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Upc2p and Ecm22p, Dual Regulators of Sterol Biosynthesis in *Saccharomyces cerevisiae*

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Sterol levels affect the expression of many genes in yeast and humans. We found that the paralogous transcription factors Upc2p and Ecm22p of yeast were sterol regulatory element (SRE) binding proteins (SREBPs) responsible for regulating transcription of the sterol biosynthetic genes *ERG2* and *ERG3*. We defined a 7-bp SRE common to these and other genes, including many genes involved in sterol biosynthesis. Upc2p and Ecm22p activated *ERG2* expression by binding directly to this element in the *ERG2* promoter. Upc2p and Ecm22p may thereby coordinately regulate genes involved in sterol homeostasis in yeast. Ecm22p and Upc2p are members of the fungus-specific Zn[2]-Cys[6] binuclear cluster family of transcription factors and share no homology to the analogous proteins, SREBPs, that are responsible for transcriptional regulation by sterols in humans. These results suggest that *Saccharomyces cerevisiae* and human cells regulate sterol synthesis by different mechanisms.

Sterols, essential for life in most, if not all, eukaryotes, are synthesized by a pathway known variously as the mevalonate pathway, the isoprenoid pathway, or the sterol biosynthetic pathway. Although sterols (which include molecules ranging from cholesterol to steroid hormones) are the major products of the sterol biosynthetic pathway, this pathway also produces many important nonsterol compounds that are necessary for cellular processes such as respiration, glycosylation, protein prenylation and photosynthesis. In the heavily studied mammalian sterol biosynthetic pathway, regulation occurs at both the transcriptional and the posttranscriptional levels. At present, the best-understood aspect of this regulation is the transcriptional response to changes in sterol levels. Sterol depletion in mammalian cells causes activation of the transcription factors known as sterol regulatory element (SRE) binding proteins (SREBPs) (8), encoded by the homologous genes SREBP-1 and SREBP-2. When sterols are abundant, the SREBPs are inactive, tethered to the endoplasmic reticulum (ER) membrane by two intrinsic transmembrane helices. When sterol levels drop, regulated proteolysis releases the transcriptional activation domains of the SREBPs from the membrane tether, allowing the activation domains to translocate to the nucleus. Once in the nucleus, these domains activate transcription of genes involved in sterol and fatty acid homeostasis.

The SREBPs bind the same DNA sequences in vitro (21). In vivo, the SREBPs activate an overlapping set of target genes but may have slightly different DNA binding specificities and/or activities (8, 22). Hence, differential activation and synthesis of the SREBPs may make it possible for mammalian cells to respond to a demand for different levels of sterol and nonsterol products.

Many genes in the sterol biosynthetic pathway in *Saccharomyces cerevisiae* are transcriptionally regulated in response to changes in sterol levels (6, 13). However, less is known about this regulatory mechanism in yeast. Also, yeast lack convincing homologues of the mammalian SREBPs. This work describes the identification of a yeast SRE shared by many genes involved in sterol biosynthesis and the identification of two SREBPs, Upc2p and Ecm22p, which regulate transcription of the sterol biosynthetic genes. Both Upc2p and Ecm22p are members of the Zn[22]-Cys[6] binuclear cluster family of fungal transcription factors (31). *UPC2* was originally identified as a semidominant allele that allows sterol uptake under aerobic conditions (11). Upc2p is also involved in the anaerobic activation of expression of the DAN/TIR mannoproteins (1). The *ECM22* gene was identified as a mutant sensitive to the cell wall perturbing agent calcofluor white (20). Our data indicated that both Ecm22p and Upc2p were involved in regulation of sterol biosynthesis and identified *ERG2* and *ERG3* as targets of both proteins.

MATERIALS AND METHODS

Strains and media. All yeast strains used were isogenic with W303-1a except as noted in Table 1. Gene deletions were made by a PCR-based gene disruption method (2) such that the entire open reading frame (ORF) was replaced by marker sequences from pRS403 (*HIS3*), pRS404 (*TRP1*), pRS405 (*LEU2*), or pRS406 (*URA3*) (29). The *Escherichia coli* strain used was ER2508 from New England Biolabs.

Yeast cells were grown in minimal medium (YM; 0.67% Difco yeast nitrogen base with 5% ammonium sulfate without amino acids) containing 2% glucose and required supplements. Solid media contained 2% agar (Bacto). A 25-mg/ml stock solution of lovastatin (a generous gift from J. Bergstrom, Merck) was made as previously described (12). Lovastatin, Geneticin (Gibco), and amphotericin B (Sigma) were used in solid media at final concentrations of 40 μg/ml, 1 mg/ml, and 100 ng/ml, respectively. All drugs were added after autoclaving. Lovastatin plates were incubated at 24°C, Geneticin plates at 30°C and amphotericin B plates at 37°C. Amphotericin B plates were used within 12 h of preparation. Bacteria were grown in LB Amp (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 100 μg of ampicillin/ml).

Plasmids. The plasmid constructions of this work made extensive use of the pRS vector series (29) as well as the Univector series (19). pJR2325 was an *ERG2-lacZ* reporter in an integrating *TRP1* plasmid based on pRS414. The CEN ARS fragment of pRS414 was removed by cutting with *Eco*0109I and religated. The plasmid contained the *ERG2* promoter fragment from −751 to +12 relative

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TABLE 1. Strains used in this study

Strain	Genotype	Source
W303-1a	*MAT***a** *ade2-1 leu2-3,112 his3-1 ura3-52 trp1-100 can1-100*	R. Rothstein
JRY7187	W303-1a *erg2*Δ::*TRP1*	This study
JRY7179	W303-1a *upc2*Δ::*HIS3*	This study
JRY7180	W303-1a *ecm22*Δ::*TRP1*	This study
JRY7181	W303-1a *ecm22*Δ::*TRP1 upc2*Δ::*HIS3*	This study
JRY7182	W303-1a *ERG2*::*pJR2320*	This study
JRY7183	W303-1a *ERG2*::*pJR2321*	This study
JRY7184	*MAT***a**/*MAT*α *ade2*/*ADE2 LYS2*/*lys2 his3*/*his3 leu2*/*leu2 trp1*/*trp1 ura3*/*ura3 suc2*::*HIS3*/*suc2*::*HIS3 ERG2*::pJR2325/*ERG2*::pJR2325 *LEU2*::pJR2326/*LEU2*::pJR2326	This study
JRY7188	W303-1a *ecm22*Δ::*TRP1 upc2*Δ::*HIS3 erg2*Δ::*TRP1 pJR2331* (*ECM22* 2μm *URA3*)	This study
JRY7189	*MAT*α *lys2*Δ *leu2-3,112 his3-1 ura3-52 trp1-100 ecm22*Δ::*TRP1 erg2*Δ::*TRP1*	This study
JRY7190	W303-1a *URA3*::*pJR2318*	This study
JRY7191	JRY7179 *URA3*::*pJR2318*	This study
JRY7192	JRY7180 *URA3*::*pJR2318*	This study
JRY7193	JRY7181 *URA3*::*pJR2318*	This study

to the ATG initiation codon and an *Xba*I site fused to the second amino acid of *lacZ*. The *PGK* terminator was subcloned 3′ of *lacZ*.

pJR2326 was an *ERG2-KanMX* reporter in an integrating *LEU2* plasmid based on YIplac128 (14). pJR2326 was made by a three-fragment ligation of the *Sph*I-*Xba*I fragment (containing the *ERG2* promoter) of pJR2325, a *KanMX* fragment amplified by PCR using primers av100 (5′-TAAACATCTAGAGGTA AGGAAAAGACTCACGTTTCG-3′) and solig191 (5′-GAAAACAAGAATTC TTTTTATTGTCAGTAC-3′) cut with *Xba*I and *Eco*RI and the YIplac128 vector backbone cut with *Sph*I and *Eco*RI.

pJR2307 was a 2μm *URA3*-marked plasmid based on pLacZi (GenBank accession no. U89671) from Clontech. The 2μm origin was PCR amplified from pRS426 using primers av249 (5′-TTTCCCCGAAAAGTGCCTGCAGAACGA AGCATCTGTGCTTC-3′) and av250 (5′-CATGATAATAATGGTTCTGCAG TATGATCCAATATCAAAGGAAATG-3′) and subcloned as a *Pst*I fragment into the *Nsi*I site of pLacZi to make pJR2307. Plasmids pJR2308-2315 and pJR2327-2329 were made by subcloning a phosphorylated double-stranded oligonucleotide into the *Eco*RI and *Xho*I sites of pJR2307. The oligonucleotides were designed to have single-stranded overhangs on either end to facilitate cloning into the *Eco*RI and *Xho*I sites. The design of the oligonucleotides was such that the *Eco*RI site was followed by an *Eco*RV site (to allow for easy identification of plasmids with inserts), followed by the relevant sequence from the *ERG2* promoter and an *Xho*I site. pJR2316 was made from pJR2307 by subcloning a fragment of the *ERG2* promoter PCR-amplified using primers av134 (5′-AAGAGAGTCGACGGTACCCATTTCGGCACTAAAATC-3′) and av67 (5′-TCTTGTCGACCATGGCGCTGCAGATCTATC-3′) into the *Eco*RI and *Xho*I sites of pJR2307. pJR2317 was made similarly as pJR2316 but used pJR2320 as a template for the PCR. The *ERG2* promoter in this plasmid therefore had a mutated SRE.

pJR2318 was an *ERG3-lacZ* reporter made by subcloning an *ERG3* promoter fragment as an *Eco*RI-*Xho*I fragment into the same sites in pLacZi (integrating plasmid carrying *URA3*) (GenBank accession no. U89671) from Clontech. The *ERG3* promoter fragment was generated by PCR amplification from genomic DNA using primers av127 (5′-ATTCCCGAATTCGTCCTGCTTTGAGTCGT TTTC-3′) and av128 (5′-CTCTAACTCGAGCCGCGATGTTTCTTTCGACC-3′).

pJR2320 contained the *ERG2* promoter from −751 to +198 as an *Eco*RI-*Xho*I fragment in pRS406 (integrating plasmid carrying *URA3*). The *ERG2* SRE was mutated (from 5′-CTCGTATAAG-3′ to 5′-ACGATATCTA-3′) by using a PCR sewing technique (2) using genomic DNA from W303-1a as a template and the primers av134 (5′-GTACTGGAATTCCTGCATTATCATCCCGATTGCT-3′), av194 (5′-AACCACGGCCACGATATCTACCGCAAGGAAAACTACCGGT-3′), av195 (5′-TTCCTTGCGGTAGATATCGTGGCCGTGGTTCGATTCTGC C-3′), and av308 (5′-CCCCGTAACTCGAGTTAAGTGCGTCTCTGACATCC TG-3′). pJR2321 was similar to pJR2320 but had a wild-type SRE. The insert was generated by PCR using the primers av134 and av308 and genomic DNA from W303-1a as a template.

pJR2330 was a 2μm *LEU2* plasmid containing a 3.6-kb fragment containing *ECM22*. The *ECM22* fragment was PCR amplified using primers av357 (5′-GA CGTTGGATCCGGTAGAGTGGCGCTAGCATAG-3′) and av358 (5′-GCAT TTAGGATCCAACGTATACCGGGTGTACCTC-3′), and the resulting fragment was subcloned into the *Bam*HI site of pRS425. pJR2331 contained *ECM22* on a 3.6-kb *Bam*HI fragment (same as in pJR2330) subcloned into the *Bam*HI site of pJR2332. pJR2332 was pRS426 (2μm, *URA3*), cut with *Eco*RI and *Sal*I and resealed by blunt-end ligation, such that the *Eco*RI-*Sal*I fragment in the multiple cloning site was missing.

pJR2322 was made from pJR2333 by *cre-lox* site-specific recombination with pHB2 as previously described (19), resulting in a GST-Ecm22$p_{1–497}$ fusion protein expressed from the *tac* promoter. pJR2333 was made by subcloning a PCR-amplified fragment carrying *ECM22* (codons 1 to 497 with a STOP codon inserted after codon 497) as an *Xho*I-*Sac*I fragment into the same sites in pUNI10 (19). The PCR fragment was generated by using DynaZyme EXT (Finnzymes) with primers av352 (5′-CTCCATCTCTCGAGAAATGACATCC GATGATGGGAATG-3′) and av390 (5′-CAAATTCAAGAGCTCTTATCTA GACAGCCCGACTAATTCAG-3′) and plasmid pJR2330 as a template.

pJR2323 was made by subcloning a PCR-amplified fragment containing Upc2$p_{1–395}$ (amino acids 1 through 395) as an *Xho*I-*Not*I fragment into the bacterial expression vector pBAT4 (23). The PCR fragment was generated using primers av570 (5′-ACAGTTCCTCGAGATGAGCGAAGTCGGTATACAGA ATC-3′) and av571 (5′-AGTAGTTGCGGCCGCTTATGATTGTAAAGGCCC TTCCTTTACC-3′).

2μm overexpression screen. The diploid host strain (JRY7184) was transformed with a 2μm *URA3* genomic (YEp24-based) library (9) using a lithium acetate-based transformation protocol (17) and allowed to recover for 6 h in liquid minimal medium lacking uracil, selecting only for uracil prototrophy provided by the plasmid. Transformants were subsequently plated on minimal medium lacking uracil and containing Geneticin (1 mg/ml) to select for transformants with elevated expression of *ERG2-KanMX*. From an estimated 100,000 initial transformants, 70 Geneticin-resistant colonies were selected, and 41 of these isolates also showed increased expression of *ERG2-lacZ*. Thirteen plasmids were recovered that conferred increased expression of *ERG2-lacZ* upon retransformation. Eleven of these contained overlapping genomic fragments that included the gene *ECM22*. A 2μm plasmid with a 3.6-kb PCR fragment containing only the *ECM22* gene (pJR2330) was sufficient to increase the expression of *ERG2*.

RNA blots. Cultures for RNA preparation were grown in minimal medium containing all necessary supplements. Cultures grown under inducing conditions contained 40 μg of lovastatin/ml. All cultures were inoculated from a fresh stationary-phase culture (grown overnight) and grown for 16 h to an optical density at 600 nm (OD_{600}) of 0.2 to 1.0. Then, 50 ml of these cultures was harvested by centrifugation, and the cell pellet was frozen at −80°C. Total RNA purification and RNA blot hybridization were performed as previously described (7) with 10 μg of total RNA used in all lanes.

β-Galactosidase assays. β-Galactosidase liquid assays and filter lifts were performed as described previously (2, 33).

Mobility shift assays. Mobility shift assays were performed essentially as described previously (4). The wild-type probe used was a 60-bp double-stranded DNA made by annealing av401 (5′-CGACCACGGCCCTCGTATAAGCCGC AAGGAAAACTACCGGTGCTATCGTTCTCGTTTGGA-3′) to av402 (5′-A GGATCCAAACGAGAACGATAGCACCGGTAGTTTTCCTTGCGGCTTA TACGAGGGCCGTG-3′). The 4-bp overhangs (indicated by underscores) were then filled in by using Klenow fragment (New England Biolabs) and radiolabeled dCTP (NEN). The probe was purified on a 3% agarose gel, recovered on a

DEAE membrane (NA45; Schleicher & Schuell), eluted in DEAE elution buffer (10 mM Tris-Cl [pH 7.9], 1 mM EDTA [pH 8.0], 1 M NaCl), ethanol precipitated, and resuspended in Tris-EDTA (pH 7.6). The extent of labeling was determined by scintillation counting. A total of 20,000 cpm were used in each reaction, which corresponded to ca. 3 ng of probe. The probe with the 10-bp SRE mutation was made in the same way by using oligonucleotides av403 (5′-CGAC CACGGCCACGATATCTACCGCAAGGAAAACTACCGGTGCTATCG TTCTCGTTTGGA-3′) and av404 (5′-AGGATCCAAACGAGAACGATAGC ACCGGTAGTTTTCCTTGCGGTAGATATCGTGGCCGTG-3′). Specific competitors were made by annealing two complementary oligonucleotides (without single-strand overhangs). The sequences of the oligonucleotides used were 5′-C GACCACGGCCCTCGTATAAGCCGCAAGGAAAA-3′ and 5′-TTTTCCTT GCGGCTTATACGAGGGCCGTGGTCG-3′, except for the point mutations shown in the figure. Sheared salmon sperm DNA was used as nonspecific competitor in all experiments.

Bacterial extracts were made as follows. A 1-ml quantity overnight in LB Amp of the bacterial strain ER2508 expressing either glutathione *S*-transferase (GST) alone (pHB2), the GST-Ecm22p$_{1–497}$ fusion protein (pJR2322), or Upc2p$_{1–395}$ (pJR2323) or carrying the plasmid pBAT4 (23) was diluted into 100 ml of LB Amp culture and grown at 37°C to an OD_{595} of 0.5. Expression of the various fusion proteins was then induced by adding 100 μl of 4 M IPTG (isopropyl-β-D-thiogalactopyranoside) and growing the culture for an additional 4 h. Cells were harvested by centrifugation at 3,000 × *g* for 5 min, and the cell pellet was frozen at −80°C. A whole-cell bacterial extract was made by adding 1 ml of buffer (2 mM Tris-Cl [pH 7.5], 10% glycerol, 2 mM $MgCl_2$, 10 μM Zn_2SO_4, 125 μg of sheared salmon sperm DNA/ml) to the frozen pellet. Lysis was achieved by six 15-s sonications, with a 30-s rest on ice between each sonication. The extract was cleared by centrifugation in an Eppendorf centrifuge for 15 min at 2,000 × *g*. Protein concentrations were determined by Bio-Rad protein assays (Bio-Rad). We used 25 μg of total protein in each reaction. The protein extracts made from strains carrying plasmids pJR2322 and pHB2 differed from those made from strains carrying plasmids pBAT4 and pJR2323 by the addition of 75 mM NaCl.

Transmembrane helix prediction. Transmembrane helix predictions were obtained using the following programs: PHDhtm (http://www.embl-heidelberg.de/predictprotein/predictprotein.html), TMAP (http://130.237.130.31/tmap/single.html) (24, 25), TMpred (http://www.ch.embnet.org/software/TMPRED_form.html), PSORT (http://psort.nibb.ac.jp/), TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/), and HMMTOP (http://www.enzim.hu/hmmtop/) (32). TMAP and TMpred predicted four transmembrane helices in both proteins. PHDhtm predicted two transmembrane helices in Ecm22p and one in Upc2p, PSORT predicted one transmembrane helix in both proteins, and TMHMM and HMMTOP both predicted no transmembrane helix in either protein.

RESULTS

Overexpression of *ECM22* and *UPC2* increased expression of *ERG2*. *ERG2*, which encodes Δ8-7 sterol isomerase, is one of many genes in the yeast sterol biosynthetic pathway that are transcriptionally regulated in response to changes in sterol levels (6, 13, 30). The proteins responsible for mediating this regulation have not been described. A genetic selection was performed to identify transcription factors responsible for the sterol-mediated regulation of *ERG2* expression. Specifically, an overexpression strategy was used to select for genes that, when overexpressed, led to increased expression of *ERG2*. *ERG2* expression was monitored by a pair of integrated reporter genes consisting of *ERG2* regulatory sequences fused to the coding regions of *KanMX* (*ERG2-KanMX*, pJR2326) and of *ERG2* regulatory sequences fused to *LacZ* (*ERG2-lacZ*, pJR2325). These reporters allowed detection of increased *ERG2* expression by increased resistance to the translational inhibitor Geneticin (conferred by *KanMX*) and by increased β-galactosidase activity (encoded by *lacZ*). Yeast transformation is itself mutagenic, and mutations at most steps of ergosterol biosynthesis can lead to elevated reporter expression due to disruption of feedback repression. Because mutations in ergosterol biosynthesis would primarily be recessive, a diploid strain was used to avoid this substantial background.

A diploid strain (JRY7184) homozygous for both *ERG2-lacZ* and *ERG2-KanMX* was transformed with a high-copy (2μm, YEp24) genomic library (9) and then plated on minimal medium lacking uracil, selecting for the plasmid-borne *URA3* gene. The medium also contained Geneticin to select for transformants with elevated expression of *ERG2-KanMX*. Eleven plasmids were recovered from this screen that had overlapping genomic fragments containing the gene *ECM22*. A plasmid with a 3.6-kb fragment containing only the *ECM22* gene (pJR2330) was sufficient to increase expression of both *ERG2* reporters.

Ecm22p is a member of the Zn[2]-Cys[6] binuclear cluster family of fungal transcription factors (31). The deduced Ecm22p protein sequence is 45% identical to that encoded by *UPC2* (Proteome, Inc. [http://www.proteome.com/databases/index.html]), a member of the same family of transcription factors. The sequence similarity between Ecm22p and Upc2p was particularly high in the carboxyl-terminal region (76% identity, 238 of 314), as well as in the amino-terminal region (70% identity, 56 of 80), which contained the presumptive DNA binding domain (77% identity, 30 of 39) (Fig. 1A). No plasmids containing *UPC2* were isolated in the initial screen. Nevertheless, overexpression of *UPC2* also led to increased expression of an *ERG2-lacZ* reporter (pJR2316) (data not shown). Thus, overexpression of either *ECM22* or *UPC2* affected expression of *ERG2*.

***ECM22* and *UPC2* had overlapping functions.** The strong sequence similarity between *ECM22* and *UPC2* and their shared ability to activate *ERG2* expression implied that these genes might have similar or overlapping functions. Evidence of overlapping functions of Ecm22p and Upc2p was also recently reported by Shianna et al. (28). The sensitivity of each of the single null mutants as well as the double null mutant to two sterol-related drugs, lovastatin and amphotericin B, was tested (Fig. 1B). These two inhibitors provide useful measures of the overall expression level of the sterol biosynthetic pathway: reduced flux through the pathway results in lovastatin sensitivity and reduced ergosterol levels in the plasma membrane result in amphotericin B resistance. The *ecm22*Δ *upc2*Δ double mutant (JRY7181) was much more resistant to amphotericin B than either of the single mutants (JRY7179 and JRY7180) or the wild-type strain (W303-1a), indicating that the double mutant had reduced levels of ergosterol. The double mutant was also much more sensitive to lovastatin than either of the single mutants or the wild type. Qualitatively, phenotypes such as these might result from a loss of expression of *ERG2* alone (as shown below). However, a deletion of the SRE in the *ERG2* promoter, to which both Ecm22p and Upc2p bound (described below), did not result in increased resistance to amphotericin B or sensitivity to lovastatin. Furthermore, whereas *erg2*Δ is viable, a recent report shows that *ecm22*Δ and *upc2*Δ are synthetically lethal in some strain backgrounds (28). This result, together with the resistance to amphotericin B and the sensitivity to lovastatin of our *ecm22*Δ *upc2*Δ double mutant relative to either single mutant, suggested that the two genes had overlapping functions that extended beyond activation of *ERG2*, as confirmed below.

Lovastatin is an inhibitor of sterol biosynthesis and causes a decrease in the production of both sterols and a variety of nonsterol products made by this pathway. The sensitivity of the

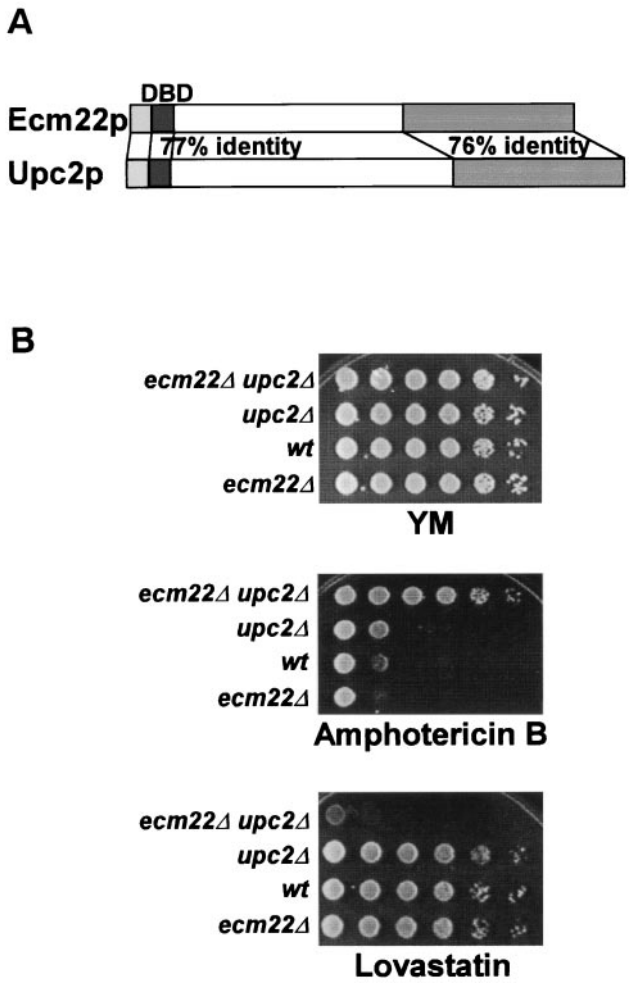

FIG. 1. (A) Ecm22p and Upc2p show strong sequence similarity. They are 77% identical in the DNA-binding domain (DBD) and 76% identical in the carboxyl-terminal region. (B) *ECM22* and *UPC2* had overlapping functions. The phenotypes of wild-type (wt; W303-1a), *ecm22*Δ (JRY7180), *upc2*Δ (JRY7179), and *ecm22*Δ *upc2*Δ (JRY7181) strains were compared by spotting 10-fold serial dilutions of each strain onto minimal medium plates containing 100 ng of amphotericin B or 40 μg of lovastatin/ml.

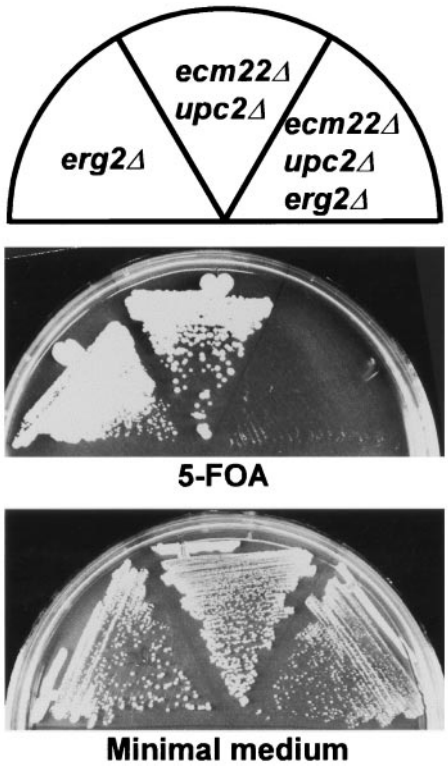

FIG. 2. *erg2*Δ was synthetically lethal with *ecm22*Δ *upc2*Δ. An *erg2*Δ (JRY7187) strain, an *ecm22*Δ *upc2*Δ (JRY7181) strain, and an *ecm22*Δ *upc2*Δ *erg2*Δ (JRY7188) strain, all carrying the plasmid pJR2331 (*ECM22, URA3*), were grown on 5-FOA. Only the *ecm22*Δ *upc2*Δ *erg2*Δ strain was unable to grow.

*ecm22*Δ *upc2*Δ double mutant might reflect either the consequences of a lack of sterols or a lack of some other product of the pathway. We distinguished between these two possibilities by inhibiting sterol synthesis after the last branch point of the pathway. A sterol-specific block can be achieved with an *erg2*Δ null mutation. *ERG2* encodes one of several nonessential enzymes in the latter steps of the sterol biosynthetic pathway that modify the basic sterol structure. In the absence of *ERG2*, cells make a sterol adequate for growth but not the preferred ergosterol. We attempted to generate an *erg2*Δ *ecm22*Δ *upc2*Δ triple mutant by crossing an *ecm22*Δ *upc2*Δ mutant (JRY7181) to an *erg2*Δ *ecm22*Δ mutant strain (JRY7189). All possible double mutant segregants from this cross (*erg2*Δ *ecm22*Δ [2], *erg2*Δ *upc2*Δ [2], and *ecm22*Δ *upc2*Δ [5]) were viable. However, all five *erg2*Δ *ecm22*Δ *upc2*Δ triple mutant segregants died shortly after spore germination, generating microcolonies of <100 cells (data not shown). The synthetic lethality of the *erg2*Δ *ecm22*Δ *upc2*Δ triple mutant was further evaluated by generating an *erg2*Δ *ecm22*Δ *upc2*Δ triple mutant (JRY7188) kept alive by a *URA3*-marked plasmid containing a wild-type copy of *ECM22* (pJR2331), as evidenced by the inability of the strain to grow on medium containing 5-fluoroorotic acid (5-FOA), which selects against *URA3* (Fig. 2). If the lovastatin sensitivity of the *ecm22*Δ *upc2*Δ double mutant were due to depletion of a nonsterol product of the pathway, the triple mutant should still be viable but lovastatin sensitive. In contrast, if the lovastatin sensitivity reflected an inability to respond to depletion of late sterol intermediates or ergosterol, the triple mutant should mimic the *ecm22*Δ *upc2*Δ double mutant in the presence of lovastatin and be dead. The synthetic lethality of the *erg2*Δ *ecm22*Δ *upc2*Δ triple mutant therefore suggested that the viable *ecm22*Δ *upc2*Δ double mutant was deficient in a regulatory response to sterol depletion.

Ecm22p and Upc2p regulated transcription of *ERG2* and *ERG3* in response to sterol levels. To establish whether or not Ecm22p and Upc2p played a role in sterol-mediated regulation of *ERG2* transcription, *ERG2* expression levels were measured in an *ecm22*Δ (JRY7180), an *upc2*Δ (JRY7179), and an *ecm22*Δ *upc2*Δ (JRY7181) mutant grown under inducing and noninducing conditions (Fig. 3A). Inducing conditions differed from noninducing by inclusion of lovastatin in the growth medium. *ERG2* expression was measured using a set of *ERG2* reporters containing *ERG2* regulatory sequences fused to the *lacZ* coding sequence as described below (see Fig. 5). Either

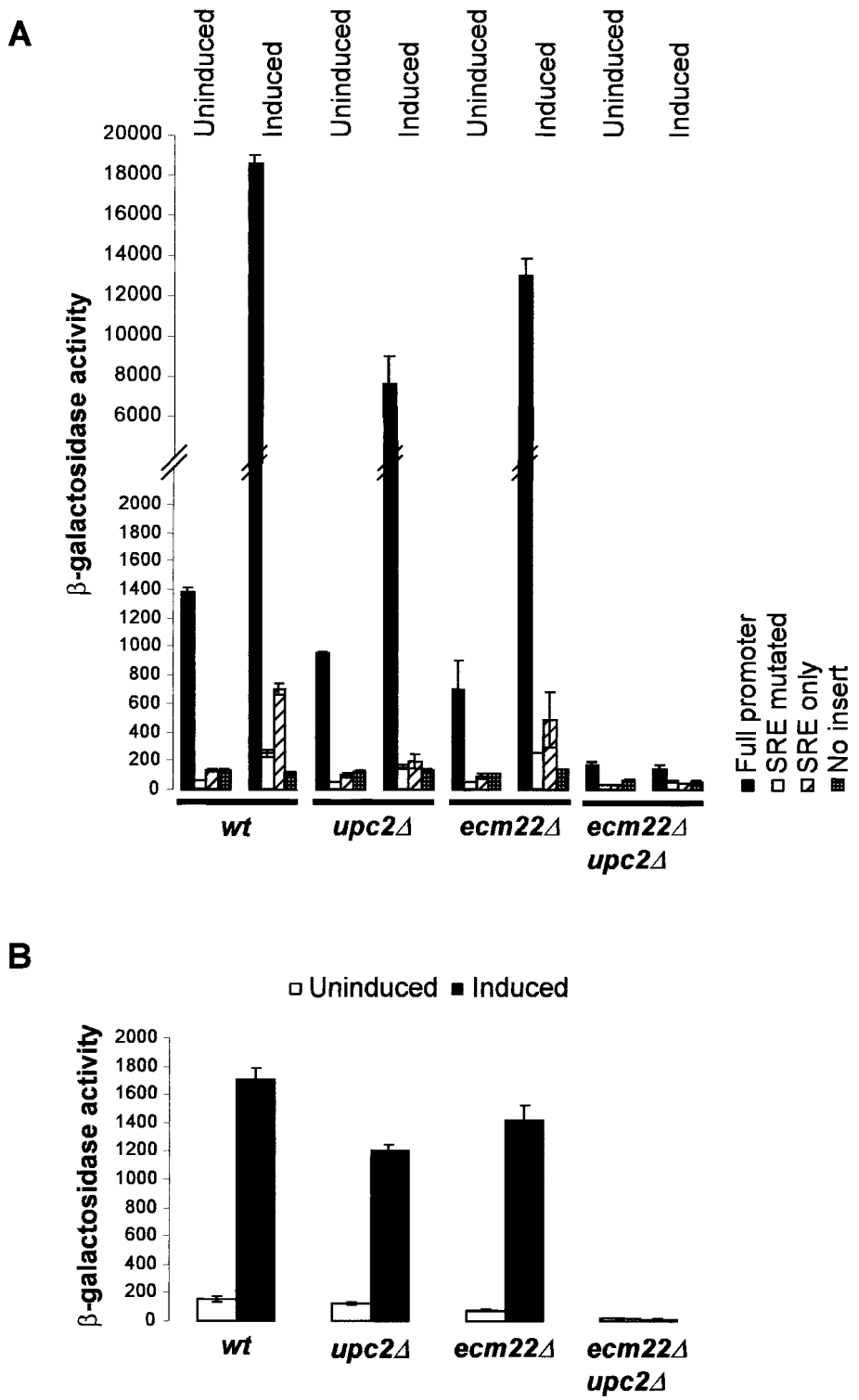

FIG. 3. (A) *UPC2* and *ECM22* were necessary for sterol-mediated regulation of *ERG2-lacZ*. *ERG2-lacZ* reporters were used to determine the contribution of each of the genes to the regulation of *ERG2*. "Full promoter" refers to a reporter having *ERG2* promoter sequences from −751 to −93 controlling transcription of *lacZ*. "SRE mutated" has the same promoter except that 10 bp of the SRE have been mutated. "SRE only" has only the 11-bp SRE controlling *lacZ*. The plasmids were transformed into wild-type (wt; W303-1a), *upc2*Δ (JRY7179), *ecm22*Δ (JRY7180), and *ecm22*Δ *upc2*Δ (JRY7181) strains. β-Galactosidase assays were performed on the transformants after they were grown for 16 h either in minimal medium (uninduced) or in minimal medium containing 40 μg of lovastatin/ml (induced). The assay values represent the average of two determinations. (B) *UPC2* and *ECM22* were necessary for sterol-mediated regulation of *ERG3-lacZ*. An *ERG3-lacZ* reporter was integrated at the *URA3* locus of wild-type (JRY7190), *upc2*Δ (JRY7191), *ecm22*Δ (JRY7192), and *upc2*Δ *ecm22*Δ (JRY7193) strains. β-Galactosidase assays were performed on the transformants following growth for 16 h in conventional medium (uninduced) or in the presence of 40 μg of lovastatin/ml (induced). The assay values represent the average of two determinations.

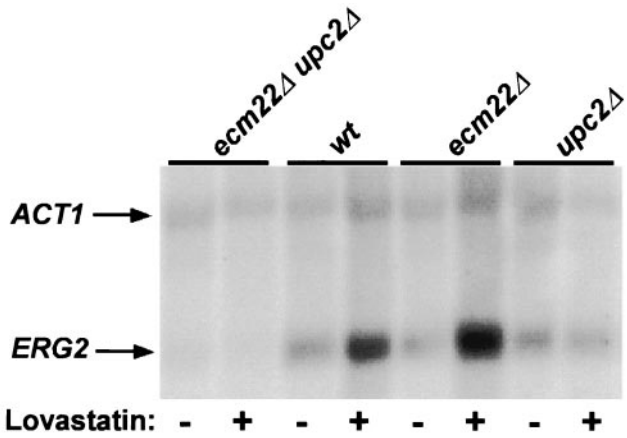

FIG. 4. UPC2 was necessary for sterol-mediated regulation of *ERG2*. *ERG2* transcript levels were monitored by RNA blot hybridization. Total RNA was prepared from wild-type (wt; W303-1a), *ecm22*Δ (JRY7180), *upc2*Δ (JRY7179), and *ecm22*Δ *upc2*Δ (JRY7181) strains grown for 16 h in minimal medium (uninduced) or in the presence of 40 μg of lovastatin/ml (induced). *ACT1* served as the loading control.

TABLE 2. Genes with SREs in their promoters

Gene	Putative SRE[a]	Coordinates	
		Begin	End
ERG1	T**TCGTATA**GCT	−383	−373
ERG2	C**TCGTATA**AGC	−186	−176
ERG3	C**TCGTATA**AGT	−287	−277
	GTG**TATACGA**G	−329	−319
ERG6	T**TCGTATA**TGG	−433	−423
ERG8	TTC**TATACGA**G	−155	−145
ERG11	TAC**TATACGA**G	−611	−601
ERG12	GCC**TATACGA**A	−675	−665
ERG13	T**TCGTATA**TAC	−215	−205
	AAT**TATACGA**G	−101	−91
ERG25	A**TCGTATA**CGG	−464	−454
	T**TCGTATA**CGG	−484	−474
	G**TCGTATA**GGA	−716	−706
LCB1	CTT**TATACGA**G	−444	−434
LCB2	TAG**TATACGA**T	−371	−361
	A**TCGTATA**TCT	−601	−591

[a] This table shows the location of the putative SREs in the promoters of genes in the sterol biosynthetic pathway, as well as two genes involved in sphingolipid biosynthesis. The seven critical base pairs are shown in boldface. Note that some sequences are of the complementary strand.

ECM22 or *UPC2* was sufficient to confer sterol-mediated regulation to a reporter containing the whole *ERG2* promoter. In the absence of both *ECM22* and *UPC2*, no *ERG2* induction was detected in response to lovastatin. This regulation was in large part dependent on the presence of an 11-bp SRE (see below) in the *ERG2* promoter. Furthermore, Upc2p was capable of activating expression through the 11-bp SRE, indicating that the SRE alone was sufficient for sterol-mediated regulation by Upc2p.

The effect of Ecm22p and Upc2p on sterol-mediated regulation of *ERG2* was further investigated by measuring *ERG2* mRNA levels in *ecm22*Δ, *upc2*Δ, and *ecm22*Δ *upc2*Δ mutants (JRY7180, JRY7179, and JRY7181) grown under inducing and noninducing conditions (Fig. 4). Under noninducing conditions, the level of *ERG2* mRNA in the *upc2*Δ mutant was comparable to the level in wild-type cells. Thus, *UPC2* was not required for expression of *ERG2* per se. In contrast, the *upc2*Δ strain did not induce *ERG2* transcription when grown in medium containing lovastatin, whereas the wild-type strain did. Therefore, *UPC2* was necessary for *ERG2* induction under low-sterol conditions. The *ERG2* mRNA levels in the *ecm22*Δ mutant were similar to the levels in wild type, under both noninducing and inducing conditions. Thus, *ECM22* was not required for uninduced expression of *ERG2* and also was not necessary for sterol-mediated regulation of *ERG2,* at least at the time point assayed. The discrepancy between the mRNA and the β-galactosidase measurements of *ERG2* expression in this mutant may simply reflect the higher stability of β-galactosidase (5) compared to *ERG2* mRNA. The β-galactosidase measurements integrated most or all of *ERG2* induction over the 16-hour induction, whereas the mRNA levels captured a regulatory snapshot at 16 h. Together, these data indicated that either Upc2p or Ecm22p was capable of mediating sterol regulation of *ERG2* transcription and suggested that Ecm22p may have a more transient effect on *ERG2* transcription in response to sterol limitation. Furthermore, because *ERG2* expression under noninducing conditions was reduced in the *ecm22*Δ *upc2*Δ double mutant compared to either of the single mutants and wild type, both proteins contributed to the uninduced level of *ERG2* transcription.

A similar experiment established that both Ecm22p and Upc2p were involved in the sterol-mediated regulation of the *ERG3* gene. As discussed below, *ERG3* is one of several sterol biosynthetic genes that had regulatory sequences identical to the *ERG2* SRE (Table 2). We used an *ERG3* reporter with *ERG3* regulatory sequences fused to *lacZ* (pJR2318) integrated at *URA3* (JRY7190, JRY7191, JRY7192, and JRY7193) to study the effect of Ecm22p and Upc2p on *ERG3* expression. No induction of *ERG3* occurred in response to lovastatin treatment in the *ecm22*Δ *upc2*Δ double mutant. Both *ECM22* and *UPC2* individually were able to confer sterol-mediated regulation of *ERG3* transcription (Fig. 3B).

An 11-bp SRE in the *ERG2* promoter conferred regulation by sterol levels. Analysis of the *ERG2* promoter led to the identification of an SRE. In these experiments, fragments 5′ to the *ERG2* coding region were subcloned into a reporter, consisting of the *CYC1* promoter directing expression of *lacZ* (pJR2307). This context allows a regulatory sequence from another gene to be recognized by its ability to activate and/or regulate transcription from the start site provided by the *CYC1* promoter. An 11-bp (5′-CTCGTATAAGC-3′) SRE, positioned between bp −186 and −176 relative to the *ERG2* ORF, conferred sterol-mediated regulation to the *CYC1-lacZ* reporter (Fig. 5).

The *ERG2* SRE was necessary for induced and uninduced expression but not for basal expression. The RNA analysis in Fig. 4 indicated that Ecm22p and Upc2p contributed to the uninduced level of *ERG2* expression. To determine whether the SRE was necessary either for basal expression of *ERG2* or just for regulation of *ERG2* expression, a 10-bp mutation in the SRE in the native *ERG2* promoter was constructed. This mutation blocked induction of *ERG2* mRNA by lovastatin (Fig. 6A). However, the uninduced level of expression of *ERG2* in

Plasmid name	Start point	End point	β-galactosidase activity Non-induced	Induced	Fold induction
pJR2316	-751	-93	1279 ± 218	11022 ± 289	8.6
pJR2308	-231	-184	100 ± 5	78 ± 5	0.8
pJR2309	-185	-137	461 ± 73	5676 ± 529	12.3
pJR2310	-136	-93	19 ± .1	19 ± 3	1.0
pJR2311	-211	-164	715 ± 15	2412 ± 34	3.4
pJR2312	-163	-117	65 ± 6	142 ± 22	2.2
pJR2313	-186	-164	90 ± 6	613 ± 27	6.8
pJR2314	-186	-176	114 ± 13	504 ± 61	4.4
pJR2317	-175	-137	238 ± 31	448 ± 52	1.9
pJR2318	-165	-137	89 ± 9	108 ± 12	1.2
pJR2319	-175	-155	322 ± 10	497 ± 20	1.5
pJR2315	10bp random		159 ± 8	117 ± 12	0.7
pJR2307	no insert		145 ± 18	152 ± 15	1.0

FIG. 5. An SRE in the *ERG2* promoter was sufficient to confer sterol-mediated regulation of expression. Fragments of the *ERG2* promoter were subcloned into a *CYC1-lacZ* reporter. The inserts are represented schematically on the left: black bars indicate inserts that conferred sterol-mediated regulation, and gray bars indicate fragments that did not. pJR2316 had the whole *ERG2* promoter from −751 to −93 (numbers refer to base pairs 5′ of the A of the initiation codon). Other sequences inserted were as follows: pJR2308 (5′-GATATCGCACATTCCTGCCCTTACGCTCCAGGGCAGAATCGAACCACGGCCCT-3′), pJR2309 (5′-GATATCGTATAAGCCGCAAGGAAAACTACCGGTGCTATCGTTCTCGTTTGGAT-3′), pJR2310 (5′-GATATCGATTTTCAGTATGGAAGAATTTGGATAGATCTGCAGCGCCATGG-3′), pJR2311 (5′-GATATCTCCAGGGCAGAATCGAACCACGGCCCTCGTATAAGCCGCAAGGAAAA-3′), pJR2312 (5′-GATATCTACCGGTGCTATCGTTCTCGTTTGGATGATTTTCAGTATGGAAGAAT-3′), pJR2313 (5′-GATATCTCGTATAAGCCGCAAGGAAAAC-3′), pJR2314 (5′-GATATCTCGTATAAG-3′), pJR2327 (5′-GATATCGCAAGGAAAACTACCGGTGCTATCGTTCTCGTTTGGAT-3′), pJR2328 (5′-GATATCACTACCGGTGCTATCGTTCTCGTTTGGAT-3′), pJR2329 (5′-GATATCGCAAGGAAAACTACCGGTG-3′), and pJR2315 (5′-GATATCGATACGATT-3′). pJR2307 had no inserted sequences. The resulting plasmids were transformed into the wild-type strain W303-1a. β-Galactosidase assays were performed on the transformants after they were grown for 16 h in conventional minimal medium (uninduced) or in the presence of 40 μg of lovastatin per ml (induced). The assay values represent the average of 2 determinations.

both mutant and wild-type strains was quite low, raising the possibility that the SRE was required for basal expression, as well as for uninduced and induced expression. Indeed, the *lacZ* data in Fig. 3A indicated that the SRE contributed to the uninduced level of expression. In the absence of expression, induction would not be reliably measured. To address this possibility, we tested whether the phenotypes of a strain with a deletion of the SRE in the *ERG2* promoter (JRY7182) were equivalent to those of a strain with a deletion of the *ERG2* gene itself (JRY7187). If the two phenotypes differed, the *ERG2* gene must be expressed at some level in the absence of a SRE. The *erg2*Δ strain was significantly more amphotericin B resistant and lovastatin sensitive than the strain with the SRE deletion (Fig. 6B). Therefore, deletion of the SRE did not eliminate expression of *ERG2*. Hence, the SRE was a mediator of *ERG2* regulation and not of its basal expression. However, the higher levels of expression in the presence of the SRE than in its absence under noninducing conditions (Fig. 3A) implied that a fraction of Ecm22p or Upc2p molecules were active under noninducing conditions.

Ecm22p and Upc2p bound the *ERG2* SRE directly. The deduced sequence of Ecm22p and Upc2p indicated that they were both members of the Zn[2]-Cys[6] binuclear cluster family of fungal transcription factors (31), raising the possibility that they were transcriptional activators that bound directly to the SRE. To test this prediction, we developed a mobility shift assay using a radioactively labeled 60-bp probe containing sequences from the *ERG2* promoter that included the SRE. A protein extract made from bacteria expressing a truncated version of Upc2p (amino acids 1 through 395) (pJR2323) produced a mobility shift that was dependent on the presence of the SRE (Fig. 7A). The shifted band could be competed away by an excess of an unlabeled double-stranded oligonucleotide that contained the SRE but not by one lacking it. A probe in which the 11 bp in the SRE were replaced by a random sequence was not shifted under the same conditions. Thus, Upc2p bound DNA directly in an SRE-dependent manner, as expected of a transcription factor controlling the expression of *ERG2*.

A similar experiment showed that Ecm22p also bound directly to the *ERG2* promoter. A protein extract made from bacteria expressing a truncated version of Ecm22p (amino acids 1 through 497) as a GST fusion protein (pJR2322) produced a mobility shift that was dependent on the presence of the SRE. Hence, Ecm22p, as well as Upc2p, bound DNA directly in a SRE-dependent manner (Fig. 7B).

Using this mobility shift assay, 7 of the 11 bp in the SRE were identified to be critical for binding by Upc2p. A series of double-stranded oligonucleotides were constructed with consecutive transversions of each of the 11 bp of the SRE. These unlabeled oligonucleotides were used as specific competitors in mobility shift assays with the radioactively labeled 60-bp probe that contained a wild-type SRE. Only oligonucleotides that could not compete with the labeled probe for Upc2p binding would allow visualization of a shifted band. The critical base pairs in the SRE were the 7-bp sequence TCGTATA (Fig. 8A). In a similar experiment testing the DNA binding specificity of Ecm22p, the same 7-bp sequence was critical for the binding by Ecm22p (Fig. 8B). Therefore, both Upc2 and Ecm22p bound the same 7-bp TCGTATA sequence.

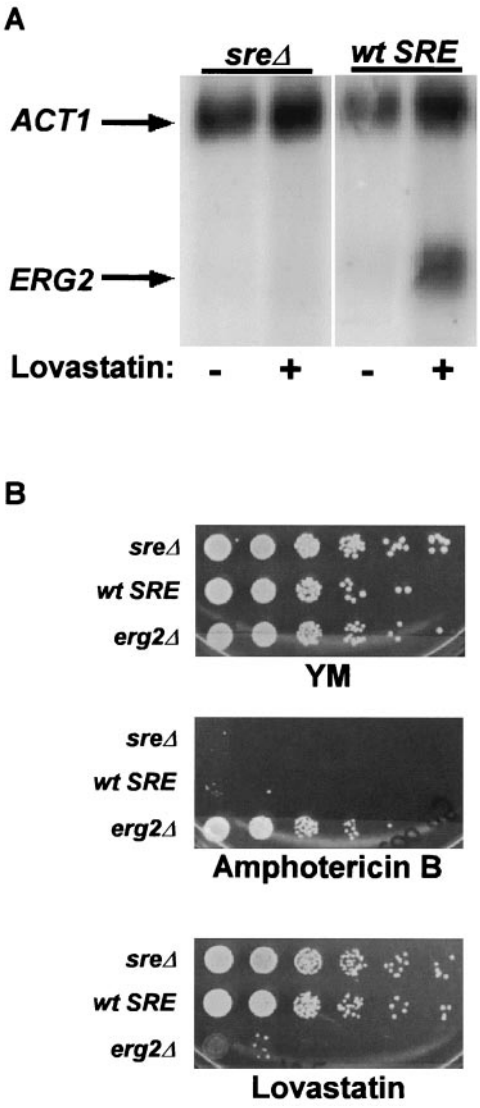

FIG. 6. (A) The SRE was necessary for sterol-mediated regulation of *ERG2*. *ERG2* transcript levels were monitored by RNA blot analysis. Total RNA was prepared from strains that had a mutated *sre*Δ (JRY7182) and wild-type *SRE* (JRY7183) grown for 16 h in minimal medium (uninduced) or in the presence of 40 μg of lovastatin/ml (induced). *ACT1* served as the loading control. (B) The SRE was not necessary for basal expression of *ERG2*. The phenotypes of strains *sre*Δ (JRY7182), wild-type (wt) SRE (JRY7183), and *erg2*Δ (JRY7187) were compared by spotting 10-fold serial dilutions of each strain onto minimal medium plates containing 100 ng of amphotericin B or 40 μg of lovastatin/ml.

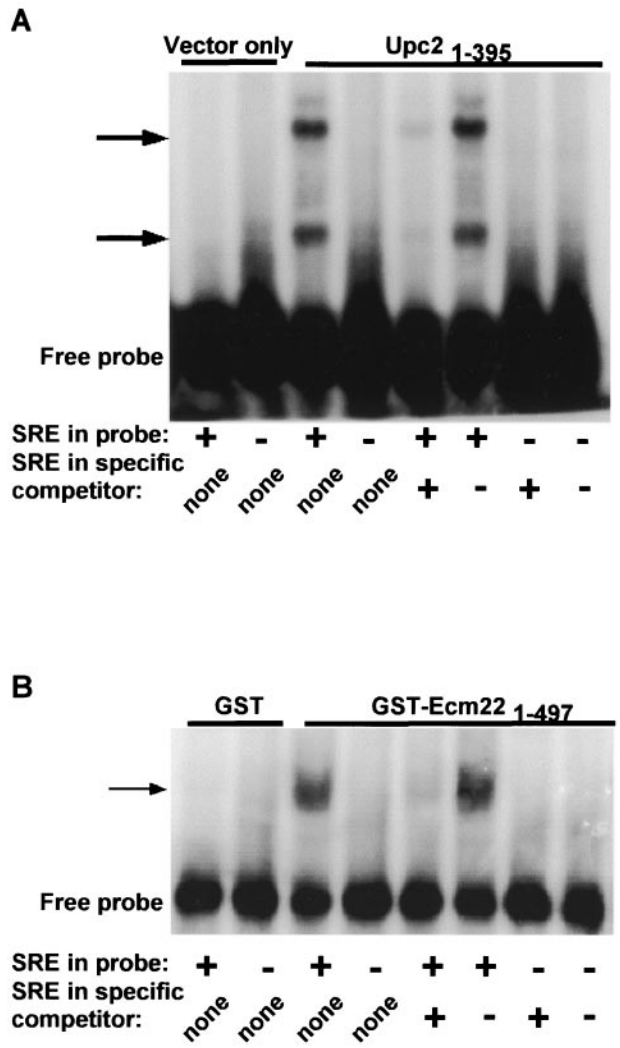

FIG. 7. (A) Upc2p bound directly to the *ERG2* promoter in a SRE-dependent manner. Mobility shift assays were performed by using a radiolabeled double-stranded DNA probe containing sequences from the *ERG2* promoter overlapping the SRE and 25 μg of bacterial protein extract from a strain expressing a truncated $Upc2p_{1–395}$ protein (amino acids 1 through 395) (pJR2323) or carrying the vector plasmid pBAT4 (23). Arrows indicate shifted bands. Lanes where the probe contained a wild-type SRE are labeled "+" (5′-CGACCACGGCCCTCGTATAAGCCGCAAGGAAAACTACCGGTGCTATCGTTCTCGTTTGGA-3′), and lanes where 10 bp of the SRE were replaced by random sequence (underlined) are labeled "−" (5′-CGACCACGGCCACGATATCTACCGCAAGGAAAACTACCGGTGCTATCGTTCTCGTTTGGA-3′). "None" indicates no competitor other than salmon sperm DNA was used. Unlabeled double-stranded oligonucleotides (5′-CGACCACGGCCCTCGTATAAGCCGCAAGGAAAA-3′ is labeled "+"; 5′-CGACCACGGCCACGATATCTACCGCAAGGAAAA-3′ is labeled "−") were used as specific competitors, where indicated, at a 250-fold molar excess to the radioactively labeled probe. (B) Ecm22p also bound directly to the *ERG2* promoter in a SRE-dependent manner. The experiment was performed as for Upc2p, but the protein extract was made from bacteria expressing either GST alone (pHB2) or GST-$Ecm22p_{1–497}$ (pJR2322).

DISCUSSION

The present study identified an SRE and two SREBPs required for the regulation of the sterol biosynthetic genes *ERG2* and *ERG3*. Upc2p and Ecm22p were necessary for the regulation of *ERG2* expression in response to changes in sterol levels, and both bound directly to the *ERG2* promoter. Phenotypes of the *ecm22*Δ *upc2*Δ double mutant further supported a role for *ECM22* and *UPC2* in sterol homeostasis.

The SRE in the *ERG2* promoter was both necessary and sufficient to confer regulated transcription in response to sterol depletion by lovastatin. Seven base pairs in the *ERG2* SRE were necessary for the binding of Upc2p and Ecm22p to DNA. These 7 bp had a perfect match in a 24-bp upstream activating sequence (UAS) found in the *ERG3* promoter that is necessary

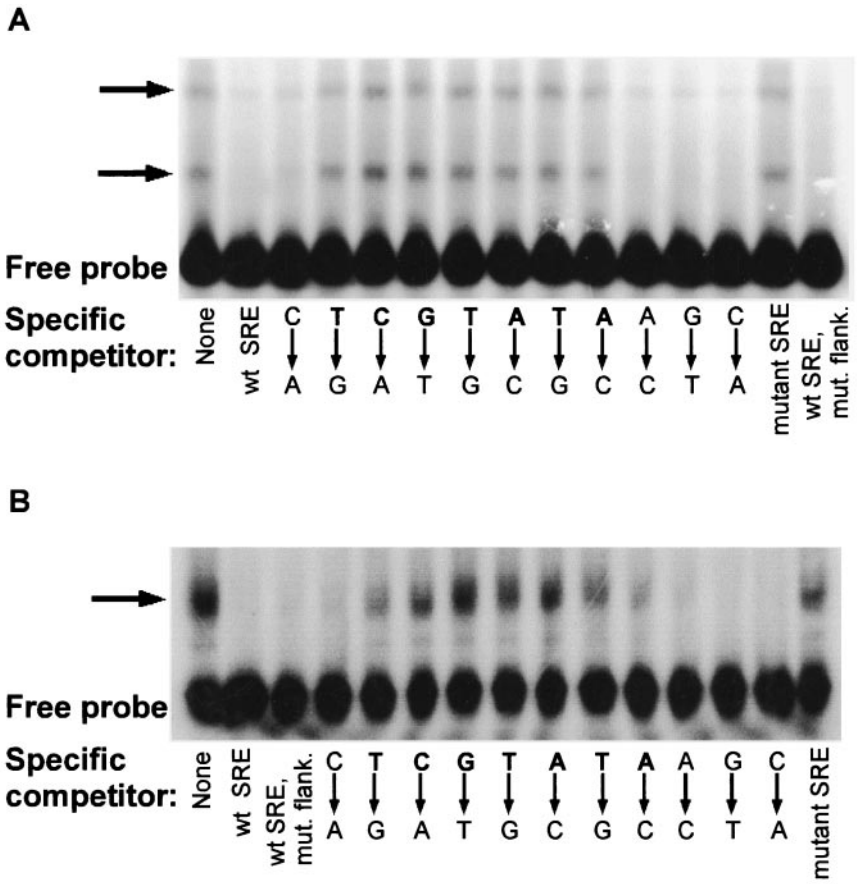

FIG. 8. (A) The 7-bp sequence TCGTATA was necessary for Upc2p binding to the *ERG2* promoter. Mobility shift assays were performed using 25 μg of protein from a bacterial extract expressing a truncated $Upc2p_{1–395}$ protein (pJR2323) and a radioactively labeled double-stranded DNA probe containing sequences from the *ERG2* promoter with a wild-type SRE as described for Fig. 7. Fourteen different specific double-stranded competitors were used. One of the competitors contained a wild-type SRE (wt SRE) and had the sequence 5′-CGACCACGGCCCTCGTATAAGCCGCAAGGAAAA-3′, one had the entire SRE mutated (5′-CGACCACGGCCACGATATCTACCGCAAGGAAAA-3′, labeled "mutant SRE"), one had a wild-type SRE but all other sequences were mutated (labeled "wt SRE, mut. flank."), and the rest contained a series of consecutive transversions in each of the base pairs of the SRE. The sequence of these competitors was 5′-CGACCACGGCCCTCGTATAAGCCGCAAGGAAAA-3′ except for the mutations noted in the figure. The competitors were used at a 250-fold excess compared to the probe. (B) Ecm22p bound the same 7-bp sequence (TCGTATA) as did Upc2p. The experiment was performed as for Upc2p except that the protein extract was made from bacteria expressing GST-$Ecm22p_{1–497}$ (pJR2322).

for the regulation of *ERG3* in response to sterols (3). *ECM22* and *UPC2* were also necessary for the sterol regulation of *ERG3*. Furthermore, the same 7-bp sequence element was found in the promoters of other yeast genes that are regulated by sterols, suggesting that these genes may be coordinately regulated by Upc2p and Ecm22p through their SREs (Table 2).

Sterols and anaerobic growth. Sterol biosynthesis is dependent on oxygen and can take place only during aerobic growth. Unlike aerobically growing cells, anaerobically growing cells can acquire sterols by taking them up from the growth medium. *UPC2* was first identified in a screen for mutants that take up sterols under aerobic conditions (11). The sterol uptake phenotype is due to a semidominant allele containing a point mutation in the carboxyl-terminal part of *UPC2*. Recently, *UPC2* was shown to be necessary for the anaerobic induction of the DAN/TIR genes (1). Due to the oxygen requirement of sterol production, this effect could in principle reflect either a response to oxygen or a response to sterol levels.

Lovastatin inhibits an early step in sterol biosynthesis. Thus, lovastatin treatment will reduce levels of early, nonsterol, products of the pathway in addition to reducing sterol levels. However, the *erg2*Δ null mutant blocks sterol synthesis specifically, leaving early products of the pathway unaffected. The synthetic lethality of *erg2*Δ in combination with the *ecm22*Δ *upc2*Δ double mutant therefore suggested that a sterol-specific block generated a signal to activate Ecm22p and Upc2p. When this signal failed to upregulate sterol biosynthesis, because the transcription factors responsible for the effect had been deleted, the cells died. This conclusion is supported by the induction of *ERG2* expression in an *erg24*Δ mutant (30). Moreover, *ERG3-lacZ* expression is induced in an *erg2*Δ mutant (3). Both of these observations pinpoint a late sterol product as the key product whose level is monitored by Ecm22p and Upc2p. We therefore propose that the principal function of Ecm22p and Upc2p is to regulate genes in response to sterol levels.

Effect of *ECM22* and *UPC2* on sterol biosynthesis. Upc2p and Ecm22p activated *ERG2* transcription in response to the

need for more sterols by binding directly to a 7-bp SRE in the *ERG2* promoter. The presence of identical sequence elements in the promoters of several other genes involved in sterol biosynthesis in yeast indicated that Upc2p and Ecm22p may regulate multiple genes in this pathway (Table 2). Sequence elements identical to the *ERG2* SRE are within 500 bp 5′ of the coding region of *ERG1, ERG2, ERG3, ERG6, ERG8, ERG13*, and *ERG25*, and within the first 1,000 bp 5′ of *ERG11* and *ERG12*. With the exception of *ERG13*, all of these genes have been shown to be transcriptionally regulated by sterols (3, 6, 13, 18, 30). It therefore seems likely that Upc2p and Ecm22p are involved in the sterol regulation of genes other than *ERG2* and *ERG3*. Hence, Upc2p and Ecm22p may be major transcriptional regulators of sterol-responsive genes in the sterol biosynthetic pathway of yeast.

The SRE motif is found in the promoters of a number of other sterol-regulated genes in yeast. Some of these genes are involved in sphingolipid biosynthesis. Both *LCB1* and *SUR2* are regulated by sterols (6), and *LCB1* contains an SRE within the first 500 bp of the 5′ untranslated region. This raises the possibility that sphingolipid biosynthesis in yeast may be coordinated with sterol biosynthesis, with Upc2p and Ecm22p providing the coordination.

Our data indicated that Upc2p and Ecm22p were transcriptional activators of *ERG2* and other genes and that the activities of the proteins were influenced by sterol levels. The glutamine-rich domain in the middle of both Upc2p and Ecm22p was consistent with this hypothesis. However, at least three of the genes with a SRE in the promoter (*CYC7*, *BAP2*, and *DIP5*) are repressed, rather than induced, by the lack of sterols (6). This paradoxical response of SRE-containing genes raises the formal possibility that Upc2p and Ecm22p may also be transcriptional repressors or recruit a repressor under certain conditions. In fact, such a dual effect is seen in the case of the transcriptional repressor Ume6p, another member of the Zn[2]-Cys[6] binuclear cluster family of transcription factors. During mitosis Ume6p recruits the Sin3p-Rpd3p histone deacetylase, resulting in repression of adjacent genes, whereas in meiosis Ume6p recruits the transcriptional activator Ime1p (27), thereby activating transcription. However, if Upc2p and Ecm22p were activators as well as repressors, the nature of their transcriptional effect would have to depend on other DNA-binding proteins in the vicinity of the SRE or upon the precise position of the SRE in the promoter. Alternatively, other proteins in yeast may mediate repression in response to sterol depletion.

Numerous genes shown to be regulated in response to changes in sterol levels (6, 13) lack promoter elements with similarity to the *ERG2* SRE. It is possible that Upc2p and Ecm22p could indirectly regulate a broader set of genes than those that have SREs in their promoters by regulating other transcription factors. Alternatively, Upc2p and Ecm22p may bind additional sequence elements different from the SRE identified here. Hap1p, another member of the Zn[2]-Cys[6] binuclear cluster family, can bind two unrelated sequences (15, 26). Moreover, in identifying the 7 bp of the SRE necessary for binding, we tested only one point mutation (a transversion) in each position. In fact, the anaerobic response element (AR1; TCGT TYAG) (10) through which Upc2p induces anaerobically expressed genes (1) differs from the SRE by 2 bp. It is still not known whether or not Upc2p binds directly to AR1. It is therefore possible that further mutations of the binding site may be functional. Hence, it is conceivable that Upc2p and Ecm22p could directly regulate genes lacking a SRE.

Why have two genes for the same function? Upc2p and Ecm22p had closely overlapping functions, and both proteins were capable of conferring sterol-mediated regulation to *ERG2* and *ERG3* by binding directly to the same 7-bp regulatory sequence. The major difference between *UPC2* and *ECM22* in our experiments was that Ecm22p failed to activate transcription from the 11-bp SRE alone, although the effect of Ecm22p on the whole promoter was SRE dependent. Ecm22p binding and/or activation therefore may be dependent on other factors that bound the *ERG2* promoter. Based on β-galactosidase assays, the effect of Ecm22p was somewhat weaker than that of Upc2p. Furthermore, Ecm22p seemed to have a more transient effect on *ERG2* expression than Upc2p. In a situation where Ecm22p and Upc2p may compete for binding to the same site, different promoters with different relative affinities for Ecm22p and Upc2p may provide a range of sterol-mediated regulatory responses.

Activation of Upc2p and Ecm22p. The central unanswered question is the mechanism by which sterols influence the activity of Upc2p and Ecm22p. Recent work by Abramova et al. (1) showed that *UPC2* transcription, although expressed aerobically, is anaerobically induced, and that the semidominant *UPC2-1* mutant, which has a point mutation in the carboxyl-terminal domain, causes constitutive aerobic expression of anaerobically induced genes. Hence, the carboxyl-terminal domain may repress transcriptional activity. Interestingly, the carboxyl-terminal domains of both Ecm22p and Upc2p were predicted to have one or more transmembrane helices, making it a possibility that Ecm22p and Upc2p directly measure sterol levels in membranes. However, the number of transmembrane helices predicted varied considerably depending upon which program was used to make the prediction.

Nevertheless, if Upc2p and Ecm22p are integral membrane proteins, then Upc2p and Ecm22p, much like the mammalian SREBPs, may be released from the membrane in response to the need for more sterols. Regulated proteolysis as a mechanism for activating a membrane-bound transcription factor has been described in yeast (16). Sterols may regulate proteolytic activation by influencing properties of the ER membrane.

Another possibility is that the carboxyl-terminal domain of Ecm22p and Upc2p is a direct sterol sensor, even though it has no similarity at the protein sequence level to any currently known sterol binding domains. Clearly, determining where the Ecm22p and Upc2p proteins reside in the cells under noninducing conditions and whether they are structurally altered upon induction will be decisive in testing these and other models of activation.

The Zn[2]-Cys[6] binuclear cluster family of transcription factors is fungus specific (31) and, as expected, we have not found any proteins with similarity to the amino-terminal DNA binding region of Upc2p and Ecm22p in any nonfungal organism. Perhaps more surprisingly, we have also found no proteins with homology to the carboxyl-terminal domains of either protein outside of fungi. Therefore, proteins homologous to Ecm22p and Upc2p are probably absent from mammals. Consequently, despite seemingly parallel sterol regulatory circuits

in yeast and mammals, the transcription factors involved share no sequence similarity and are clearly not homologous proteins. Understanding the mechanism of activation of Ecm22p and Upc2p may therefore reveal a new dimension to how organisms regulate sterol synthesis.

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