Transcriptional Profiling Identifies Two Members of the ATP-binding Cassette Transporter Superfamily Required for Sterol Uptake in Yeast*

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Lisa J. Wilcox‡§, Dina A. Balderes‡, Brook Wharton‡, Arthur H. Tinkelenberg‡, Govinda Rao§, and Stephen L. Sturley‡¶**

From the ‡Institute of Human Nutrition and §Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York 10032 and ¶Affymetrix, Inc., Santa Clara, California 95051

In contrast to lipoprotein-mediated sterol uptake, free sterol influx by eukaryotic cells is poorly understood. To identify components of non-lipoprotein-mediated sterol uptake, we utilized strains of Saccharomyces cerevisiae that accumulate exogenous sterol due to a neomorphic mutation in the transcription factor, UPC2. Two congenic upc2-1 strains, differing quantitatively in aerobic sterol uptake due to a modifying mutation in the HAP1 transcription factor, were compared using DNA microarrays. We identified 9 genes as responsive to UPC2 that were also induced under anaerobiosis, when sterol uptake is essential. Deletion mutants in these genes were assessed for sterol influx in the upc2-1 background. UPC2 itself was up-regulated under these conditions and was required for aerobic sterol influx. Deletion of the ATP-binding cassette transporters YOR011c (AUS1) or PDR11, or a putative cell wall protein encoded by DAN1, significantly reduced sterol influx. Sodium azide and vanadate inhibited sterol uptake, consistent with the participation of ATP-binding cassette transporters. We hypothesized that the physiological role of Aus1p and Pdr11p is to mediate sterol uptake when sterol biosynthesis is compromised. Accordingly, expression of AUS1 or PDR11 was required for anaerobic growth and sterol uptake. We proposed similar molecules may be important components of sterol uptake in all eukaryotes.

Unesterified sterol is an essential component of all eukaryotic membranes where it influences membrane fluidity and the activity and localization of many proteins. In mammalian cells, the processes that control the supply of this commodity are precisely regulated, primarily at the transcriptional level. Although the endogenous synthesis and storage (as ester) of cholesterol are critical aspects of this homeostasis, it is the uptake of exogenous cholesterol that presents the greatest challenge to this equilibrium. The endocytosis of cholesterol-containing low density lipoprotein particles by the low density lipoprotein receptor or the selective uptake of cholesteryl ester from high density lipoproteins by the scavenger receptor type B1 are transcriptionally regulated events (1–3). The reverse process of sterol efflux to high density lipoproteins requires ABCA1, a member of the ATP-binding cassette (ABC) family of membrane transporters which is also regulated transcriptionally (4). In contrast, the mechanisms and regulation of non-lipoprotein-mediated free sterol influx are poorly understood. This saturable process is of major relevance to the uptake of dietary cholesterol from bile acid micelles into the brush border membranes of enterocytes (5). However, the molecular components of this phenomenon have yet to be identified despite the observation that it can be inhibited by small molecules such as sterol glycosides (6).

The budding yeast, Saccharomyces cerevisiae, exhibits many aspects of sterol homeostasis in common with higher eukaryotes and has been a useful model for the study of sterol trafficking (7, 8). Normal or “wild-type” strains of S. cerevisiae do not significantly accumulate exogenous sterol from the media when grown aerobically but, instead, satisfy their sterol requirements by way of ergosterol biosynthesis. This phenomenon is referred to as aerobic sterol exclusion (9). However, when sterol biosynthesis is compromised, for example by anaerobic growth, sterol uptake is required for viability (10). Completely anaerobic systems are difficult to maintain and allow for little experimental manipulation. For this reason, studies of sterol influx in yeast have primarily employed sterol auxotrophs or mutants in heme synthesis which accumulate sterol from the media when grown aerobically (11). In the background of heme and sterol competence, aerobic sterol influx has been achieved using the upc2-1 mutant strain (12), the molecular basis of which is a single nucleotide change in the UPC2 (UPtake Control) open reading frame (13). UPC2 encodes a member of a fungal regulatory family containing the Zn(II)2Cys6 binuclear cluster DNA binding domain. Upc2p activates transcription via binding to sterol regulatory elements of some enzymes of ergosterol biosynthesis (14). Deletion of the UPC2 gene does not result in sterol uptake, suggesting that the upc2-1 allele (a glycine to aspartic acid change at residue 888) is a gain-of-function mutation (13).

We hypothesized that sterol uptake in yeast results from altered transcriptional regulation of one or more targets of UPC2. We therefore conducted a genome-wide transcriptional analysis of normal and upc2-1 mutant strains grown under aerobic conditions. By virtue of this expression profiling, we...
Deletion mutant strains were generated by homologous recombination with PCR products generated using the K. lactis URA3 sequence of the pRS408 plasmid and “KO” gene was identified by PCR using oligonucleotides (Table I) that contained 45–50 bp of gene-specific sequence (denoted by capital letters) and 21 bp of K. lactis URA3 sequence (denoted by lowercase letters). The upper and lower case letters indicate sequences specific to the target gene or replacing gene, respectively.

have identified a subset of 9 genes regulated by UPC2 and induced by anaerobiosis that are putative components of free sterol influx. Based on the null phenotypes of each candidate gene, the major determinants of sterol uptake are two members of the ABC superfamily of membrane transporters, AUS1 and PDR11.

**EXPERIMENTAL PROCEDURES**

**General**—Complete (YEFPD) and synthetic complete (SCD) media were prepared as described (15). Yeast extract, yeast nitrogen base, Bacto-peptone, and Bacto-agar were from Difco. D-Dextrose, lyticase, sodium azide, and sodium orthovanadate were from Sigma. [4-14C]cholesterol, [9,10-3H]palmitic acid, and [α-32P]dCTP were from PerkinElmer Life Sciences. Molecular biology and genetic procedures were performed according to conventional protocols (15). Gene-specific oligonucleotides were synthesized by GeneLink or Invitrogen.

**Yeast Strains and Molecular Techniques**—Yeast strains used in this study are isogenic with the strain W303-1A (MATα ade2-1, can1-1, trp1-1, ura3-1, his3-11, 15, leu2-3, 112 (16)). Strain UP20 carrying the upc2-1 allele (12) in the background of strain X2180-1A was a generous gift of Drs. Leo Parks and Andy Keesler. The upc2-1 allele was monitored by quantitative sterol uptake and by PCR-mediated detection of an allele-specific restriction endonuclease site (17). Strains SCT325 (normal, MATa), SCY1012 (upc2-1 lo, MATa), and SCY591 (upc2-1 hi, MATa) were used for the microarray studies and were derived by standard genetic techniques (15). Deletion mutant strains were generated by homologous recombination with PCR products generated using the Kluyveromyces lactis URA3 gene and oligonucleotides (Table I) that contained 45–50 bp of gene-specific sequence and 21 bp of K. lactis URA3 sequence (18). Yeast transformation was performed with lithium acetate (19) followed by selection on SCD-ura media. Deletion mutants were confirmed by PCR using oligonucleotides annealing upstream and downstream of the specific gene sequences. The presence of a Ty transposon insertion at the 3′ end of the HAP1 gene was identified by PCR using oligonucleotides specific to HAP1 and the Ty1 transposon (CTTCTTTTATCAAATTTATTATAACATCTGAGTCCATTGAAAATTTTG, respectively).

**RNA Isolation and cDNA Synthesis**—Total RNA was isolated from strains grown to an A600 = 0.5–0.6 in 200 ml of YEFPD as described (20). Poly(A) + RNA was purified from total RNA by oligotex oligo(dT) selection (Qiagen). The Poly(A) + RNA was converted into double-stranded cDNA by using a modified oligo(dt) primer (T7-(dT)24, GGCAGGT- GAATTGTATACGACTCTATAGGGGCGCG(T7)24) with a T7 RNA polymerase promoter sequence at the 5′ end and the Superscript Choice system (Invitrogen) for cDNA synthesis. The cDNA was purified with phenol/chloroform/isoamyl alcohol. The purified cDNA was precipitated with 7.5 M ammonium acetate and 95% ethanol for 20 min at 12,000 × g and resuspended in diethyl pyrocarbonate-treated H2O to achieve a final concentration between 0.25 and 0.65 μg/μl.

**In Vitro Transcription**—Synthesis of biotin-labeled cRNA was carried out by in vitro transcription using Enzo Bioarray RNA transcript labeling kit (Enzo Diagnostics), according to the manufacturer’s instructions. The biotin-labeled cRNA was purified using RNasey spin columns (Qiagen). The biotin-labeled cRNA was fragmented in a 40-μl reaction mixture containing 40 μm Tris acetate (pH 8.1), 100 mM potassium acetate, and 30 mM magnesium acetate and incubated at 94°C for 35 min.

**GeneChip Hybridization and Analysis**—The biotin-labeled and fragmented cRNA was hybridized to the YE6100 or S98 Yeast GeneChip arrays (Affymetrix), washed, and stained according to the manufacturer’s instructions. The arrays were scanned using the Hewlett-Packard Gene Array Scanner (Affymetrix) at a wavelength of 570 nm and analyzed using Microarray Suite 5.0 software (Affymetrix).

**Data Mining and Analysis**—The analysis of transcriptional results was carried out using the data base manager, Microsoft Access. Clustering analysis was performed by the method of self-organizing maps (21) using the program GeneCluster (Whitehead Institute for Biomedical Research/MIT Center for Genome Research). Aerobic and anaerobic growth expression profiles were examined using the data base available at wwwMP.Leidenuniv.nl/yeast (22). The analysis was completed using two comparisons, one with the YE6100 GeneChip series and the other with the S98 yeast array.

**Northern Blotting**—RNA was isolated as described above and resolved on a 1.2% agarose, formaldehyde gel. The membrane was hybridized with a random oligonucleotide-primed (Stratagene) [32P]dCTP-labeled probe, made from each PCR-generated insert of each gene examined. The hybridization was carried out in QuikHyb buffer (Stratagene) for 1 h at 65°C. The membrane was washed twice with 2× SSC, 0.1% SDS at room temperature and once with 0.1× SSC, 0.1% SDS at 65°C.

**Analysis of Exogenous Sterol Accumulation and Esterification**—Cells were grown for 18 h to mid-log phase in YEFPD media containing 1% tryptone/ethanol (1:1) and 0.01 μg/ml [4-14C]cholesterol. Anaerobic growth was achieved using BD PharMingen, CO2-generating gas packs and jars as described previously (17). Attainment of anaerobiosis was monitored by the inability of normal strains to grow in the absence of exogenous sterol. Sterol uptake and esterification was determined by the inclusion of 0.01 μg/ml [4-14C]cholesterol in the presence of fatty acids (TWEEN 80) and unlabeled ergosterol (20 μg/ml) as described (17). The net accumulation of [4-14C]cholesterol in the cells was measured by scintillation counting. The energetics of sterol transport were investigated by the inclusion of increasing concentrations of sodium orthovanadate and sodium azide for a 20-min pretreatment period followed by co-incubation with [4-14C]cholesterol and [9,10-3H]palmitic acid for 1 h.

**RESULTS AND DISCUSSION**

**Transcriptional Changes in Constitutive Sterol Influx Mutants**—Sterol uptake by yeast is a physiological response to compromised sterol biosynthesis, such as occurs during anaerobic growth. In normal strains, this manifests as sterol exclusion in the aerobic phase with an −350-fold increase upon
induction of anoxia. A genetic approach to determine the basis for this phenomenon identified the UPC2 transcription factor as a key regulator of sterol uptake (13). Congenic variants of a “gain-of-function” allele of UPC2 (upc2-1) facilitating aerobic sterol uptake in the W303-1A background were derived by a 6-generation back-crossing protocol with the UPC20 mutant strain originally obtained from L. Parks (S288c background). During this process two quantitatively different phenotypes with regard to sterol uptake were observed, suggesting the presence of a modifier locus for sterol uptake in the original UPC20 strain. Compared with the normal W303 parent strain, the upc2-1 low uptake strain (upc2-1 lo) accumulates ~6-fold more exogenous sterol, whereas the upc2-1 high uptake strain (upc2-1 hi) accumulates 68-fold more sterol (not shown). The segregation of these phenotypes indicated the high uptake variant to result from unlinked mutations in two genes (upc2-1 and an unlinked modifier), whereas the low variant resulted solely from the upc2-1 mutation.

We decided to take advantage of the two variants that differ quantitatively in sterol influx to facilitate identification of candidate sterol transporters. We hypothesized that sterol influx in yeast results from altered transcriptional regulation of one or more targets of Upc2p. The transcriptional changes in 6213 open reading frames (ORFs) were identified using DNA microarrays. Genes with altered expression were determined using the statistical algorithm in MAS 5.0 (Affymetrix) which included probability values (statistical significance) associated with detection and comparisons. Only genes with expression determined to be significantly altered in lo and hi strains relative to wild-type (duplicate analysis for normal, determined to be significantly altered in lo and hi strains with detection and comparisons. Only genes with expression ing the statistical algorithm in MAS 5.0 (Affymetrix) which croarrays. Genes with altered expression were determined us-

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TABLE II

**Genes with altered expression in upc2-1 mutants**

| Genes | Carbohydrate Metabolism |
|-------|--------------------------|
| DAM, PAU and TIR Gene Family | |
| **CRF** | **Genes** | **upc2-1 lo** | **upc2-1 hi** |
| **CRF** | **upc2-1 lo** | **upc2-1 hi** |
| **CRF** | **upc2-1 lo** | **upc2-1 hi** |

Genome-wide changes in gene expression were determined in aerobically grown yeast using DNA microarrays. The genes listed exhibited altered expression levels in the upc2-1 strains relative to the normal strain. Changes in expression are indicated as signal log ratio (base 2). Asterisks indicate genes that contain a characteristic motif (CGTTT) in their 5'-untranslated region previously identified as the core of a Upc2p consensus binding site. A subset of 19 genes (underlined) were identified as candidates for a role in sterol uptake based on the correlation of expression and sterol uptake. Functional descriptions are assigned based on entries at the Yeast Proteome Database (www.proteome.com/databases/YPD).

The upc2-1 mutants exhibit a number of sterol-related phenotypes including elevated sterol influx and esterification (12, 25). Accordingly, the second largest category of genes responsive to the upc2-1 mutation was involved in sterol metabolism. This consists of the following: HES1, encoding a putative oxysterol-binding protein; ERG11, ERG3, ERG24, ERG25, ERG26, CYB5, and NCP1 encoding proteins involved in sterol biosynthesis; ATP2, encoding a protein shown to detoxify steroids; and ARE1, encoding a sterol esterification enzyme. The upc2-1 mutants are characterized by constitutive sterol influx, and thus genes encoding transporters are of particular interest. Transporters with elevated expression in these mutants include YOR011w, PDR11, FIT2, and SFP1. The proteins encoded by FIT2 and SFP1 are involved in the transport of iron and calcium, respectively. Therefore, they are unlikely to be directly involved in sterol transport. However, the substrates for the transporters encoded by YOR011w and PDR11 are currently unknown.

Two transcription factors UPC2 and HAP1 were up-regulated in the mutant strains. The increased expression of UPC2 in the upc2-1 strains is consistent with the autoregulation demonstrated previously (23). The induction of HAP1, a well characterized, heme-responsive, transcriptional regulator of hypoxic genes (26), suggests a regulatory interaction between UPC2 and HAP1. The goal of this study was to specifically identify genes involved in sterol influx. We have therefore made no determination of whether the genes altered in these strains represent direct targets of the Upc2p transcription factor or are secondary changes. However, it is of interest to note that 54 of the 82 induced genes (indicated by asterisks in Table II) contain a characteristic motif (CGTTT) in their 5’-untranslated region, previously identified as the core of a Upc2p consensus binding site (24). This includes 20 DAN/PAU genes, the 5 ERG genes, and the transporters YOR011w and PDR11.

**Characterization of the Difference between upc2-1 lo and upc2-1 hi Strains**—The upc2-1 lo and upc2-1 hi variants provide a useful model for the transcriptional analysis of constitutive sterol uptake; however, it was unclear what mechanisms(s) contributed to the differential sterol uptake between the two strains. Specifically, the identity of the second mutation in the upc2-1 hi strain was unknown. Comparison of upc2-1 lo and upc2-1 hi strains revealed genes with altered expression levels of 2-fold change or greater (signal log ratios >1.0, Table III). One of the genes induced in the upc2-1 hi strain relative to the low uptake variant is ARV1, which encodes a protein essential for anaerobic viability in yeast with a proposed role in sterol trafficking (17). Indeed, deletion of ARV1 from upc2-1 mutants results in a 50% decrease in sterol influx. Interestingly, the modifier locus present in upc2-1 hi variant displayed genetic linkage to the ARV1 gene (YLR242C)
PCR was used to confirm this mutant, and the normal and upc2-1/H11032 a Ty1 element disrupting the 3' upc2-1 found on chromosome XII (28). The original strain carrying the HAP1 ROX1 be regulated by the transcriptional repressor, DAN1, and DAN3, DAN4, and TIR4 encode proteins of the seripauperin family; and YGR131w and YOR011w encode proteins of the oxysterol-binding protein family; and ARV1 sequence of the seripauperin family; and CYC1 are known to be regulated by oxygen status (22). A subset of 8 genes was determined to conduct analysis of microarray data from aerobically and aerobic transcript pattern of these 19 genes using a previously induced under anaerobic conditions (10), we examined the aerobic sterol uptake of a mutant do not carry the mutant allele. Our results indicate the modification factor identifying affect on aerobic sterol uptake of a mutant upc2-1 hi strain. The normal and upc2-1/H11032 a Ty1 element disrupting the 3' upc2-1 found on chromosome XII (28). The original strain carrying the HAP1 ROX1 be regulated by the transcriptional repressor, ROX1 (27), which is itself regulated by the transcriptional regulator factor HAP1 found on chromosome XII (28). The original strain carrying the upc2-1 mutation is derived from S288c, a strain shown to carry a Ty1 element disrupting the 3' region of the HAP1 ORF (29). PCR was used to confirm this mutant HAP1 allele in the upc2-1 hi strain. The normal and upc2-1 lo strains used in this study do not carry the mutant allele. Our results indicate the modifying effect on aerobic sterol uptake of a mutant HAP1 in the presence of the upc2-1 mutation.

Identifying Candidate Genes Required for Sterol Influx—The differential sterol influx between normal, upc2-1 lo, and upc2-1 hi strains was used as a basis for clustering analysis by the method of self-organizing maps, using the program GeneCluster (Whitehead Institute for Biomedical Research/MIT Center for Genome Research). This approach was carried out to identify those genes whose expression reflected the pattern of sterol uptake in the upc2-1 strains. This revealed a subset of 19 genes (Table II, underlined genes) initially identified as candidates for a role in sterol uptake. Because sterol influx in yeast is induced under anaerobic conditions (10), we examined the anaerobic transcript pattern of these 19 genes using a previously conducted analysis of microarray data from aerobically and anaerobically grown yeast (22). A subset of 8 genes was determined which exhibit increased expression in normal yeast grown anaerobically. This subset is composed of the transcription factor UP2; HES1 is a member of the oxysterol-binding protein family; DAN1, DAN3, DAN4, and TIR4 encode proteins of the seripauperin family; and YGR131w and YOR011w encode proteins of the previously uncharacterized proteins. The largest induction observed was in DAN1 (increased 76-fold in upc2-1 lo and 411-fold in upc2-1 hi). Northern blotting was carried out to confirm the induction of the 8 genes (Fig. 1). All 8 genes were found to be induced in the upc2-1 lo strain and to a greater extent in the upc2-1 hi strain, relative to normal yeast. Expression was essentially negligible in the normal strain grown aerobically. In addition, the closest paralog of YOR011w, PDR11, was regulated in a similar fashion to these genes. These proteins represent a sub-group of cluster 1 of the ABC transporter superfamily in yeast and are 68% identical. Although PDR11 did not achieve statistical significance in the cluster analysis, our Northern analysis confirmed this expression profile (Fig. 1). PDR11 was therefore included in subsequent analyses. Actin expression was unchanged in upc2-1 lo and upc2-1 hi strains relative to normal yeast.

Assessing Candidate Genes Required for Sterol Influx—Deletion mutations in the 9 induced genes were created in the upc2-1 hi strain by homologous recombination. Sterol uptake was assessed by the accumulation in cells of [4-14C]cholesterol supplied to the media of aerobically grown cultures (Fig. 2). To ensure that the cholesterol uptake assay reflects internalization of [4-14C]cholesterol, rather than merely binding to the cell wall, we assayed the cellular esterification of the exogenously supplied sterol. At the end of the labeling period, 40 ± 6% of cell-associated [4-14C]cholesterol was esterified by the labeling enzyme. When compared with normal yeast, we found that deletion of UPC2 abolished the induced sterol influx in the upc2-1 strain. These results indicate the upc2-1 mutation to represent a gain-of-function mutation. Despite the loss of UPC2, this deletion mutant retains the hap1 Ty1 mutation. Therefore, the mutant hap1 allele does not confer constitutive sterol influx but rather enhances uptake in the presence of the upc2-1 mutation.

Deletion of HES1 or YGR131w (Fig. 2) did not significantly

**TABLE III**

Genes with altered expression between upc2-1 hi and lo strains

| ORF | Gene | Signal log ratio | Functional description |
|-----|------|-----------------|------------------------|
| Increases | | | |
| YJR047c | ANB1 | 3.1 | Translation elongation factor |
| YLR242c | ARV1 | 1.1 | Possible role in sterol transport |
| YER067w | | 1.1 | Unknown |
| YHL044w | | 1.1 | Unknown |
| Decreases | | | |
| YKR046c | | −3.5 | Unknown |
| YDL037c | | −1.9 | Unknown |
| YDL038c | | −1.9 | Unknown |
| YLJ052c | AQP2 | −1.9 | Aquaporin water channel |
| YPR065w | ROX1 | −1.8 | Hypoxia gene repressor |
| YJR048w | CYC1 | −1.8 | Cytochrome c isofrom 1 |
| YOR065w | CYT1 | −1.7 | Cytochrome c1 |
| YDR222w | | −1.6 | Unknown |
| YCR098c | GIT1 | −1.6 | Permease for glycerophosphoinositol |
| YEL024w | RIP1 | −1.4 | Iron–sulfur protein of cytochrome c1 |
| YKR071c | | −1.4 | Unknown |
| YMR145c | NDE1 | −1.3 | NADH dehydrogenase |
| YBR095c | PHOS | −1.3 | Acid phosphatase |
| YNR019w | ARB2 | −1.3 | Sterol ester synthetase |
| YGL065c | LEU1 | −1.2 | Isopropylmalylase isomerase |
| YKR039w | GAP1 | −1.2 | General amino acid permease |
| YFR033c | QCR6 | −1.1 | Ubiquinol-cytochrome c oxidoreductase |
| YLR153c | ACS2 | −1.1 | Acetyl-coenzyme A synthetase |
| YKL029c | MAE1 | −1.1 | Malic enzyme of mitochondria |
| YKL129w | OAC1 | −1.1 | Oxaloacetate carrier |
| YLR142w | PUT1 | −1.1 | Proline oxidase |
| YML075c | HMG1 | −1.1 | HMG-CoA reductase |
| YBR296c | PHOS9 | −1.0 | Phosphate permease homolog |
| YPR191w | QCRR2 | −1.0 | Ubiquinol-cytochrome c reductase |
act in a tempting to speculate that these cell wall mannoproteins might proteins. The yeast cell wall can bind cholesterol (31). Thus it is serine content relative to the related serine-rich Srp proteins, about their function. Named PAU (seripauperins) for their low PAU family of anaerobically expressed genes, little is known suggests a role in the process of sterol influx. Although there have gests a role in the process of sterol influx. Due to the high degree of similarity in this family of proteins, it is quite likely that sufficient redundancy exists to mask an important role in sterol influx by single deletion of proteins, the expression pattern of the PAU genes sug- Despite the obvious difficulty in assessing the role of this fam- which is greater than 95% identical to 12 other PAU proteins. To characterize further the process of sterol uptake as deter- To make sterol accessible for import further determined that PDR11, which is the most closely related ABC transporter to AUS1, was similarly regulated in response to the upc2-1 mutation and thus may have some overlap in substrates. Deletion of PDR11 had a significant effect on sterol uptake in the upc2-1 strain, reducing it by 60%. The combined deletion of PDR11 and AUS1 in the upc2-1-hi strain negated sterol uptake, resulting in a level of uptake indistinguishable from that in control strains (Fig. 3A). There were no apparent viability consequences to losing these activities under aerobic conditions.

To characterize further the process of sterol uptake as deter- Fig. 2. Aerobic sterol uptake in deletion mutants. Deletion mutants at the indicated loci were generated by homologous recombination in the upc2-1 hi strain. Cells were grown to mid-log phase in media containing 0.01 μCi/ml [4-14C]cholesterol. Net accumulation of [4-14C]cholesterol in the cells was measured as described under “Experimental Procedures” and reflects the mean % ± S.E. of the upc2-1 hi strain from at least 3 separate experiments. *, p < 0.01; **, p < 0.0001. The upc2-1 hi strain accumulated 1663.9 ± 103.0 dpm [4-14C]cholesterol per mg dry weight.

Further, we measured aerobic sterol uptake in several deletion mutants of ABC transporters, and found that deletion of PDR11 had a significant effect on sterol uptake, reducing it by 60% compared with normal strains. The combined deletion of PDR11 and AUS1 in the upc2-1 hi strain negated sterol uptake, resulting in a level of uptake indistinguishable from that in control strains (Fig. 3A). There were no apparent viability consequences to losing these activities under aerobic conditions.

To characterize further the process of sterol uptake as deter- YOR011w and PDR11 Encode ABC Proteins Required for Sterol Uptake—Deletion of the previously uncharacterized ORF, YOR011w, resulted in a dramatic decrease in sterol ac- cumulation (Fig. 2, −85%; p < 0.0001) and esterification (not shown). Examination of this 1394-amino acid ORF revealed a putative ABC protein belonging to the cluster 1 superfamily of yeast ABC transporters. YOR011w has the structural characteristics of two nucleotide binding domains and two trans- membrane domain regions, suggesting it acts as a full trans- porter (32). Based on our findings we have named this gene AUS1 for ABC protein involved in Uptake of Sterol 1. We further determined that PDR11, which is the most closely related ABC transporter to AUS1, was similarly regulated in response to the upc2-1 mutation and thus may have some overlap in substrates. Deletion of PDR11 had a significant effect on sterol uptake in the upc2-1 strain, reducing it by 60%. The combined deletion of PDR11 and AUS1 in the upc2-1-hi strain negated sterol uptake, resulting in a level of uptake indistinguishable from that in control strains (Fig. 3A). There were no apparent viability consequences to losing these activities under aerobic conditions.

The Physiological Role of Aus1p and Pdr11p—Sterol uptake under aerobic growth conditions, as studied here in the upc2-1 strains, is an artificial situation that nevertheless provided a powerful molecular genetic approach to identifying a key transporter. To identify the physiological role of these ABC family members in sterol uptake, we assayed this phenomenon in normal, AUS1, and PDR11 deletion mutants grown under an aerobic growth conditions where sterol uptake normally occurs and is essential. Although the aus1Δ strains are viable anaerobi- cally (Fig. 4A), they are significantly compromised for sterol uptake compared with normal strains (Fig. 4B). Surprisingly, deletion of PDR11 had little effect on anaerobic sterol uptake or growth; however, the combined loss of both transporters (in an aus1Δ pdr11Δ strain) abolished sterol uptake. The redundancy in these transporters is further demonstrated by the finding that either transporter alone can supply sufficient sterol to support anaerobic growth. Deletion of both proteins, however,
results in a dramatic reduction of sterol influx and severely compromises growth during anaerobiosis (Fig. 4). The anaerobic growth defect of \( \text{aus1} / \text{H9004} \) \( \text{pdr11} / \text{H9004} \) strains is essentially identical to normal yeast grown anaerobically under sterol-deprived conditions, indicating the essential role for these transporters for sterol influx. The growth defect of the \( \text{aus1} / \text{H9004} \) \( \text{pdr11} / \text{H9004} \) strain could not be rescued by inclusion of either cholesterol or ergosterol at levels up to 25-fold higher than the concentrations used in our studies (not shown).

The discovery of a pair of ABC proteins that together totally account for sterol influx in yeast can be appreciated within the context of recent progress made in the study of sterol efflux in mammals. Mutations in the ABC transporter, ABCA1, were shown to be the genetic basis of Tangier disease (33–35), a disease characterized by defective cellular cholesterol efflux. Furthermore, the ABC transporters, ABCG5 and ABCG8, promote biliary excretion of plant sterols such as sitosterol. Mutations in these genes result in the disease \( \text{sitosterolemia} \) (36, 37). We demonstrate here that an energy-dependent, vanadate-sensitive reaction mediates the opposite (i.e. inward) transport of sterol in yeast that is dependent on two putative ABC transporters. This raises the possibility that such transporters may

FIG. 4. Anaerobic growth and sterol uptake in ABC transporter deletion strains. A, \( \text{AUS1} \) and \( \text{PDR11} \) deletion strains were created in strain SCY325 (normal control) and grown anaerobically for 3 days on YEPD plates supplemented with 20 \( \mu \text{g/ml} \) ergosterol and 0.5% Tween 80 as an oleate source. 5-Fold dilutions are shown left to right. B, net accumulation (dpm/mg dry weight) of sterol in the same strains was measured in liquid media containing 0.01 \( \mu \text{Ci/ml} \) [\( 4\text{-}^{14}\text{C}\)] cholesterol as described under "Experimental Procedures" and reflects the mean \( \pm \) S.E. from at least three separate experiments. *, \( p < 0.02 \); **, \( p < 0.0001 \).

The discovery of a pair of ABC proteins that together totally account for sterol influx in yeast can be appreciated within the context of recent progress made in the study of sterol efflux in mammals. Mutations in the ABC transporter, ABCA1, were shown to be the genetic basis of Tangier disease (33–35), a disease characterized by defective cellular cholesterol efflux. Furthermore, the ABC transporters, ABCG5 and ABCG8, promote biliary excretion of plant sterols such as sitosterol. Mutations in these genes result in the disease \( \text{sitosterolemia} \) (36, 37). We demonstrate here that an energy-dependent, vanadate-sensitive reaction mediates the opposite (i.e. inward) transport of sterol in yeast that is dependent on two putative ABC transporters. This raises the possibility that such transporters may
also function in sterol influx in mammals. Although Aus1p and Pdr11p have significant homology to a number of human ABC transporters including ABCG1, breast cancer resistance protein, and ABCA1, it is not known which, if any, human ABC transporter shares its functional role. The upc2-1, aus1Δ pdr11A strains in yeast provide a powerful model system to study the role of ABC transporters in eukaryotic sterol influx.

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