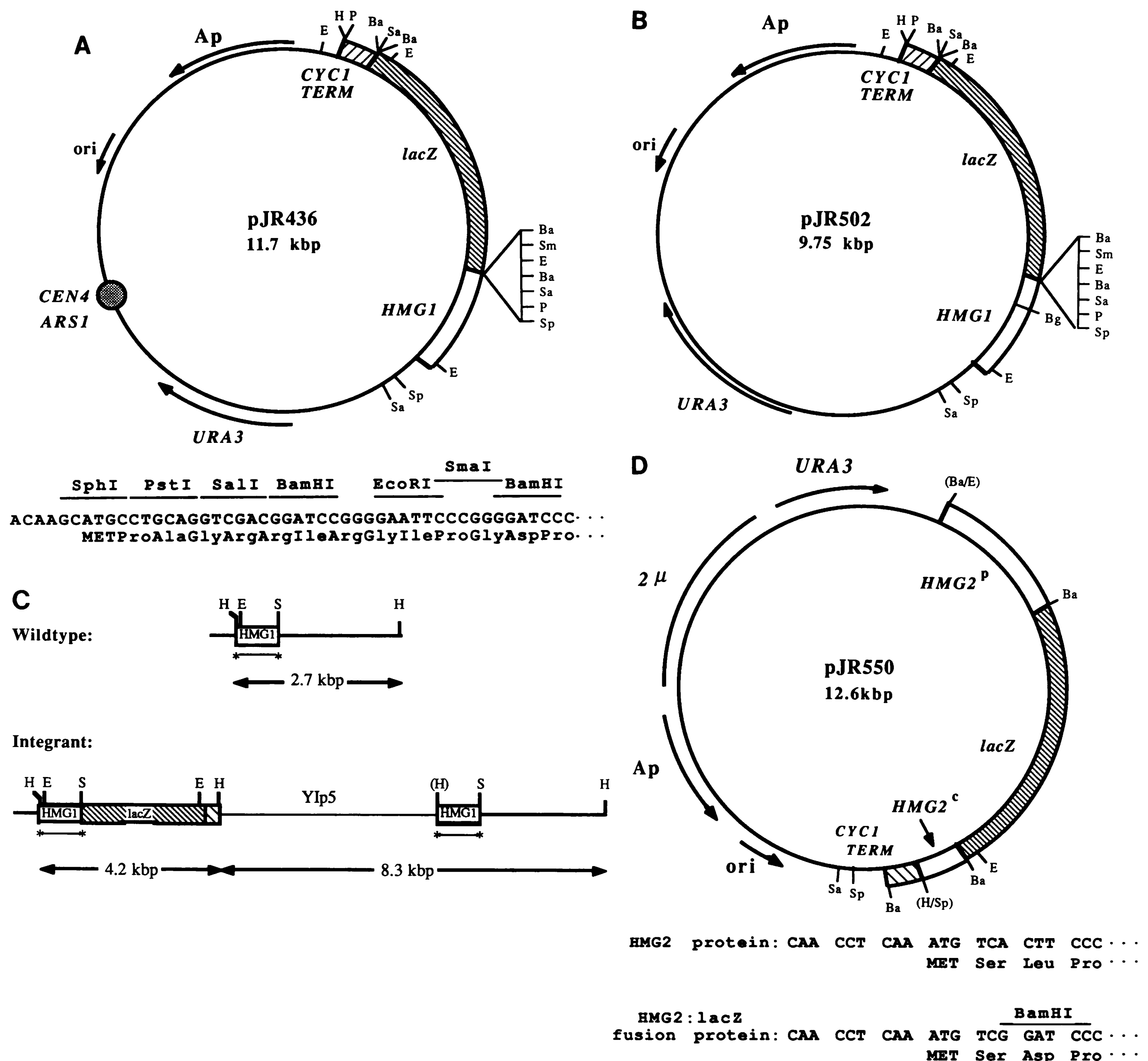

FIG. 1. Description of *lacZ* fusions. Details of plasmid construction are given in Materials and Methods. All integrations were monitored by gel transfer hybridization experiments.

of plasmid pJR59, extending from nucleotide −957 of the *HMG1* promoter to nucleotide +4 at the first ATG of the *HMG1* open reading frame (5, 44), was isolated and inserted into the *Hin*dIII-*Sph*I sites of the polylinker of pEMBL18 (provided by R. Levis [12]) to generate plasmid pJR420. Next, the 3.0-kbp *Sal*I fragment carrying the *Escherichia coli lacZ* gene was isolated from pMC1871 (9) and inserted into the *Sal*I site of pJR420 to generate plasmid pJR425. This insertion created an in-frame fusion between *HMG1* and *lacZ* at the first ATG of the *HMG1* coding sequence. Plasmid pJR425 was then cut with *Hin*dIII and *Xba*I, liberating a 4.1-kbp *HMG1-lacZ* fusion fragment, and treated with the Klenow fragment of *E. coli* DNA polymerase I to make the ends flush. The *HMG1-lacZ* fragment was inserted into plasmid pJR422, which had been linearized by cutting with *Bam*HI and treated with the Klenow fragment of *E. coli* polymerase I to make the ends flush. The resulting centromere plasmid, pJR436, carried the *HMG1-lacZ* fusion immediately followed by the *CYC1* transcription terminator (Fig. 1A). The sequence of the fusion junction between *HMG1* and *lacZ* was verified by sequencing across the junction by using the universal primer, which hybridized to *lacZ* near the fusion junction (47).

For construction of pJR502, an integrating plasmid carrying the *HMG1-lacZ* fusion at the first ATG of *HMG1*, plasmid pJR436 was cut to completion with *Hin*dIII and then partially digested with *Sph*I. A 4.55-kbp *Sph*I-*Hin*dIII fragment containing the *HMG1-lacZ*-TERM fusion was isolated and inserted into the *Hin*dIII-*Sph*I sites of plasmid YIp5 (49) to generate pJR502 (Fig. 1B). pJR502 carried the yeast selectable marker *URA3* but did not contain sequences that allowed autonomous replication in yeast cells. Therefore,

this plasmid was used to direct integration of the *HMG1-lacZ* fusion into the yeast chromosome at the *HMG1* locus. pJR502 was linearized in vitro by cleavage at the unique *Bgl*II site in the *HMG1* promoter sequences, and this linear plasmid was used to transform the yeast strain JRY527. The ends of the linear plasmid directed integration of the plasmid at *HMG1* by homologous recombination (46), resulting in a partial duplication of *HMG1* promoter sequences. The insertion was placed between the chromosomal sequence normally 5′ of the first ATG and the coding sequence of *HMG1* (Fig. 1C). The structure of the *HMG1* locus was verified by gel transfer hybridization analysis of DNA from one Ura$^+$ transformant, JRY1443, and from a tetrad derived from a cross of JRY1443 with JRY528, a strain with a wild-type *HMG1* locus. Integration of this *HMG1-lacZ* fusion at the *HMG1* locus was shown not to eliminate *HMG1* expression by crossing JRY1443 with JRY986, a strain that carries the *hmg2::HIS3* allele of *HMG2* (6). Of 21 tetrads from this cross, 16 had four viable spores, and several HIS$^+$ URA$^+$ segregants were recovered. If the integration of *HMG1-lacZ* eliminated *HMG1* expression, no HIS$^+$ URA$^+$ segregants would be recovered, since at least one functional isozyme of HMG-CoA reductase is essential for growth. Thus, this integration event did not disrupt *HMG1*.

For construction of pJR550, a multicopy plasmid construct carrying the *HMG2-lacZ* fusion, site-directed mutagenesis was used to create a *Bam*HI site in plasmid pJR479, a deletion derivative of pJR418 constructed as previously described (5). pJR479 carried a 1,810-bp fragment of the *HMG2* gene in the vector pEMBL18. The insert in pJR479 extended from the *Eco*RI site at nucleotide −1442 in the upstream region of *HMG2* to nucleotide +368 of the *HMG2* coding sequences. The oligonucleotide 5′-CGTTTTTAAGG GATCCGACATTTGAGGT-3′ was used to introduce three nucleotide changes (underlined) in pJR479 as described above. This mutation created a *Bam*HI site at nucleotide +7 of the *HMG2* sequence, overlapping the second and third codons of the *HMG2* open reading frame. Plasmid pJR538 was identified as a derivative of pJR479 that had acquired a *Bam*HI site at the correct location. Next, a 3.0-kbp *Bam*HI fragment containing the *lacZ* gene was isolated from pMC1871 and inserted into the *Bam*HI site of pJR538 to generate plasmid pJR541, in which the *lacZ* gene was fused in frame to the initiating ATG of *HMG2*. pJR541 was then cut to completion with *Sph*I, treated with T4 DNA polymerase to make the ends flush, and then partially digested with *Eco*RI. The 5.0-kbp *HMG2-lacZ* fusion fragment was isolated and inserted into pJR543 (described above), which had been digested with *Hin*dIII, treated with the Klenow fragment of *E. coli* DNA polymerase I to make the ends flush, and then digested with *Eco*RI. This step generated plasmid pJR545. Next, plasmid pJR545 was partially digested with *Eco*RI. Linear molecules were isolated and treated with the Klenow fragment of *E. coli* DNA polymerase I to make the ends flush and then digested to completion with *Sph*I. The 5.25-kbp *HMG2-lacZ*-TERM fragment was isolated and inserted into plasmid YEp24, which had been cleaved with *Bam*HI, treated with the Klenow fragment of *E. coli* DNA polymerase I to make the ends flush, and then digested with *Sph*I. This step generated plasmid pJR550, which consisted of the *HMG2-lacZ*-TERM fusion carried on the yeast multicopy plasmid YEp24 (Fig. 1D). All standard recombinant DNA methods were performed by procedures found in reference 34. *E. coli* DH-1 (18) was used for the propagation of plasmids unless otherwise noted.

β-Galactosidase assays. The procedure used for β-galactosidase assays was from Hagen and Sprague (16) as modified from Miller (34). Assays were done on 1.5-ml portions in duplicate from several transformants for each plasmid under each condition. Units were normalized to cell density. In strains containing either multicopy plasmids or integrated *lacZ* fusions, the activity assays agreed within 15%. In the case of centromere plasmids containing *lacZ* fusions, the activity levels varied by as much as a few fold among isogenic transformants, presumably because of variation in copy number (45). Therefore, the integrated and multicopy plasmids were used for all experiments reported.

Preparation of cell extracts. Cell cultures (50 ml) were grown in YM medium with vigorous shaking to an optical density at 600 nm of 0.8 to 2. The cells were pelleted by centrifugation, washed twice with ice-cold 50 mM Tris hydrochloride (pH 7.5), and transferred to a 1.5-ml microcentrifuge tube. The cell pellet was suspended in an equal volume of 50 mM Tris hydrochloride (pH 7.5). Glass beads (0.5 mm; Biospec Products, Bartlesville, Okla.) were added until the total volume was four times that of the original cell pellet, and the cells were broken by vigorous vortexing for five 1-min intervals separated by 1-min periods of cooling on ice. The supernatant fraction was transferred to a new microcentrifuge tube, and the glass beads were washed by brief vortexing with 2 volumes of 50 mM Tris hydrochloride (pH 7.5). This supernatant fraction was combined with the first supernatant fraction, and the extract was centrifuged at 2,000 × *g* for 2 min at 4°C. The supernatant fraction was removed and frozen in a dry ice-ethanol bath and stored at −70°C.

HMG-CoA reductase activity assays. The assay for determining HMG-CoA reductase activity by measuring the conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonate was modified from the procedure of Alberts et al. (1). Activity in cell extracts was assayed in 50 mM Tris hydrochloride (pH 7.5)–5 mM dithiothreitol–200 μM NADPH–300 μM DL-[^{14}C] HMG-CoA (1 μCi/μmol) together with 20 mM glucose 6-phosphate and 9.75 mU of glucose 6-phosphate dehydrogenase per ml for regeneration of NADPH. The reaction was carried out in a final volume of 100 μl and was started by the addition of cell extract. The reaction mixture was incubated for 5 min at 37°C, the reaction was stopped by addition of 20 μl of 6 N HCl, and the mixture was incubated for an additional 15 min at 37°C to allow conversion of the reaction product, mevalonate, to mevalonolactone. To separate the product from the substrate, 100 μl of the reaction mixture was applied to a 1.5-ml Bio-Rex 5 column (100-200 mesh; Bio-Rad Laboratories, Richmond, Calif.) poured in a Pasteur pipette, and the mevalonolactone was eluted with three 1-ml water washes. Each 1-ml eluant was mixed with 10 ml of Scint-A (Packard Instrument Co., Inc., Rockville, Md.), and the amount of radiolabeled product was determined by scintillation counting. The enzyme activity was linear with respect to the amount of extract added. Protein determinations were made by using bovine serum albumin as the standard (31). Assays were performed at least in duplicate for each of two different amounts of extract, and duplicate determinations agreed within 15%.

HMG-CoA synthase and acetoacetyl-CoA thiolase assays. Enzyme activities were determined by minor modifications of the procedures described previously (53). The critical differences were using a 100,000 × *g* supernatant fraction for the assays and replacing the 50 mM phosphate buffer with 50 mM Tris hydrochloride buffer (pH 7.5), thus avoiding the formation of a magnesium phosphate precipitate during the assay.

TABLE 2. Expression of *HMG1-lacZ* and *HMG2-lacZ* fusions

Strain[a]	Plasmid	Fusion	Locus	β-Galactosidase sp act[b]
JRY1314	pJR436	1st ATG *HMG1*	YCp50	3.7
JRY1443	pJR502	1st ATG *HMG1*	*HMG1*	6.5
JRY1775	pJR550	1st ATG *HMG2*	YEp24	0.6

[a] All strains were derived from JRY527 and hence were isogenic.
[b] Expressed in Miller units (37).

TABLE 3. *HMG1-lacZ* and *HMG2-lacZ* expression in *hem1* cells

Strain	*HEM1* genotype	*lacZ* fusion	β-Galactosidase activity[a]		
			High δ-ALA	Low δ-ALA	Hemin I
JRY2041	+	*HMG1-lacZ*	2.7	2.3	3.2
JRY2042	−	*HMG1-lacZ*	2.4	0.1	5.9
JRY2060	+	*HMG2-lacZ*	0.2	0.2	0.4
JRY2063	−	*HMG2-lacZ*	4.4	100.5	5.8

[a] High- and low-δ-ALA media contained δ-ALA at 50 and 0.5 μg/ml, respectively; hemin I was added to media at 13 μg/ml. Activity is expressed in Miller units (37).

Analysis of yeast mRNA. Yeast mRNA was isolated as described previously (52). Aerobic cultures (100 ml) were grown in YM and harvested during logarithmic growth. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography, with minor modifications of a previously described procedure (2). Poly(A)$^+$ RNA samples were analyzed by gel transfer hybridization analysis (32). Quantitative estimates of the abundance of individual mRNA species were based upon multiple exposures of autoradiograms and, in some cases, comparisons of band intensities resulting from loading different amounts of RNA.

Analysis of yeast genomic DNA. Yeast genomic DNA was prepared as described previously (22). The DNA was digested to completion with the indicated restriction enzymes and evaluated by a gel transfer hybridization analysis (32).

RESULTS

***HMG1-lacZ* fusion constructions.** Two different fusions of *lacZ* to the *HMG1* promoter and 5′ untranslated region were constructed. In the first, the fusion junction between *HMG1* and *lacZ* was at an *Sph*I site that overlaps the first ATG of the *HMG1* coding sequence. Therefore, this fusion contained the *HMG1* promoter and 5′ untranslated sequences but contained no HMG1 protein-coding sequences. This fusion was introduced into yeast cells both on a centromere plasmid and integrated into the yeast chromosome at *HMG1*. The structure of the fusion integrated at *HMG1* is shown in Fig. 1C. The integration at *HMG1* created a *URA3* strain that precluded the opportunity to select for the propagation of *URA3*-marked plasmids.

Evaluation of *HMG1-lacZ* and *HMG2-lacZ* fusions. Plasmid pJR436 carried the fusion of *lacZ* to the first ATG of *HMG1* on the yeast centromere vector YCp50, which is maintained at one to two copies per cell. The expression of *HMG1-lacZ* on YCp50 was promoted by a 0.95-kbp *HMG1* promoter fragment, whereas expression of the fusion integrated at *HMG1* was promoted by all of the chromosomal sequences normally upstream of *HMG1*. Levels of expression of the chromosomal and plasmid-borne fusions were similar (Table 2), suggesting that the 0.95-kbp *HMG1* promoter fragment present on pJR436 contained all sequences necessary for normal *HMG1* expression. There were, however, slight differences in expression of the fusion in these two contexts. Expression of the fusion on the plasmid YCp50 was somewhat more variable than expression of the fusion integrated into the chromosome, presumably because of copy number variations of the plasmid in different populations of cells.

Since the *HMG1* open reading frame contained a second in-frame ATG codon 10 codons 3′ of the first ATG, there was some ambiguity regarding whether fusions to the first ATG would measure all expression of the *HMG1* gene (24). Therefore, a fusion was made of *lacZ* to a restriction site placed after the second ATG by methods similar to those described above. Since this construction produced significantly less β-galactosidase activity than did the fusion to the first ATG (unpublished observations), the protein produced from the fusion to the first ATG presumably reflects all of the expression from the *HMG1* gene.

Plasmid pJR550 carried a fusion of *lacZ* to the *HMG2* promoter and 5′ untranslated sequences on the yeast multicopy plasmid YEp24. Strains carrying the fusion typically expressed 0.5 to 1.0 U of β-galactosidase activity. Since an integrated *HMG2-lacZ* fusion would be expected to produce even lower levels of β-galactosidase activity, the experiments with an *HMG2-lacZ* fusion all used plasmid pJR550.

Heme depletion and *lacZ* fusion expression. The effect of heme depletion on expression of the *HMG1-lacZ* and *HMG2-lacZ* fusions was tested. Heme depletion had opposite effects on the expression of the two fusions: expression of *HMG1-lacZ* was decreased, whereas expression of *HMG2-lacZ* was increased (Table 3). β-Galactosidase activity expressed from *HMG1-lacZ* was approximately 20-fold lower in *hem1* strains grown in low-δ-ALA medium (heme deficient) than in *HEM1* strains. Activity was restored to wild-type levels by addition either of high concentrations of δ-ALA or of hemin I. These results indicated that decreased expression of *HMG1* was due to deficiency of heme itself rather than of an intermediate in the heme biosynthetic pathway.

In contrast to its effect on *HMG1-lacZ*, starvation for heme resulted in increased expression of *HMG2-lacZ*. β-Galactosidase activity expressed from *HMG2-lacZ* was 100-fold or more higher in *hem1* strains grown in low-δ-ALA medium than in a *HEM1* strain. Growth of *hem1* strains in medium containing either high δ-ALA or hemin type I resulted in dramatically reduced expression of *HMG2-lacZ*, although expression was not reduced to wild-type levels. If levels of δ-ALA were increased further (100 to 200 μg/ml), expression of *HMG2-lacZ* was closer to wild-type levels (data not shown), suggesting that cells grown in high δ-ALA (50 μg/ml) were still partially depleted of heme. These results indicated that heme was a negative regulator of *HMG2* expression.

Rate of induction of *HMG1* and *HMG2*. The rate of heme-mediated induction of *HMG1-lacZ* expression was examined by shifting *HEM1* and *hem1* cells from low-δ-ALA medium to high-δ-ALA medium and assaying β-galactosidase activity expressed from the fusion gene at various times after the shift. Heme acted rapidly to induce *HMG1* expression. Two hours after the shift, β-galactosidase activity in *hem1* cells had increased dramatically (Fig. 2A). Furthermore, at early times after the shift, the level of β-galactosidase activity in *hem1* cells carrying *HMG1-lacZ* was induced to a level higher than the steady-state level in wild-type cells. *HEM1* cells shifted to high-δ-ALA medium showed a similar initial increase of β-galactosidase activity. After 24 h, how-

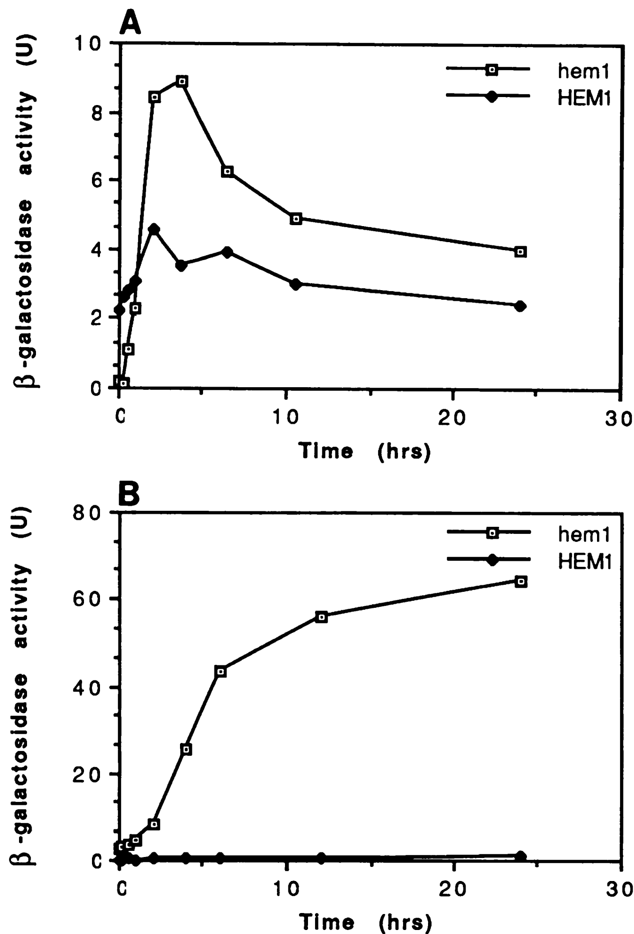

FIG. 2. Induction of *HMG1-lacZ* and *HMG2-lacZ* expression in *hem1* cells. (A) The *HEM1 HMG1-lacZ* strain JRY2041 and the *hem1 HMG1-lacZ* strain JRY2042 were grown in low-δ-ALA medium. Cells were harvested during logarithmic growth, washed with medium lacking δ-ALA, and suspended in high-δALA medium. β-Galactosidase activity was determined at various times after the shift to high-δ-ALA medium. (B) The *HEM1 HMG2-lacZ* strain JRY2060 and the *hem1 HMG2-lacZ* strain JRY2063 were grown in high-δ-ALA medium. Cells were harvested during logarithmic growth, washed with medium lacking δ-ALA, and suspended in low-δ-ALA medium. β-Galactosidase activity was determined at various times after the shift.

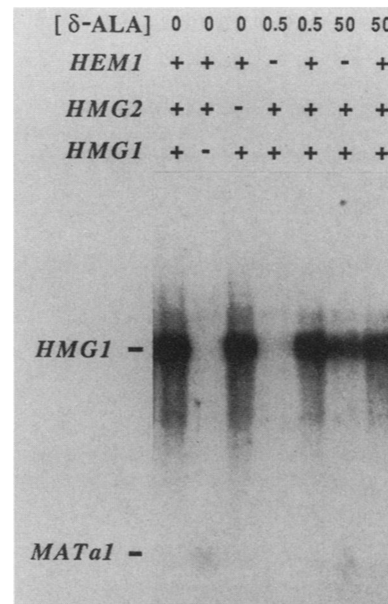

FIG. 3. RNA blot hybridization analysis of *HMG1* mRNA in *hem1* cells. Poly(A)$^+$ RNA was isolated from JRY527 (*HMG1 HMG2 HEM1*), JRY1159 (*hmg1::LYS2 HMG2 HEM1*), and JRY1160 (*HMG1 hmg2::HIS3 HEM1*) grown in medium lacking δ-ALA and from JRY2092 (*HMG1 HMG2 hem1*) and JRY2093 (*HMG1 HMG2 HEM1*) grown in either high- or low-δ-ALA medium. A 10-μg sample of poly(A)$^+$ RNA from each culture was fractionated by electrophoresis on a 1.2% agarose gel and transferred to nitrocellulose. The filter was hybridized to a 2.46-kbp *HMG1* fragment and to a fragment that contained the *MAT*a locus. Longer exposures of this blot showed that the intensity of the *MAT*a*1* mRNA was approximately the same in each lane.

ever, *HMG1-lacZ* expression in both *HEM1* and *hem1* strains had returned to wild-type levels. The rate of induction of *HMG2-lacZ* expression upon shift from high-δ-ALA medium to low-δ-ALA medium was also examined (Fig. 2B). In a *hem1* strain, induction of *HMG2* expression began within 2 h after the shift and was essentially complete at 12 h after the shift. Expression of *HMG2-lacZ* in a *HEM1* strain was not affected by the shift to low-δ-ALA medium. Judging from the rapidity of *HMG2* induction, *hem1* cells were apparently depleted of heme fairly quickly after the shift to low-δ-ALA medium.

***HMG1* and *HMG2* mRNA levels in a *hem1* mutant.** The effect of heme starvation on HMG-CoA reductase mRNA levels was examined by analyzing poly(A)$^+$ RNA obtained from the *hem1* strain JRY2092 and the *HEM1* strain JRY2093, each grown in both high- and low-δ-ALA media. A 2.46-kbp fragment of the *HMG1* gene was used as a probe in an RNA gel transfer hybridization experiment (Fig. 3). This probe hybridized to a 3.25-kbp mRNA in *HMG1* strains but did not detect a cross-hybridizing mRNA in JRY1159, which carried the *hmg1::LYS2* disruption. The probe was thus specific for the *HMG1* message. The steady-state level of the *HMG1* message was lower in *hem1* strains than in *HEM1* strains. This reduction in the *HMG1* message level was greater in cells grown in low-δ-ALA medium but was apparent even in *hem1* strains grown in high-δ-ALA medium, again suggesting that *hem1* cells grown in high-δ-ALA medium were somewhat depleted of heme. On the whole, these data indicated that the reduced expression of the *HMG1-lacZ* fusion in heme-starved cells was caused primarily by changes in the steady-state level of the *HMG1* message.

The effect of the *hem1* mutation on the steady-state level of *HMG2* mRNA was examined by hybridizing a 1.0-kbp fragment of the *HMG2* gene to the same filter used in the previous RNA gel transfer hybridization experiment. This probe hybridized to a 3.2-kbp mRNA in strains that carried a wild-type *HMG2* allele and was missing in JRY1160, which carried the *hmg2::HIS3* disruption (Fig. 4). Thus, the probe was specific for the *HMG2* message. A 1.9-kbp mRNA was abundant in cells that carried the *hmg2::HIS3* disruption and presumably represented a polyadenylated RNA species produced from the *hmg2::HIS3* allele. The steady-state level of *HMG2* message was greater in a *hem1* mutant strain than in a *HEM1* strain, and the level of *HMG2* message in *hem1* cells was reduced by growth in high-δ-ALA medium. It was strikingly evident, however, that the extent of induction of *HMG2* mRNA was not nearly as great as the induction of

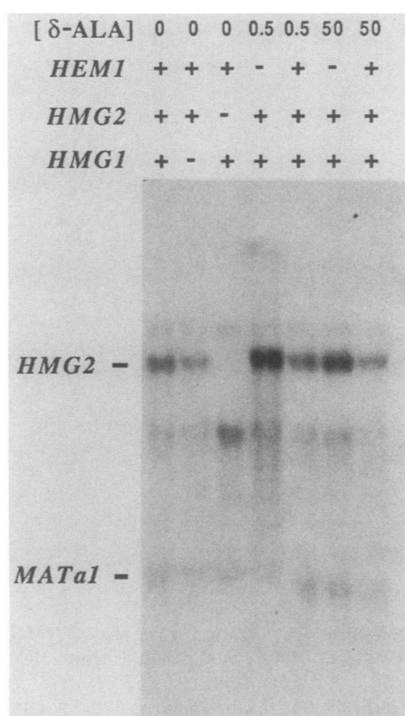

FIG. 4. RNA blot hybridization analysis of *HMG2* mRNA in *hem1* cells. A 1.0-kbp *HMG2* fragment and a fragment that contained the *MAT***a** locus were radiolabeled and hybridized to the filter used for Fig. 3. Longer exposures of this blot showed that the intensity of the *MAT***a***1* mRNA was approximately the same in each lane. Additional details are provided in the legend to Fig. 3.

β-galactosidase activity expressed from *HMG2-lacZ*. Indeed, *HMG2* message levels in *hem1* strains grown in low-δ-ALA medium were approximately threefold higher than in a *HEM1* strain (unpublished observations), whereas β-galactosidase activity from *HMG2-lacZ* was typically 100-fold higher under these conditions. The quantitative difference between the results of the two assays was surprising and is discussed below. These results suggested that heme levels regulated the expression of *HMG2* at the transcriptional level, with higher heme levels leading to decreased expression. The *HMG2-lacZ* fusion provided a qualitatively reliable, but quantitatively imprecise, monitor of influences on *HMG2* expression.

HMG-CoA reductase activity in *hem1* cells. In mammals, HMG-CoA reductase activity is regulated by both transcriptional and posttranscriptional mechanisms. Therefore, it was important to determine whether the effects of heme level on *HMG1* and *HMG2* transcription were paralleled by changes in enzyme activity. The effect of heme depletion on total cellular HMG-CoA reductase activity was studied in strains carrying the *hem1*::*LEU2* mutation. A diploid heterozygous for *hem1*::*LEU2* was made by mating JRY527 with JRY1236. The two *HEM1* and two *hem1* segregants from one tetrad of this diploid were grown in low-δ-ALA medium, and the specific activity of HMG-CoA reductase in cell extracts was determined. The specific activity of HMG-CoA reductase in the two *hem1* segregants was the same as in the two *HEM1* segregants (Table 4), indicating that heme depletion did not alter total cellular HMG-CoA reductase activity. These four strains had the same specific activity of HMG-CoA reductase when grown in high-δ-ALA medium (data not shown).

TABLE 4. HMG-CoA reductase specific activity in *hem1* cells grown in medium containing 0.5 μg of δ-ALA per ml

Strain	Genotype			Sp act[a]
	HEM1	*HMG1*	*HMG2*	
JRY2090	+	+	+	0.58
JRY2091	−	+	+	0.60
JRY2092	−	+	+	0.66
JRY2093	+	+	+	0.62
JRY2110	+	+	−	0.25
JRY2111	−	+	−	0.03
JRY2112	−	+	−	0.03
JRY2113	+	+	−	0.33
JRY2100	+	−	+	0.34
JRY2101	−	−	+	0.74
JRY2102	+	−	+	0.33
JRY2103	−	−	+	0.96

[a] Expressed as nanomoles of mevalonate formed per minute per milligram of protein.

Since heme depletion had opposite effects on the expression of *HMG1* and *HMG2*, it was essential to distinguish the effects of heme depletion on the activity of the HMG1 and HMG2 isozymes. The influence of heme concentration on the enzymatic activities encoded by the two genes was determined by assaying HMG-CoA reductase activity in strains carrying either the *hmg1*::*LYS2* or the *hmg2*::*HIS3* allele.

The effect of heme depletion on the activity of the HMG1 isozyme was studied in segregants from a diploid heterozygous for *hem1*::*LEU2* and homozygous for *hmg2*::*HIS3* (JRY1160 × JRY2108). The four spore clones from one tetrad of this cross were grown in low-δ-ALA medium, and the HMG-CoA reductase activity was determined. HMG-CoA reductase activity was 10-fold lower in the *hem1* segregants than in the *HEM1* segregants. *HMG1*-derived HMG-CoA reductase activity in the *hem1* strain was restored to wild-type levels when cells were grown in high-δ-ALA medium (data not shown). The magnitude of the change in the activity of the HMG1 isozyme in heme-depleted cells correlated well with both the change in expression of the *HMG1-lacZ* fusion and the change in the level of *HMG1* mRNA. These data extended the conclusion that heme was a positive regulator of *HMG1* expression and that the induction of *HMG1*-dependent activity occurred at the level of mRNA accumulation.

The effect of heme depletion on the activity of the HMG2 isozyme was studied in strains with the *hmg1*::*LYS2* allele and the *hem1*::*LEU2* allele. For this experiment, the four spore clones from one complete tetrad from a diploid heterozygous for the *hem1*::*LEU2* disruption and homozygous for *hmg1*::*LYS2* (JRY1159 × JRY2098) were grown in low-δ-ALA medium, and the HMG-CoA reductase activity was determined. Under these growth conditions, the activity of the HMG2 isozyme was two- to threefold higher in the *hem1* strains than in the *HEM1* strains (Table 4). HMG-CoA reductase activity derived from the HMG2 isozyme was restored to wild-type levels when the *hem1* strain was grown in high-δ-ALA medium (data not shown). The induction of HMG-CoA reductase activity correlated well in magnitude with the induction of *HMG2* mRNA in *hem1* strains grown in low-δ-ALA medium. Thus, heme depletion caused a threefold increase in *HMG2* message level, and this increase resulted in a comparable increase in HMG-CoA reductase activity contributed by the HMG2 isozyme. Because of the quantitative agreement between the analysis of *HMG2*

TABLE 5. Expression of *lacZ* fusions in *hapl* and *hap2* cells

Strain	Genotype		*lacZ* fusion	β-Galactosidase activity[a]
	hapl	*hap2*		
JRY2076	+	+	*HMGl-lacZ*	4.6
JRY2079	−	+	*HMGl-lacZ*	0.2
JRY2082	+	−	*HMGl-lacZ*	8.3
JRY2077	+	+	*HMG2-lacZ*	1.8
JRY2080	−	+	*HMG2-lacZ*	3.9
JRY2083	+	−	*HMG2-lacZ*	1.1

[a] Determined for each strain in three cultures grown from independent single colonies. Values are averages of these three determinations, expressed in Miller units (37).

mRNA and enzyme activity, the magnitude of the *HMG2-lacZ* induction was considered artifactually high.

Roles of HAP1 and HAP2 in HMG-CoA reductase gene expression. The *HAPl* gene product mediates the effects of heme and oxygen levels on transcription of several yeast genes. In addition, HAP2 participates in expression of some heme-regulated genes (14). The *HMGl-lacZ* and *HMG2-lacZ* fusions were used to determine whether the *HAPl* and *HAP2* genes also regulated the expression of *HMGl* and *HMG2*. The *lacZ* fusion plasmids pJR502 (*HMGl-lacZ*; linearized by cleavage with *Bgl*II) and pJR550 (*HMG2-lacZ*) were transformed into a *HAPl HAP2* strain (BWG1-7a), a *hapl*::*LEU2 HAP2* strain (BWG1-7a *hapl*::*LEU2*), and a *HAPl hap2*::*LEU2* strain (BWG1-7a *hap2*::*LEU2*). All strains transformed with the *HMGl-lacZ* fusion were shown to have a single copy of the *HMGl-lacZ* fusion integrated at *HMGl* by a gel transfer hybridization experiment (data not shown). Expression of *HMGl-lacZ* was reduced 20- to 30-fold in a strain carrying the *hapl*::*LEU* disruption (Table 5). The level of β-galactosidase activity in this *hapl*::*LEU2* strain was comparable to the activity in a *heml*::*LEU2* strain, suggesting that the effect of heme on *HMGl* transcription was mediated by the HAP1 protein. In contrast, the *hapl*::*LEU2* disruption caused a twofold increase in the expression of *HMG2-lacZ*. The magnitude of the increase in *HMG2-lacZ* expression in the *hapl* strain was much lower than in the *heml* strain. The significance of this slight increase was unclear. The *hap2*::*LEU2* disruption caused a twofold increase in the expression of *HMGl-lacZ* and had no effect on the expression of *HMG2-lacZ*. The small effect of the *hap2* mutation on expression of *HMGl* suggested that *HAP2* did not play a major role in the regulation of HMG-CoA reductase gene expression.

Heme independence of acetoacetyl-CoA thiolase and HMG-CoA synthase expression. Studies with mammalian cells have provided evidence that the first three enzymes of the sterol biosynthetic pathway, acetoacetyl-CoA thiolase, HMG-CoA synthase, and HMG-CoA reductase, are coordinately regulated (10, 11). There is suggestive evidence that these enzymes are also coordinately regulated in yeast cells (48). Two experiments were performed to address whether the regulation of acetoacetyl-CoA thiolase and HMG-CoA synthase activity was also influenced by heme. The effect of heme depletion on the steady-state level of HMG-CoA synthase mRNA was examined by an RNA gel transfer hybridization experiment. A 2.2-kbp fragment of the HMG-CoA synthase gene (*ERG13*; from pSOC481, provided by K. Jarman and J. Proffitt, Amoco Corp.) was hybridized to the filter used in the experiments shown in Fig. 3 and 4. This probe detected a 1.6-kbp HMG-CoA synthase mRNA. The steady-state level of this mRNA was reduced approximately twofold in a *heml* mutant strain relative to the level in a wild-type strain. However, the level of the HMG-CoA synthase mRNA in the *heml* strain was not affected by the concentration of δ-ALA in the medium (data not shown). To determine whether this slight reduction in message level was biochemically significant, the levels of HMG-CoA synthase and acetoacetyl-CoA thiolase activity were measured in *HEMl* and *heml* strains. The assays revealed no effect of the *heml* mutation on either of these activities (Table 6). Therefore, in yeast cells, HMG-CoA synthase and acetoacetyl-CoA thiolase do not appear to be coordinately regulated with HMG-CoA reductase in response to heme levels.

TABLE 6. Lack of response of acetoacetyl-CoA thiolase and HMG-CoA synthase activity to heme levels

Strain	*HEMl* genotype	Growth medium	Sp act (μmol/min per mg of protein)	
			Acetoacetyl-CoA thiolase	HMG-CoA synthase
JRY438	+	YM	0.17	0.016
JRY1236	−	YM high δ-ALA	0.13	0.017
	−	YM low δ-ALA	0.13	0.014

DISCUSSION

The identification of two yeast isozymes for HMG-CoA reductase caused us to explore aspects of HMG-CoA reductase regulation that might provide clues as to why two genes exist for this enzyme (4). Since a single isozyme exists in mammalian cells, and the mammalian enzyme is functionally equivalent to either of the yeast enzymes, it seemed possible that such an exploration might lead to an understanding of the subtleties of the regulation of this pathway that have previously been elusive.

The results of this study indicated that the in vivo heme level regulated the expression of both yeast HMG-CoA reductase genes. Expression of the *HMGl* gene was studied in heme-depleted *heml* mutants by analysis of enzyme activity, mRNA levels, and *HMGl-lacZ* fusion expression. The results of these analyses were clear: heme depletion resulted in an approximately 10-fold decrease in HMG1 enzyme activity, *HMGl* mRNA level, and β-galactosidase activity expressed from the *HGMl-lacZ* fusion gene. These results established that the *HMGl-lacZ* fusion provided a qualitatively and quantitatively accurate means by which to measure *HMGl* regulation by heme. The *hapl* mutation, but not the *hap2* mutation, resulted in decreased *HMGl* expression. These data implied that heme stimulated transcription of *HMGl* through the action of the *HAPl* transcriptional activator. Since the mRNA measurements reported here reflect steady-state levels, changes in the mRNA levels could reflect changes in mRNA stability, the rate of synthesis, or both. The data were interpreted as changes in the rate of transcription due to the involvement of *HAPl*.

The effects of heme on *HMGl* and *HMG2* expression were rather different. As in several previously studied examples (for example, references 28 and 29), heme was found to act as a negative regulator of *HMG2* expression. Expression of *HMG2* was studied under heme-depleted conditions by three types of analysis: enzyme activity, mRNA levels, and *HMG2-lacZ* fusion expression. Growth of a *heml* mutant in low-δ-ALA medium resulted in an approximately threefold increase in the level of *HMG2* mRNA, a threefold increase in *HMG2*-encoded HMG-CoA reductase activity, and a dra-

matic increase in β-galactosidase activity produced from the *HMG2-lacZ* fusion. Thus, in heme-deficient *hem1* cells, there was excellent quantitative and qualitative agreement between the increases in *HMG2*-derived HMG-CoA reductase enzyme activity and *HMG2* mRNA levels and a qualitative agreement with the *HMG2-lacZ* fusion expression. Repression by heme was *HAP1* and *HAP2* independent, which was not surprising since these two genes encode known transcriptional activators. The reason for the discrepancy between the magnitude of the *HMG2::lacZ* induction and the magnitude of the enzyme and mRNA induction is unclear. It is unlikely that differences between the half-lives of *HMG2* protein and β-galactosidase could account for the discrepancy, since the induction of *HMG2* mRNA corresponds in magnitude to the induction of enzyme activity. Furthermore, the β-galactosidase produced from the *HMG2::lacZ* gene was unlikely to be more stable than that produced from the *HMG1::lacZ* gene. It was possible, however, that the *HMG2::lacZ* mRNA was much more stable than the *HMG2* mRNA, thus leading to a disproportionate induction of β-galactosidase activity.

The opposing effects of heme levels on the expression of the two HMG-CoA reductase genes were not indirect effects caused by changes in HMG-CoA reductase activity. Null alleles of *HMG1* had no effect on *HMG2* expression and vice versa. Furthermore, both induction of *HMG2* expression and loss of *HMG1* expression in response to changes in heme level could occur in the absence of the other isozyme. Thus, the effects of heme on expression of each isozyme were independent of effects on the other isozyme. The involvement of *HAP1* in *HMG1* transcription suggested that the effect of heme levels on *HMG1* expression was direct. However, it remains possible that *HAP1* controlled another gene that was responsible for *HMG1* transcription. There was no information that would indicate whether the effect of heme on *HMG2* was direct or was indirect due to some other effect of heme deficiency.

Although heme did modulate the expression of the *HMG1* and *HMG2* genes and the activities of the HMG1 and HMG2 isozymes, heme depletion did not affect the total cellular activity of HMG-CoA reductase. In fact, the pattern of the heme-mediated effects acted to buffer against changes in the total HMG-CoA reductase activity level. The lack of an effect on total cellular HMG-CoA reductase activity by heme depletion is in apparent conflict with an earlier report of heme depletion resulting in a substantial decrease in HMG-CoA reductase activity (27). There are important differences between the experiments reported here and the earlier ones that may explain this discrepancy. In this work, heme-deficient cells were produced by growth of a *hem1* mutant on low-δ-ALA medium, whereas in the earlier study heme-deficient cells were produced by growing a *hem1* mutant in the complete absence of heme (supplemented with ergosterol, unsaturated fatty acids, and methionine). In particular, the presence or absence of ergosterol in the growth medium may have affected HMG-CoA reductase activity. It is worth noting that heme-deficient *hem1* cells are not significantly more sensitive than wild-type cells to compactin, an inhibitor of HMG-CoA reductase, as would be expected if the total HMG-CoA reductase levels were significantly decreased under these conditions.

The regulation of HMG-CoA reductase synthesis by heme may not be specific to yeast cells. A variant Chinese hamster ovary cell line has been described whose growth properties are those expected of a heme auxotroph, although this notion was not tested directly. In this cell line, the induction of HMG-CoA reductase activity normally observed in wild-type cells upon a shift to delipidated medium failed to occur. A spontaneous revertant of this variant restored normal induction of HMG-CoA reductase (26). It would be interesting to determine whether heme supplementation could restore normal regulation to this cell line.

Heme level did not appear to affect the activities of other early enzymes of the sterol biosynthetic pathway. In the case of HMG-CoA synthase, mRNA levels were reduced only slightly in heme-deficient *hem1* cells, and enzyme activity was not altered by heme deficiency. In the case of acetoacetyl-CoA thiolase, there was no effect of heme depletion on enzyme activity level. No probe existed with which to measure the level of acetoacetyl-CoA thiolase mRNA. In contrast to HMG-CoA reductase, both acetoacetyl-CoA thiolase and HMG-CoA synthase appear to be encoded by a single gene, eliminating the possibility of compensatory changes obscuring an effect of heme level. Thus, although the analysis of HMG-CoA synthase and acetoacetyl-CoA thiolase remains somewhat incomplete, heme did not appear to coordinately regulate the early steps in the sterol biosynthetic pathway.

The physiological rationale for why heme-mediated regulation of *HMG1* and *HMG2* was different is not immediately clear. It is likely that heme levels served to reflect the relative level of oxygen available to cells. In fact, growth of cells in semianaerobic conditions reduced the level of *HMG1*-encoded activity threefold and increased the level of *HMG2*-encoded activity fourfold (unpublished observations). Recent work indicates that the two isozymes are located in different places within the cell (R. Wright and J. Rine, manuscript in preparation), raising the possibility that mevalonate made by the two isozymes is not equally available to all subsequent branches of the pathway. In this regard, two nonsterol products of the pathway are important in oxygen utilization. Ubiquinone is a component of the electron transport chain involved in respiration and is derived in part from farnesyl pyrophosphate, an intermediate of the sterol biosynthetic pathway (36). The side chain of heme **a**, a cofactor of cytochrome *c* oxidase, is also derived from farnesyl pyrophosphate (23). The demand for both of these products would be higher during aerobic growth, with high heme levels, than during semianaerobic growth, with low heme levels. Perhaps the two genes respond differently to demands for different products of the pathway.

In some respects, the transcriptional regulation of the yeast HMG-CoA reductase genes by heme parallels the transcriptional regulation of mammalian HMG-CoA reductase genes by low-density lipoprotein. Circulatory low-density lipoprotein levels reflect the available supply of cholesterol to individual cells in mammals. The yeast *S. cerevisiae*, being a unicellular eucaryote, in all probability does not respond to the level of sterols in its niche. Rather, yeast cells may monitor intracellular sterol levels. Molecular oxygen is required both in heme biosynthesis and in two steps of sterol biosynthesis, cyclization of squalene and demethylation of lanosterol. Oxygen level may therefore be an effective monitor of the availability of sterols. Thus, transcriptional control of HMG-CoA reductase by low-density lipoproteins in mammals and by heme in yeast cells may reflect similar biological needs. To extend the analogy, the nonsterol regulators of HMG-CoA reductase synthesis in mammals operate at the translational level. We have found that feedback regulation of *HMG1* by products made from mevalonate, in aerobically grown cells, also operates at the translational level (M. Thorsness and J. Rine, manuscript in

preparation). Thus, there are striking similarities between the patterns of HMG-CoA reductase regulation in yeast cells and mammals. Finally, *HMG1* and *HMG2* join the growing list of genes whose expression is regulated by heme.

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