DOI: 10.1534/genetics.105.053025

Cumulative Mutations Affecting Sterol Biosynthesis in the Yeast *Saccharomyces cerevisiae* Result in Synthetic Lethality That Is Suppressed by Alterations in Sphingolipid Profiles

Martin Valachovic,*,[1,2] Bart M. Bareither,*,[1] M. Shah Alam Bhuiyan,*,[1] James Eckstein,[†] Robert Barbuch,[†] Dina Balderes,[‡] Lisa Wilcox,[‡] Stephen L. Sturley,[‡] Robert C. Dickson[§] and Martin Bard*,[3]

**Department of Biology, Indiana University–Purdue University, Indianapolis, Indiana 46202,* [†]*Department of Drug Disposition, Eli Lilly, Lilly Corporate Center, Indianapolis, Indiana 46285,* [‡]*Institute of Human Nutrition, Columbia University Medical Center, New York, New York 10032 and* [§]*Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, Kentucky, 40536*

Manuscript received November 28, 2005
Accepted for publication May 15, 2006

ABSTRACT

UPC2 and *ECM22* belong to a Zn(2)–Cys(6) family of fungal transcription factors and have been implicated in the regulation of sterol synthesis in *Saccharomyces cerevisiae* and *Candida albicans*. Previous reports suggest that double deletion of these genes in *S. cerevisiae* is lethal depending on the genetic background of the strain. In this investigation we demonstrate that lethality of *upc2*Δ *ecm22*Δ in the S288c genetic background is attributable to a mutation in the *HAP1* transcription factor. In addition we demonstrate that strains containing *upc2*Δ *ecm22*Δ are also inviable when carrying deletions of *ERG6* and *ERG28* but not when carrying deletions of *ERG3*, *ERG4*, or *ERG5*. It has previously been demonstrated that *UPC2* and *ECM22* regulate *S. cerevisiae ERG2* and *ERG3* and that the *erg2*Δ *upc2*Δ *ecm22*Δ triple mutant is also synthetically lethal. We used transposon mutagenesis to isolate viable suppressors of *hap1*Δ, *erg2*Δ, *erg6*Δ, and *erg28*Δ in the *upc2*Δ *ecm22*Δ genetic background. Mutations in two genes (*YND1* and *GDA1*) encoding apyrases were found to suppress the synthetic lethality of three of these triple mutants but not *erg2*Δ *upc2*Δ *ecm22*Δ. We show that deletion of *YND1*, like deletion of *GDA1*, alters the sphingolipid profiles, suggesting that changes in sphingolipids compensate for lethality produced by changes in sterol composition and abundance.

THE yeast *Saccharomyces cerevisiae* has provided a powerful model system to study the biochemistry of lipid biosynthesis. Many genes encoding the enzymes for sterol, fatty acid, phospholipid, and sphingolipid synthesis have been isolated, their products characterized, and the orthologous genes in human cells identified (Daum *et al.* 1998; Kelley and Herman 2001). However, less is known about the regulation of lipid synthesis and transport between organelles. Recently, two transcription factors, Upc2p and Ecm22p, were implicated in the coordination of these processes in this model organism (Vik and Rine 2001; Wilcox *et al.* 2002).

Upc2p and Ecm22p are members of the Zn[2]–CyS[6] binuclear cluster family of transcription factors (Todd and Andrianopoulos 1997) and share significant amino acid sequence identity (45%). A semidominant allele of *UPC2* (*upc2-1*) was demonstrated to confer aerobic sterol influx, a process normally restricted to anaerobiosis (Lewis *et al.* 1988; Crowley *et al.* 1998), while a lesser role of Ecm22p in sterol transport has also been reported (Shianna *et al.* 2001). Microarray analyses of the *upc2-1* mutant identified novel genes involved in sterol influx, including *AUS1* and *PDR11*, which encode ABC transporters required for anaerobic sterol influx (Wilcox *et al.* 2002).

In addition to a role in sterol transport, an involvement of Upc2p and Ecm22p in regulating sterol biosynthesis has been suggested (Vik and Rine 2000). Both transcription factors have been referred to as sterol regulatory element (SRE)binding proteins (SREBPs), responsible for regulating the transcription of the ergosterol biosynthetic genes *ERG2* and *ERG3* via binding to an 11-bp SRE (Vik and Rine 2001). However, these proteins lack sequence conservation with the analogous mammalian or recently identified *Schizosaccharomyces pombe* SREBPs (Brown and Goldstein 1997; Hughes *et al.* 2005); whether they exhibit functional similarity remains to be established. Despite this, a large number of ergosterol biosynthetic genes, such as *ERG1*, *ERG6*, *ERG8*, *ERG12*, *ERG13*, and *ERG25* contain this SRE and

[1]These authors contributed equally to this work.

[2]*Present address:* Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Moyzesova 61, 90028 Ivanka pri Dunaji, Slovak Republic.

[3]*Corresponding author:* Department of Biology, Indiana University–Purdue University, 723 W. Michigan St., Indianapolis, IN 46202.
E-mail: mbard@iupui.edu

are likely under *UPC2/ECM22* transcriptional control. In *Candida albicans*, loss of *UPC2* resulted in an inability to upregulate *ERG2* and *ERG11* in response to azole treatment. Similarly, a 7-bp SRE core element also appears in many of the same ergosterol biosynthetic genes in *C. albicans* (Silver *et al.* 2004).

Upc2p has further been proposed to regulate the transcription of a number of anaerobically expressed genes (Kwast *et al.* 1998). For example, Upc2p induces the expression of the anaerobic cell wall-related genes in the *PAU* family (Abramova *et al.* 2001; Wilcox *et al.* 2002). Combined with the induction of sterol influx, a largely anaerobic process, these results suggest that Upc2p and Ecm22p play a role in coordinating the transcriptional response to hypoxia. The role of Ecm22p under anaerobic conditions has not been documented. Furthermore, the transcriptional targets of Upc2p and Ecm22p under oxygen-limited conditions are largely unknown.

In this article we demonstrate synthetic lethality between genes encoding transcription factors that decrease sterol biosynthesis (*UPC2*, *ECM22*, and *HAP1*) and mutations in ergosterol biosynthetic genes that by themselves are viable (*ERG2*, *ERG6*, and *ERG28*). In addition, we identify mutations in *GDA1* and *YND1* as suppressors of this synthetic lethality. Gda1p was shown previously to play a role in mannosylation of sphingolipids (Abeijon *et al.* 1993). Ynd1p is related to Gda1p and we demonstrate that it also plays a role in sphingolipid synthesis. These results demonstrate that inviability caused by changes in sterols can be suppressed by compensatory changes in sphingolipids.

MATERIALS AND METHODS

General: Yeast complete (YEPD), synthetic complete (CSM), and bacterial media were prepared as described (Ausubel *et al.* 1998). Yeast extract, yeast nitrogen base, Bacto-peptone, and Bacto-agar were from Difco. Molecular biology and genetic procedures were performed according to conventional protocols (Ausubel *et al.* 1998). Gene-specific oligonucleotides were synthesized by Invitrogen.

Yeast strains and molecular techniques: Yeast strains used in this study are isogenic with the strain W303-1A (*MAT***a** *ade2-1, can1-1, trp1-1, ura3-1, his3-11, 15, leu2-3, 112*) (Thomas and Rothstein 1989). Deletion mutant strains were generated by homologous recombination with PCR products (Erdeniz *et al.* 1997) generated using the *S. cerevisiae* pRS303, -304, -305, and -306 vectors to generate *HIS3*, *TRP1*, *LEU2*, and *URA3* selectable markers (Sikorski and Hieter 1989) and oligonucleotides that contained 45–50 bp of gene-specific sequence. Disruptions containing the kanamycin (G418) resistance marker were obtained using the plasmid pFA6 as template. Oligonucleotides used to create various deletions are listed in Table 1. All disruptions were verified by PCR. The Ty transposon insertion at the 3′ end of the *HAP1* gene was identified by PCR using oligonucleotides specific to *HAP1* and the Ty1 transposon (CTTCCTTTTATCAAAGCAT CTTG and CGAG GATTTAGGAATCCATAAA, respectively). The *hap1*Δ mutation was created as follows: An integrating plasmid PdAG1-HS (kindly provided by A. Gower and J. L. Pinkham) containing a 1347 deletion within *HAP1* spanning the *Hin*dIII site to *Sal*I site was digested with *Eco*RI for targeted integration into the *HAP1* locus of SCY325. *URA3* transformants were selected and colonies placed on 5-fluoroorotic acid (FOA) medium to select for a strain (SCY2127) containing the *hap1*Δ allele. PCR screening primers for the characterization of the *HAP1* locus are indicated in Table 1. Plasmid pU6ΔsacI containing the normal *UPC2* gene was used as the rescue plasmid in all suppressor screens (A. Tinklenberg and S. L. Sturley, unpublished data). Standard protocols for matings, sporulation, tetrad analyses, and transformation are as described (Burke *et al.* 2000).

Strains used in this study were derived from W303. SCY325 and SCY328 are common Sturley laboratory strains. All other strains listed in Table 2 were derived for this study and are deletions, with the exception of *hap1Ty*.

Transposon mutagenesis: Fifteen pools of the mTn-lacZ/LEU2 transposon library were received as a generous gift from Michael Snyder's lab (Burns *et al.* 1994). UltraMAX DH5α-FT Competent Cells were transformed with the pools of DNA according to standard bacterial transformation protocols. Transformants were selected on LB medium containing 60 μg/ml ampicillin. On average 250,000 colonies could be harvested from each pool. DNA purification was performed according to standard DNA midi-prep protocols (Ausubel *et al.* 1998). *S. cerevisiae* DNA from amplified pools containing the 6.6-kb mTN-*lacZ/LEU2* cassette was restricted with *Not*I and transformed into the appropriate strains containing the *UPC2* rescue plasmid pU6ΔSacI. For *hap1*Δ *upc2*Δ *ecm22*Δ /pUΔSacI, 33,754 *LEU2* transformants were obtained. For the *erg2*, *erg6*, and *erg28* mutants in the *upc2*Δ *ecm22*Δ background, 10,804, 39,771, and 1095 *LEU2* transformants were obtained, respectively. Transformants that were able to lose the rescue plasmid on FOA medium became candidates for containing suppressor mutations. To locate the site of genomic insertion inverse PCR was used as described on the Gottschling web site (http://www.fhcrc.org/science/labs/gottschling/misc/ipcr.html) and in Ochman *et al.* (1988). Genomic DNA from *S. cerevisiae* from putative suppressor strains was extracted according to standard protocols (Ausubel *et al.* 1998) and digested with *Aci*I, *Alu*I, *Hae*III, *Hpa*II, *Rsa*I, or *Taq*I that corresponded to known restriction sites in the mTn-*LacZ/LEU2* transposon cassette. The restriction site defined the sequence for which both the inverse PCR and sequencing primers would be used. Inverse PCR was performed using primers InPCR1, -2, -7, and -8. A PCR product using primers InPCR7 and InPCR 8 was used as a template for a second round of PCR with the primers InPCR1 and InPCR2. Using this method, PCR products were produced that were amenable to DNA sequencing. DNA purification for nucleotide sequencing was performed using the QIAquick Method (QIAGEN). Sequencing was performed at the Indiana University Biochemistry and Biotechnology Facility using mTn3-SEQ1, which anneals at position 15–42 of the mTn-*lacZ/LEU2* transposon cassette.

Sterol analyses: Sterols were extracted following published protocols (Gachotte *et al.* 1999). Gas chromatography analysis of sterols was conducted on an HP5890 series II GC, using a DB-5 capillary column (15 m × 0.25 mm i.d., 0.2 μm film thickness) with nitrogen as carrier gas (30 cm/sec) and was programmed from 195° to 300° (195° for 3 min, 5.5°/min to 300° then held for 10 min). Gas-chromatography–mass spectrometry analyses were performed with an HP5890 GC coupled to an HP5972 mass selective detector. Electron impact MS (70 eV, scanning from 40 to 700 or 650 atomic mass units, at 1-sec intervals) was performed using the following conditions: DB-5MS column (20 m × 0.18 mm i.d., 0.18 μm film thickness), helium as carrier gas (30 cm/sec), detector temperature 180°, column temperature 100°–300° (100° for

TABLE 1

Primers used in this study

Primer name	Primer sequence	Purpose
frwUPC2	CGGTAAACGTAAATTCCATAACAAATCAA AGAATGGGTGCGATAACTGTAAAAGAAG AAGTggcgggtgtcggggctggc	*UPC2* disruption
revUPC2	CTATCAGGTTCAGATTGCCTTTGGTAGAA AGATCTAAAAGCTTAGCGATGTTACTGGT ACttgccgatttcggcctattg	
frwECM22	CCGATGATGGGAATGCTGGACAAGAAAG AGAGAAGGATGCTGAACTGATTGAGGTTG Gggcgtacgctgcaggtcgac	*ECM22* disruption
revECM22	CGCGATGCAGTTTGTCCAAATATGCTAAA GTTATCAAGTACGGTGAATCAATTTCTAC Ggatcgatgaattcgagctcg	
frwERG2	CCACTCCTTTTGTTGATTGGTGTTGTAGGC TACATTATGAACGTATTGTTCACTACCTG GTggcgggtgtcggggctggc	*ERG2* disruption
revERG2	CAAGTTCTTACCCATGTCCCTGGCAGTCA GGTAGACAGTTCTATATAGAGTGTATAAA TCttgccgatttcggcctattg	
frwERG6	GGCCCAATTCACTAGGGAGTTACATGGTG ATGATATTGGTAAAAAGACAGGTTTGAGT GCTGGCGGGTGTCGGGGCTGGC	*ERG6* disruption
revERG6	CGTTTTCTGGCTTCCTAGCGACGAAAAGC ATCATTGGAGTGAATAACTTGGACTTACC ACTTGCCGATTTCGGCCTATTG	
frwERG28	CTTTACGGTATTACCGATAGGAAACTTCT ATTTTATGATTTTTTCGTTCGGGGACGGAA CTGGCGGGTGTCGGGGCTGGC	*ERG28* disruption
revERG28	CGTTAGAGGAGAGAGGTAGGGATACTTA CAGAAATGAAGCCAAATTGGCAGCTTTTT GAGTTGCCGATTTCGGCCTATTG	
frwHDA3	GATTTACTACGCATTTTAGACACGAAACC AATACCTACAATTGTTGATGCTACTACTCT Gtggcgggtgtcggggctggc	*HDA3* disruption
revHDA3	CGTGGAATTGTTAAGTTTTCCAAGTTGTCC ATCGTCTTCGATAACTGTCCCACTAACTCC ttgccgatttcggcctattg	
frwTUP1	GGGAAGAAAGAAATCAGCTTTCCATCCAA ACCAATATGACTGCCAGCGTTTCGAATAC GCtggcgggtgtcggggctggc	*TUP1* disruption
revTUP1	GTGTGGTGGCCACGGACAATACAAAGTCT TTATGCCCGATATACGTAACTTCACAAGT GCttgccgatttcggcctattg	
frwYND1	ATTTCCCCGTCTGCCCTCTTATGCTCATAG AAAACACTAATGATCGGTTTGGTATCGTC Atggcgggtgtcggggctggc	*YND1* disruption
revYND1	CAGGTCGTAAGTTTGACGGAGCGAGATTA GCGGACTGACTTGCTGTCCTTTGTGGTTCG Tttgccgatttcggcctattg	
frwIES1	GTATTGGAAACAGTTCATGATTAACTAAG TAAAATCGAAAGCTAAATAAAGGCGACA CTCtggcgggtgtcggggctggc	*IES1* disruption
revIES1	CTTAAACATGGCGGTTGCTTCTTCGTCATC AATTTCCGCGCGTTCGATTTTATTTAAAAG ttgccgatttcggcctattg	

(*continued*)

TABLE 1

(Continued)

Primer name	Primer sequence	Purpose
MNN11fwd	GAAGAAAGCCAATTTTCAACTTGGAATCA TCCTGCCGGTTTGTTGTTGGTGTTAGCTGT Gtggcgggtgtcggggctggc	*MNN11* disruption
MNN11rev	GTAAAGAAAGATCGAGATTCATCAATAA AGCATCTTGATCAACAAAATGAATATACT TGGttgccgatttcggcctattg	
UME6fwd	CAGCGCACAGGAACTAGGACACTACCGC ACTCAAACCATTTGCATGGACCTTAACTC ACGtggcgggtgtcggggctggc	*UME6* disruption
UME6rev	GTCTTTCCTCGGTACACTTCTTTTTCCTTA ATCTACAAATCCAGCAACCAGTACGGGAC Cttgccgatttcggcctattg	
MNN9fwd	AATTTTAGTATTTTTCAAACAGAAGAATT TAAAAGAGCTAGAATAAAAAGTTAGGAA ACAtggcgggtgtcggggctggc	*MNN9* disruption
MNN9rev	ATTAATGGCCTATAAGTGGCAATTTCTGC ATAACCCTCGACAATAATCTCGTCATCAC CCttgccgatttcggcctattg	
GDA1fwd	CAATCTTTATTGGCGAACAGTTAAGGGTC CTCTCGAGAAGAAACATTAAGACATCATC GCtggcgggtgtcggggctggc	*GDA1* disruption
GDA1rev	AACAATTTGTGTAGACCCTCCGCCTAAGT CAAAAACGGCAGCAGTAGGTAACTTGGG GCCttgccgatttcggcctattg	
GDA1-5′GAL	CGGGATCCCACAAAAAACATGGCGCCCA	pYES2-GDA1 plasmid
GDA1-3′GAL	CGGAATTCGAAAAGCGGTGTCCATGTTC	
inPCR1	5′-TAAGTTGGGTAACGCCAGGGTTTTC-3′	Nested/inverse PCR for Rsa*I*, Taq*I*
inPCR2	5′-TTCCATGTTGCCACTCGCTTTAATG-3′	
inPCR7	CCTCAGGAAGATCGCACTCC	Inverse PCR for Taq*I*
inPCR8	GTGATGGTGCTGCGTTGGAG	
MTn3-SEQ1	CCCCCTTAACGTGAGTTTTCGTTCCACT	Sequencing InPCR
HAP1-frw	ACTTCCTTTTATCAAAGCATCTTG	*hap1*Δ check in SCY2127
HAP1-2rev	GACGTATCCCATTCTGAAACGCAAC	
LW1	ATTCCAACGGTTCCATCGTTAACC	*Hap1* wild-type check
LW2	TATCCCATTCTGAAACGCAACG	
LW1	ATTCCAACGGTTCCATCGTTAACC	*Hap1Ty* check
LW3	CGAGGATTTAGGAATCCATAAA	

In the disruption primers, lowercase letters correspond to conserved regions bordering the *TRP1*, *KAN*^{MX}, *HIS3*, *LEU2*, and *NAT*^{R} genes in the pRS304, pFA6, pRS303, pRS315, and pAG36 vectors, respectively. All primers are designed so that left to right corresponds to 5′ → 3′.

1 min, 10°/min to 300° then held for 15 min); and DB-5 (10 m × 0.25 mm i.d., 0.25 μm film thickness), helium as carrier gas (50 cm/sec), column temperature 40°–300° (40° for 1 min, 30°/min to 300° then held for 4 min). All injections were run in splitless mode.

Sphingolipid and membrane microdomain analyses: Lipids were extracted from cells radiolabeled with [^{3}H]*myo*-inositol, separated by chromatography on Whatman HP-K plates and detected by using a BioScan apparatus (Dickson *et al.* 1997). The chromatography solvent was 55:45:10 (chloroform: methanol:0.25% KCl) (Puoti *et al.* 1991). Detergent-insoluble fractionations were carried out according to Bagnat *et al.* (2000) with a few minor alterations. A total of 10 OD_{600} units of exponential phase cells were lysed in TNE (50 mM Tris–HCl, pH 7.4/150 mM NaCl/5 mM EDTA) buffer by bead-beating mechanical disruption at 4° in the presence of protease inhibitors. The supernatant (450 μl) was incubated for 30 min on ice with pre-chilled Triton X-100 at final concentration of 1% v/v. A total of 400 μl of this mixture with 2 volumes of 60% OPTIPREP were then overlaid with 2.7 ml of 30% OPTIPREP and finally with 0.5 ml TNE protease buffer and centrifuged for 3 hr at 45,000 rpm in a SW50.1 rotor at 4°. A total of 500 μl fractions 1–8 were removed from the top of the gradient. The detergent-insoluble (raft-associated) proteins reside predominately in fraction 2 and sometimes in fraction 1. Fractions were stored at −80°. All immunoblots were performed on 8%

TABLE 2

Strains used in this study

Strain	Genotype
W303	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1, ura3-1 can1*
SCY325	*MAT*α *ade2-1 his3-11,15 leu2-3,112,trp1-1, ura3-1 can1*
SCY328	*MAT***a** *ade2-1 his3-11,15 leu2-3,112,trp1-1ura3-1 can1*
SCY1832	*MAT*α *upc2*Δ*::URA3 hap1Ty*
SCY1987	*MAT*α *upc2*Δ*::URA3*
SCY1996	*MAT*α ecm22Δ*::LEU2*
SCY2127	*MAT*α *hap1*Δ
MVS36	*MAT***a** *erg28*Δ*::HIS3*
MVS40	*MAT***a** *erg2*Δ*::HIS3*
MVS41	*MAT*α *erg6*Δ*::HIS3*
MH1-3B	*MAT*α *hap1*Δ *upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI[a]
M28-2D	*MAT***a** *erg28*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
M6-3B	*MAT***a** *erg6*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
M2-7A	*MAT*α *erg2*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
MVS139	*MAT*α *ynd1*Δ*::LEU2*
M5B-3	*MAT***a** *upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
MVS3	*MAT***a** *erg3*Δ*::TRP1 upc2*Δ*::URA3 ecm22*Δ*::LEU2*
MVS14	*MAT***a** *erg5* Δ*::TRP1 upc2*Δ*::URA3 ecm22*Δ*::LEU2*
MVS18	*MAT***a** *erg4*Δ*::TRP1 upc2*Δ*::URA3 ecm22*Δ*::LEU2*
BBY102	*MAT***a** *ynd1*Δ*::LEU2 erg2* Δ*::HIS3 upc2* Δ*::TRP1 ecm22* Δ*::KANMX4/*pU6ΔSacI
BBY103	*MAT***a** *ynd1*Δ*::LEU2 hap1*Δ *upc2* Δ*::TRP1 ecm22* Δ*::KANMX4/*pU6ΔSacI
BBY105	*MAT*α *ynd1* Δ*::LEU2 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY107	*MAT***a** *ynd1*Δ*::LEU2 erg2*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY112	*MAT***a** *ynd1*Δ*::LEU2 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY113	*MAT***a** *ynd1*Δ*::LEU2 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY114	*MAT***a** *gda1*Δ*::HIS3*
BBY125	*MAT*α *gda1*Δ*::HIS3 hap1*Δ *upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY131	*MAT*α *ynd1*Δ*::LEU2 erg28*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
BBY137	*MAT***a** *gda1*Δ*::HIS3 erg28*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY29	*MAT*α *gda1*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY31	*MAT***a** *gda1*Δ*::HIS3 erg6*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY34	*MAT***a** *gda1*Δ*::HIS3 erg6*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY38	*MAT*α *gda1*Δ*::HIS3 erg2*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY47	*MAT*α *ynd1*Δ*::LEU2 erg6*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI
SBY56	*MAT*α *ynd1*Δ*::LEU2 hap1*Δ*::HIS3 upc2*Δ*::TRP1 ecm22*Δ*::KANMX4/*pU6ΔSacI

All strains are derived from W303. SCY325 and SCY328 are common laboratory strains. All others were generated in this study.

[a] pU6ΔSacI is a complementing plasmid containing UPC2.

SDS–PAGE gels with a 5% stacking gel. α-Gas1p or α-Pma1p antibody was used at a 1:2500 dilution followed by α-goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:4000 dilution). Amersham ECL assays were used to detect the proteins.

RESULTS

The *upc2*Δ *ecm22*Δ double mutant exhibits synthetic lethality in combination with various mutations affecting sterol synthesis: In contrast to other findings, Parks' group (Shianna *et al.* 2001) found that the double mutant *upc2*Δ *ecm22*Δ was inviable in their genetic background. The original *upc2-1* allele (Lewis *et al.* 1988) was derived from strain S288C, in which a Ty1 element disrupts the 3′ region of the *HAP1* ORF (Gaisne *et al.* 1999). The *HAP1* gene encodes a heme-responsive transcription factor that regulates sterol biosynthesis (Turi and Loper 1992; Kennedy *et al.* 1999). In a *upc2-1* gain-of-function mutant, *HAP1* is upregulated and furthermore acts as a modifier of sterol uptake (Wilcox *et al.* 2002). Given the roles of Upc2p and Ecm22p in the regulation of sterol biosynthesis, we further investigated the cross-talk between the *HAP1* and *UPC2/ECM22* pathways by attempting to generate a triple mutant by crossing an *ecm22*Δ mutant to a *upc2*Δ *hap1Ty* strain. Whereas every possible double mutant segregant from the cross was recovered, all triple mutant segregants (*hap1Ty upc2*Δ *ecm22*Δ) died (50 tetrads dissected). However, triple *hap1Ty upc2*Δ *ecm22*Δ mutants containing a *URA3*-marked plasmid with a wild-type copy of *UPC2* (pU6ΔSacI) were viable. *upc2*Δ *ecm22*Δ strains containing a deletion allele of *HAP1* were also inviable; dissection of ~39 tetrads also failed to yield a viable *hap1*Δ *upc2*Δ *ecm22*Δ strain. Thus both the *hap1TY* and

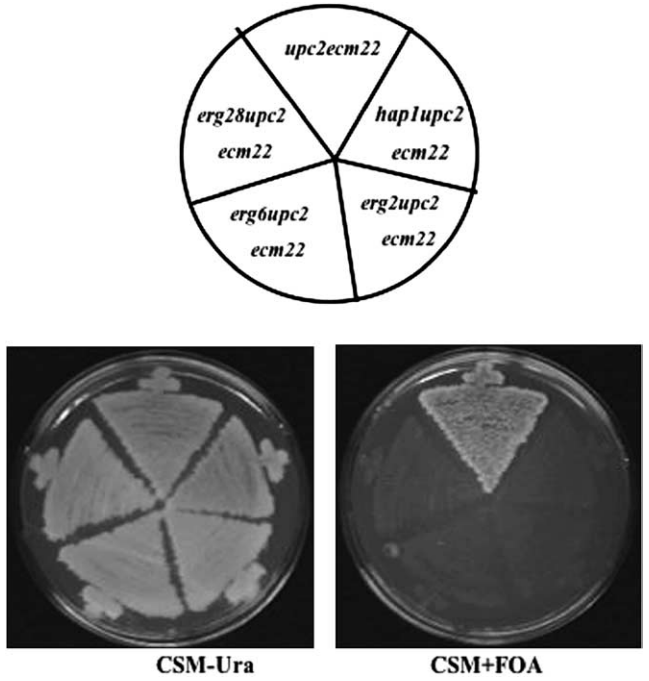


FIGURE 1.—The triple mutants *hap1*Δ *upc2*Δ *ecm22*Δ, *erg2*Δ *upc2*Δ *ecm22*Δ, erg6Δ upc2Δ ecm22Δ, and erg28Δ upc2Δ ecm22Δ contain a *UPC2* rescue plasmid (pUΔSacI) and are able to grow on CSM-Ura medium but cannot grow on CSM + FOA, which negatively selects against plasmid retention. The *upc2*Δ *ecm22*Δ control strain grows on both media.

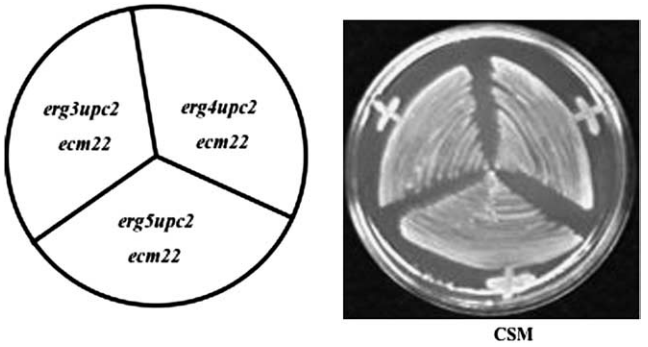


FIGURE 2.—The triple mutants *erg3*Δ *upc2*Δ *ecm22*Δ, *erg4*Δ *upc2*Δ *ecm22*Δ, and *erg5*Δ *upc2*Δ *ecm22*Δ are all able to grow on CSM medium without a rescue plasmid.

*hap1*Δ alleles in combination with *upc2*Δ *ecm22*Δ were inviable. The inability of *hap1Ty* (or *hap1*Δ) *upc2*Δ *ecm22*Δ*/pU6*Δ*SacI* strains to grow on medium containing 5-FOA, which selects against the *URA3* containing plasmid, further supports the inviability of the triple mutant combination (Figure 1).

In previous studies, we and others have demonstrated that the combined deletion of *UPC2*, *ECM22*, and *ERG2* was synthetically lethal (VIK and RINE 2001; VALACHOVIC *et al.* 2004). However, this lethality could be suppressed by a mutation in *ELO3*, which is necessary for synthesis of the C_{26} fatty acids found in sphingolipids (VALACHOVIC *et al.* 2004). In the current study, we investigated whether *upc2*Δ *ecm22*Δ was synthetically lethal in combination with other viable ergosterol deletion mutants, such as *erg3*Δ, *erg4*Δ, *erg5*Δ, *erg6*Δ, and *erg28*Δ. Our results demonstrate that *ERG6* and *ERG28* but not *ERG3*, *ERG4*, or *ERG5* are essential genes in the absence of *UPC2* and *ECM22* (Figures 1 and 2). These results are summarized in Table 3, in which we demonstrate that viable triple mutants of *hap1*Δ (40 tetrads dissected), *erg6*Δ (33 tetrads dissected), and *erg28*Δ (38 tetrads dissected) with *upc2*Δ *ecm22*Δ were not observed. Triple mutants containing *hap1*Δ *upc2*Δ *ecm22*Δ, *erg6*Δ *upc2*Δ *ecm22*Δ, and *erg28*Δ *upc2*Δ *ecm22*Δ were, however, recovered from crosses containing the rescue plasmid (pU6ΔSacI) with a normal *UPC2* allele. These strains grew on CSM-Ura but failed to grow on FOA medium as expected if the triple mutants are inviable (Figure 1). Finally, attempts to disrupt *HAP1*, *ERG6*, or *ERG28* wild-type alleles in a *upc2*Δ *ecm22*Δ strain were unsuccessful, supporting the hypothesis that mutating these genes in the double deletion background mutant is a lethal event.

Deletion of *upc2* and *ecm22* alters sterol profile: An 11-bp SRE responsible for *UPC2*-mediated sterol regulation of *ERG2* was found in eight other sterol biosynthetic genes in *S. cerevisiae* and a similar element was found in several ergosterol biosynthetic genes in *C. albicans* (VIK and RINE 2001; SILVER *et al.* 2004). The *upc2*Δ *ecm22*Δ double mutant accumulates large amounts of two sterol intermediates, episterol and ergosta-5,7-dien-ol, at the expense of end-product sterol (VALACHOVIC *et al.* 2004). The single deletion mutants *upc2*Δ and *ecm22*Δ had sterol profiles essentially like that of the wild type. Initially, we looked at *erg3*Δ, *erg4*Δ, and *erg5*Δ alone and in a *upc2*Δ *ecm22*Δ genetic background to determine exactly how the sterol profiles were altered. The principal sterol that accumulates in *erg3*Δ is ergosta-7,22,-dienol (70% of total sterol), but in a *erg3*Δ *upc2*Δ *ecm22*Δ mutant the principal sterol is episterol (41%) followed by fecosterol (13.9%) and ergosta-7-enol(13.3%), not a dramatic difference in sterol composition. Similar results were observed in an *erg4*Δ strain

TABLE 3

Genetic analyses to determine viability *of hap1*Δ, *erg2*Δ, *erg6*Δ, and *erg28*Δ in a *upc2*Δ *ecm22*Δ mutant background

Mating	No. of tetrads dissected	Viable triple mutants[a]
*erg3*Δ *upc2*Δ × *upc2*Δ *ecm22*Δ	23	19
*erg4*Δ *upc2*Δ × *upc2* Δ *ecm22*Δ	24	24
*erg5*Δ *upc2*Δ × *upc2*Δ *ecm22*Δ	18	10
erg6 Δ *upc2*Δ × *upc2* Δ *ecm22*Δ	33	0
*erg28*Δ *upc2*Δ × *upc2*Δ *ecm22*Δ	38	0
*hap1*Δ × *upc2*Δ *ecm22*Δ	40	0

[a] Number of viable triple mutant isolates in a *upc2ecm22* background.

TABLE 4

Genes disrupted by mTn3 in multiple backgrounds

	M2-7A: *erg2upc2ecm22*	M6-3B: *erg6upc2ecm22*	MH1-3B: *hap1upc2ecm22*	M28-2D: *erg28upc2ecm22*
YND1	1	1	1	—
YER049w	1	2	1	—
HDA3	—	1	1	—
RPN9	—	1	1	—
IES1	1	2	—	—
SSN2	1	—	1	—
BCK2	—	2	—	—
RDN25-1	—	3	—	3
RDN37-1	—	3	—	3
TAR1	—	3	—	1
YNL011C	1	4	—	—
YNL010W	1	4	—	—

M2-7A [*erg2*Δ*upc2*Δ*ecm22*Δ*p(U,U)*], M6-3B [*erg*Δ*upc2*Δ*ecm22*Δ*p(U,U)*], *MH1-3B* [*hap1*Δ*upc2*Δ*ecm22*Δ*(U,U)*], and *M28-2D* [*erg28*Δ*upc2*Δ*ecm22*Δ*p(U,U)*] were all transformed with the mTn3 library resulting in several genes disruped in multiple backgrounds.

compared to the *erg4*Δ *upc2*Δ *ecm22*Δ triple mutant. The former accumulates almost exclusively ergosata-5,7,22, 24(28) sterols but the triple mutant accumulates mostly episterol, two closely related *erg4*Δ-type sterols, ergosta-5,7,24(28), ergosta-5,7,22, 24(28), as well as zymosterol. Finally, the sterol profiles of *erg5*Δ and the *erg5*Δ *upc2*Δ *ecm22*Δ were essentially the same, accumulating principally ergosta-5,7 diene (74.6% and 67.6%), respectively. These results suggest that these triple mutants are likely viable because the sterols produced are not significantly different from those that occur in the *erg3*Δ, *erg4*Δ, and *erg5*Δ single mutants.

Isolation of suppressors of inviable *upc2*Δ *ecm22*Δ triple mutants: Previously we isolated a UV-induced suppressor mutation of an *erg2*Δ *upc2*Δ *ecm22*Δ triple mutant. Genetic analysis of the suppressor showed that it was resistant to the morpholine tridemorph (Baloch *et al.* 1984), suggesting that the suppressor may be *elo2* or *elo3* (Valachovic *et al.* 2004). This suppressor was identified as *elo3*, which has been demonstrated to suppress lethal mutations of *erg2* (Silve *et al.* 1996), although in the majority of genetic backgrounds *erg2*Δ mutants are not lethal (Ashman *et al.* 1991).

Similar attempts to identify viable suppressors of triple mutants of *hap1*, *erg6*, *erg2*, or *erg28* in the *upc2*Δ *ecm22*Δ background were unsuccessful. Therefore transposon mutagenesis using *hap1*Δ *upc2*Δ *ecm22*Δ, *erg2*Δ *upc2*Δ *ecm22*Δ, *erg6*Δ *upc2*Δ *ecm22*Δ, and *erg28*Δ *upc2*Δ *ecm22*Δ strains containing the *UPC2* rescue plasmid pU6ΔSacI were transformed with a mutagenized transposon library-mTn-*lacZ/LEU2*. Transformants were subsequently plated onto synthetic complete medium containing FOA to select loss of the pU6ΔSacI plasmid. Colonies purified on CSM-leu media that were able to grow on FOA medium were then considered as candidates for carrying a suppressor mutation. These colonies were then subjected to PCR analysis to ensure that a wild-type version of the *UPC2* was not present. Approximately 40,000 transformants were obtained for triple mutants of *erg6*, 34,000 for *hap1*, 11,000 for *erg2*, and 1100 for *erg28*. Transposon screening by PCR allowed us to identify 116 different candidate suppressor genes. The largest category (14.7%) involved genes encoding transcription factors and the next largest class (10.3%) involved genes required for chromatin remodeling and chromatin structure. In total, 15 categories of genes were identified and the ones that were isolated multiple times are listed in Table 4.

Putative transposon suppressors were confirmed by independently creating gene deletions: While the transposon mutagenesis screen indicated many putative suppressors, it was necessary to independently confirm these mutations by creating knockouts of putative genes and crossing these into our strains to generate viable quadruple mutants (*e.g.*, *mutx*Δ *hap1*Δ *upc2*Δ *ecm22*Δ). We chose several categories of genes to independently disrupt: *TUP1* and *HDA3* are two genes involved in chromatin repression; transposon mutagenesis indicated that in the *upc2*Δ *ecm22*Δ background, *tup1* rescued *hap1* and *hda3* suppressed both *hap1* and *erg6*. A mutation in *OSH1* encoding an oxysterol-binding protein suppressed *erg6*Δ and a mutation in *UME6* (a transcriptional regulator) suppressed *erg2*Δ in *upc2*Δ *ecm22*Δ strains. Various crosses of these independently derived disrupted genes to the triple mutants containing a rescue plasmid indicated that *tup1*Δ was not a suppressor of *hap1*Δ; *hda3*Δ was not a suppressor of *hap1*Δ, *erg2*Δ, or *erg6*Δ; *osh1*Δ was not a suppressor of *erg6*Δ; and *ume6*Δ was not a suppressor of *erg2*Δ. However, *ynd1*Δ that was initially identified as a suppressor of *hap1*Δ, *erg2*Δ, and *erg6*Δ mutants by transposon mutagenesis was confirmed by independently derived

TABLE 5

Synthetically lethal backgrounds in which *ynd1*Δ was tested as a suppressor

Mating	Tetrads dissected[a]	Viable quadruples[b]	Inviable quadruples[c]	Viable triples[d]
hap1upc2ecm22/p × *ynd1upc2ecm22*/p[e]	41	44	0	43
erg2upc2ecm22/p × *ynd1erg2upc2ecm22*/p	18	0	9	16
ynd1erg2upc2ecm22/p × *ynd1upc2ecm22*/p	46	0	65	20
erg6upc2ecm22/p × *ynd1upc2ecm22*/p	54	28	0	15
erg28upc2ecm22/p × *ynd1upc2ecm22*/p	50	30	0	54

Matings were made to produce quadruple mutants composed of a synthetically lethal triple mutant background plus *ynd1*. All mutations are deletions.

[a] Number of tetrads dissected to produce the quadruple mutants.

[b] Number of quadruple mutants where *ynd1* suppressed lethality as indicated by the ability to lose the accompanying rescue containing *UPC2* plasmid on FOA medium.

[c] Number of quadruple mutants in which *ynd1* did not suppress lethality as indicated by the inability to grow on FOA medium.

[d] Number of synthetically lethal triple mutants produced by the cross as a control and not plated on FOA medium.

[e] URA3-containing rescue plasmid carrying UPC2 allele.

disruptions to suppress *hap1*Δ, *erg6*Δ, and *erg28*Δ mutants but unexpectedly not the *erg2*Δ triple mutant (summarized in Table 5). In various independent crosses involving the generation of triple or quadruple mutants, segregants containing the pU6ΔSacI rescue plasmid were plated onto FOA medium. For *hap1*Δ, *erg6*Δ, and *erg28*Δ mutants that also contained the *ynd1*Δ allele in the *upc2*Δ *ecm22*Δ background, viability (suppression) was indicated as the ability to lose the rescue plasmid. However, in two distinct matings in which *ynd1*Δ *erg2*Δ *upc2*Δ *ecm22*Δ quadruple mutants were generated, no quadruple mutant strain was able to lose the rescue plasmid. *YND1* encodes an apyrase (nucleoside diphosphatase and nucleoside triphosphatase activity) and has 20% amino acid identity to *GDA1*, which also encodes a nucleoside diphosphatase. *GDA1* is required for transporting GDP-mannose into the Golgi lumen (Gao *et al.* 1999). *GDA1* mutants are defective in mannosylation of both proteins and sphingolipids (Yanagisawa *et al.* 1990; Ashman *et al.* 1991; Abeijon *et al.* 1993). The *ynd1*Δ *gda1*Δ double mutant grows slowly and demonstrates defects in cell morphology and protein glycosylation. An independently derived *gda1*Δ mutant strain was similarly mated to all four triple mutants and similar results were obtained. The *gda1*Δ mutation suppressed *hap1*Δ, *erg6*Δ, and *erg28*Δ triple mutants but not the *erg2*Δ *upc2*Δ *ecm22*Δ triple mutant (Table 6). Figure 3 demonstrates that both *ynd1* and *gda1* deletions suppress lethality of *hap1*Δ, *erg6*Δ, and *erg28*Δ triple mutants but not the *erg2*Δ triple mutant. To determine whether *GDA1* expression would reverse the suppression of the *gda1*Δ-suppressed quadruple mutant, the *GDA1* open reading frame was introduced into the pYES2 expression vector that contains the galactose-inducible promoter *GAL1*. On media containing glucose both empty vector control (pYES2) and pYES2-GDA1 did not adversely affect growth of the *hap1*Δ, *erg6*Δ, and *erg28*Δ mutants in *gda1*Δ *upc2*Δ *ecm22*Δ strains. However, on galactose-containing media in which expression of the *GDA1* is induced, the quadruple mutants grew worse than when *GDA1* was not expressed (Figure 4, compare B_1 and B_2, D_1 and D_2, and F_1 and F_2) indicating that *GDA1* expression did reverse suppression. Minimal *GDA1* expression under noninducible

TABLE 6

Synthetically lethal backgrounds in which *gda1*Δ was tested as a suppressor

Mating	Tetrads dissected[a]	Viable quadruples[b]	Inviable quadruples[c]	Viable triples[d]
hap1upc2ecm22/p × *gda1upc2ecm22*/p[e]	35	30	0	17
erg2upc2ecm22/p × *gda1upc2ecm22*/p	45	0	24	20
erg6upc2ecm22/p × *gda1upc2ecm22*/p	37	30	0	16
erg28upc2ecm22/p × *gda1upc2ecm22*/p	39	34	0	30

Matings were made to produce quadruple mutants composed of a synthetically lethal triple mutant background plus *gda1*. All mutations are deletions.

[a] Number of tetrads dissected to produce the quadruple mutants.

[b] Number of quadruple mutants where *gda1* suppressed lethality as indicated by the ability to lose the accompanying rescue containing *UPC2* plasmid on FOA medium.

[c] Number of quadruple mutants in which *gda1* did not suppress lethality as indicated by the inability to grow on FOA medium.

[d] Number of synthetically lethal triple mutants produced by the cross as a control and not plated on FOA medium.

[e] URA3-containing rescue plasmid carrying *UPC2* allele.

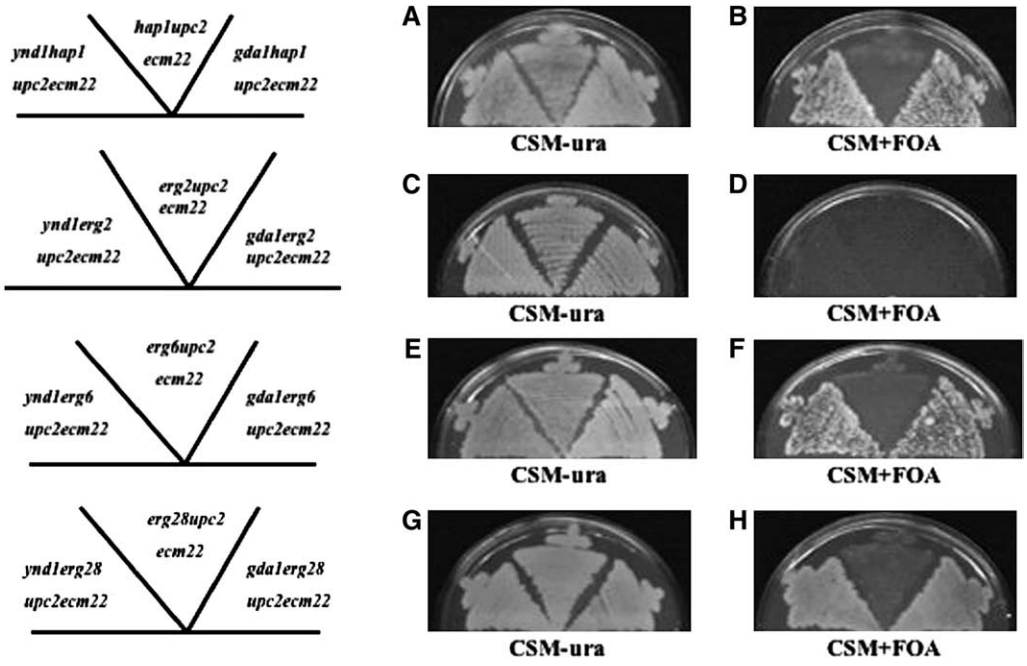


FIGURE 3.—(A and B) Suppression of *hap1*Δ *upc2*Δ *ecm22*Δ by *ynd1 and gda1* mutations. The quadruple mutants *ynd1hap*Δ *upc2*Δ *ecm22*Δ and *gda1hap*Δ *upc2*Δ *ecm22*Δ will grow on CSM-Ura or CSM + FOA medium, which selects against plasmid retention of the rescue plasmid, pUΔSacI. Only the triple mutant *hap1*Δ *upc2*Δ *ecm22*Δ cannot grow without the rescue plasmid on CSM + FOA medium. (C and D) The *erg2*Δ *upc2*Δ *ecm22*Δ triple mutant is not suppressed by *ynd1* and *gda1* as loss of plasmid results in inviability. (E and F) Suppression of *erg6*Δ *upc2*Δ *ecm22*Δ by *ynd1* and *gda1* mutations. The quadruple mutants *ynd1erg6*Δ *upc2*Δ *ecm22*Δ and *gda1erg6*Δ *upc2*Δ *ecm22*Δ will grow on CSM-Ura or CSM + FOA medium, which selects against plasmid retention of a rescue plasmid. *erg6*Δ *upc2*Δ *ecm22*Δ cannot grow without the rescue plasmid on CSM + FOA medium. (G and H) Suppression of *erg28*Δ *upc2*Δ *ecm22*Δ by *ynd1 and gda1* mutations. The quadruple mutants *ynd1erg28*Δ *upc2*Δ *ecm22*Δ and *gda1erg28*Δ *upc2*Δ *ecm22*Δ will grow on CSM-Ura or CSM+FOA medium. *erg28*Δ *upc2*Δ *ecm22*Δ cannot grow without the rescue plasmid on CSM + FOA medium.

conditions (glucose medium) showed similar growth in the absence or presence of wild-type *GDA1* (Figure 4, compare A_1 and A_2, C_1 and C_2, and E_1 and E_2). Unexpectedly, the inducible expression of *GDA1* was also able to prevent growth of *ynd1 hap1*Δ (or *erg6*Δ or *erg28*Δ) *upc2*Δ *ecm22*Δ strains. These results suggest that overexpression of *GDA1* can reverse the suppression due to a mutation of the related gene *YND1*, suggesting lesions in both *GDA1* and *YND1* genes are suppressing through a similar mechanism.

Sterol analyses of the *ynd1*- and *gda1*-suppressed strains: We were interested in determining what the sterol profiles of the suppressed quadruple mutants might look like since these strains might be regarded as having a sterol composition that was minimally adequate for viability. The sterol profiles of the *hap1*Δ, *erg6*Δ, *erg28*Δ single mutants, the *upc2*Δ *ecm22*Δ double mutant, and the *ynd1*Δ- and *gda1*Δ-suppressed quadruple mutants are given in Tables 7–9. The *hap1* sterol profile is essentially that of wild type (as are the single *ynd1* and *gda1* mutants). The *upc2*Δ *ecm22*Δ sterol profile indicated a decrease in ergosterol and increase in sterol intermediates. *ynd1*Δ *hap1 upc2*Δ *ecm22*Δ and *gda1*Δ *hap1upc2*Δ *ecm22*Δ are capable of synthesizing ergosterol at very low levels (3.5 and 3.1%, respectively) and also have nearly identical sterol profiles in which the predominant sterol is zymosterol, similar to that of the *erg6*Δ single mutant. *gda1*Δ *erg6*Δ *upc2*Δ *ecm22*Δ and *ynd1*Δ *erg6*Δ *upc2*Δ *ecm22*Δ are also nearly identical (Table 8). The differences between *erg28*Δ strains in the suppressed state are also substantial, with the quadruple mutants synthesizing much less ergosterol than an *erg28* strain alone (Table 9). Quadruple mutants lacking either *YND1* or *GDA1* synthesize more ergosta-5,7,24(28) sterols and substantially more 4-methylzymosterol and 4,4-dimethylzymosterol than the *erg28* single mutant. It is unlikely that these latter two would function as membrane sterols due to the C-4 methyl groups.

Altered sphingolipid composition in a *ynd1* mutant: Since Ynd1p appears to be related to the guanosine diphosphatase encoded by *GDA1* and since a *gda1* mutation alters the sphingolipid composition of cells (ABEIJON *et al.* 1993), we examined the sphingolipid composition of a *ynd1* mutant. Sphingolipids were radiolabeled to equilibrium with [^{3}H] *myo*-inositol, extracted from cells, and analyzed by thin-layer chromatography. Deletion of *gda1* caused a twofold reduction in the level of the major sphingolipid species, mannose-(inositol-P)$_2$-ceramide [M(IP)$_2$C] (Table 10). In contrast, deletion of ynd1p did not change the level of M(IP)$_2$C (Table 10); however, it did cause the mannose-inositol-P-ceramide (MIPC) and the inositol-P-ceramides to increase ~50%, similar to what was observed in the *gda1* mutant (Table 10). We conclude from these results that Ynd1 and Gda1 play roles in sphingolipid synthesis and their loss of function leads to changes in the species of sphingolipids produced in cells.

Loss of Ynd1p and Gda1p activity normalizes cold detergent-insoluble proteins: The ratio of various lipids, particularly sterols and glycosphingolipids, in the plasma membrane and other organellar membranes is directly responsible for the sequestration or complexing

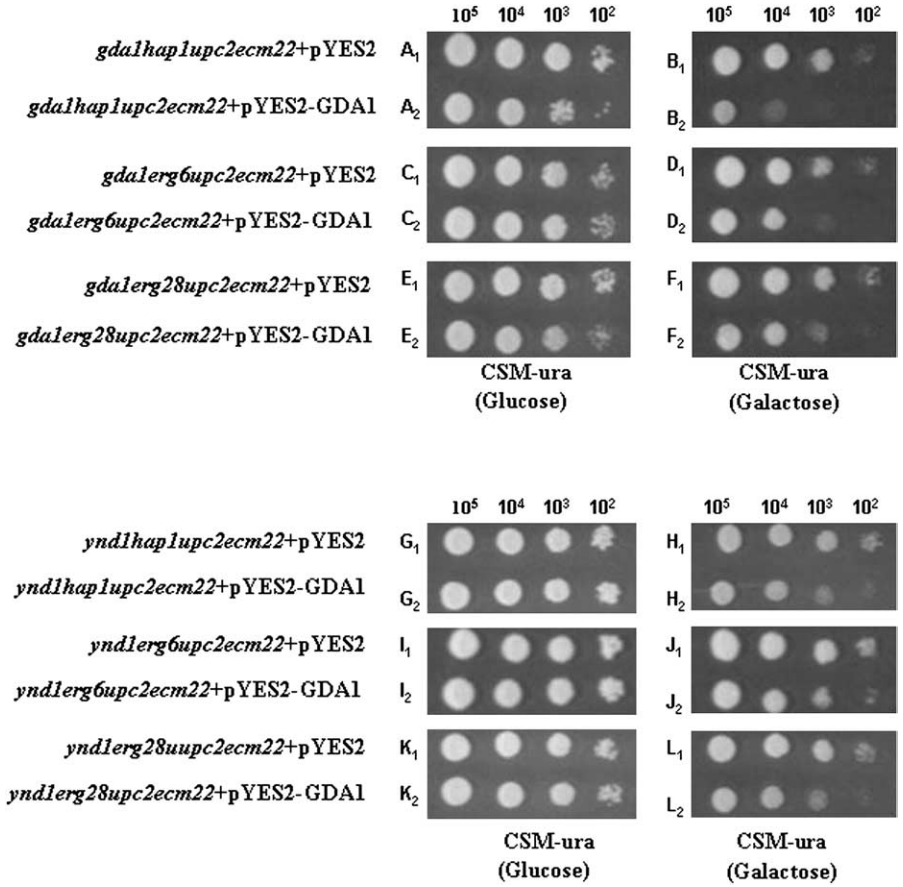


FIGURE 4.—Overexpression of a plasmid containing the *GDA1* ORF under the control of a galactose-inducible *GAL1* promoter. Expression of *GDA1* reverses suppression due to *gda1* and *ynd1* mutations. Expression of *GDA1* in a *gal1 hap1*Δ *upc2*Δ *ecm22*Δ background reduces viability on galactose medium more than on glucose medium (B_2 *vs.* A_2) as expected. Similar growth was observed with vector controls (A_1 and B_1). However, *GDA1* expression on galactose medium also reduced suppression of the *ynd1hap1*Δ *upc2*Δ *ecm22*Δ quadruple mutant (H_2 *vs.* H_1 but not on glucose medium (G_1 and G_2). Similar results were observed for suppression of the *gda1erg6*Δ *upc2*Δ *ecm22*Δ, *ynd1erg6*Δ *upc2*Δ *ecm22*Δ, *gda1erg28*Δ *upc2*Δ *ecm22*Δ, and *ynd1erg2*Δ *upc2*Δ *ecm22*Δ strains.

of certain proteins into membrane microdomains known as rafts. These are experimentally defined by their degree of solubility and thus extraction by cold detergents, such as 1% Triton X-100, and their flotation properties following density gradient fractionation. In mutants deficient in sphingolipid biosynthesis due to a temperature-sensitive allele of the *LCB1* gene allele, raft formation is absent by this assay at the temperature nonpermissive for growth (BAGNAT *et al.* 2000). We reasoned that the combined loss of the sterol biosynthetic genes and their transcription factors was a lethal event due to aberrant lipid biosynthesis and that this would produce a membrane incompatible with raft localization and viability. Deletion of *YND1* or *GDA1*

TABLE 7

Sterol analysis of *ynd1hap1upc2ecm22* and *gda1hap1upc2ecm22*

Sterols	*hap1*	*upc2ecm22*	*ynd1hap1upc2ecm22*	*gda1hap1upc2ecm22*
Squalene	1.4	5.3	21.4	16.4
Zymosterol	5.6	11.5	6.8	1.7
Ergosterol	69.4	10.8	3.5	3.1
Ergosta-7,22-dienol	0	1.5	0.6	2.9
4-methyl zymosterol and fecosterol	4.2	10.7	12.3	11.0
Ergosta-5,7,24(28) trien-3ol and ergosta-5,7-dienol	0	0	6.7	6.9
Episterol	9.7	21.9	18.7	31.5
Ergosta 5,7-dienol	5.5	24.6	0	0
Ergosta 7-enol	0	0.8	7.8	5.8
4-methyl fecosterol	0.5	0	3.0	2.4
Lanosterol	1.0	3.9	1.2	2.8
4,4-dimethyl zymosterol	2.6	9.1	17.9	15.4

All mutations are deletions. Values indicate sterol as percentage of total sterol. Sterol analyses of these strains represent the average of three independent experiments.

TABLE 8

Sterol analysis of *ynd1erg6upc2ecm22* and *gda1erg6upc2ecm22*

Sterol	*erg6*	*ynd1erg6upc2ecm22*	*gda1erg6upc2ecm22*
Squalene	0.3	8.7	17.1
Cholesta-5,8,24-trienol	2.1	0	0
Zymosterol	46.7	53.2	34.9
Cholesta-5,7,24-trienol	24.9	16.4	18.2
Cholesta-7,24-dienol	3.6	7.9	10.9
Cholesta-5,7,22,24-tetraenol	20.8	0.4	0.9
4-methylzymosterol	0.8	3.5	4.7
Lanosterol	0.2	3.8	5.3
4,4-dimethyl-zymosterol	0.8	6.2	8.0

Sterol analyses of these strains represent the average of three independent experiments.

could compensate for this situation by modulation of sphingolipid composition, and thus raft association, to produce a viable cell. We therefore assessed the localization of two known raft-associated proteins, the *PMA1* and *GAS1* gene products, to detergent-insoluble microdomains (rafts) using established protocols (Figure 5, A and B). Cell lysates were treated with cold Triton X-100 followed by density gradient centrifugation (Bagnat *et al.* 2000) and resolution by SDS–PAGE and immunoblot. In extracts from *erg28*Δ or *upc2*Δ *ecm22*Δ double mutants (Figure 5, A and B) and normal cells (not shown) both Pma1p and Gas1p localize to detergent-insoluble fractions 1 and 2 (peaking in fraction 2). By contrast, the same proteins partially relocalize to nonraft domains (*i.e.*, detergent soluble), such that the proportion of these proteins in rafts is markedly diminished in the viable *ynd1*Δ *erg28*Δ *upc2*Δ *ecm22*Δ or *gda1*Δ *erg28*Δ *upc2*Δ *ecm22*Δ strains. We assume that the inviable triple mutants lack raft-associated proteins.

DISCUSSION

In this article we describe the genetic interactions of two transcription factors (*UPC2* and *ECM22*) that affect sterol biosynthesis and demonstrate that in conjunction with mutations in other ergosterol biosynthetic genes or transcription factors this can lead to inviability. We further demonstrate that such mutants can be suppressed by additional mutations in the sphingolipid biosynthetic pathway. The *upc2*Δ *ecm22*Δ double mutant strain was initially determined to be viable in one genetic background but not in another. The *HAP1* gene in strain S228C has a Ty insertion at the C terminus resulting in a *hap1* mutant phenotype, as indicated by loss of cytochrome c expression (Gaisne *et al.* 1999). However, the *hap1Ty* allele still allows for growth under heme-limiting conditions. In this investigation, we demonstrate that *hap1Ty upc2*Δ *ecm22*Δ or *hap1*Δ *upc2*Δ *ecm22*Δ represent synthetically lethal combinations and

TABLE 9

Sterol analysis of *ynd1erg28upc2ecm22* and *gda1erg28upc2ecm22*

Sterols	*erg28*	*ynd1erg28upc2ecm22*	*gda1erg28upc2ecm22*
Squalene	4.1	9.2	13.6
Zymosterol	3.0	0.9	0.8
Ergosterol	77.4	5.6	4.9
Ergosta 7,22-dienol	0.7	0.9	0.3
4-methyl zymosterol	4.0	25.5	14.9
Ergosta 5,7,24(28) trien-3-ol and 4-methyl cholesta-trienol	0	13.9	22.1
Episterol	3.3	9.9	13.1
4-methyl fecosterol	0	1.1	1.2
4-methyl cholesta-8,24-dien-3-one	0.6	0	0
4-methyl cholestasterols[a]	5.1	6.2	8.5
Lanosterol	1.3	5.1	7.1
4,4-dimethyl zymosterol	0.5	21.6	13.5

Sterol analyses of these strains represent the average of three independent experiments.
[a] These 4-methylcholestasterols could not be unambiguously identified.

TABLE 10

Changes in the sphingolipid composition of *gda1*Δ and ynd1Δ cells

[^{3}H] *myo*-inositol phospholipids	Wild-type SPH/GPI	*gda1*Δ SPH/GPI	*gda1*Δ/wild type	*ynd1*Δ SPH/GPI	*ynd1*Δ/wild type
GPI	1.00	1.00	1.00	1.00	1.00
$M(IP)_2C$	1.89 (2.10, 1.68)	1.01 (1.07, 0.96)	0.53 ↓	1.89 (2.01, 1.77)	1.00
MIPC + IPC	1.34 (1.25, 1.43)	2.01 (1.88, 2.14)	1.50 ↑	1.96 (1.68, 2.24)	1.46 ↑

Ratios are the average of two experiments and were determined by dividing the counts per minute in each radioactive lipid peak by the counts per minute in the GPI peak. Values in parentheses represent data from two separate experiments. SPH, sphingolipid; GPI, glycerolphospho-inositol; IPC, inositolphosphorylceramide; $M(IP)_2C$, mannosyldiphosphorylinositolceramide; MIPC, mannosylphosphorylinositolceramide.

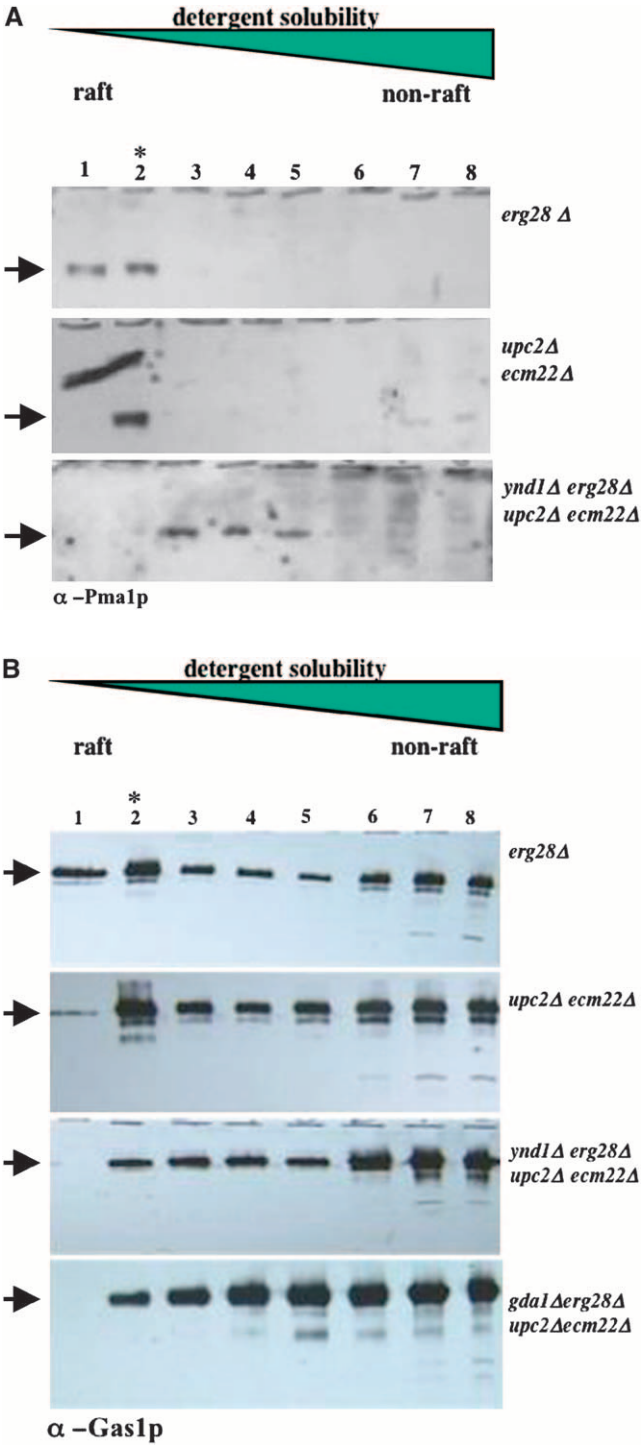
that the presence of the hap1Ty allele in the S288C background was likely responsible for the diverse finding that *upc2*Δ *ecm22*Δ was viable in one genetic background but not in another. Hap1p is also a Zn(2)–Cys(6) fungal transcription factor known to affect the sterol biosynthetic pathway, in particular the *ERG11* (Turi and Loper 1992) and *ERG9* genes (Kennedy *et al.* 1999) and the sterol esterification gene *ARE2* (Jensen-Pergakes *et al.* 2001). In addition, levels of HMG1, encoding the major isoform of HMG-CoA reductase, are decreased more than twofold in a *hap1*Δ mutant as determined by microarray (Ter Linde and Steensma 2002). However, *HAP1* may affect the sterol pathway indirectly as it also is required for repression by *ROX1*, a repressor of aerobic gene expression. A number of sterol biosynthetic genes are also known to be regulated by *ROX1* (Zitomer and Lowry 1992), among them *ERG11*, NADPH cytochrome P450 reductase, and *HMG2* (Ter Linde and Steensma 2002). Thus it is not surprising that loss of *HAP1* in conjunction with deletion of *UPC2 ECM22* would be lethal.

Mutations in *UPC2* and *ECM22* have a broad effect on sterol biosynthesis. When sterol levels are reduced due to the addition of Lovastatin, the expression of *ERG2* and *ERG3* substantially increase. Both gene products bind to the same regulatory elements in the *ERG2* and *ERG3* promoters (Vik and Rine 2001). A model has been presented in which the activation domains of Upc2p and Ecm22p are targeted by a repressor and in response to sterol depletion binding of the repressor is reversed (Davies *et al.* 2005). Several other ergosterol biosynthetic genes may be regulated by these transcription factors such as *ERG1*, *ERG6*, *ERG8*, *ERG11*, *ERG12*, *ERG13*, and *ERG25*, since all contain a consensus 11-bp sterol regulatory element in their respective promoters (Vik and Rine 2001). The *UPC2* deletion in *C. albicans* renders cells highly susceptible to antifungals that target the sterol pathway, such as ketoconazole and fluconazole (MacPherson *et al.* 2005). Thus *UPC2* and *ECM22* appear to be global regulators of sterol biosynthesis in fungi. While not directly relevant to this investigation, a gain-of-function semidominant allele of *UPC2*, designated *upc2-1*, allows for aerobic sterol uptake (Lewis *et al.* 1988).

Deletion of *UPC2* and *ECM22* is synthetically lethal with ergosterol biosynthetic mutants: We were curious to determine which other viable ergosterol mutations resulted in synthetic lethality in *upc2*Δ *ecm22*Δ strains. Our results suggested that *erg6*Δ and *erg28*Δ were also synthetically lethal with *upc2*Δ *ecm22*Δ but that *erg3*, *erg4*, and *erg5* were not. Mutations in *ERG2* and *ERG6* are the most deleterious of the viable ergosterol deletions (Bard *et al.* 1978). *erg6* mutants have altered permeability characteristics, often are unable to utilize respiratory energy sources, have reduced mating capability, and are unable to transport tryptophan (Gaber *et al.* 1989). Due to their inability to methylate the side chain, the sterols accumulated have a diminished mobility. Mutations in *ERG2* and *ERG28* also compromise growth (Ashman *et al.* 1991; Gachotte *et al.* 2001) as do mutations in *ERG3*. However, *erg4* and *erg5* mutants grow nearly as well as wild type and accumulate sterols that differ from ergosterol only in the inability to reduce double bonds in the sterol side chain (Lees *et al.* 1999). Both *erg4* and *erg5* strains contain the conjugated double bonds in the sterol B ring, as do wild-type cells. These mutants have almost wild-type sensitivities to the antifungal nystatin (Molzahn and Woods 1972). While it was expected that *erg4*Δ *upc2*Δ *ecm22*Δ and *erg5*Δ *upc2*Δ *ecm22*Δ strains would be viable, the viability of *erg3*Δ *upc2*Δ *ecm22*Δ was less obvious because the *ERG3* lesion precludes the addition of a double bond at the C-5 position in the sterol B ring.

A clue to the effect on viability of the *erg2*Δ *upc2*Δ *ecm22*Δ triple mutant was obtained when a suppressor of this strain was found to contain the *elo3* mutation. Virtually all *erg2* mutant strains in *S. cerevisiae* are viable, with the single exception of an *erg2* strain (Silve *et al.* 1996) that could be suppressed by a mutation in the sphingolipid genes *ELO2* or *ELO3*, which encode fatty acid elongases that are necessary for synthesis of C_{26}-fatty acyl chain found in yeast sphingolipids (Oh *et al.* 1997). We isolated a suppressor of *erg2*Δ *upc2*Δ *ecm22*Δ, which was subsequently identified as *elo3* (Valachovic *et al.* 2004). Thus an *erg2*Δ *upc2*Δ *ecm22*Δ not only would have reduced sterol levels as a result of the transcription factor mutations but also would have an altered sterol

profile due to loss of sterol C-8 isomerase activity. The restoration of viability in *erg2 elo3 upc2*Δ *ecm22*Δ suggests that *elo3*Δ suppression of *erg2*Δ essentially converts the quadruple mutant strain to a *upc2*Δ *ecm22*Δ double mutant strain. For reasons not yet clear the sphingolipid mutation suppressing the sterol mutation may result in an alteration of lipid raft function, thereby restoring viability. We also speculate that an inviable *erg2* isolate may accumulate a lower level of sterol than is found in most *erg2* genetic backgrounds. Finally, the sterol defect in a *upc2*Δ *ecm22*Δ double mutant results in an accumulation of episterol (45%). This sterol could not accumulate in an *erg2 upc2*Δ *ecm22*Δ background because of the *erg2* mutation.



Transposon mutagenesis and suppression due to *ynd1* and *gda1* mutations: Of 116 total transformants containing suppressors involving all four triple mutant strains (*upc2*Δ *ecm22*Δ with *hap1*Δ, *erg2*Δ, *erg6*Δ, or *erg28*Δ), 25% of the mutations implicated genes involved in transcription or in chromatin remodeling. Other categories of suppressor mutations were in genes involved in endocytosis (7%), ubiquitination (6%), and ~5% each involved genes in phosphate metabolism, the cytoskeleton, DNA repair, metabolism, mitochondria, or RNA metabolism. Upon designing disruptions of several of these genes to independently confirm suppression we found that most did not confer suppression when mated to the triple mutants. It is likely that mutations in genes involved in repression mechanisms, such as *TUP1*, *HDA2*, *HDA3*, *IES1*, and *SSN2*, enhanced survival of the triple mutants long enough for other suppressors to accumulate during the transformation process and during growth on FOA medium. For this reason we confined ourselves to mutants that indicated suppression by independent disruptions and matings.

The observation that the *ynd1* mutation (from the transposon library) suppressed *hap1*Δ, *erg2*Δ, and *erg6*Δ mutants suggested a common mechanism in the context of *upc2*Δ *ecm22*Δ. We suggest that the *upc2*Δ *ecm22*Δ double mutant reduces sterol biosynthesis to a minimal level that is still compatible with viability but introduction of ergosterol mutations into this background lowers the threshold of functional sterol below this threshold. Thus *hap1*Δ *upc2*Δ *ecm22*Δ strains would be inviable because *HAP1* is itself a transcription factor affecting sterol biosynthesis and its loss would further diminish expression of the pathway. We subsequently demonstrated by genetic crosses that *ynd1*Δ suppresses the *erg6*Δ, *erg28*Δ, and *hap1*Δ triple mutants but not the *erg2*Δ triple mutant.

Not finding that *ynd1* suppressed *erg28*Δ *upc2*Δ *ecm22*Δ was likely due to the fact that only 1095 transposon-generated transformants were screened, whereas for

Figure 5.—Cold detergent-insoluble protein localization. Protein extracts were prepared from strains of the indicted genotypes and resolved by density gradient fractionation after solubilization (or otherwise) with cold detergent. Eight fractions were collected, resolved by SDS–PAGE, and visualized following immunoblotting with antisera against the PMA1 or GAS1 proteins (A and B, respectively). Fraction 2 (indicated by the asterisk) represents the peak of detergent-insoluble protein characteristic of membrane microdomains. Uniquely in the quadruple *erg28*Δ *upc2*Δ *ecm22*Δ *gda1*Δ and *erg28*Δ *upc2*Δ *ecm22*Δ*ynd1*Δ strains, detergent-insoluble proteins are underrepresented relative to the other mutants and normal strains. Arrows indicate fraction localization of Pma1p (A) and Gsa1p (B).

the other screens the total number of transformants screened was significantly higher. *YND1* is a protein of 630 amino acids that functions as an apyrase, enzymes that hydrolyze both di- and triphosphate nucleotides. *GDA1* is a homolog of *YND1* and shares 20% amino acid identity. Both *ynd1*Δ and *gda1*Δ single mutants have decreased N-linked glycosylation and O-linked glycosylation (Gao *et al.* 1999). The *ynd1*Δ *gda1*Δ double mutant results in slow growth. Gda1p is involved in the import of mannose into the Golgi (Abeijon *et al.* 1993) and fuels the Golgi antiporter Vrg4p by cleaving GDP into GMP, thus facilitating import of GDP mannose into the Golgi as GMP is simultaneously exported (Gao and Dean 2000). Once in the Golgi, GDP-mannose becomes a substrate for various reactions involving sphingolipid synthesis and cell wall remodeling. Abeijon *et al.* (1993) demonstrated that elongation of O-linked carbohydrate chains is blocked at the mannobiose step, N-linked carbohydrates of carboxypeptidase are not elongated beyond the phosphatidyl inositol stage, and biosynthesis of MIPC is severely impaired in *gda1* mutants. Even though we did not obtain *gda1* suppressors in our transposon screening, our results indicate that a *gda1*-deleted strain mated to give the four different types of quadruple mutants showed the exact same profile of suppression as did *ynd1*. This investigation implicates for the first time a role of Ynd1p, like Gda1p, in sphingolipid biosynthesis.

Lipid rafts are mixtures of sterols and sphingolipids that form separate domains in the plasma membrane. These rafts possess specific proteins and are the targets for signaling, channels, and other functions (Bagnat *et al.* 2000; Brown and London 2000). In this investigation we extend what is known regarding synthetic lethal and suppressor interactions that occur between the sterol and sphingolipid pathways. Inviable isolates of *erg2* and *erg24* are suppressed by both *elo2* and *elo3* (Silve *et al.* 1996; Baudry *et al.* 2001). ARV1, which harbors defects in trafficking of sterols, accumulates novel sterol intermediates, and synthesizes higher levels of sterol esters, also shows defects in sphingolipids (Tinkelenberg *et al.* 2000; Swain *et al.* 2002). A temperature-sensitive mutant of *ERG26* also had sphingolipid defects (Baudry *et al.* 2001), and finally *elo3* is synthetically lethal with *erg6* (Eisenkolb *et al.* 2002). One model of lipid raft organization is based on the relative sizes of the polar and nonpolar regions of sterols and sphingolipids (Fantini *et al.* 2002). In this model, both molecules have a cylindrical or pyramidal shape. Sterols have a small polar head region relative to their nonpolar region, and sphingolipids have a large polar region relative to their nonpolar region. In lipid rafts, these two molecules interdigitate via their complementing polar and nonpolar regions. A hypothesis for the synthetic lethality in this study is that nonviability is a result of sterol intermediates fitting poorly in lipid rafts in combination with an overall lack of bulk sterols. We hypothesize that sterol intermediates produced by the *erg2*, *erg6*, *erg28*, and *hap1* mutations have alterations such that their polar regions, nonpolar regions, or both poorly complement the wild-type sphingolipid polar and nonpolar regions. In the case of single *erg* mutations there is impairment of membrane function, but not enough to prevent viability. The *upc2*Δ *ecm22*Δ double mutation causes a decrease of sterol synthesis, but this does not prevent viability. However, when these two phenomena are combined, as in *ergX upc2*Δ *ecm22*Δ, they produce synthetic lethality.

If synthetic lethality is caused in part by poor complementation of the sterol intermediates with wild-type sphingolipids, suppression of lethality by mutating *YND1* or *GDA1* may be due to alterations in sphingolipids that allow better interactions with sterol intermediates. Of the known suppressors, *ynd1* and *gda1* mutants cause an alteration in the polar head group of sphingolipids, while the *elo3* mutants cause an alteration in the nonpolar region. The commonality we found in *ynd1* and *gda1* mutants was an increase in sphingolipids with a smaller polar head group (MIPC + IPC, Table 10) compared to $M(IP_2)C$, which has the largest polar head group. Having a smaller polar head group would make the sphingolipid more cylindrical and less conical, which would fit better with a sterol that was less cylindrical and more conical. The quadruple mutant strains in which *ynd1* and *gda1*suppress inviability have an increase in sterol intermediates that give a more cylindrical and less conical structure. Our results confirm a previous demonstration that *gda1* mutants have reduced levels of $M(IP)_2C$ (Abeijon *et al.* 1993) but differ in that we also see an increase rather than a decrease in MIPC. The difference may be due to the way in which sphingolipids were radiolabeled. They labeled cells for 10 min with [^{3}H] *myo*-inositol and chased with nonradioactive inositol for 90 min and thus measured the rate of sphingolipid synthesis. We labeled for >10 cell divisions and thus measured the relative abundance of sphingolipids.

In summary, strains suppressed by *ynd1* and *gda1* are likely to have sphingolipids that are narrower near the apex, which can complement sterols that are wider near the apex and narrower at the base. To test this hypothesis, we investigated the localization of two known raft-associated proteins, Pma1p and Gas1p, in the various viable mutant combinations. Consistent with our hypothesis, we observed a unique redistribution of these raft-associated proteins in the suppressed strains that lacked the *ERG28* gene product. This redistribution was not complete; a minor proportion of the proteins remain raft associated, presumably a context that is sufficient for viability. These results confirm the altered nature of membrane microdomains due to loss of *GDA1* or *YND1* in the context of disturbed sterol composition due to the *erg28*Δ *upc2*Δ *ecm22*Δ mutations. Interestingly, we did not observe the same relocalization of Pma1p or Gas1p due to loss of *ERG6*, suggesting alternate modes of suppression for these pathways.

This work was supported by National Institutes of Health grants GM-62104 (M.B.), GM-41302 (R.C.D.), and DK-54320 (S.L.S). S.L.S. also acknowledges the American Heart Association (National and Heritage) and the Ara Parseghian Medical Research Foundation.

LITERATURE CITED

Abeijon, C., K. Yanagisawa, E. C. Mandon, A. Hausler, K. Moremen *et al.*, 1993 Guanosine diphosphatase is required for protein and sphingolipid glycosylation in the Golgi lumen of Saccharomyces cerevisiae. J. Cell Biol. **122:** 307–323.

Abramova, N., O. Sertil, S. Mehta and C. V. Lowry, 2001 Reciprocal regulation of anaerobic and aerobic cell wall mannoprotein gene expression in Saccharomyces cerevisiae. J. Bacteriol. **183:** 2881–2887.

Ashman, W. H., R. J. Barbuch, C. E. Ulbright, H. W. Jarrett and M. Bard, 1991 Cloning and disruption of the yeast C-8 sterol isomerase gene. Lipids **26:** 628–632.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1998 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Bagnat, M., S. Keranen, A. Shevchenko, A. Shevchenko and K. Simons, 2000 Lipid rafts function in biosynthetic delivery of proteins to the cell surface in yeast. Proc. Natl. Acad. Sci. USA **97:** 3254–3259.

Baloch, R. I., E. I. Mercer, T. E. Wiggins and B. C. Baldwin, 1984 Inhibition of ergosterol biosynthesis in Saccharomyces cerevisiae and Ustilago maydis by tridemorph, fenpropiomorph, and fenpropidin. Phytochemistry **23:** 2219–2226.

Bard, M., N. D. Lees, L. S. Burrows and F. W. Kleinhans, 1978 Differences in crystal violet uptake and cation-induced death among yeast sterol mutants. J. Bacteriol. **135:** 1146–1148.

Baudry, K., E. Swain, A. Rahier, M. Germann, A. Batta *et al.*, 2001 The effect of the erg26–1 mutation on the regulation of lipid metabolism in Saccharomyces cerevisiae. J. Biol. Chem. **276:** 12702–12711.

Brown, D. A., and E. London, 2000 Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J. Biol. Chem. **275:** 17221–17224.

Brown, M. S., and J. L. Goldstein, 1997 The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell **89:** 331–340.

Burke, D., D. Dawson and T. Stearns, 2000 *Methods in Yeast Genetics, 2000 Edition*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Burns, N., B. Grimwade, P. B. Ross-Macdonald, E. Y. Choi, K. Finberg *et al.*, 1994 Large-scale analysis of gene expression, protein localization, and gene disruption in Saccharomyces cerevisiae. Genes Dev. **8:** 1087–1105.

Crowley, J. H., F. W. Leak, Jr., K. V. Shianna, S. Tove and L. W. Parks, 1998 A mutation in a purported regulatory gene affects control of sterol uptake in Saccharomyces cerevisiae. J. Bacteriol. **180:** 4177–4183.

Daum, G., N. D. Lees, M. Bard and R. Dickson, 1998 Biochemistry, cell biology and molecular biology of lipids of Saccharomyces cerevisiae. Yeast **14:** 1471–1510.

Davies, B. S., H. S. Wang and J. Rine, 2005 Dual activators of the sterol biosynthetic pathway of Saccharomyces cerevisiae: similar activation/regulatory domains but different response mechanisms. Mol. Cell. Biol. **25:** 7375–7385.

Dickson, R. C., E. E. Nagiec, G. B. Wells, M. M. Nagiec and R. L. Lester, 1997 Synthesis of mannose-(inositol-P)2-ceramide, the major sphingolipid in Saccharomyces cerevisiae, requires the IPT1 (YDR072c) gene. J. Biol. Chem. **272:** 29620–29625.

Eisenkolb, M., C. Zenzmaier, E. Leitner and R. Schneiter, 2002 A specific structural requirement for ergosterol in long-chain fatty acid synthesis mutants important for maintaining raft domains in yeast. Mol. Biol. Cell **13:** 4414–4428.

Erdeniz, N., U. H. Mortensen and R. Rothstein, 1997 Cloning-free PCR-based allele replacement methods. Genome Res. **7:** 1174–1183.

Fantini, J., N. Garmy, R. Mahfoud and N. Yahi, 2002 Lipid rafts: structure, function and role in HIV, Alzheimers and prion diseases. Expert Rev. Mol. Med. **2002:** 1–22.

Gaber, R. F., D. M. Copple, B. K. Kennedy, M. Vidal and M. Bard, 1989 The yeast gene ERG6 is required for normal membrane function but is not essential for biosynthesis of the cell-cycle-sparking sterol. Mol. Cell. Biol. **9:** 3447–3456.

Gachotte, D., S. E. Sen, J. Eckstein, R. Barbuch, M. Krieger *et al.*, 1999 Characterization of the Saccharomyces cerevisiae ERG27 gene encoding the 3-keto reductase involved in C-4 sterol demethylation. Proc. Natl. Acad. Sci. USA **96:** 12655–12660.

Gachotte, D., J. Eckstein, R. Barbuch, T. Hughes, C. Roberts *et al.*, 2001 A novel gene conserved from yeast to humans is involved in sterol biosynthesis. J. Lipid Res. **42:** 150–154.

Gaisne, M., A. M. Becam, J. Verdiere and C. J. Herbert, 1999 A 'natural' mutation in Saccharomyces cerevisiae strains derived from S288c affects the complex regulatory gene HAP1 (CYP1). Curr. Genet. **36:** 195–200.

Gao, X. D., and N. Dean, 2000 Distinct protein domains of the yeast Golgi GDP-mannose transporter mediate oligomer assembly and export from the endoplasmic reticulum. J. Biol. Chem. **275:** 17718–17727.

Gao, X. D., V. Kaigorodov and Y. Jigami, 1999 YND1, a homologue of GDA1, encodes membrane-bound apyrase required for Golgi N- and O-glycosylation in Saccharomyces cerevisiae. J. Biol. Chem. **274:** 21450–21456.

Hughes, A. L., B. L. Todd and P. J. Espenshade, 2005 SREBP pathway responds to sterols and functions as an oxygen sensor in fission yeast. Cell **120:** 831–842.

Jensen-Pergakes, K., Z. Guo, M. Giattina, S. L. Sturley and M. Bard, 2001 Transcriptional regulation of the two sterol esterification genes in the yeast Saccharomyces cerevisiae. J. Bacteriol. **183:** 4950–4957.

Kelley, R. I., and G. E. Herman, 2001 Inborn errors of sterol biosynthesis. Annu. Rev. Genomics Hum. Genet. **2:** 299–341.

Kennedy, M. A., R. Barbuch and M. Bard, 1999 Transcriptional regulation of the squalene synthase gene (ERG9) in the yeast Saccharomyces cerevisiae. Biochim. Biophys. Acta **1445:** 110–122.

Kwast, K. E., P. V. Burke and R. O. Poyton, 1998 Oxygen sensing and the transcriptional regulation of oxygen-responsive genes in yeast. J. Exp. Biol. **201:** 1177–1195.

Lees, N. D., M. Bard and D. R. Kirsch, 1999 Biochemistry and molecular biology of sterol synthesis in Saccharomyces cerevisiae. Crit. Rev. Biochem. Mol. Biol. **34:** 33–47.

Lewis, T. L., G. A. Keesler, G. P. Fenner and L. W. Parks, 1988 Pleiotropic mutations in Saccharomyces cerevisiae affecting sterol uptake and metabolism. Yeast **4:** 93–106.

MacPherson, S., B. Akache, S. Weber, X. De Deken, M. Raymond *et al.*, 2005 Candida albicans zinc cluster protein Upc2p confers resistance to antifungal drugs and is an activator of ergosterol biosynthetic genes. Antimicrob. Agents Chemother. **49:** 1745–1752.

Molzahn, S. W., and R. A. Woods, 1972 Polyene resistance and the isolation of sterol mutants in Saccharomyces cerevisiae. J. Gen. Microbiol. **72:** 339–348.

Ochman, H., A. S. Gerber and D. L. Hartl, 1988 Genetic applications of an inverse polymerase chain reaction. Genetics **120:** 621–623.

Oh, C. S., D. A. Toke, S. Mandala and C. E. Martin, 1997 ELO2 and ELO3, homologues of the Saccharomyces cerevisiae ELO1 gene, function in fatty acid elongation and are required for sphingolipid formation. J. Biol. Chem. **272:** 17376–17384.

Puoti, A., C. Desponds and A. Conzelmann, 1991 Biosynthesis of mannosylinositolphosphoceramide in Saccharomyces cerevisiae is dependent on genes controlling the flow of secretory vesicles from the endoplasmic reticulum to the Golgi. J. Cell Biol. **113:** 515–525.

Shianna, K. V., W. D. Dotson, S. Tove and L. W. Parks, 2001 Identification of a UPC2 homolog in Saccharomyces cerevisiae and its involvement in aerobic sterol uptake. J. Bacteriol. **183:** 830–834.

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.

Silve, S., P. Leplatois, A. Josse, P. H. Dupuy, C. Lanau *et al.*, 1996 The immunosuppressant SR 31747 blocks cell proliferation by inhibiting a steroid isomerase in Saccharomyces cerevisiae. Mol. Cell. Biol. **16:** 2719–2727.

Silver, P. M., B. G. Oliver and T. C. White, 2004 Role of Candida albicans transcription factor Upc2p in drug resistance and sterol metabolism. Eukaryot. Cell **3:** 1391–1397.

Swain, E., J. Stukey, V. McDonough, M. Germann, Y. Liu *et al.*, 2002 Yeast cells lacking the ARV1 gene harbor defects in sphingolipid metabolism: Complementation by human ARV1. J. Biol. Chem. **277:** 26177–26184.

Ter Linde, J. J., and H. Y. Steensma, 2002 A microarray-assisted screen for potential Hap1 and Rox1 target genes in Saccharomyces cerevisiae. Yeast **19:** 825–840.

Thomas, B. J., and R. Rothstein, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

Tinkelenberg, A. H., Y. Liu, F. Alcantara, S. Khan, Z. Guo *et al.*, 2000 Mutations in yeast ARV1 alter intracellular sterol distribution and are complemented by human ARV1. J. Biol. Chem. **275:** 40667–40670.

Todd, R. B., and A. Andrianopoulos, 1997 Evolution of a fungal regulatory gene family: the Zn(II)2Cys6 binuclear cluster DNA binding motif. Fungal Genet. Biol. **21:** 388–405.

Turi, T. G., and J. C. Loper, 1992 Multiple regulatory elements control expression of the gene encoding the Saccharomyces cerevisiae cytochrome P450, lanosterol 14 alpha-demethylase (ERG11). J. Biol. Chem. **267:** 2046–2056.

Valachovic, M., L. I. Wilcox, S. L. Sturley and M. Bard, 2004 A mutation in sphingolipid synthesis suppresses defects in yeast ergosterol metabolism. Lipids **39:** 747–752.

Vik, A., and J. Rine, 2000 Membrane biology: membrane-regulated transcription. Curr. Biol. **10:** R869–R871.

Vik, A., and J. Rine, 2001 Upc2p and Ecm22p, dual regulators of sterol biosynthesis in Saccharomyces cerevisiae. Mol. Cell. Biol. **21:** 6395–6405.

Wilcox, L. J., D. A. Balderes, B. Wharton, A. H. Tinkelenberg, G. Rao *et al.*, 2002 Transcriptional profiling identifies two members of the ATP-binding-cassette transporter superfamily required for sterol uptake in yeast. J. Biol. Chem. **277:** 32466–32472.

Yanagisawa, K., D. Resnick, C. Abeijon, P. W. Robbins and C. B. Hirschberg, 1990 A guanosine diphosphatase enriched in Golgi vesicles of Saccharomyces cerevisiae. Purification and characterization. J. Biol. Chem. **265:** 19351–19355.

Zitomer, R. S., and C. V. Lowry, 1992 Regulation of gene expression by oxygen in Saccharomyces cerevisiae. Microbiol. Rev. **56:** 1–11.

Communicating editor: B. J. Andrews