PDR16 and PDR17, Two Homologous Genes of Saccharomyces cerevisiae, Affect Lipid Biosynthesis and Resistance to Multiple Drugs*

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The Saccharomyces cerevisiae open reading frame YNL231C was recently found to be controlled by the multiple drug resistance regulator Pdr1p. Here we characterize YNL231C (PDR16) and its homologue YNL264C (PDR17). Deletion of PDR16 resulted in hypersensitivity of yeast to azole inhibitors of ergosterol biosynthesis. While no increase in drug sensitivity was found upon deletion of PDR17 alone, a Δpdr16,Δpdr17 double mutant was hypersensitive to a broad range of drugs. Both mutations caused significant changes of the lipid composition of plasma membrane and total cell extracts. Deletion of PDR16 had pronounced effects on the sterol composition, whereas PDR17 deletion mainly affected the phospholipid composition. Thus, Pdr16p and Pdr17p may regulate yeast lipid synthesis like their distant homologue, Sec14p. Theazole sensitivity of the PDR16-deleted strain may be the result of imbalanced ergosterol synthesis. Impaired plasma membrane barrier function resulting from a change in the lipid composition appears to cause the increased drug sensitivity of the double mutant strain Δpdr16,Δpdr17. The uptake rate of rhodamine-6-G into de-energized cells was shown to be almost 2-fold increased in a Δpdr16,Δpdr17 strain as compared with wild-type and Δpdr5 strains. Collectively, our results indicate that PDR16 and PDR17 control levels of various lipids in various compartments of the cell and thereby provide a mechanism for multidrug resistance unrecognized so far.

The yeast Saccharomyces cerevisiae has, like many other organisms, the ability to acquire multiple drug resistance, i.e. become less sensitive to a broad range of chemically and functionally unrelated cytotoxic compounds (1, 2). In yeast this phenomenon can be provoked by a regulatory disorder, namely a mutation in the transcription factors Pdr1p or Pdr3p. Pdr1p and Pdr3p are homologous Zn2Cys6 DNA-binding proteins which control the expression of drug efflux pump-encoding genes (3, 4). Gain-of-function mutations in the PDR1 or PDR3 genes may result in increased production of these efflux pumps, leading to drug resistance (5, 6). Neither Pdr1p or Pdr3p nor the drug efflux pumps which they regulate are required for growth of yeast in the absence of drugs. It is not known whether the true physiological function of these drug resistance determinants is to protect the cell from external toxic compounds or whether they may play other roles. In order to get more insight into the physiological role of Pdr1p, we recently screened for target genes regulated by this transcription factor. This screening resulted in the identification of a broad range of novel Pdr1p target genes, one of which was the open reading frame YNL231C (PDR16).

Expression of the PDR16 gene is five times higher in strains carrying pdr1–3, a strong constitutive allele of PDR1, as compared with isogenic wild-type strains or strains deleted for PDR1.1 The PDR16 gene encodes for a protein of 351 amino acids. This protein is 49% identical and 75% similar to the product of the YNL264C (PDR17) gene of S. cerevisiae. Neither PDR16 nor PDR17 has been functionally characterized. The Pdr16p is also 23% identical and 54% similar to the product of the S. cerevisiae SEC14 gene. This homology is spread throughout the protein sequence. However, three sequence blocks which are strongly conserved among the SEC14 proteins from different yeasts (around amino acid positions 55–60, 205–210, and 235–240) (7) are also the most conserved areas between these proteins and PDR16 and PDR17.

Sec14p was initially identified as a PtdIns2 transfer protein (PTP) which can perform transfer of phospholipids between membranes in vitro (8, 9). Subsequently, it was shown that Sec14p/PTP is required for transport of proteins through the Golgi complex (10). It has been proposed that in vivo Sec14p senses the levels of PtdIns and PtdCho in the Golgi complex and exerts negative feedback on PtdCho synthesis through the Kennedy pathway (11). More recently, it was suggested that Sec14p/PTP may also regulate formation of secretory vesicles from the Golgi by stimulating the turnover of phospholipids

1 H. B. van den Hazel and A. Goffeau, unpublished observation.

2 The abbreviations used are: PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine; YPD medium, yeast extract/potato/dextrose medium; YPM medium, yeast/potato/glycerol medium; GLC/MS, gas-liquid chromatography/mass spectrometry; TMA-DPH, trimethylammonium diphenylhexatriene; Mes, 4-morpholinethanesulfonic acid.
(12, 13). The involvement of PTP in signal transduction of higher eukaryotes has been discussed (14–16).

In the present work, we analyze the role of the distant yeast SEC14 homologues, PDR16 and PDR17, in drug resistance and lipid biosynthesis/sorting in a wild-type and a pdr1–3 background. We find that deletion of PDR16 leads to a strongly increased sensitivity toazole antifungals. Deletion of both PDR16 and PDR17 leads to reduced resistance to a broad range of drugs. We, furthermore, show that the mutations affect the phospholipid and sterol composition of the plasma membrane, and that they change the total yeast lipid composition. We propose that the azole sensitivity of the △pdr16 single mutant is mainly due to impaired sterol synthesis and that the broad increase in drug sensitivity of the double mutant is a result of a more general change in plasma membrane composition. The possible roles of Pdr16p and Pdr17p in lipid biosynthesis and sorting are discussed.

MATERIALS AND METHODS

Strains and Deletions of Genes—Escherichia coli strain DH5α (17) was used for plasmid propagation. S. cerevisiae strain US50–18c (MATα his3Δ1) (3), its derivative (MATα pdr1–3 ura3 his1Δ1 pdr3 his1Δ5-hisG1) (18), and strain FY1679–28C (MATα ura3–52 leu2Δ1 his3α2500 trp1Δ63 GAL2–1+) (derivative of FY1679) (19) were used for the construction of strains deleted for the PDR16, PDR17, and YOR1 genes, as well as reference strains in drug sensitivity assays. The US50–18c derivatives AD1 (MATα pdr1–3 ura3 his1Δ1 hisG1) (18) and AD13 (MATα pdr1–3 ura3 his1Δ1 pdr3 hisG1) (19) were used as reference strains in drug sensitivity assays. PDR5-deleted strain FYMK–1/1 (20) was used for drug uptake experiments.

A construct for the disruption of the YOR1 gene (pDK30) (21) was kindly supplied by Dr. W. Scott Moyle-Rowley. All deletions of the PDR16 gene and the deletions of the PDR17 gene in US50–18c and its derivatives were constructed using the procedure described by Alani et al. (22). A DNA fragment corresponding to the 5′-flanking region of the gene was generated by PCR and cloned into pSK–

PDR16 and PDR17 of Yeast

(1935) and pBVH1451. The pBVH1451 plasmid was subsequently treated with SacI and EcoRI and transferred to the E. coli strain US50–18c. Cells in which recombination between the linearized plasmid and the chromosomal locus had generated a circular centromeric plasmid containing the entire gene were selected on media lacking uracil. Plasmids were isolated from yeast, transformed into E. coli, and restriction analysis of plasmid preparations was performed in order to verify the presence of the gene. A multicopy plasmid containing PDR16 was generated by insertion of a SacI-SacI fragment from the single-copy plasmid containing the gene into pRS426 (25).

Growth Media and Drug Resistance Assays—E. coli was grown in standard Luria broth medium (17). Yeast was grown on standard rich glucose (YPD) or glycerol (YPG) media, or on SC medium lacking appropriate amino acids for plasmid maintenance (26). For drug resistance tests on solid media, drugs were added to the media immediately prior to pouring. The drug concentrations tested were the following: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 μg of rhodamine-6-G (Merck, stock in ethanol) per ml of YPD; 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 40, 50, and 100 μg of nystatin per ml of YPD; 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, 20, 40, and 100 μg of cycloheximide (Sigma, stock in ethanol) per ml of YPD; 0.25, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 μg of cycloheximide (Sigma, stock in ethanol) per ml of YPD; 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 μg of miconazole (kindly supplied by Janssen Pharmaceutica, stock in dimethyl sulfoxide) per ml of YPD; 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, and 20 μg of miconazole (kindly supplied by Janssen Pharmaceutica, stock in dimethyl sulfoxide) per ml of YPD; 2, 5, 10, 15, 20, 25, 30, 40, 50, and 100 μg of nystatin per ml of YPD; 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10, 20, and 50 μg of ketoconazole (kindly supplied by Janssen Pharmaceutica, stock in dimethyl sulfoxide) per ml of YPD; 0.5, 1, 2, 5, 10, 25, 50, and 100 μg of itraconazole (kindly supplied by Janssen Pharmaceutica, stock in dimethyl sulfoxide) per ml of YPD; 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, and 1 μg of antimony A (Sigma, stock in dimethyl sulfoxide) per ml of YPD; 10 μg of 4-nitroquinoline oxide (Sigma, stock in dimethyl sulfoxide) per ml of YPD; 100 μg of kuromycin (Sigma, stock in dimethyl sulfoxide) per ml of YPD; 10 μg of oxadiazole (Sigma, stock in dimethyl sulfoxide) per ml of YPD; and 100 μg of diphenylpropylamine (kindly supplied by Janssen Pharmaceutica, stock in dimethyl sulfoxide) per ml of YPD.

Drug preparations were made on aseptic procedure. Phosphate buffer (10 mM) per ml of YPD; 2, 5, 10, 20, 50, and 100 μg of antimycin A (Sigma, stock in dimethyl sulfoxide) per ml of YPD; and 10 μg of rhodamine-6-G (Sigma, stock in dimethyl sulfoxide) per ml of YPD.

Rhodamine-6-G Uptake Assay—Approximately 10 7 cells from an overnight culture were grown in 10 ml of YPD to an final OD600 of 4.0 and resuspended in 20 ml of YPD and grown for 3 h at 30 °C. About 5.6 × 10 10 cells were pelleted and washed three times with buffer A (50 mM HEPES/NaOH, pH 7.0). The cells were subsequently resuspended in 4 ml of de-energization buffer (1 μM antimycin A, 5 mM 2-deoxy-r-glucose in buffer A), incubated for 2 h at 30 min at 30 °C, and transferred to a water bath at 20 °C. A 200-μl aliquot of the de-energized cell suspension was pelleted, washed once with 200 μl of cold buffer A, and resuspended in 2 ml of the same cold buffer. Cell fluorescence background was measured using an SLM Aminco 48000 S spectrophotometer. The excitation wavelength was 529 nm (4 nm slit), and the emission wavelength was 553 nm (4 nm slit). Rhodamine-6-G was added to the remaining cell suspension to a final concentration of 5 μM, and 200-μl aliquots were taken every 5 min up to 1 h. The cells of each aliquot were pelleted, washed, and resuspended as described above and immediately subjected to fluorescence measurements. The cell fluorescence background values after 0- and 1-h incubation were averaged, and this average was subtracted from each fluorescence value.

Isolation of Yeast Subcellular Fractions—Cells were grown on YPD medium containing 2% glucose, 2% peptone (Oxoid), and 1% yeast extract (Oxoid) under aerobic conditions at 30 °C. Growth of yeast cells was determined by measurement of the OD600 nm.

Highly purified plasma membrane was isolated as follows. The pellet of crude plasma membrane (28) was suspended in 5 mM Mes, 0.2 mM EDTA, pH 6.0, with 10 strokes in a loose-fitting Dounce homogenizer and layered on top of a sucrose density gradient made of 10 ml of 38% (w/w), 10 ml of 43% (w/w), and 10 ml of 53% (w/w) sucrose in 5 mM Mes, 0.2 mM EDTA, pH 6.0. Centrifugation was carried out at 100,000 × g for 2.4 h in a Beckman L5-65B ultracentrifuge at 4 °C.
Cells were homogenized for 3 min with CO\textsubscript{2} cooling in the presence of 30–33\% tent and quality of the preparations as well as cross-contamination with 100,000 \times g supernatant contains the cytosolic proteins. Protein content and quality of the preparations as well as cross-contamination with other organelle membranes were assessed as described previously (30–33).

Lipid Analysis of Whole Cell Extracts and Subcellular Fractions—Cells were homogenized for 3 min with CO\textsubscript{2} cooling in the presence of glass beads using a Merckenshager Homogenizer. Lipids of whole yeast cells and organelle preparations were extracted by the procedure of Folch et al. (34). Analysis of individual phospholipids and neutral lipids was carried out by published procedures (35, 36). Alkaline hydrolysis of lipid extracts was carried out as described elsewhere (37). Individual lipids were analyzed by gas-liquid chromatography (GLC) on an HP 5890 Series II Plus GC equipped with electronic pressure control and an HP chemstation software package. An HP 5972 mass selective detector and authentic standards were used for identification of steroids. Injector and interface were kept at 250 and 300 °C, respectively. GLCMS analysis was performed on a capillary column, HP-SMS 30 m \times 0.25 mm \times 0.25-\mu m film thickness, programmed from 150 °C to 320 °C at 20 °C/min after a 2-min hold at 150 °C. Finally, the column was kept at 320 °C for 10 min. All analyses were carried out in the constant flow mode. Helium was used as carrier gas with a linear velocity of 34.1 cm/s. One-\mu l aliquots of the samples were injected with an HP 7673 autosampler in splitless mode. Electron impact ionization with 70 eV ionization energy was used for mass spectrometry. Data were collected by scanning from 150 to 600 atomic mass units at 1.6 scans/s.

Alternatively, GLC was performed on an HP 5890 equipped with a flame ionization detector (FID) operated at 320 °C using a capillary column (HP5, 30 m \times 0.32 mm \times 0.25-\mu m film thickness). After a 1-min hold at 50 °C the temperature was increased to 310 °C at 10 °C/min. The final temperature was held for 10 min. Nitrogen was used as carrier gas and 1-\mu l aliquots of samples were injected cool on column. Relative retention times of sterols were similar as described previously (38–40).

Determination of Lipid Transfer Activity—Lipid transfer activity of cytosolic fractions and peripheral organelle membrane proteins was measured according to Ceolotto et al. (41). Integral and peripheral membrane proteins were separated by treatment of organelle membranes with 0.25 M KCl for 20 min on ice. Insoluble membrane components were sedimented by centrifugation at 100,000 \times g for 1 h, whereas solubilized proteins were recovered in the supernatant.

The rate of protein-catalyzed transfer of fluorescently labeled phospholipids from small unilamellar donor vesicles to unlabeled unilamellar acceptor membranes was measured as described previously (9, 40–42). The phospholipid transfer activity was measured using a Shimadzu RF-5301 spectrophotometer. The excitation wavelength was set at 342 nm (1.5 nm slit) and the emission wavelength was set at 380 nm (3 nm slit). The assay was performed for 7 min with measurements taken every 0.5 s. Fluorescently labeled PtdCho (44), PtdIns (45), PtdSer (46), and N-trinitrophenyl phosphatidylethanolamine (47) were synthesized by published procedures.

Anisotropy Measurement—Fluidity of the plasma membrane was determined in vitro by measuring the fluorescence anisotropy of TMA-DPH. Samples containing 100 \mu g of membrane protein were incubated with 2.7 nmol of TMA-DPH for 30 min at 30 °C. Fluorescence measurements were carried out using a Shimadzu RF 5301 spectrophotometer as described previously (48).

RESULTS

Drug Sensitivity of StrainsDeleted for PDR16 and/or Its Homologue PDR17—We recently identified PDR16 (YNL231C) as one of several novel genes controlled by the yeast multiple drug resistance regulator Pdr1p.\textsuperscript{1} To investigate whether PDR16 like other Pdr1p targets is involved in multiple drug resistance, we deleted the gene and studied the effects on drug sensitivity. In order to maximize the possible effects, the deletion was made in a pdr1–3 genetic background (strain US50–18c) which led to overexpression of Pdr16p.

Deletion of PDR16 had no effect on yeast growth in the absence of drugs. However, the PDR16-deleted strain (Δpdr16) exhibited a strongly increased sensitivity to miconazole and ketoconazole as compared with the parental strain US50–18c (Table I). For miconazole, the sensitivity of Δpdr16 was increased approximately 20-fold over the control: while the minimal inhibitory concentration was 2 \mu g/ml for the parental strain, it was only 0.1 \mu g/ml for the Δpdr16 strain. Sensitivity to ketoconazole increased about 10 times. Similar results were obtained with itraconazole (data not shown). The Δpdr16 strain was also slightly more sensitive to nystatin than the parental strain. No significant changes in sensitivity to any of the other drugs tested were observed (Table I).

To verify whether the drug sensitivity phenotype was indeed due to loss of PDR16 gene function, we introduced a single-copy plasmid carrying the intact PDR16 gene in the Δpdr16 mutant. The resulting transformant had a level of miconazole resistance identical to that of the US50–18c parental strain, indicating that the mutant phenotype was indeed due to loss of PDR16 function (data not shown).

The PDR16 gene has a close homologue in S. cerevisiae termed PDR17 (YNL264C). To test whether there is a functional relationship between these two genes, we generated an isogenic strain deleted for PDR17, and a double mutant deleted for both PDR16 and PDR17. The single PDR16-deleted strain (Δpdr16) did not exhibit a growth defect as compared with US50–18c. Moreover, the Δpdr17 strain did not show increased drug sensitivity, except for a minor increase in sensitivity to 4-nitroquinoline-N-oxide (Table I). The growth rate of the double mutant strain Δpdr16,Δpdr17, on the other hand, was slightly decreased on rich media as compared with the parental and the single mutant strains (data not shown). Growth of the various strains was also tested on non-fermentable carbon sources, at high pH, osmolality, and temperature. While most of these adverse growth conditions did not differentially affect the growth of the parental and mutant strains, growth of the double-deleted strain Δpdr16,Δpdr17 was severely reduced as compared with the parental and the single-deleted Δpdr16 and Δpdr17 strains at 37 °C on potassium phosphate-buffered pH 7 plates containing 0.5 M potassium chloride (data not shown). Furthermore, the Δpdr16,Δpdr17 strain was even more sensitive to the azole antifungals miconazole and ketoconazole than the Δpdr16 strain (Table I). Moreover, the double mutant also displayed increased sensitivities to cycloheximide, rhodamine-6-G, oligomycin, 4-nitroquinoline-N-oxide, antymycin A, and

| Drug sensitivity of yeast strain US50–18c and various derivatives after 3 days of growth at 30 °C | Minimal inhibitory drug concentration (\mu g/ml of media) |
|---|---|
| **US50–18c** | **US50–18c** |
| **Δpdr16** | **Δpdr16** |
| **Δpdr17** | **Δpdr17** |
| **Miconazole** | 2 | 0.1 | 2 | 0.025 |
| **Ketoconazole** | 10 | 10 | 10 | 0.25 |
| **Nystatin** | 25 | 40 | 25 | 20 |
| **Cycloheximide** | 1.5 | 1.5 | 1.5 | 0.5 |
| **Rhodamine-6-G** | >200 | >200 | >200 | 200 |
| **Oligomycin** | 1 | 1 | 1 | 0.5 |
| **4-NQO\textsuperscript{*}** | 5 | 5 | 2 | 2 |
| **Antimycin A** | 0.1 | 0.1 | 0.1 | 0.1 |
| **Ethidium bromide** | 2 | 2 | 2 | 2 |
| **Crystal violet** | >20 | >20 | >20 | 20 |

\* 4-NQO, 4-nitroquinoline-N-oxide.
crystal violet (Table I). The increase in sensitivity as compared with the \( \Delta pdr16 \) single-deleted strain was about 2–4-fold for most drugs; only sensitivities for ethidium bromide and nysta-tatin were not increased. The increased drug sensitivity phenomenons of the \( \Delta pdr16, \Delta pdr17 \) strain were indeed due to loss of \( PDR17 \) function, because introducing a single-copy plasmid carrying \( PDR17 \) restored the miconazole and cycloheximide resistance of the \( \Delta pdr16, \Delta pdr17 \) strain to the levels of the single \( PDR16 \)-deleted strain.

The \( PDR16 \)- and \( PDR17 \)-related phenotypes that we described thus far were observed in the US50–18c genetic background. US50–18c is highly drug resistant due to the \( pdr1–3 \) mutation which results in a strong overexpression of \( Pdr5p \)-regulated drug efflux pumps such as \( Pdr5p \), \( Snq2p \), and \( Yor1p \) (5, 49). In order to test whether the \( PDR16 \)- and \( PDR17 \)-related phenotypes were specific for this particular genetic background, or whether they also occurred in an otherwise wild-type context, we deleted these two genes in the wild-type strain FY1679–28c and studied drug sensitivity phenotypes.

As expected, due to lack of overexpression of the drug efflux pumps, the FY1679–28c strain was generally much more sensitive to most drugs than the US50–18c strain. As can be seen from Table II, however, the effects of deletion of \( PDR16 \) and/or \( PDR17 \) in wild-type were roughly the same as in US50–18c. The sensitivity to miconazole and ketoconazole was increased about 10–20-fold upon deletion of \( PDR16 \), and most drug sensitivities were increased 2–5-fold upon additional deletion of \( PDR17 \). Thus, the \( PDR16/PDR17 \)-dependent drug sensitivity phenomenons are not specific for US50–18c, but can also be observed in a wild-type genetic background. The lower resistance of FY1679–28c toward crystal violet and rhodamine-6-G allowed detection of a slightly increased sensitivity to these drugs in the \( \Delta pdr16 \) strain as compared with the parental strain (Table II).

### Deletions of \( PDR16 \) and \( PDR17 \) Rather Affect Drug Uptake Than Drug Efflux—Two yeast drug efflux pumps known to mediate resistance to azoles are \( Pdr5p \) and \( Yor1p \) (50). In order to investigate whether the effects of \( PDR16 \) onazole resistance were due to reduced \( Pdr5p \) and \( Yor1p \) function, we constructed strains deleted for \( PDR16 \) as well as for \( PDR5 \) and/or \( YOR1 \), and investigated their drug sensitivities. As can be seen in Table III, a triple mutant \( \Delta pdr16, \Delta pdr5, \Delta yor1 \) is more sensitive against some drugs, e.g. ketoconazole and miconazole, than a \( \Delta pdr5, \Delta yor1 \) strain, indicating that at least part of the effect of the \( PDR16 \) gene on drug resistance is independent of \( Pdr5p \) and \( Yor1p \) function. Furthermore, Table III shows a comparison of the drug sensitivities of strains deleted for \( PDR5 \) and/or \( YOR1 \) to those of the \( \Delta pdr16, \Delta pdr17 \) strain. The \( \Delta pdr16, \Delta pdr17 \) strain is more resistant to cycloheximide and rhodamine-6-G, two typical substrates for \( Pdr5p \), than a \( \Delta pdr5 \) strain, and more resistant to oligomycin, a typical \( Yor1p \) substrate, than a \( \Delta yor1 \) strain. This strongly suggests that \( Pdr5p \) and \( Yor1p \) are at least partially active in the \( \Delta pdr16, \Delta pdr17 \) strain, and that deletion of \( PDR16 \) and \( PDR17 \) does not lead to loss of function of these drug efflux pumps.

To test whether a difference in passive drug transport was the reason for the reduced drug resistance of the \( PDR16 \)- or \( PDR17 \)-deleted strains, we investigated drug uptake into cells in which active transport was blocked by energy depletion. As a probe for drug uptake we used rhodamine-6-G, a toxic pink colored fluorescent dye to which a probe for drug uptake we used rhodamine-6-G, a toxic pink colored fluorescent dye to which we added 2-deoxy-D-glucose and antimycin A. After 2.5 h at 30 °C, rhodamine-6-G was added and its cellular uptake was followed (Fig. 1). The wild-type and the \( \Delta pdr5 \) strain showed similar rates of rhodamine-6-G uptake indicating that \( Pdr5p \), a strong rhodamine-6-G pump, was not active under these conditions and energy depletion was complete. Under the same conditions, the \( \Delta pdr16, \Delta pdr17 \) strain showed an almost 2-fold higher rate of rhodamine-6-G uptake. These data indicate that the increased rhodamine-6-G sensitivity of the \( \Delta pdr16, \Delta pdr17 \) strain is at least partially due to an increased passive drug uptake into these cells.

### Plasma Membrane Lipid Composition of Strains Deleted for \( PDR16 \) and/or \( PDR17 \)—The fact that \( \Delta pdr16, \Delta pdr17 \) mutations appear to affect the uptake of drugs into yeast led us to investigate some properties of the plasma membrane of the respective mutants. Homologies of the \( PDR16 \) and \( PDR17 \) gene

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**Table II**

| Drug sensitivity of yeast strain FY1679–28c and various derivatives after 3 days of growth at 30 °C |
|-----------------------------------------------|
| Minimal inhibitory drug concentrations |
| FY1679–28c | FY1679–28c | FY1679–28c | FY1679–28c | FY1679–28c |
| Miconazole | 0.1 | 0.01 | 0.1 | 0.005 |
| Ketoconazole | 5 | 0.25 | 5 | 0.1 |
| Nystatin | 15 | 15 | 15 | 15 |
| Cycloheximide | 0.5 | 0.5 | 0.5 | 0.05 |
| Rhodamine-6-G | 100 | 50 | 100 | 2.5 |
| Oligomycin | 1 | 1 | 1 | 0.5 |
| 4-NQO | 2 | 2 | 2 | 1 |
| Antimycin A | NA | <0.01 | <0.01 | <0.01 |
| Ethidium bromide | 2 | 2 | 2 | 1 |
| Crystal violet | 10 | 5 | 10 | 1 |

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**Table III**

| Drug sensitivity of derivatives of yeast strain US50–18c after 3 days of growth at 30 °C |
|-----------------------------------------------|
| Minimal inhibitory drug concentrations |
| \( \Delta pdr16, \Delta pdr17 \) |
| Miconazole | 0.025 | 0.05 | 0.005 | 2 | 0.1 | 0.025 |
| Ketoconazole | 0.25 | 0.5 | 0.1 | 10 | 1 | 0.25 |
| Nystatin | 20 | 40 | 30 | 25 | 15 | 20 |
| Cycloheximide | 0.5 | 0.5 | 0.5 | 1.5 | 1.5 | 0.05 |
| Rhodamine-6-G | 200 | 50 | 50 | >200 | >200 | NA |
| Oligomycin | 0.5 | 2 | 1 | 0.25 | 0.1 | NA |
| 4-NQO | 2 | 5 | 5 | 5 | 5 | 5 |
| Antimycin A | 0.05 | 0.025 | 0.025 | 0.05 | 0.05 | 0.05 |
| Ethidium bromide | 2 | 2 | 2 | 2 | 2 | 2 |
| Crystal violet | 20 | 10 | 5 | >20 | >20 | NA |

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\[ ^a \] NA, not analyzed.
\[ ^b \] 4-NQO, 4-nitroquinoline-N-oxide.
products to Sec14p also suggested that perhaps lipid synthesis
and/or sorting might be controlled by these genes.

Tables IV and V show that deletion of PDR16 and PDR17 in
the FY1679–28c and US50–18c background caused several
alterations of the plasma membrane lipid composition.
Whereas the amount of total phospholipids was similar in all
strains tested and the pdr1–3 mutation did not have marked
effects, the pattern of individual phospholipids was signifi-
cantly changed in the plasma membranes of Δpdr16, Δpdr17,
and the double mutant (Table IV). In plasma membrane
preparations of Δpdr16,Δpdr17 strains concentrations of PtdCho
and PtdIns were markedly increased as compared with the
control strains FY1679–28c and US50–18c, whereas the level
of PtdEtn was dramatically reduced. Furthermore, the amount
of PtdSer was increased in the plasma membrane of the double
mutant in wild-type, but remained constant in the pdr1–3
background. Changes in the PtdCho and PtdIns levels appear
to be a cumulative effect of both Δpdr16 and Δpdr17, whereas
alterations in PtdEtn and PtdSer seem to be more clearly
expressed in the Δpdr17 strain. It is noteworthy that the
Δpdr17 deletion causes a major increase in the amount of
negatively charged phospholipids, PtdSer and PtdIns, in the
plasma membrane. This fact may influence surface properties
and/or function of membrane bound proteins.

The total sterol content of the plasma membrane (Table V)
was significantly reduced by the Δpdr16,Δpdr17 mutations in the
background of the FY1679–28c strain. This effect was not
seen in US50–18c which bears a pdr1–3 mutation. In both
backgrounds, however, ergosterol precursors were observed in
the plasma membrane of the double mutant. Especially the
unusual presence of 4,4-dimethylzymosterol and lanosterol in
the plasma membrane deserves our attention. It is well known
that Erg11p, the cytochrome P450-dependent lanosterol 14-
demethylase which uses lanosterol as a substrate, is a most
sensitive target to azole inhibitors (for a review, see Ref. 51). A
possible effect on enzymes of ergosterol biosynthesis of the
Δpdr16,Δpdr17 mutations might cause increased sensitivity to
azoles as shown in this study (see Tables I-III). As an alterna-
tive, the PDR16 and PDR17 deletions might cause mistarget-
ing of the sterol precursor which is normally found at signifi-
cant amounts only in microsomal membranes and, in the form
of fatty acyl esters, in lipid particles (52).

Despite the marked changes of the plasma membrane lipid
composition caused by the Δpdr16,Δpdr17 mutations the bulk
fluidity of the plasma membrane appears to be largely pre-
served. Measurement of anisotropy using the fluorescent
marker TMA-DPH as a probe revealed that membrane fluidity
was not changed (data not shown). Thus, the mutant cells
obviously compensate in that respect almost perfectly for the
above mentioned alterations.

**Total Lipid Composition of Strains Deleted for PDR16
and/or PDR17—To elucidate the possible role of PDR16 and
PDR17 in maintaining a distinct lipid composition of yeast**

![Graph](http://www.jbc.org)

**TABLE IV**

| Total phospholipid content was calculated from quantification of lipid phosphorus. |
|--------------------------------------------------|
| Phospholipids\(^a\) (mg/mg protein) | US50–18c | US50–18c Δpdr16 | Δpdr17 | FY1679–28c | FY1679–28c Δpdr16 | Δpdr17 | FY1679–28c Δpdr16 | Δpdr17 |
|--------------------------------------|---------|----------------|--------|------------|-----------------|--------|-----------------|--------|
| PtdCho                              | 10.0 ± 3.3 | 24.4 ± 1.2 | 6.4 ± 3.0 | 12.5 ± 4.5 | 12.5 ± 1.6 | 15.9 ± 0.6 |
| PtdEtn                              | 41.8 ± 4.3 | 18.9 ± 1.3 | 38.9 ± 2.9 | 35.9 ± 0.3 | 19.4 ± 2.6 | 23.8 ± 0.5 |
| PtdIns                              | 20.3 ± 2.1 | 22.1 ± 1.0 | 24.7 ± 2.1 | 20.8 ± 2.7 | 35.2 ± 0.2 | 30.4 ± 1.6 |
| PA                                  | 7.9 ± 0.8 | 19.0 ± 1.0 | 8.8 ± 1.4 | 7.5 ± 1.3 | 9.8 ± 2.0 | 11.5 ± 1.3 |
| Lyso-PtdEtn                         | 3.5 ± 1.8 | 4.1 ± 1.9 | 5.7 ± 1.0 | 6.8 ± 1.2 | 5.9 ± 0.4 | 9.1 ± 0.4 |
| Others                              | 12.6 ± 1.6 | 4.8 ± 0.7 | 9.8 ± 1.8 | 10.5 ± 0.2 | 10.3 ± 3.0 | 3.8 ± 0.7 |
| (PtdIns + PtdSer): (PtdCho + PtdEtn): | 3.92 ± 2.2 | 6.7 ± 1.7 | 5.7 ± 1.0 | 6.1 ± 1.1 | 6.9 ± 0.3 | 5.5 ± 1.6 |

\(^a\) PA, phosphatidic acid; lyso-PtdEtn, lysophosphatidylethanolamine.
plasma membrane by either regulating biosynthesis or sorting of various lipids we compared the lipid composition of plasma membrane preparations to that of total membranes.

Both in wild-type and in the pdr1–3 background the phospholipid composition of total cellular membranes was changed significantly upon deleting PDR16 and PDR17 (Table VI). While the PtdCho content was increased, the PtdEtn content was decreased in Δpdr16,Δpdr17 compared with the control strains. The level of PtdSer in total membranes was hardly affected by deleting the two genes, and only a minor decrease of PtdIns was observed. Thus, apart from PtdIns, the changes in phospholipid composition of total membranes corresponded very well to the alterations of plasma membrane phospholipid composition (see Tables IV and VI).

Deletion of PDR16 and PDR17 also changed the sterol pattern of total cells in the pdr1–3 and wild-type background (Table VII). Especially the accumulation of 4,4-dimethylzymosterol and lanosterol in the double mutants can be correlated with the appearance of these two ergosterol precursors in the plasma membrane of Δpdr16,Δpdr17 strains (see Tables V and VII). The increased total sterol content in US50–18c Δpdr16,Δpdr17 relative to US50–18c may be attributed to the slower growth of the former strain that is accompanied by accumulation of sterol esters.

Lipid Transfer Activities of PDR16 and PDR17 Gene Products in Vitro—Since the PDR16 and PDR17 genes are distant homologues of the SEC14 gene which encodes the yeast PITP, it was tempting to speculate that Sec14p and the PDR16 and PDR17 gene products may have similar functions. Therefore we investigated whether the levels of PtdIns, PtdCho, and PtdSer transfer activities in isolated subcellular fractions differed between the various strains used in this study. Cytosolic Sec14p/PITP and membrane-bound lipid transfer proteins prefer PtdIns as a substrate (Fig. 2). In the US50–18c background the lipid transfer activity of the cytosol was only affected to a minor extent by mutations of the PDR16 and PDR17 genes. In contrast, the effect of these mutations on the membrane-bound lipid transfer activity was pronounced. Especially in microsomal fractions (Fig. 2) of Δpdr16, Δpdr17 strains the PtdIns transfer activities were largely reduced as compared with the control strain US50–18c. Reduced transfer activities were also observed for microsomal fractions of both single mutants. Effects of the Δpdr16 and Δpdr17 single mutations on PtdIns transfer activity were not additive, which might be an indication that the products of PDR16 and PDR17 somehow interact and deletion of one gene reduces the transfer activity conferred by the other. However, absolute rates of phospholipid transfer activities can only be measured with a mean standard deviation of ±15–30% and should be therefore interpreted with caution. The levels of transfer activity in mitochondrial and plasma membrane fractions were low, and no significant difference was observed between any of the strains (data not shown). In subcellular fractions of FY1679–28c, the level of PtdIns transfer activity was similar to the Δpdr16, Δpdr17 double mutant in the pdr1–3 background, and deletion of PDR16 and PDR17 did not further reduce the transfer activities (data not shown). In summary, these data suggest that the increase of the in vitro transfer activities due to the

### Table V
Sterol composition of purified plasma membrane

|                 | US50–18c | US50–18c Δpdr16 | FY1679–28c Δpdr16 | FY1679–28c Δpdr17 | FY1679–28c Δpdr16,Δpdr17 |
|-----------------|----------|-----------------|------------------|------------------|--------------------------|
| Total sterols   | 0.40 ± 0.04 | 0.39 ± 0.06 | 0.45 ± 0.19 | 0.45 ± 0.12 | 0.63 ± 0.02 | 0.28 ± 0.07 |
| % of total sterols | 99 ± 1 | 76 ± 2 | 94 ± 2 | 79 ± 4 | 93 ± 1 | 63 ± 3 |
| Ergosterol      | 2.3 ± 0.8 | 3.7 ± 1.1 | 4.9 ± 1.2 | 5.3 ± 1.3 | 5.7 ± 1.4 | 6.0 ± 1.5 |
| Lanosterol      | 3.1 ± 1.2 | 4.5 ± 1.6 | 5.7 ± 1.8 | 6.1 ± 2.0 | 6.5 ± 2.1 | 6.8 ± 2.2 |
| Zymosterol      | 4.6 ± 2.0 | 6.0 ± 2.4 | 7.3 ± 2.6 | 7.7 ± 2.8 | 8.0 ± 3.0 | 8.3 ± 3.2 |
| Episterol       | 0.7 ± 0.1 | 0.9 ± 0.1 | 1.1 ± 0.1 | 1.3 ± 0.1 | 1.5 ± 0.1 | 1.7 ± 0.1 |
| Fecosterol      | 0.3 ± 0.0 | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.0 | 0.8 ± 0.0 |
| 4,4-Dimethylzymosterol | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.6 ± 0.0 | 0.7 ± 0.0 | 0.8 ± 0.0 | 0.9 ± 0.0 |
| Others          | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 |

### Table VI
Phospholipid composition of total cellular membranes

| Phospholipids (mg/g CDW)* | US50–18c | US50–18c Δpdr16 | FY1679–28c Δpdr16 | FY1679–28c Δpdr17 |
|---------------------------|----------|-----------------|------------------|------------------|
| PtdCho                    | 42.2 ± 1.5 | 48.2 ± 2.3 | 37.7 ± 0.9 | 49.0 ± 0.1 |
| PtdEtn                    | 17.3 ± 2.8 | 11.6 ± 0.3 | 22.6 ± 0.5 | 14.0 ± 0.1 |
| PtdSer                    | 5.4 ± 0.6 | 7.6 ± 0.1 | 6.8 ± 0.1 | 7.5 ± 2.8 |
| PtdIns                    | 20.2 ± 0.3 | 19.1 ± 0.8 | 19.4 ± 0.1 | 14.3 ± 0.7 |
| PA                        | 4.9 ± 0.7 | 5.3 ± 1.0 | 2.5 ± 0.5 | 4.5 ± 1.6 |
| DM-PtdEtn                 | 2.4 ± 1.4 | 1.7 ± 0.1 | 7.4 ± 0.3 | 4.2 ± 0.1 |
| Lyso-PL                   | 2.3 ± 0.1 | 2.9 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 1.1 |
| Lyso-PtdEtn               | 1.1 ± 0.2 | 0.3 ± 0.1 | ND | 0.7 ± 0.1 |
| CL                        | 4.3 ± 1.8 | 2.0 ± 0.2 | 2.3 ± 0.9 | 1.9 ± 0.9 |
| Others                    | 0.1 ± 0.1 | 1.3 ± 0.2 | 0.4 ± 0.3 | 2.0 ± 0.2 |

* CDW, cell dry weight.
* DM-PtdEtn, dimethylphosphatidylethanolamine; Lyso-PL, lysosphospholipids; CL, cardiolipin. For other abbreviations see Table IV.
* ND, not detectable.
**PDR16 and PDR17 of Yeast**

**DISCUSSION**

Pdr1p has been known for several years as a transcriptional regulator controlling yeast multiple drug resistance (for review, see Ref. 2). Pdr1p together with its homologue Pdr3p regulates the expression of the drug efflux pumps Pdr5p, Snq2p, Yor1p, Pdr10p, and Pdr15p (18, 53). Recently, it was found that Pdr1p and Pdr3p control transport of phospholipids across the plasma membrane via Pdr5p and Yor1p (18, 54), raising the possibility that such transport is a physiological function of the network of PDR proteins. Furthermore, Pdr1p and Pdr3p regulate the expression of two hexose transporter-encoding genes (6). Thus, Pdr1p controls plasma membrane function by regulating the level of various active transport systems. The present work suggests that Pdr1p may also affect structure and function of the plasma membrane and drug resistance of the yeast through another mechanism, namely by controlling the expression of the PDR16 gene.

The **PDR16** gene is specifically required for resistance of yeast cells to miconazole and ketoconazole (see Tables I and II). These two drugs have a similar mode-of-action: they affect ergosterol biosynthesis at the level of the ERG11 gene product, the cytochrome P450-dependent lanosterol 14α-demethylase. One possible explanation for the increasedazole sensitivity of the Δpdr16 strain is that the intracellular levels of azoles are increased due to a change in structure and/or function of the plasma membrane of the mutant strain. However, although plasma membrane composition was found to be altered in the mutant, deletion of PDR16 alone had little effect on resistance of yeast cells to other drugs. Thus, it is not likely that the permeability of the plasma membrane has changed severely in the single mutant. The accumulation of precursor sterols in the plasma membrane of the Δpdr16 mutant in the absence of azoles rather suggests that the activity of enzymes that play a role in ergosterol biosynthesis is affected in this strain, making it more sensitive to inhibition by azoles. The finding that the level of precursor sterols is not only elevated in the plasma membrane, but also in total cell extracts of the double mutant Δpdr16Δpdr17 supports that hypothesis.

Drug resistance phenotypes due to deletion of **PDR17** were only seen in the absence of **PDR16**. The double-deleted strain **Δpdr16Δpdr17** exhibits a broad drug sensitivity spectrum, although the most dramatic effects were observed with miconazole and ketoconazole. The increased sensitivity to a broad range of drugs, including mutagens, inhibitors of protein synthesis and mitochondrial energy production, rather points toward a general change in intracellular drug concentrations due to the mutations than to an impairment of all the individual drug target functions. Most drugs for which increased sensitivity was found are hydrophobic and are believed to enter the cell by passive diffusion. However, the yeast plasma membrane contains a number of protein pumps which can extrude drugs from the cell in an ATP-dependent manner. Thus, a change in intracellular drug concentrations could be due to increased passive uptake of the drugs through the plasma membrane or to reduced active, protein-mediated drug efflux out of the yeast. The **Δpdr16Δpdr17** strain was more resistant to cycloheximide and rhodamine-6-G than a **Δpdr5** strain, and more resistant to oligomycin than a **Δyor1** strain, indicating that Pdr5p and Yor1p are active in the **Δpdr16Δpdr17** strain. Moreover, deletion of **PDR16** further reduced drug resistance of a **Δpdr5Δyor1** strain, indicating that at least part of the reduced resistance is independent of Pdr5p and Yor1p. Thus, there is no indication that active protein-mediated drug efflux is reduced in strains deleted for **PDR16** and/or **PDR17**. We cannot exclude that Pdr5p and Yor1p function is partially affected, or that other yet unidentified drug efflux systems are less active in the **Δpdr16Δpdr17** strain. The observation, however, that energy-depleted **Δpdr16Δpdr17** cells exhibit an increased rate of rhodamine-6-G uptake as compared with wild-type and **Δpdr5** cells suggests, that at least part of the difference in drug sensitivity is due to a difference in passive transport (see Fig. 1). Thus, the increased sensitivity of the double mutant strain **Δpdr16Δpdr17** is at least partially, and perhaps entirely, due to an increased passive uptake of drugs into the cell.

Increase in passive uptake of drugs into the cell might be explained by the changed lipid composition of the plasma membrane of the mutants. Both **PDR16** and **PDR17** appear to affect the lipid composition of the plasma membrane, although in a different manner. Whereas deletion of the **PDR16** gene mostly affects the sterol composition (Table V) deletion of **PDR17** rather alters the phospholipid composition of the plasma membrane (Table IV). Most strikingly, the ratio of the negatively charged phospholipids, PtdIns and PtdSer, to the uncharged phospholipids, PtdCho and PtdEtn, is dramatically increased.

| Sterol composition of total cellular membranes determined by GLC | US50–18c | US50–18c Δpdr16 | FY1670–28c | FY1670–28c Δpdr16 |
|---|---|---|---|---|
| Ergosterol | 68 ± 5 | 70 ± 2 | 55 ± 2 | 49 ± 3 |
| Lanosterol | 0.5 ± 0.5 | 3 ± 1 | 8 ± 1 | 14 ± 2 |
| Zymosterol | 17 ± 4 | 6 ± 1 | 6 ± 2 | 6 ± 2 |
| Episterol | 3 ± 2 | 4 ± 2 | 6 ± 2 | 5 ± 3 |
| Fecosterol | 2 ± 1 | 3 ± 1 | 13 ± 2 | 7 ± 4 |
| 4,4-Dimethylzymosterol | 5 ± 2 | 9 ± 2 | 1 ± 1 | 9 ± 2 |
| Squalene | ND<sup>a</sup> | ND | 5 ± 1 | 5 ± 1 |
| Others | 4 ± 2 | 5 ± 3 | 7 ± 2 | 5 ± 2 |

<sup>a</sup> CDW, cell dry weight.

<sup>b</sup> ND, not detectable.

**Fig. 2.** Phosphatidylinositol transfer activity in cytosol and 0.25 M KCl extracts of cellular membrane fractions of yeast strains US50–18c and derivatives thereof. **CYT**: cytosol; **M20**: 20,000 × g microsomes; **M30**: 30,000 × g microsomes; **M40**: 40,000 × g microsomes; **M100**: 100,000 × g microsomes. Values of at least three independent experiments with a standard deviation of ±15% are shown.
in the Pdr17 deletion strain. To distinguish between the influence of both mutations on lipid synthesis and transport to the plasma membrane, the bulk membrane lipid composition was compared with that of the plasma membrane. The changes in plasma membrane lipid composition reflect to a large extent those of total membranes. Thus, mutation of PDR16 and PDR17 rather appear to influence synthesis than transport of lipids.

In rich medium the changes of the lipid composition in the plasma membrane in the double mutant do not cause a severe growth defect. Local replacement of certain lipids appears to compensate for deficiencies caused by the Pdr16, Pdr17 mutations. Since the level of total membrane PtdCho in the Pdr16, Pdr17 strain is also increased it is most likely that the mutations cause alterations in the biosynthesis of PtdCho, probably by regulating the pathway in an as yet unknown way.

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REFERENCES

1. Balzi, E., and Goffeau, A. (1994) Biochim. Biophys. Acta 1187, 152–162
2. Balzi, E., and Goffeau, A. (1995) J. Biener. Biomembr. 27, 71–76
3. Balzi, E., Chen, W., Usaszewski, S., Capieux, E., and Goffeau, A. (1987) J. Biol. Chem. 262, 16871–16879
4. Delaveau, T., Delahodde, A., Carvajal, E., Sibtk, J., and Jacq, C. (1994) Mol. Gen. Genet. 244, 501–512
5. Carvajal, E., van den Hazel, H. B., Cybulski-Kolaczkowska, A., Balzi, E., and Goffeau, A. (1997) Mol. Gen. Genet. 256, 406–415
6. Nourani, A., Papajova, D., Delahodde, A., Jacq, C., and Subik, J. (1997) Mol. Gen. Genet. 256, 397–405
7. Montebliva, L., Sanchez, M., Pla, J., Gil, C., and Nombela, C. (1996) Yeast 12, 1097–1105
8. Daum, G., and Paltauf, F. (1984) Biochim. Biophys. Acta 794, 385–391
9. Zsolti, G., Hermetter, A., Paltauf, F., and Daum, G. (1989) Biochim. Biophys. Acta 996, 101–209
10. Bankaitis, V. A., Aiken, J. R., Cleves, A. E., and Dowhan, W. (1990) Nature 347, 561–562
11. Skinner, H. B., McGee, T. P., McMaster, C. R., Fry, M. R., Bell, R. M., and Daum, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 112–116
12. Akears, B. G., McGee, T. P., Mayninger, P., Gledville, A., Phillips, S. E., Kagwada, S., and Bankaitis, V. A. (1997) Nature 387, 101–105
13. Patton-Yogt, J. L., Griep, E., Steenwijk, A. B. V., Dovel, S., Swede, M. J., and Henry, S. A. (1997) J. Biol. Chem. 272, 20873–20883
14. Cunningham, E., Tan, S. K., Swigart, P., Hsuan, J., Bankaitis, V., and Cockeroff, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6589–6593
15. Cockeroff, S., Ball, A., Fensome, A., Hara, S., Jones, D., Prosser, S., and Swigart, P. (1997) Biochem. Soc. Trans. 25, 1125–1131
16. Cockeroff, S. (1997) FEBS Lett. 410, 44–48
17. Sambo, J., Pritsch, E. F., and Manfis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
18. Decottignies, A., Grant, A. M., Balzi, E., and Goffeau, A. (1995) J. Biol. Chem. 271, 31543–31549
19. PDR16 and PDR17 genes exhibit homology to SEC14. In contrast to the sec14 mutation, however, deletions of PDR16 and PDR17 are not lethal. Furthermore, the cellular level of Sec14p in wild-type and pdr1–3 background is the same independent of the intactness of PDR16 and/or PDR17 indicating that the expression of Sec14p is not regulated at the same level as that of the PDR16 gene product. Sec14p is a regulator of PtdCho synthesis through the Kennedy pathway (11, 55) or PtdCho turnover (12, 13). The imbalance of the PtdCho level in the Golgi caused by SEC14 dysfunction was shown to negatively influence the formation of Golgi-to-plasma membrane secretory vesicles. Further investigation will be needed to demonstrate whether Pdr16p and Pdr17p have functions similar to Sec14p, i.e. modulation of lipid levels in subcellular compartments.

3. H. Pickler, unpublished observation.
PDR16 and PDR17, Two Homologous Genes of *Saccharomyces cerevisiae*, Affect Lipid Biosynthesis and Resistance to Multiple Drugs

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