MOLECULAR AND CELLULAR BIOLOGY, Aug. 1996, p. 3981–3989 Vol. 16, No. 8
0270-7306/96/$04.00+0

Transcriptional Regulation of a Sterol-Biosynthetic Enzyme by Sterol Levels in *Saccharomyces cerevisiae*

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Received 15 March 1996/Returned for modification 19 April 1996/Accepted 1 May 1996

Sterols and all nonsterol isoprenoids are derived from the highly conserved mevalonate pathway. In animal cells, this pathway is regulated in part at the transcriptional level through the action of sterol response element-binding proteins acting at specific DNA sequences near promoters. Here we extend at least part of this regulatory paradigm to the *ERG10* gene, which encodes a sterol-biosynthetic enzyme of *Saccharomyces cerevisiae*. Specifically, the discovery of sterol-mediated feedback control of *ERG10* transcription is reported. Deletion analysis of the *ERG10* promoter region identified sequences involved in the expression of *ERG10*. This regulatory axis appeared to involve sterol levels, as a late block in the pathway that depletes sterol, but not nonsterol isoprenoids, was able to elicit the regulatory response.

Cholesterol homeostasis in animal cells is maintained in part through transcriptional feedback regulation of certain genes involved in cholesterol metabolism. These genes include those encoding proteins involved in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase, as well as the gene encoding the low-density lipoprotein (LDL) receptor gene involved in cholesterol uptake (16). When cholesterol levels are high, transcription of these genes is low, resulting in reduced sterol synthesis and reduced uptake of serum cholesterol in the form of LDL particles. In turn, when the intracellular cholesterol levels drop, the transcription of these genes is coordinately induced, allowing increased flux through the mevalonate pathway and increased cellular uptake of serum LDL particles.

Mutational analysis of the promoter regions of the HMG-CoA synthase, HMG-CoA reductase, and LDL receptor genes has defined the DNA sequences required for sterol-mediated regulation of transcription (7, 34, 42, 46, 47). An alignment of these sequences reveals a conserved element within the regulatory regions of these promoters, termed sterol response element 1 (SRE-1) (16). This element is involved in the sterol-mediated transcriptional regulation of the HMG-CoA synthase and LDL receptor genes (42, 43). Furthermore, mutation of SRE-1 results in constitutively low levels of expression, indicating that this element acts in a positive fashion that activates transcription when intracellular sterol levels are low.

Some aspects of transcriptional regulation of the HMG-CoA reductase gene in response to sterol levels suggest a mechanism that may be different from that of the HMG-CoA synthase and LDL receptor genes. SRE-1 lies in the reverse orientation in the HMG-CoA reductase promoter and contains one nucleotide change in the core sequence compared with SRE-1 of the LDL receptor promoter. When this nucleotide change is incorporated into the LDL receptor promoter SRE-1, all sterol regulation is lost. Moreover, the two additional nucleotides adjacent to the 8-bp SRE-1 sequence that are required for regulation of the HMG-CoA synthase and LDL receptor genes are different in the HMG-CoA reductase promoter. Mutational analysis of the SRE-1 sequence of the HMG-CoA reductase promoter result in constitutively high levels of expression (33, 34), suggesting that in this context either SRE-1 acts as a repressor of expression or the site overlaps with another site required for repression. In fact, mutational analysis of the HMG-CoA reductase promoter defines two regions involved in sterol regulation (33). One region contains a sequence not previously known to be involved in regulation. The other region encompasses the 5′ half of the SRE-1 sequence and extends six nucleotides upstream. The pattern of expression of these mutant promoters is complex and suggests that the mechanism of HMG-CoA reductase transcriptional regulation might be similarly complex.

Mutational analysis of the promoter of the rat farnesyl diphosphate synthase gene has identified a novel 6-bp sequence involved in the sterol-mediated transcriptional regulation of that gene (44, 45). Moreover, the consensus SRE-1s present in the promoter region of this gene are dispensable for sterol-mediated regulation. These data suggest that animal cells have two, and perhaps more, mechanisms to set transcription rates in response to sterol levels. This notion also lends credence to the idea that HMG-CoA reductase transcriptional regulation may be distinct from HMG-CoA synthase and LDL receptor gene regulation. However, there appears to be some overlap in the regulatory machineries. Mutant cell lines have been isolated on the basis of their constitutively high levels of cholesterol production (8, 31) or their failure to synthesize cholesterol (13). These mutant cell lines lose either transcriptional activation or repression in a coordinated fashion for all three genes, suggesting that the three genes share at least some regulators.

Recently, the first components of the sterol-mediated transcriptional regulation machinery were identified (reviewed in reference 15). Two nuclear proteins, SREBP-1 and SREBP-2, were purified on the basis of their ability to bind SRE-1 in vitro (5, 51). There is a striking correlation between the ability of these SREBPs to bind promoters bearing various mutant SRE-1s in vitro and their ability to activate transcription in vivo, suggesting that these proteins are indeed responsible for the transcription of these genes in vivo. The sequences of SREBP-1 and SREBP-2 reveal them to be highly homologous

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TABLE 1. Strains used in this study

Strain (reference)	Genotype
JRY 527	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52*
JRY1590	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52 hmg1*::*LYS2*
JRY1443 (49)	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52 HMG1-lacZ*(*URA3*)
JRY2138 (37a)	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52 erg13*::*HIS3*
JRY4002 (12)	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52 HMG1-lacZ*(*URA3*) *erg13*::*HIS3*
JRY5032	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1706 (*ERG10-lacZ URA3* CEN)
JRY5033	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52-ERG10-lacZ-URA3*
JRY5034	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1707 (*ERG10P*$_{\Delta[-411\ to\ -176]}$-*lacZ URA3* CEN)
JRY5035	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1708 (*ERG10P*$_{\Delta[-204\ to\ -171]}$-*lacZ URA3* CEN)
JRY5036	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1709 (*ERG10P*$_{\Delta[-269\ to\ -139]}$-*lacZ URA3* CEN)
JRY5037	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1710 (*ERG10P*$_{\Delta[-413\ to\ -365]}$-*lacZ URA3* CEN)
JRY5038	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1711 (*ERG10P*$_{\Delta[-413\ to\ -303]}$-*lacZ URA3* CEN)
JRY5060	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1714 (*ERG10P*$_{\Delta[both\ SREs]}$-*lacZ URA3* CEN)
JRY5061	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1715 (*ERG10P*$_{\Delta[5'\ SRE]}$-*lacZ URA3* CEN)
JRY5062	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1716 (*ERG10P*$_{\Delta[3'\ SRE]}$-*lacZ URA3* CEN)
JRY5112	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52-GPD-HMG2*(*ura3*) *hmg1*::*LYS2 hmg2*::*HIS3*
JRY5113	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52-GPD-HMG2*(*ura3*) *hmg1*::*LYS2 hmg2*::*HIS3* + pJR1378 (*ERG10 URA3* 2μm)
JRY5114	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52 hmg1*::*LYS2* + pJR1378 (*ERG10 URA3* 2μm)
JRY5115	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1744 (*ERG10P*$_{\Delta[-262\ to\ -231]}$-*lacZ URA3* CEN)
JRY5132	*MAT***a** *ade2-101 his3Δ200 lys2-801 met*$^-$ *ura3-52* + pJR1754 (*ERG10*$_{[-411]}$-*lacZ URA3* CEN)

to each other and to be members of the basic region–helix-loop-helix–leucine zipper (bHLH-ZIP) family of transcription factors (20, 56). Both proteins contain carboxy-terminal extensions beyond the bHLH-ZIP domain, which contain potential membrane-spanning regions. Full-length SREBP-1 is localized to membranes outside the nucleus and is proteolytically processed to a soluble amino-terminal fragment containing the transcriptional activation and bHLH-ZIP domains (52). The smaller processed form is able to enter the nucleus and stimulate transcription. When sterols are limiting, the SREBPs are cleaved and the regulatory domains are able to enter the nucleus and activate transcription, resulting in increased sterol synthesis and uptake. When sterols are abundant, SREBP processing is inhibited, thereby leading to a reduction of transcription. Three distinct mutant cell lines that each fail to repress transcription when sterols are abundant (SRD-1, -2, and -3 CHO) all have mutant alleles that truncate SREBP-2 (54, 55). These truncated forms of the protein are able to enter the nucleus and activate transcription constitutively, providing additional evidence that the sterol-sensitive processing of the SREBPs is physiologically relevant to sterol-mediated transcriptional control.

Here, we describe the transcriptional feedback regulation of a yeast gene *ERG10* involved in sterol biosynthesis. This gene encodes the enzyme acetoacetyl-CoA thiolase, which catalyzes an early step in sterol biosynthesis. Reduced flux through the mevalonate pathway, by either genetic or pharmacological methods, resulted in an induction of *ERG10* mRNA. The regulation involved a quantitative, rather than a qualitative, change in the mRNA, and the regulatory sequences were mapped to a small region of the promoter. This response appeared to result from altered sterol levels because a late block in the pathway that depletes sterol, but not nonsterol, isoprenoids, also caused an induction of *ERG10* expression.

MATERIALS AND METHODS

Materials. [α-^{32}P]dCTP (400 Ci/mmol), [1-^{14}C]sodium acetate, and the Multiprime DNA labeling system were purchased from Amersham Corporation (Arlington Heights, Ill.). *o*-Nitrophenyl-β-D-galactopyranoside and DL-mevalonic acid lactone were purchased from Sigma (St. Louis, Mo.). The polyAT track mRNA isolation kit was purchased from Promega (Madison, Wis.). The Bradford protein assay kit was purchased from Bio-Rad (Richmond, Calif.). The Sequenase DNA sequencing kit was purchased from United States Biochemical Corporation (Cleveland, Ohio). Nitrocellulose was purchased from Schleicher & Schuell, Inc. (Keene, N.H.). Lovastatin was the gift of A. Alberts (Merck, Rahway, N.J.). Zaragozic acid was provided by J. Bergstrom (Merck).

Media and genetic methods. *Saccharomyces cerevisiae* strains were grown at 30°C in yeast minimal medium containing 2% glucose as described previously (1). Amino acid and base supplements were added, when needed, at the following concentrations: adenine, 30 μg/ml; methionine, histidine, and uracil, 20 μg/ml; and lysine, 40 μg/ml, from stock solutions in distilled H_2O. Solid medium contained 2% agar (Difco, Detroit, Mich.). Yeast cells were transformed by the lithium acetate method (21, 38). Stock solutions of lovastatin, zaragozic acid, and mevalonic acid lactone were prepared as described previously (12). Unless stated otherwise, cells were treated with lovastatin at a concentration of 40 μg/ml for at least 8 h.

Plasmid and strain constructions. All strains used in this study were derived from JRY527 (53) and are isogenic except where indicated. The strains used in this study are described in Table 1.

Strains containing altered levels of HMG-CoA reductase and/or acetoacetyl-CoA thiolase were constructed in the following manner. Strain JRY1590 was obtained by sporulating a diploid derived from a rare mating between JRY1159 (*MAT***a** *ade2-101 his3Δ200 lys 2-801 met ura3-52 hmg1*::*LYS2 HMG2*) and JRY1160 (*MAT***a** *ade2-101 his3Δ200 lys 2-801 met ura3-52 HMG1 hmg2*::*HIS3*) (3). This strain contains the *hmg1*::*LYS2* allele and expresses roughly 20% HMG-CoA reductase activity relative to the wild type (2). Acetoacetyl-CoA thiolase activity was overexpressed in this strain by transforming it with a 2μm plasmid bearing the *ERG10* gene (pJR1378 [19]), yielding JRY5114. Alternately, a high level of HMG-CoA reductase activity was achieved with strain JRY5112. This strain contains disruptions of both *HMG1* and *HMG2* and has the *HMG2* gene under the transcriptional control of the *GPD* promoter integrated at the *URA3* locus (18). Acetoacetyl-CoA thiolase activity was similarly overexpressed in this strain by transforming it with the multicopy *ERG10*-containing plasmid (pJR1378), yielding JRY5113. Expression of *ERG10* from this 2μm plasmid has been shown to result in greater than 10-fold-increased acetoacetyl-CoA thiolase activity (19).

The *ERG10-lacZ* reporter gene construct was made in two steps. First, an *Eco*RI fragment consisting of the *lacZ* gene fused to the transcriptional terminator from the *CYC1* gene was isolated from a partial digest of pJR502 (49). This fragment was then ligated into the complementary *Mun*I site of pJR1377, which contains the *Kpn*I-*Xba*I fragment of the *ERG10* gene in pEMBL19 (19). The resulting plasmid (pJR1704) contained an in-frame *ERG10-lacZ* fusion that included the first 15 codons of the *ERG10* gene and the *ERG10* promoter region extending 541 bp upstream of the initiator methionine. The *Kpn*I-*Hin*dIII fragment containing the *ERG10-lacZ* fusion including the *CYC1* terminator was then subcloned into the respective sites of the yeast integrating vector pRS306 (40), yielding pJR1705, and into the centromere-containing vector pRS316 (40), yielding pJR1706. These two plasmids bearing the *ERG10-lacZ* reporter gene fusion were then introduced into yeast cells by transformation to uracil prototrophy. The centromere-containing *ERG10-lacZ* plasmid (pJR1706) was transformed into JRY527, yielding JRY5032. Alternately, the integrating plasmid (pJR1705) was linearized by digestion with *Stu*I and then used for transformation of JRY527, yielding JRY5033. The *Stu*I site is in the *URA3* gene, thereby targeting

the integration of the *ERG1-lacZ* fusion gene to the *URA3* chromosomal locus. The chromosomal structure of JRY5033 was confirmed by genomic hybridization experiments.

A derivative of the *ERG10-lacZ* reporter gene fusion containing a smaller region of the promoter sequences was constructed by digesting pJR1706 with *Kpn*I and *Bst*EII, followed by treatment with T4 DNA polymerase to create blunt ends and religation. The resulting plasmid (pJR1757) contains only 411 bp of the *ERG10* promoter region immediately upstream of the initiating methionine. Transformation of JRY527 with this plasmid yielded strain JRY5132. A second derivative of the *ERG10-lacZ* reporter gene containing an internal deletion of the *ERG10* promoter region was constructed by digesting pJR1706 with *Bst*EII and *Msc*I, followed by treatment with T4 DNA polymerase to create blunt ends and religation. The resulting plasmid (pJR1707) lacked the region of the *ERG10* promoter from nucleotides −411 to −176 (where the A of the initiating methionine is designated +1). This plasmid was then transformed into JRY527, yielding JRY5034.

An additional set of *ERG10* promoter deletions of the *ERG10-lacZ* reporter gene was created by a PCR-based strategy. This strategy consisted of generating regions of the *ERG10* promoter (including *Bst*EII and *Msc*I sites that had been engineered into the ends of the primers) by PCR and then replacing the *Bst*EII-*Msc*I region of the promoter with these fragments. By generating PCR products of subregions within this *Bst*EII-*Msc*I fragment, it was possible to create deletions within this region once the fragments were replaced. First, a deletion of the region from −205 to −170 was created by using the oligonucleotides DAD13 (5′-CCG$_{-417}$AGGTCACCTCTCATC$_{-402}$-3′) and DAD18 (5′-CCTGGC$_{-206}$CAGCGAAAAAACCGGC$_{-222}$-3′) as PCR primers. The nucleotides that are not complementary to the *ERG10* promoter (engineered restriction sites) are underlined, and the region of the promoter complementary to the primers is indicated by the subscript numbers. Following PCR amplification, the product was digested with *Bst*EII and *Msc*I and ligated to the large vector fragment of pJR1706 that had been digested with *Bst*EII and *Msc*I. The resulting plasmid (pJR1708) contained a novel sequence at the junction (G$_{-206}$CC$_{-169}$), confirmed by double-stranded DNA sequencing. The same methodology was used to create a deletion from positions −269 to −139, using the oligonucleotides DAD13 and DAD19 (5′-CCTGGCCAG$_{-270}$GAAACGATAAACCCG$_{-285}$-3′). Sequencing of pJR1709 revealed there had been an additional deletion of the G at position −284. The sequence of the junction was C$_{-270}$TGGCC$_{-138}$. The −413 to −365 deletion (pJR1710) was created with oligonucleotides DAD14 (5′-CCGGTCAC$_{-364}$CTGTAAATAGAAACAGC$_{-347}$-3′) and DAD17 (5′-GGC$_{-165}$CTGGCCACGAACGTG$_{-180}$-3′). The sequence of the junction was C$_{-414}$AGTG$_{-364}$. Finally, the −413 to −303 deletion (pJR1711) was created with oligonucleotides DAD17 and DAD15 (5′-CCGGTCAC$_{-302}$CATTGTTGTGC CCTG$_{-287}$-3′). The sequence of the junction was C$_{-414}$AGTG$_{-302}$. An additional mutation was evident from the sequencing: the sequence C$_{-290}$CT had been changed to TC. These constructs bearing various deletions of the *ERG10* promoter within the *ERG10-lacZ* reporter gene were introduced into JRY527, yielding JRY5035(pJR1708), JRY5036(pJR1709), JRY5037(pJR1710), and JRY5038(pJR1711).

A set of smaller deletions within the *ERG10* promoter region was created by oligonucleotide-directed mutagenesis as described previously (28). The oligonucleotide DAD29 (5′-AACCGGCTTGGG$_{-230}$AAGCTTC$_{-263}$CTGTTAGGAA ACG-3′) allowed the replacement of the region from −262 to −231 with a *Hin*dIII site (underlined sequences). This region encompasses one of two SRE-like sequences present in the *ERG10* promoter region and includes the adjacent 24 bp. The resulting construct (pJR1744) was introduced into JRY527, yielding JRY5115. In addition, smaller deletions were constructed in order to remove these two SRE-like elements, either singularly or in combination. The oligonucleotide DAD26 (5′-CCTGGCCACGAACG$_{-178}$AAGCTTG$_{-186}$GCTAGTAG TACCG-3′) was used to replace the region from −185 to −179 containing the more promoter-proximal SRE-like element with a *Hin*dIII site (underlined). Alternately, the oligonucleotide DAD27 (5′-GGCACCTCGTTATAA$_{-254}$TCTA GAC$_{-163}$CTGTTAGGAAACG-3′) allowed the replacement of the more distal SRE-like element in the region from −262 to −255 with an *Xba*I site (underlined). Plasmid pJR1714 has both SRE-like sequences removed, while pJR1715 has the distal site removed and pJR1716 has the proximal site removed. All three of these plasmids were introduced into strain JRY527, yielding strains JRY5060(pJR1714), JRY5061(pJR1715), and JRY5062(pJR1716).

To create a disruption of the *TYE7* gene, it was necessary to first clone the gene. This cloning was achieved by performing genomic PCR on yeast cells, using the primers DAD23 (5′-CGTAAAGCACCACAAGCAGT-3′) and DAD24 (5′-GTCTGCTATGTTATGGCCTTG-3′), which are complementary to the promoter region and the 3′ untranslated region of the *TYE7* gene, respectively (29). The resulting PCR fragment was subsequently blunt-end cloned into the *Sma*I site of the vector pUC18, yielding pJR1712. Next, the *Bam*HI fragment from pJR984 (also called pJJ215 [23]) containing the yeast *HIS3* gene was cloned into the *Bgl*II site within the *TYE7* coding region. The orientation of the resulting clone (pJR1713) was such that transcription of the *HIS3* gene was in the opposite orientation of the *TYE7* gene. It had been previously shown that *TYE7* is not essential for growth (29). Therefore, the *tye7*::*HIS3* disruption was introduced directly into the haploid strain JRY5033 by transformation of the PCR fragment generated by the primers DAD23 and DAD24, using pJR1713 as the template DNA. The chromosomal structure of the *TYE7* locus was confirmed in the resulting strain (JRY5059) by genomic PCR analysis.

Acetate labeling of cells. Measurements of sterol synthesis were determined by the incorporation of [1-^{14}C]acetate into sterols as described previously (18, 35). Band intensities were quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

β-Galactosidase assays. The protocol for β-galactosidase assays was essentially that of Hagen and Sprague (17) as modified from that of Miller (32). The assays were performed in a volume of 1.5 ml in duplicate, and the units were normalized to cell density. The assays were performed in at least three independent cultures, and the standard deviations are indicated.

Acetoacetyl-CoA thiolase enzyme assays. Extracts were prepared from 50 ml of logarithmically growing cells as previously described (49) with the exception that a 50 mM potassium phosphate buffer (pH 7.5) was used. The protein concentration of the extract was determined with a Bradford protein assay kit (Bio-Rad). Extract aliquots were stored at −80°C. The reaction conditions for the acetoacetyl-CoA thiolase assay were as described previously (19). The assays were performed at two different extract concentrations to ensure the linearity of the reaction.

Poly(A) RNA measurements. Total RNA was prepared from each sample as described previously (27). Poly(A) RNA was isolated with a polyAT tract mRNA isolation kit (Promega). Equal amounts of mRNA were separated on a 1.2% agarose-formaldehyde gel, blotted onto nitrocellulose, and probed with gene fragments radiolabeled with [^{32}P]dCTP by random priming (Amersham). Hybridization mixtures were incubated overnight in 50% formamide–5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–5× Denhardt's solution–1% glycine–0.1 mg of salmon sperm DNA per ml at 42°C. The blots were washed once in 2× SSPE–0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature and again with 0.1× SSPE–0.1% SDS for 30 min at 42°C. Band intensities were visualized by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics). In the Northern (RNA) blot in Fig. 3, hybridization of a *MAT***a***1* probe revealed that all lanes contained equal amounts of mRNA (data not shown).

The procedure for measuring mRNA half-lives was essentially that of Caponigro et al. (6). Briefly, an exponentially growing culture of a strain with a temperature-sensitive mutation in the large subunit of RNA polymerase II (JRY3823) was split; one half was treated with lovastatin (40 μg/ml), and the other half was left untreated. After 12 h of additional growth at the permissive temperature (26°C), the cells were concentrated and allowed to recover at 26°C for 10 min. Transcription was terminated by transferring the cells to an empty 37°C prewarmed flask, and an equal volume of medium at 56°C was added. This allowed rapid equilibration of the culture to 37°C. Cells were removed at various times, pelleted, and quickly frozen in liquid nitrogen. Total RNA was then prepared and subjected to hybridization experiments as described above. *MAT***a***1* transcript served as a control mRNA with a short half-life, and *HMG1* transcript served as a control with a long half-life. Neither of these mRNAs were affected by lovastatin treatment (data not shown).

To map the sites of transcription initiation of the *ERG10* mRNA, primer extension analysis was performed. One-hundred-milliliter cultures of JRY527 were grown in medium with and without lovastatin (40 μg/ml). After 8 h of growth, total RNA was isolated and then poly(A) RNA was isolated. Then 5 μg of poly(A) RNA was hybridized to 1.85 ng of ^{32}P end-labeled primer (HISER031, 5′-GAGACATTTTGAGTACG-3′, 5.6×10^6 cpm/pmol) in hybridization buffer (2 mM Tris [pH 7.6], 0.2 mM EDTA, 250 mM KCl) in a volume of 10 μl. The reaction mixture was placed at 74°C for 2 min and then at 50°C for 1 h. Extension of the primer was initiated by the addition of 40 μl of reverse transcriptase buffer (20 mM Tris [pH 8.7], 10 mM $MgCl_2$, 100 μg of actinomycin D per ml, 5 mM dithiothreitol, 0.33 mM deoxynucleoside triphosphates) and 0.4 μl of reverse transcriptase. The extension reaction was placed at 37°C for 20 min and then terminated with the addition of 300 μl of ethanol. The nucleic acids were pelleted and then resuspended in 5 μl of formamide loading buffer–0.1 M NaOH (2:1) (30). The reaction products were then separated on an 8% polyacrylamide-urea sequencing gel along with a set of double-stranded sequencing reactions of pJR1706, using HISER031 as a primer in order to size the products, and visualized by autoradiography.

RESULTS

Feedback regulation of acetoacetyl-CoA thiolase. Previous work indicated that in *S. cerevisiae*, the activity of acetoacetyl-CoA thiolase, the first enzyme of the sterol-biosynthetic pathway, was induced in strains compromised in the ability to synthesize ergosterol, the fungal equivalent of cholesterol (39). This observation was confirmed in assays using an *erg13* mutant strain, which is a mevalonate auxotroph. The gene *ERG13* encodes HMG-CoA synthase, and its disruption results in a strain that requires exogenous mevalonate supplementation for viability (Fig. 1). The degree of flux through the mevalonate pathway can be modulated to some extent in such a strain by altering

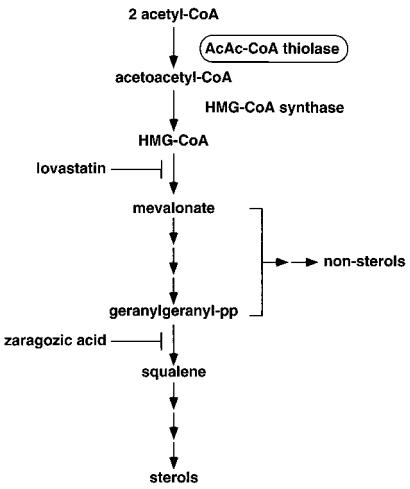

FIG. 1. The mevalonate pathway. Key intermediates of the pathway are shown, including the formation of acetoacetyl (AcAc)-CoA, catalyzed by the enzyme acetoacetyl-CoA thiolase. The sterol and nonsterol products of the pathway are distinguished. The sites of action of the drugs lovastatin (an inhibitor of HMG-CoA reductase) and zaragozic acid (an inhibitor of squalene synthase) are indicated. Multiple arrows denote multiple steps in the pathway.

the amount of mevalonate supplementation. The mevalonate auxotroph exhibited a threefold increase in acetoacetyl-CoA thiolase activity when supplemented with a low amount of exogenous mevalonate (5 mg/ml) (Fig. 2). In addition, the acetoacetyl-CoA thiolase activity returned to wild-type levels with an increase in the amount of exogenous supplementation (40

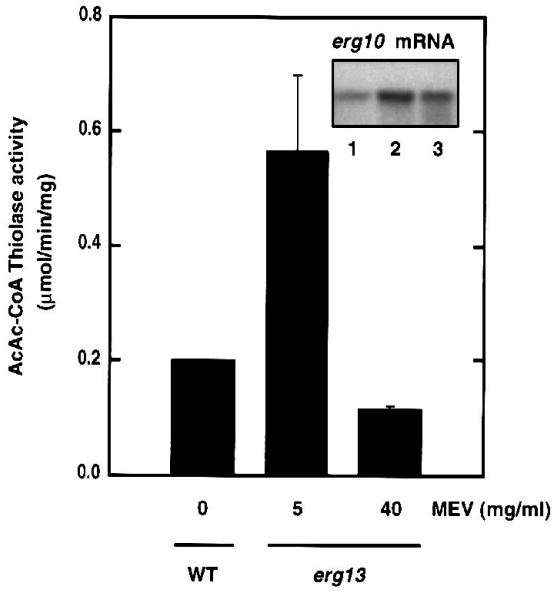

FIG. 2. Effect of mevalonate limitation on acetoacetyl-CoA thiolase enzyme activity. A wild-type (WT) strain (JRY1443) and a mevalonate auxotrophic strain (JRY4002) were grown with the indicated amount of exogenous mevalonate (MEV) supplementation. During logarithmic-phase growth, samples were taken, crude cell extracts were prepared, and acetoacetyl (AcAc)-CoA thiolase enzyme assays were performed. (Inset) *ERG10* mRNA analysis. Poly(A) RNA was isolated from a wild-type strain (JRY527; lane 1) and a mevalonate auxotrophic strain (JRY2138) grown in either a low amount of exogenous mevalonate (5 mg/ml; lane 2) or a high amount of exogenous mevalonate (40 mg/ml; lane 3). Three micrograms of poly(A) RNA was separated on an agarose-formaldehyde gel and blotted onto nitrocellulose. The blot was probed with an 800-bp *Nsi*I-*Eco*RI fragment of the *ERG10* gene and then subjected to autoradiography.

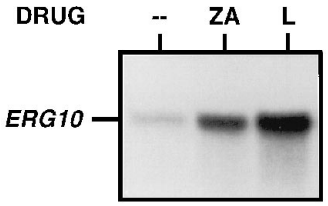

FIG. 3. Analysis of *ERG10* mRNA. A wild-type strain (JRY527) was treated with either 40 μg of lovastatin (L) per ml for 4 h or 60 μg of zaragozic acid (ZA) per ml for 3 h or left untreated (– –). Three micrograms of poly(A) RNA was then separated by gel electrophoresis and blotted onto nitrocellulose. The blot was probed with an 800-bp *Nsi*I-*Eco*RI fragment of the *ERG10* gene and then subjected to autoradiography.

mg/ml). Acetoacetyl-CoA thiolase is encoded by the *ERG10* gene (19), and this observation raised the possibility that *ERG10* expression was subject to feedback regulation. Examination of the steady-state levels of *ERG10* mRNA revealed quantitative changes that could account for the changes in enzyme levels (Fig. 2, inset).

Treatment of cells with competitive inhibitors of enzymes involved in isoprenoid biosynthesis provided an alternate approach to altering flux through the mevalonate pathway. Lovastatin is a competitive inhibitor of an early pathway enzyme, HMG-CoA reductase, and its action results in the depletion of mevalonate, which in turn causes the depletion of all isoprenoids. In contrast, treatment with zaragozic acid, a competitive inhibitor of squalene synthase, results in the depletion of squalene and the sterol compounds produced from squalene but does not alter the nonsterol isoprenoid pools. Interestingly, treatment with both of these drugs resulted in increased steady-state levels of *ERG10* mRNA (Fig. 3). Two important points can be drawn from this observation. First, the changes in mRNA levels were quantitatively similar to the changes in enzyme activity and mRNA levels observed in the mevalonate auxotroph, providing independent evidence of the regulation of the steady-state *ERG10* mRNA level. Second, the induction of *ERG10* in response to zaragozic acid treatment revealed that this feedback regulation was cued to a late (squalene or later) product of the pathway (Fig. 1). The differences in the level of *ERG10* induction between the lovastatin and zaragozic acid treatments may be biologically relevant (see below). Effects on stability could not account for the altered *ERG10* mRNA levels (not shown).

To map the transcriptional start sites of the *ERG10* mRNA and to assess whether the quantitative changes in mRNA level were associated with any qualitative differences in transcription start sites, primer extension analysis was performed. The *ERG10* transcripts produced under both mevalonate-sufficient and mevalonate-limited conditions exhibited the same relative distribution of start sites (Fig. 4). Moreover, the majority of start sites were quantitatively more abundant upon mevalonate limitation, indicating an overall quantitative induction of *ERG10* mRNA production in response to mevalonate limitation without any apparent qualitative differences.

One would expect that the reason cells induced *ERG10* upon lowered sterol pools would be to increase sterol production. However, HMG-CoA reductase catalyzes the rate-limiting step in sterol synthesis in animal cells (41), and HMG-CoA reductase levels are subject to feedback regulation in yeast cells (12, 18). Therefore, we tested whether increased levels of acetoacetyl-CoA thiolase activity could result in increased sterol synthesis by using two strains in which HMG-CoA reductase levels were set lower or higher than that in wild-type cells. One of the two strains contained a disruption of the *hmg1* gene

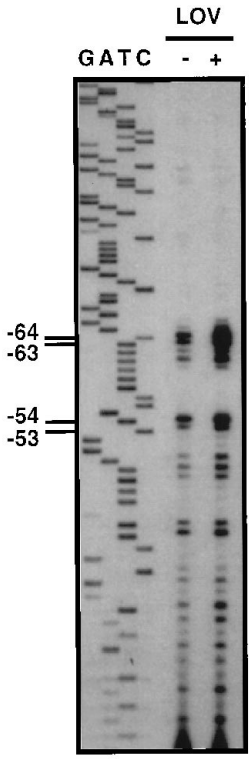

FIG. 4. Primer extension analysis of the *ERG10* transcript. A wild-type strain (JRY527) was grown in either the absence or the presence of 40 μg of lovastatin (LOV) per ml for 8 h, and then poly(A) selection of isolated total RNA was performed. A ^{32}P end-labeled oligonucleotide (HISER031) complementary to a region of the *ERG10* transcript spanning the initiator codon was used in a primer extension reaction. The reaction products were electrophoretically separated along with a set of sequencing reactions (leftmost four lanes). The numbers to the left indicate the positions of four strong transcriptional start sites (where the A of the initiation codon is designated +1).

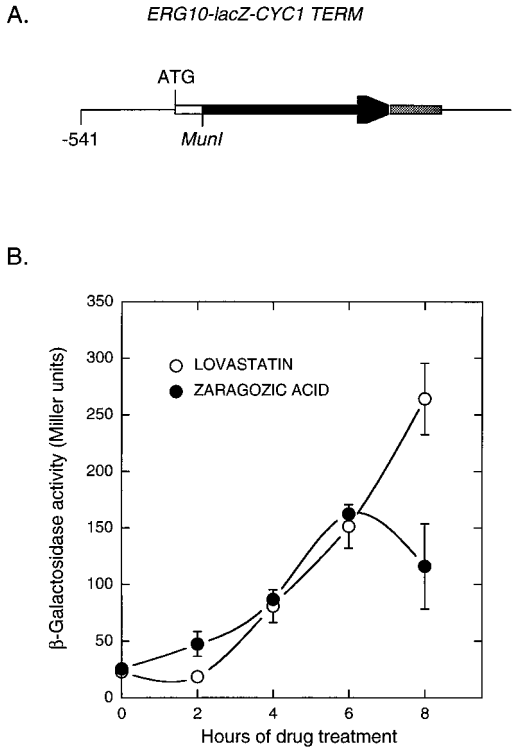

FIG. 5. Feedback regulation of an *ERG10-lacZ* reporter gene fusion. (A) Schematic representation of the *ERG10-lacZ* construct. The *ERG10* promoter including the first 15 codons of the *ERG10* coding region was fused to the bacterial *lacZ* gene, followed by the *CYC1* terminator (*TERM*). The promoter region extends 541 bp upstream of the initiator methionine. (B) Induction of reporter gene expression by inhibition of squalene synthesis. A strain harboring the *ERG10-lacZ* reporter gene integrated at the *URA3* chromosomal locus (JRY5033) was treated with either lovastatin (40 μg/ml) or zaragozic acid (60 μg/ml). At various times following the addition of drug, samples were removed and β-galactosidase assays were performed.

(JRY1590) and hence had only the level of enzyme produced by *HMG2*. The other (JRY5112) contained the *HMG2* coding sequence fused to the powerful *GPD* promoter. The level of HMG-CoA reductase activity in the second strain was 20-fold higher than that in the first. Both strains contained a wild-type copy of the *ERG10* gene. In addition, we constructed transformants of both strains that contained a multicopy plasmid with the *ERG10* gene that increased thiolase levels 10-fold (JRY5113 and JRY5114). Although the strain with greater HMG-CoA reductase activity synthesized more sterols, elevated levels of acetoacetyl-CoA thiolase had no effect on sterol synthesis (Table 2). Therefore, the biological role for feedback regulation of *ERG10* remained unclear (see Discussion).

Feedback regulation of an *ERG10-lacZ* reporter gene construct. To facilitate the analysis of *ERG10* feedback regulation, an *ERG10-lacZ* reporter gene fusion was constructed. This fusion gene contains the *ERG10* promoter region (541 bp upstream of the initiator codon) and includes the first 15 codons of *ERG10* followed by the bacterial *lacZ* gene and the transcriptional terminator element from the yeast *CYC1* gene (Fig. 5A). This reporter gene was integrated into the *URA3* chromosomal locus, and β-galactosidase activity expressed from the fusion gene in response to altered flux through the mevalonate pathway was monitored. As observed for the wild-type *ERG10* gene, treatments with both lovastatin and zaragozic acid resulted in an induction of β-galactosidase activity expressed from the reporter gene (Fig. 5B). Similarly, increasing concentrations of exogenous mevalonate supplementation of a mevalonate auxotroph harboring the reporter gene reduced the level of expression (not shown). The level of induction observed with the reporter gene was quantitatively greater than that observed by either acetoacetyl-CoA thiolase enzyme activity measurements or *ERG10* mRNA analysis (10-fold versus 3-fold).

Interestingly, the final level of induction achieved was greater with lovastatin treatment than with zaragozic acid treatment, as was observed for the *ERG10* mRNA. This might simply be the result of cell death following prolonged exposure to zaragozic acid. Zaragozic acid appears to be more cytotoxic to yeast cells than lovastatin (11a). An alternate explanation was that there are two intermediates of the pathway, a nonsterol and a sterol product, that independently set the expression level of *ERG10*. Lovastatin treatment would cause the

TABLE 2. Acetate labeling of sterols with overexpression of early pathway genes *HMG2* and *ERG10*

Strain	Relevant genotype	Labeled sterols (arbitrary PhosphorImager units)
JRY1590	*hmg1*::*LYS2*	72.2
JRY5112	*GPD-HMG2*	239.2
JRY5113	*ERG10* (2μm)	62.4
JRY5114	*GPD-HMG2 ERG10* (2μm)	256.3

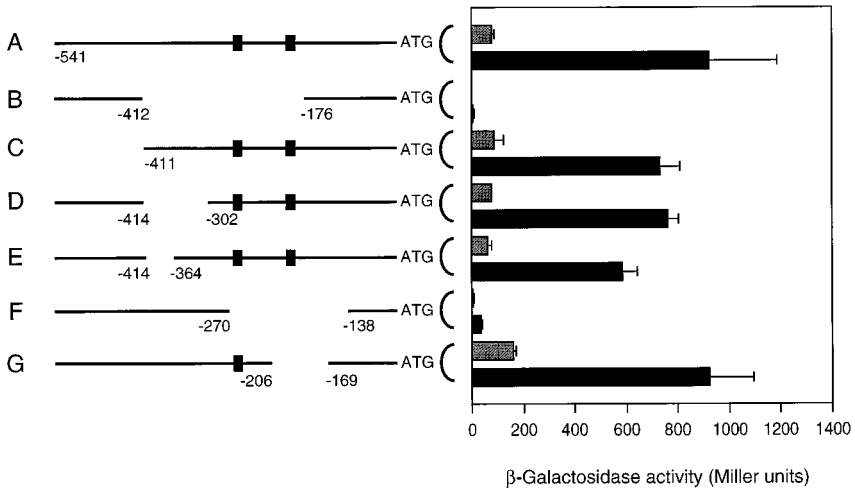

FIG. 6. Deletion analysis of the *ERG10* promoter. A series of deletions was constructed within the promoter region of the *ERG10-lacZ* reporter gene. On the left are schematic representations of the various constructs. Two sequences consisting of a 7-of-8-bp match with the core SRE element consensus are indicated (black boxes). The boundaries of the deletions are denoted by the numbers (where the A of the initiation codon is designated +1). Strains harboring these various constructs were grown in either the absence (gray bars) or the presence (black bars) of lovastatin (40 μg/ml). After 16 h of growth, samples were taken and β-galactosidase assays were performed (shown on the right). The uninduced-induced numerical values plotted are as follows: A, 80-921; B, 1.1-6.7; C, 88-731; D, 75-762; E, 61-587; F, 7.5-36; and G, 158-922.

depletion of both, whereas zaragozic acid would cause depletion of only the sterol product and thereby result in a lower level of induction. However, since the relative levels of induction at the earlier time points are the same, the toxicity explanation was most parsimonious.

***ERG10* promoter deletion analysis.** To gain some insight into the sequence elements mediating the observed regulation of the reporter gene, a deletion analysis of the promoter region was initiated (Fig. 6). A truncated version of the reporter gene containing only 411 bp of the 5′ region (Fig. 6, construct C) exhibited regulated expression similar to that of a reporter with 541 bp of the promoter region (Fig. 6, construct A). This result indicated that the 411-bp region of the promoter was sufficient to confer regulated expression. Removal of a 236-bp region of the promoter (Fig. 6, construct B) resulted in a complete loss of expression, both noninduced and induced. This result implied that elements required for expression and regulation reside within this region. Two smaller deletions, construct D (−413 to −303) and construct E (−413 to −365) (Fig. 6), resulted in expression levels essentially identical to that of the full-length promoter. Assuming that there were not any redundant elements, these data suggest that the regulatory element(s) resides within the 127-bp region from −302 to −176. A deletion of a portion of this region, construct G (−205 to −170 [Fig. 6]), did not result in altered expression, narrowing the location of the regulatory element(s) to the 96-bp region from −302 to −206. One final deletion, construct F (−269 to −139 [Fig. 6]), removed a portion of this region and resulted in a marked decrease in expression. This result suggested that a positive-acting element had been removed. Therefore, the deletions suggested the presence of a positive-acting element within a 63-bp region of the *ERG10* promoter from positions −269 to −206, which was responsible for the majority of expression under both inducing and noninducing conditions. However, the observation that lovastatin still caused some induction of expression of construct F (Fig. 6) raised the possibility that there was a second element residing in the adjacent region, from positions −302 to −270.

The observation that deletions resulting in an impaired ability to induce expression also caused a decrease in the noninduced levels of expression suggested that regulated expression might require the same promoter element(s) for both noninduced and induced expression. Examination of the sequence of the *ERG10* promoter revealed two sequences, one at −185 to −179 and another at −262 to −255, matching seven of the eight nucleotides of the core SRE-1 consensus sequence (Fig. 7A). These sequence similarities raised the interesting possibility that these elements are involved in the sterol-mediated regulation of *ERG10* expression, analogous to the role of SREs in the promoters of animal genes. One of these elements was within the region from −269 to −206, the region that appeared to contain an element responsible for the majority of *ERG10* expression. Exact deletions of the proximal and/or distal SRE-like sequences were constructed in order to assess their potential involvement in *ERG10* expression. Deletion of the proximal SRE-like sequence caused a small but significant increase in reporter gene expression (Fig. 7B, construct D). Therefore, it appeared that this sequence may act as a negative element within the context of the *ERG10* promoter. However, removal of either the distal SRE-like sequence (Fig. 7B, construct C) or both elements (Fig. 7B, construct E) resulted in essentially wild-type expression. In contrast, deletion of a slightly larger region, including the distal SRE-like sequence and the adjacent 24 bp (from −262 to −231), resulted in a marked decrease in expression (Fig. 7B, construct B). In fact, the levels of both noninduced and induced expression were essentially the same as those observed for the larger deletion (Fig. 6, construct F). These data indicated that this small 32-bp region of the *ERG10* promoter was required for the majority of expression.

The possible involvement of SRE-like sequences in sterol-mediated transcriptional regulation of *ERG10* was intriguing. As mentioned in the introduction, two transcription factors have recently been identified by virtue of their ability to bind SRE-1 (5, 51). These two proteins, SREBP-1 and SREBP-2, belong to the bHLH-ZIP family of transcription factors (20, 56). A comparison search of the protein database with the University of Wisconsin Genetics Computer Group analysis package (11) identified a yeast protein, Tye7p, with significant homology to the DNA binding, bHLH-ZIP region of the

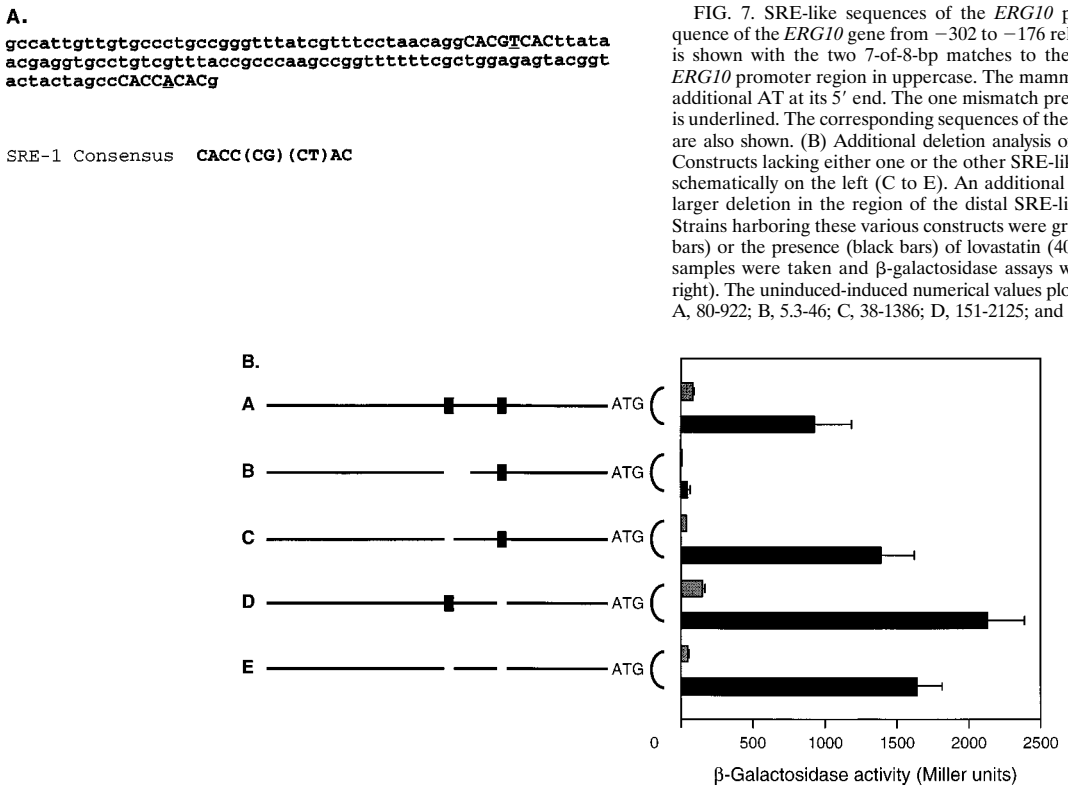

FIG. 7. SRE-like sequences of the *ERG10* promoter region. (A) The sequence of the *ERG10* gene from −302 to −176 relative to the start of translation is shown with the two 7-of-8-bp matches to the SRE-1 consensus within the *ERG10* promoter region in uppercase. The mammalian SRE-1 consensus has an additional AT at its 5′ end. The one mismatch present in each SRE-like element is underlined. The corresponding sequences of the two *ERG10* SRE-like elements are also shown. (B) Additional deletion analysis of the *ERG10* promoter region. Constructs lacking either one or the other SRE-like element, or both, are shown schematically on the left (C to E). An additional construct containing a slightly larger deletion in the region of the distal SRE-like element is also shown (B). Strains harboring these various constructs were grown in either the absence (gray bars) or the presence (black bars) of lovastatin (40 μg/ml). After 16 h of growth, samples were taken and β-galactosidase assays were performed (shown on the right). The uninduced-induced numerical values plotted in the figure are as follows: A, 80-922; B, 5.3-46; C, 38-1386; D, 151-2125; and E, 49-1635.

SREBPs. Tye7p exhibits 48% identity with SREBP-1a in the basic-helix 1 region and 74% identity in the helix 2 region (Fig. 8A). Tye7p is also a putative member of the bHLH-ZIP family of transcription factors and was identified by a mutation that altered Ty1-mediated gene expression of the *ADH2* gene (29). However, loss of Tye7p function did not affect *ERG10* expression (Fig. 8B).

DISCUSSION

Mammalian cells coordinately regulate the transcription of a number of genes involved in sterol metabolism in order to maintain proper levels of intracellular sterols (16). The paradigm of transcriptional feedback regulation by a sterol product appears to extend to the budding yeast, *S. cerevisiae*. A series of experiments described here led to the discovery that the transcription of the *ERG10* gene, encoding the first enzyme of the sterol-biosynthetic pathway, was regulated by the sterol levels in cells. Disruption of the HMG-CoA synthase gene (*ERG13*) was used to create a mevalonate auxotroph. These mevalonate auxotrophic cells supplemented with a low amount of mevalonate contained threefold more acetoacetyl-CoA thiolase enzyme activity relative to a wild-type cell. This effect was consistent with previous reports of induced acetoacetyl-CoA thiolase activity in mutant cells unable to synthesize ergosterol, the bulk end product of the mevalonate pathway in *S. cerevisiae* (39). This induced enzyme level could be reversed by increasing the amount of supplemented mevalonate, consistent with the notion that enzyme levels are sensitive to flux through the pathway.

We extended the earlier observations on induced enzyme activity to changes in the expression of the structural gene. Specifically, expression of the gene encoding acetoacetyl-CoA thiolase, *ERG10*, was induced upon mevalonate depletion by either genetic or pharmacological means. In fact, the increase in *ERG10* mRNA levels was sufficient to account for the increased enzyme activity. Therefore, there was no evidence for multiple mechanisms of regulation. The stability of the *ERG10* message was the same in mevalonate-sufficient and mevalo-

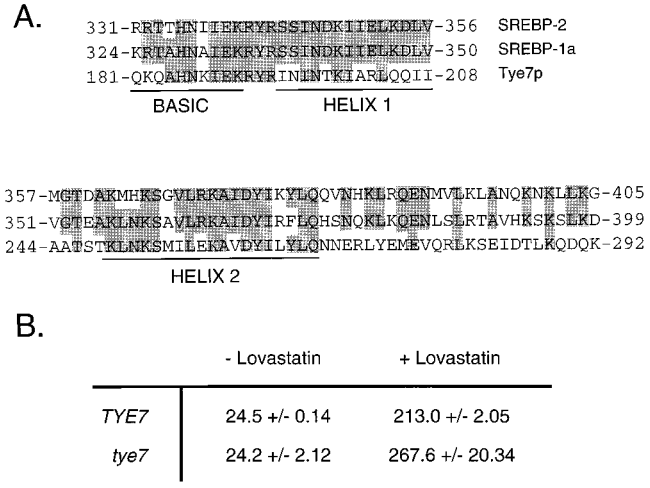

FIG. 8. Tye7p, a yeast SREBP homolog. (A) Sequence homology between two mammalian SREBPs and the yeast transcription factor Tye7p within the bHLH-ZIP domain. Tye7p has an extended loop region that is not depicted. Identical amino acid residues are indicated by the gray shading. (B) Comparison of *ERG10-lacZ* expression levels in wild-type cells and cells containing a disruption allele of *TYE7*. Both strains were either left untreated or treated with lovastatin (40 μg/ml) for >12 h. β-Galactosidase assays were then performed on exponentially growing cultures, and results are expressed in Miller units.

nate-limited conditions, indicating that the induced level of expression was due to increased transcription.

The utilization of inhibitors of different steps of the pathway allows for the depletion of subpopulations of isoprenoids. Zaragozic acid is a competitive inhibitor of squalene synthase (4). Therefore, treatment of cells with this drug depletes both the squalene pools and the sterol compounds derived from squalene. Because zaragozic acid caused increased *ERG10* expression, *ERG10* regulation responded to sterol (or squalene) depletion. Ergosterol is an obvious candidate for the sterol responsible for regulating *ERG10* expression. Altered levels of exogenous ergosterol have been reported to alter acetoacetyl-CoA thiolase activity in aerobically grown cells (50). Because of the requirement for oxygen in the synthesis of sterols, it would be interesting to examine *ERG10* expression as cells undergo the transition from aerobic to anaerobic growth. Presumably, as oxygen becomes limiting and sterol production is impaired, *ERG10* expression would be induced.

The biological rationale for the feedback regulation of *ERG10* was not clear. Usually, feedback regulation serves to adjust the level of expression of a gene in order to satisfy the cellular demand for a particular product. Because the product involved in *ERG10* regulation appeared to be a sterol, altering *ERG10* expression levels would be predicted to increase the sterol pools. However, when the *ERG10* gene was overexpressed, there was no increase in radiolabeled acetate incorporation into total sterols. It is possible that the purpose of the regulation is to increase nonsterol isoprenoid production, although it would be difficult to understand why regulation was sensitive to sterol levels if that were the case. A more satisfying explanation is that another enzyme was rate limiting for sterol production under conditions in which acetoacetyl-CoA thiolase was overproduced, and that the other enzyme must be induced with acetoacetyl-CoA thiolase in order to achieve increased sterol synthesis. *ERG9*, the structural gene encoding squalene synthase, is a good candidate for a second potentially rate-limiting enzyme because lovastatin treatment induces elevated levels of *ERG9* mRNA (36).

Deletion analysis of the *ERG10* promoter identified two regions involved in expression. The 32-bp region (−262 to −231) appeared to be required for the majority of both expression and regulation. The adjacent 33-bp region (−302 to −270) was deduced to also contribute to expression. Comparison of these two regions with the promoter region of the *ERG9* gene revealed some striking similarities. There are three blocks of sequence (termed I, II, and III) that are present in both promoters in the same orientation and relative order. Sequences I and II (TTG-T-TGCCC and TTT-TC-TTTCC, respectively) are present in the −302 to −270 region of the *ERG10* promoter. In fact, these two sequences comprise 23 of the 33 nucleotides of this region. The third sequence (GTTTA--GCC-AA, −235 to −223) partially overlaps the −262 to −231 region. All three of these sequences are present in the *ERG9* promoter region.

Although the data present here indicated that loss of Tye7p function does not affect *ERG10* expression, it is possible that its function is redundant in the cell. It is worth noting that Tye7p has three conserved amino acids within the DNA binding domain (His, Glu, and Arg) that are present in other bHLH-ZIP family members. In fact, these three residues of the Max protein have been found to bind the DNA sequence CAC (14), and these residues are required for the DNA binding activity of SREBP-1 (37). Therefore, the binding site of Tye7p would be predicted to contain a CAC sequence. Both SRE-like elements in the *ERG10* promoter contain a CAC sequence. The discovery reported here of natural sterol-mediated control of gene transcription in yeast cells offers the promise of bringing genetics to bear on this important facet of regulation in the cholesterol pathway. Although a mechanistic picture for sterol-mediated transcriptional regulation of some of these genes is beginning to emerge, there are still many details that are unclear. For example, it is not known whether sterol levels directly influence the putative protease responsible for SREBP processing, or whether the mechanism is indirect involving some other sterol-sensing protein. It has been postulated that an oxygenated sterol, rather than cholesterol itself, is the feedback regulator (24). An oxysterol-binding protein (OSBP) has been identified (25). Intriguingly, the binding affinities of OSBP for a variety of oxysterols correlate with their ability to repress expression of HMG-CoA reductase (26, 48). OSBP has been purified and cloned (9, 10). However, there is no direct evidence for its involvement in feedback regulation. It is interesting to note that *S. cerevisiae* appears to encode a family of OSBP homologs, although their involvement in isoprenoid metabolism is unclear (22). Furthermore, the transcriptional regulation of the farnesyl diphosphate synthase gene in response to sterol levels does not appear to utilize SRE-1 (44, 45), suggesting that at least one other mechanism of transcriptional regulation exists in animal cells. Clearly, to the extent that the regulatory circuitry of yeast and human sterol-biosynthetic pathways overlap, the facile manipulative genetic analysis of *S. cerevisiae* will lead to a rapid resolution of some of these outstanding issues.

ACKNOWLEDGMENTS

We thank A. Alberts (Merck) for providing lovastatin and J. Bergstrom (Merck) for providing zaragozic acid. We also thank M. ("Nick") Ashby and Randy Hampton for critical review of the manuscript as well as numerous helpful discussions.

D.D.-D. was supported by a National Science Foundation predoctoral fellowship. This work was further supported by a grant from the National Institutes of Health (GM-35827 to J.R.) and core support from a National Institute of Environmental Health Sciences Mutagenesis Center grant (P30 ESO 1896).

REFERENCES

1. **Barnes, G., W. J. Hansen, C. L. Holcomb, and J. Rine.** 1984. Asparagine-linked glycosylation in *Saccharomyces cerevisiae*: genetic analysis of an early step. Mol. Cell. Biol. **4:**2381–2388.
2. **Basson, M. E., R. L. Moore, J. O'Rear, and J. Rine.** 1987. Identifying mutations in duplicated functions in Saccharomyces cerevisiae: recessive mutations in HMG-CoA reductase genes. Genetics **117:**645–655.
3. **Basson, M. E., M. Thorsness, M. J. Finer, R. M. Stroud, and J. Rine.** 1988. Structural and functional conservation between yeast and human 3-hydroxy-3-methylglutaryl coenzyme A reductases, the rate-limiting enzyme of sterol biosynthesis. Mol. Cell. Biol. **8:**3797–3808.
4. **Bergstrom, J. D., M. M. Kurtz, D. J. Rew, A. M. Amend, J. D. Karkas, R. G. Bostedor, V. S. Bansal, C. Dufresne, F. L. VanMiddlesworth, O. D. Hensens, et al.** 1993. Zaragozic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase. Proc. Natl. Acad. Sci. USA **90:** 80–84.
5. **Briggs, M. R., C. Yokoyama, X. Wang, M. S. Brown, and J. L. Goldstein.** 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. J. Biol. Chem. **268:**14490–14496.
6. **Caponigro, G., D. Muhlrad, and R. Parker.** 1993. A small segment of the *MATα1* transcript promotes mRNA decay in yeast: a stimulatory role for rare codons. Mol. Cell. Biol. **13:**5141–5148.
7. **Dawson, P. A., S. L. Hofmann, D. R. van der Westhuyzen, T. C. Sudhof, M. S. Brown, and J. L. Goldstein.** 1988. Sterol-dependent repression of low density lipoprotein receptor promoter mediated by 16-base pair sequence adjacent to binding site for transcription factor Sp1. J. Biol. Chem. **263:**3372–3379.
8. **Dawson, P. A., J. E. Metherall, N. D. Ridgway, M. S. Brown, and J. L. Goldstein.** 1991. Genetic distinction between sterol-mediated transcriptional and posttranscriptional control of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Biol. Chem. **266:**9128–9134.
9. **Dawson, P. A., N. D. Ridgway, C. A. Slaughter, M. S. Brown, and J. L. Goldstein.** 1989. cDNA cloning and expression of oxysterol-binding protein, an oligomer with a potential leucine zipper. J. Biol. Chem. **264:**16798–16803.

10. **Dawson, P. A., D. R. Van der Westhuyzen, J. L. Goldstein, and M. S. Brown.** 1989. Purification of oxysterol binding protein from hamster liver cytosol. J. Biol. Chem. **264:**9046–9052.
11. **Devereux, J., P. Haeberli, and O. Smithies.** 1994. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
11a. **Dimster-Denk, D., and R. Hampton.** Unpublished observations.
12. **Dimster-Denk, D., M. K. Thorsness, and J. Rine.** 1994. Feedback regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in Saccharomyces cerevisiae. Mol. Biol. Cell **5:**655–665.
13. **Evans, M. J., and J. E. Metherall.** 1993. Loss of transcriptional activation of three sterol-regulated genes in mutant hamster cells. Mol. Cell. Biol. **13:** 5175–5185.
14. **Ferre-D'Amare, A. R., G. C. Prendergast, E. B. Ziff, and S. K. Burley.** 1993. Recognitin by Max of its cognate DNA through a dimeric b/HLH/Z domain. Nature (London) **363:**38–45.
15. **Gasic, G.** 1994. Basic-helix-loop-helix transcription factor and sterol sensor in a single membrane-bound molecule. Cell **77:**17–19.
16. **Goldstein, J. L., and M. S. Brown.** 1990. Regulation of the mevalonate pathway. Nature (London) **343:**425–430.
17. **Hagen, D. C., and G. F. Sprague, Jr.** 1984. Induction of the yeast α-specific *STE3* gene by the peptide pheromone **a**-factor. J. Mol. Biol. **178:**835–852.
18. **Hampton, R. Y., and J. Rine.** 1994. Regulated degradation of HMG-CoA reductase, an integral membrane protein of the endoplasmic reticulum, in yeast. J. Cell Biol. **125:**299–312.
19. **Hiser, L., M. E. Basson, and J. Rine.** 1994. *ERG10* from Saccharomyces cerevisiae encodes acetoacetyl-CoA thiolase. J. Biol. Chem. **269:**31383–31389.
20. **Hua, X., C. Yokoyama, J. Wu, M. R. Briggs, M. S. Brown, J. L. Goldstein, and X. Wang.** 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc. Natl. Acad. Sci. USA **90:**11603–11607.
21. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells with alkali cations. J. Bacteriol. **153:**163–168.
22. **Jiang, B., J. L. Brown, J. Sheraton, N. Fortin, and H. Bussey.** 1994. A new family of yeast genes implicated in ergosterol synthesis is related to the human oxysterol binding protein. Yeast **10:**341–353.
23. **Jones, K. A., and L. Prakash.** 1990. Yeast Saccharomyces cerevisiae selectable markers in pUC18 polylinkers. Yeast **6:**363–366.
24. **Kandutsch, A. A., H. W. Chen, and H. J. Geiniger.** 1978. Biological activity of some oxygenated sterols. Science **201:**498–501.
25. **Kandutsch, A. A., H. W. Chen, and E. P. Shown.** 1977. Binding of 25-hydroxycholesterol and cholesterol to different cytoplasmic proteins. Proc. Natl. Acad. Sci. USA **74:**2500–2506.
26. **Kandutsch, A. A., and E. B. Thompson.** 1980. Cytosolic proteins that bind oxygenated sterols. J. Biol. Chem. **255:**10813–10826.
27. **Kohrer, K., and H. Domdey.** 1991. Preparation of high molecular weight RNA. Methods Enzymol. **194:**398–405.
28. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:**367–382.
29. **Lohning, C., and M. Ciriacy.** 1994. The *TYE7* gene of Saccharomyces cerevisiae encodes a putative bHLH-ZIP transcription factor required for Ty1-mediated gene expression. Yeast **10:**1329–1339.
30. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Metherall, J. E., J. L. Goldstein, K. L. Luskey, and M. S. Brown.** 1989. Loss of transcriptional repression of three sterol-regulated genes in mutant hamster cells. J. Biol. Chem. **264:**15634–15641.
32. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
33. **Osborne, T. F.** 1991. Single nucleotide resolution of sterol regulatory region in promoter for 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. **266:**13947–13951.
34. **Osborne, T. F., G. Gil, J. L. Goldstein, and M. S. Brown.** 1988. Operator constitutive mutation of 3-hydroxy-3-methylglutaryl coenzyme A reductase promoter abolishes protein binding to sterol regulatory element. J. Biol. Chem. **263:**3380–3387.
35. **Parks, L. W., L. D. K. Bottema, R. J. Rodriguez, and T. A. Lewis.** 1985. Yeast sterols: yeast mutants as tools for the study of sterol metabolism. Methods Enzymol. **111:**333–346.
36. **Robinson, G. W., Y. H. Tsay, B. K. Kienzle, C. A. Smith-Monroy, and R. W. Bishop.** 1993. Conservation between human and fungal squalene synthetases: similarities in structure, function, and regulation. Mol. Cell. Biol. **13:** 2706–2717.
37. **Sato, R., J. Yang, X. Wang, M. J. Evans, Y. K. Ho, J. L. Goldstein, and M. S. Brown.** 1994. Assignment of the membrane attachment, DNA binding, and transcriptional activation domains of sterol regulatory element-binding protein-1 (SREBP-1). J. Biol. Chem. **269:**17267–17273.
37a. **Schafer, W. R., R. Kim, R. Sterne, J. Thorner, S.-H. Kim, and J. Rine.** 1989. Genetic and pharmacological suppression of oncogenic mutations in Ras genes of yeast and humans. Science **245:**379–385.
38. **Schiestl, R. H., and R. D. Gietz.** 1989. High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. **16:**339–346.
39. **Servouse, M., and F. Karst.** 1986. Regulation of early enzymes of ergosterol biosynthesis in Saccharomyces cerevisiae. Biochem. J. **240:**541–547.
40. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
41. **Siperstein, J. R., and V. M. Fagan.** 1966. Feedback control of mevalonate synthesis by dietary cholesterol. J. Biol. Chem. **241:**602–609.
42. **Smith, J. R., T. F. Osborne, M. S. Brown, J. L. Goldstein, and G. Gil.** 1988. Multiple sterol regulatory elements in promoter for hamster 3-hydroxy-3-methylglutaryl-coenzyme A synthase. J. Biol. Chem. **263:**18480–18487.
43. **Smith, J. R., T. F. Osborne, J. L. Goldstein, and M. S. Brown.** 1990. Identification of nucleotides responsible for enhancer activity of sterol regulatory element in low density lipoprotein receptor gene. J. Biol. Chem. **265:**2306–2310.
44. **Spear, D. H., J. Ericsson, S. M. Jackson, and P. A. Edwards.** 1994. Identification of a 6-base pair element involved in the sterol-mediated transcriptional regulation of farnesyl diphosphate synthase. J. Biol. Chem. **269:**25212–25218.
45. **Spear, D. H., S. Y. Kutsunai, C. C. Correll, and P. A. Edwards.** 1992. Molecular cloning and promoter analysis of the rat liver farnesyl diphosphate synthase gene. J. Biol. Chem. **267:**14462–14469.
46. **Sudhof, T. C., D. W. Russell, M. S. Brown, and J. L. Goldstein.** 1987. 42 bp element from LDL receptor gene confers end-product repression by sterols when inserted into viral TK promoter. Cell **48:**1061–1069.
47. **Sudhof, T. C., D. R. Van der Westhuyzen, J. L. Goldstein, M. S. Brown, and D. W. Russell.** 1987. Three direct repeats and a TATA-like sequence are required for regulated expression of the human low density lipoprotein receptor gene. J. Biol. Chem. **262:**10773–10779.
48. **Taylor, F. R., S. E. Saucier, E. P. Shown, E. J. Parish, and A. A. Kandutsch.** 1984. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase. J. Biol. Chem. **259:**12382–12387.
49. **Thorsness, M., W. Schafer, L. D'Ari, and J. Rine.** 1989. Positive and negative transcriptional control by heme of genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**5702–5712.
50. **Trocha, P. J., and D. B. Sprinson.** 1976. Location and regulation of early enzymes of sterol biosynthesis in yeast. Arch. Biochem. Biophys. **174:**45–51.
51. **Wang, X., M. R. Briggs, X. Hua, C. Yokoyama, J. L. Goldstein, and M. S. Brown.** 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. J. Biol. Chem. **268:**14497–14504.
52. **Wang, X., R. Sato, M. S. Brown, X. Hua, and J. L. Goldstein.** 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell **77:**53–62.
53. **Wright, R., M. Basson, L. D'Ari, and J. Rine.** 1988. Increased amounts of HMG-CoA reductase induce "karmellae": a proliferation of stacked membrane pairs surrounding the yeast nucleus. J. Cell Biol. **107:**101–114.
54. **Yang, J., M. S. Brown, Y. K. Ho, and J. L. Goldstein.** 1995. Three different rearrangements in a single intron truncate sterol regulatory element binding protein-2 and produce sterol-resistant phenotype in three cell lines. Role of introns in protein evolution. J. Biol. Chem. **270:**12152–12161.
55. **Yang, J., R. Sato, J. L. Goldstein, and M. S. Brown.** 1994. Sterol-resistant transcription in CHO cells caused by gene rearrangement that truncates SREBP-2. Genes Dev. **8:**1910–1919.
56. **Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, J. L. Goldstein, and M. S. Brown.** 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell **75:**187–197.