Multiple or pleiotropic drug resistance often occurs in the yeast *Saccharomyces cerevisiae* through genetic activation of the Cys6-Zn(II) transcription factors Pdr1p and Pdr3p. Hyperactive alleles of these proteins cause overproduction of target genes that include drug efflux pumps, which in turn confer high level drug resistance. Here we provide evidence that both Pdr1p and Pdr3p act to regulate production of an enzyme involved in sphingolipid biosynthesis in *S. cerevisiae*. The last step in formation of the major sphingolipid in the yeast plasma membrane, mannosyldiinositol phosphorylceramide, is catalyzed by the product of the *IPT1* gene, inositol phosphotransferase (Ipt1p). Transcription of the *IPT1* gene is responsive to changes in activity of Pdr1p and Pdr3p. A single Pdr1p/Pdr3p response element is present in the *IPT1* promoter and is required for regulation by these factors. Loss of *IPT1* has complex effects on drug resistance of the resulting strain, consistent with an important role for mannosyldiinositol phosphorylceramide in normal plasma membrane function. Direct assay for lipid contents of cells demonstrates that changes in sphingolipid composition correlate with changes in the activity of Pdr3p. These data suggest that Pdr1p and Pdr3p may act to modulate the lipid composition of membranes in *S. cerevisiae* through activation of sphingolipid biosynthesis along with other target genes.

Saccharomyces cerevisiae* cells contain three major classes of inositol-containing sphingolipids: inositol phosphorylceramide (IPC),* mannosylinositol phosphorylceramide (MIPC), and mannosylinositol phosphorylceramide (M(I)P)C. These lipids compose 30% of the total phospoholipids present in the yeast plasma membrane, with M(IP)C accounting for 75% of these yeast sphingolipids (1). Control of the biosynthesis of these inositol-containing sphingolipids (Fig. 1) is critical to maintain normal function of the plasma membrane even though production of MIPC or M(I)P)C is not required for viability (2, 3).

Although maintenance of normal sphingolipid levels is crucial for *S. cerevisiae* cell function, little information is available detailing the mechanisms that regulate their biosynthesis. Recently, microarray experiments have provided insight into a possible means of regulation of sphingolipid biosynthetic enzyme production. DeRisi et al. (4) profiled the genomic expression pattern of cells containing hyperactive forms of the Pdr1p and Pdr3p transcription factors. Pdr1p and Pdr3p are Cys6-Zn(II) transcription factors that act to modulate expression of genes involved in multiple or pleiotropic drug resistance in *S. cerevisiae* (see Refs. 5 and 6 for reviews). Previous data have demonstrated that single amino acid substitution mutations in Pdr1p (7) and Pdr3p (8) can lock these proteins into a hyperactive state, leading to high level expression of downstream target genes that include ATP-binding cassette transporters like Pdr5p (9, 10) and Yor1p (11). The work of DeRisi et al. (4) identified genes that are transcriptionally up-regulated in the presence of hyperactive alleles of Pdr1p and/or Pdr3p. These loci included previously known Pdr1p/Pdr3p target genes like *PDR5* and *YOR1* as well as new members of this regulon such as the *IPT1* locus.

*IPT1* encodes the last step in biosynthesis of sphingolipids, inositol phosphotransferase, that produces M(IP)C from MIPC (3). Microarray analysis indicated that levels of *IPT1* mRNA increased by 2-fold in the presence of hyperactive forms of Pdr1p or Pdr3p (4). Examination of the 5′-noncoding region of *IPT1* suggested the presence of a Pdr1p/Pdr3p response element (PDRE), the binding site for these transcriptional regulatory proteins (12). In the work described here, we provide evidence that this PDRE is required for the previously observed induction of *IPT1* gene expression by hyperactive alleles of *PDR1* and *PDR3*. Additionally, we have found that loss of the mitochondrial genome (ρ0 cell) leads to activation of Pdr3p, but not Pdr1p (13). Transcription of *IPT1* is induced in ρ0 cells in a fashion parallel to that of *PDR3*. Finally, the effects on biosynthesis of phospholipids are assayed in response to loss of the mitochondrial genome alone or simultaneous to loss of *PDR3*. These data strongly suggest that sphingolipid biosynthesis is a physiological target of Pdr1p/Pdr3p regulation and provide the first description of how production of these important lipids is transcriptionally regulated.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Media, and Methods**

*S. cerevisiae* strains (Table I) were grown in YPD medium (2% yeast extract, 1% peptone, and 2% dextrose) or minimal medium (14) supplemented with casamino acids (synthetic complete medium) at 30 °C. Drug resistance assays were performed by addition of compounds to solid medium at the indicated concentrations or by use of gradient plates as described (15).
**Sphingolipids and Multidrug Resistance in *S. cerevisiae***

**Plasmids**

A fragment of the IPT1 promoter extending from -621 to +4 was generated by PCR using the primer pair GCG GAT CCA TGC TTT CTT AGA CGT TGA AAG AC and CCG AAT TCC GCA TGG GGA AAT GCA AGG CAA GC. This DNA fragment was digested with EcoRI and BamHI and inserted into pBluescript KS II to form pL11. This same EcoRI/BamHI fragment was also transferred into pSEVC102 (16) to produce an IPT1-lacz gene fusion (pL2L2). Using pL11 as a template, a mutant form of the IPT1 PDRE was generated by PCR employing the primer pair CTT CCT GAG GAA CAA AAA TGT GAA CCG and GTA AAA CGA CGG CCA GT (M13 forward primer) along with the primer pair GGC TGC AAG AAG CGG CG and AAC AGC TAT GAC CAT G (M13 reverse primer). These products were isolated, and aliquots were pooled and reamplified with M13 forward and reverse primers. The resulting PCR fragment was cloned as an EcoRI/BamHI fragment into pBluescript SK and was transferred to E. coli DH5α, which was used to transform cells to Ura3 selection. Preparation of whole cell lipid extracts was performed according to protocol IIIB as described (23). If necessary, lipids were deacylated by mild alkaline hydrolysis (24) and afterward partitioned between butanol and water as described (25). The dried lipid extracts were resuspended in 100 µl (pulse-labeled cells) or 30 µl (steady-state labeled cells) of chloroform/methanol/water (10:10:3, v/v/v) per 107 cells. Quantification of [32P]-labeled lipids was performed using a Fuji FLA2000 phosphoimager (Fuji Photo Film, Tokyo, Japan). All bands were identified using authentic standards, except for ceramide, which was identified due to its sensitivity to fumonisin B1 in combination with its Rp value. Authentic sphingolipid standards were provided by Robert L. Lester (University of Kentucky, Lexington, KY).

**RESULTS**

**IPT1 mRNA Levels Respond to Changes in Pdr1p Activity**

A possible regulatory influence of the Pdr1p and/or Pdr3p transcription factor on sphingolipid biosynthesis was suggested by two complementary observations. First, inspection of the IPT1 promoter sequence indicated the presence of a sequence element identical to known PDREs located from -454 to -445 base pairs upstream of the IPT1 ATG codon. The IPT1 PDRE has the sequence TCCCGGGAA, which is identical to known PDREs located in PDR3 (26) and PDR5 (12). Second, microarray experiments identified IPT1 as a potential target gene of hyperactive forms of both Pdr1p and Pdr3p (4). To confirm that IPT1 mRNA levels respond to alterations in the activity of Pdr1p, we examined the expression of IPT1 mRNA by Northern analysis. Total RNA was isolated from cells carrying five different alleles of PDR1. These alleles covered a range of activity varying from no Pdr1p (pdr1Δ2Δ) to the hyperactive alleles (PDR1-1, PDR1-3, and PDR1-6), which produce higher levels of drug resistance and target gene expression than the wild-type gene. IPT1 mRNA was analyzed along with ACT1 as a control for loading (Fig. 2).

This analysis is entirely consistent with the previous microarray data and demonstrates that IPT1 mRNA levels were highest in the cells carrying the hyperactive alleles and were reduced in wild-type or pdr1Δ2Δ-containing cells. We conclude from these data that the expression of the genomic copy of IPT1 is sensitive to the level of Pdr1p activity. To determine if the
influence of Pdr1p was exerted at the level of the IPT1 promoter, we analyzed the regulation of an IPT1-lacZ fusion gene.

**Pdr1p Control of IPT1 Expression Is Mediated through the IPT1 Promoter**—To facilitate measurement of IPT1 gene expression, we fused a DNA fragment extending from the ATG codon, is required for this regulatory effect, but that this single PDRE also mediates the response of IPT1 to Pdr3p as discussed below.

**Pdr1p Directly Interacts with the PDRE at -450**—The presence of a PDRE in the promoter of IPT1 suggests that Pdr1p (and likely Pdr3p) will directly interact with this DNA element as seen in a variety of other Pdr1p/Pdr3p-regulated genes. To test this idea, DNase I protection experiments were carried out examining the ability of bacterially produced Pdr1p and Pdr3p to bind to the IPT1 promoter (Fig. 3).

DNase I reactions performed in the absence of protein or the presence of protein extract prepared from bacterial cells containing the empty expression vector were indistinguishable. Extracts prepared from cells expressing the DNA-binding domains of Pdr1p or Pdr3p were able to inhibit DNase I cleavage over sequences corresponding to the IPT1 PDRE. Both factors protect approximately equal regions of the promoter and induce strong DNase I-hypersensitive cleavage sites on both DNA strands between the PDRE and the IPT1 ATG codon. This analysis demonstrates that both Pdr1p and Pdr3p can directly bind to the IPT1 PDRE and supports the view that this binding is important in transcriptional control of IPT1.

**IPT1 Responds to Pdr3p and Mitochondrial Status**—Recently, we have found that the activity of Pdr3p is strongly regulated by the mitochondrial status of cells (13). Loss of the mitochondrial genome or the F_1F_0-cytochrome oxidase assembly factor Oxa1p leads to strong activation of PDR5 transcription in a Pdr3p-dependent fashion. To determine if IPT1 expression might also be regulated in response to loss of mitochondrial function, we examined the expression of the IPT1-lacZ fusion gene in ρ^0 cells (Table III).

Loss of the mitochondrial genome led to an increase in IPT1-lacZ expression from 15 units/OD in wild-type cells to 31 units/OD in ρ^0 cells. Introduction of the hyperactive allele of PDR3, PDR3-11, into wild-type cells along with the IPT1-lacZ fusion gene led to production of 28 units/OD of β-galactosidase activity. These data argue that Pdr3p can influence expression of IPT1 and that this gene is induced in response to loss of the mitochondrial genome. This finding led us to examine if changes in sphingolipid composition/synthesis could be detected in ρ^0 cells.

**Sphingolipid Metabolism Is Altered in ρ^0 Cells**—The above

| Strain   | Genotype | Source  |
|----------|----------|---------|
| SEY6210  | MATa leu2-3-112 ura3-52 his3Δ200 trp1Δ190 lys2-801 suc2-Δ9 Mel^* | Scott Emr |
| PB2      | MATa leu2-3-112 ura3-52 his3Δ200 trp1Δ190 lys2-801 suc2-Δ9 Mel^* pdr3Δ1::hisG | Ref. 10 |
| PB3      | MATa leu2-3-112 ura3-52 his3Δ200 trp1Δ190 lys2-801 suc2-Δ9 Mel^* pdr1Δ2::hisG | Ref. 10 |
| PB4      | MATa leu2-3-112 ura3-52 his3Δ200 trp1Δ190 lys2-801 suc2-Δ9 Mel^* pdr1Δ2::hisG pdr3Δ1::hisG | Ref. 10 |
| IL125-2B | MATa his1 (parent of DR19-T8) | Ref. 49 |
| DR19-T8  | MATa his1 PDR1-3 | Ref. 50 |
| US50-18C | MATa ura3 his1 PDR3-3 | Ref. 49 |
| D1-3/3   | MATa ura3 his1 pdr1Δ1::URA3 | Ref. 49 |
| US50-ipt1| MATa ura3 his1 PDR3-1 ipt1Δ1::URA3 | This work |
| US54-17B | MATa ura3 his1 PDR1-2 | Ref. 49 |
| US54-ipt1| MATa ura3 his1 PDR1-2 ipt1Δ1::URA3 | This work |
| D298-2A  | MATa ade1 his1 | Ref. 51 |
| BOR2-XI  | MATa ade1 his1 PDR1-6 | Ref. 51 |

**TABLE I**
*S. cerevisiae* strains used

**Fig. 2. IPT1 mRNA responds to increases in PDR1 function.** Total RNA was prepared from strains expressing the indicated alleles of **PDR1**. Equal amounts (30 μg) of RNA were electrophoresed through a denaturing agarose gel, transferred to nylon membranes, and probed with radiolabeled fragments from the genes indicated on the left. The **ACT1** signal served as a control to ensure equal loading of each lane.
data indicate that \textit{IPT1} gene expression is both responsive to Pdr1p/Pdr3p control and induced in response to loss of the mitochondrial genome. These findings suggest the possibility that \textit{PDR3} activation following loss of the mitochondrial genome might alter the efficiency of the sphingolipid biosynthetic pathway. To examine this idea, we analyzed the rates of sphingolipid synthesis by short-term labeling of the cells with \[^{3}H\]DHS or \[^{3}H\]serine. In contrast to endogenously synthesized DHS, exogenously added DHS is efficiently phosphorylated upon uptake (28) and must be dephosphorylated before it will be converted to phytocholine and phosphatidylethanolamine, and phosphatidylcholine were also labeled from DHS via conversion of DHS 1-phosphate into palmitate, due to the action of the sphingoid base phosphate lyase Dpl1p (27). The loss of the mitochondrial genome in the \(\rho^{0}\) strain led to a different labeling pattern compared with the wild type. The amount of MIPC was reduced, whereas pyrimidine cleavage is noted by CT. The location of the PDRE on each strand is shown on the sides of the panels by the open boxes.

### Table II

**Pdr1p control of IPT1 expression**

The indicated lacZ fusion genes were introduced into several different strains with different complements of \textit{PDR1} and \textit{PDR3}. Transformants were grown to mid-log phase, and \(\beta\)-galactosidase activities were determined in protein extracts as described (21). \textit{IPT1}-lacZ is a gene fusion between the wild-type \textit{IPT1} promoter and lacZ, whereas mPDRE-IPT1-lacZ is the same construct, but with the PDRE altered by site-directed mutagenesis as described under “Experimental Procedures.” PDR5-lacZ is a control for a known Pdr1p/Pdr3p-responsive gene (10).

| Strain | \textit{PDR1}/\textit{PDR3} alleles | IPT1-lacZ | mPDRE-IPT1-lacZ | PDR5-lacZ |
|--------|-----------------------------------|-----------|-----------------|-----------|
| SEY6210 | \textit{PDR1}/\textit{PDR3} | 29 \pm 8 | 21 \pm 6 | 166 \pm 40 |
| PB2   | \textit{PDR1}/\textit{pdr3-}\(\Delta\)::hisG | 33 \pm 7 | 21 \pm 5 | 134 \pm 37 |
| PB3   | \textit{pdr1}\(\Delta\)::hisG/\textit{PDR3} | 30 \pm 9 | 22 \pm 9 | 151 \pm 28 |
| PB4   | \textit{pdr1}\(\Delta\)::hisG/\textit{pdr3-}\(\Delta\)::hisG | 30 \pm 6 | 26 \pm 4 | 12 \pm 3 |
| PB4/PDR1 | \textit{PB4}/pRS315-PDR1 | 32 \pm 3 | 23 \pm 6 | 103 \pm 17 |
| PB4/PDR1-3 | \textit{PB4}/pRS315-PDR1-3 | 166 \pm 29 | 20 \pm 4 | 4210 \pm 809 |
| PB4/PDR1-6 | \textit{PB4}/pRS315-PDR1-6 | 131 \pm 26 | 18 \pm 4 | 3096 \pm 320 |

### Table III

**\textit{IPT1} expression responds to \textit{PDR3} and mitochondrial status**

Wild-type (SEY6210 (\(\rho^{+}\))) or mitochondrial genome-deficient (SEY6210 (\(\rho^{0}\)) versions of SEY6210 were transformed with the indicated lacZ gene fusions and \textit{PDR3} expression plasmids. Transformants were grown to mid-log phase and assayed for \(\beta\)-galactosidase activity using permeabilized cells (22).

| Strain | \textit{IPT1}-lacZ | mPDRE-IPT1-lacZ |
|--------|-----------------|-----------------|
| SEY6210(\(\rho^{+}\)) | 15 \pm 4 | 15 \pm 5 |
| SEY6210(\(\rho^{0}\)) | 31 \pm 6 | 16 \pm 5 |
| SEY6210(\(\rho^{0}\))/\textit{pRS315} | 15 \pm 3 | 10 \pm 1 |
| SEY6210(\(\rho^{0}\))/\textit{pRS315-PDR3-11} | 28 \pm 2 | 10 \pm 1 |

\(S. Schorling, B. Vallée, W. P. Barz, H. Reizman, and D. Oesterhelt, submitted for publication.\)
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the increase in IPC-IV and M(IP)2C and again showed the decreased MIPC amounts in the ρ⁰ strain compared with those in the wild type. Again, introduction of the Δpdr3 allele into the ρ⁰ background produced a strain that had near-normal MIPC and M(IP)2C levels, but still exhibited increased production of IPC-IV. These data confirm that sphingolipid synthesis is altered in ρ⁰ cells in a Pdr3p-dependent fashion.

Finally, to confirm that the altered sphingolipid biosynthetic rates result in a change in steady-state sphingolipid composition, phosphate labeling was employed. Cells were labeled with [32P]orthophosphate for an extended period of time, and total lipids were extracted. After mild alkaline treatment, the phosphate-labeled lipids were resolved by thin-layer chromatography and analyzed using the phosphoimager. This analysis confirmed that levels of M(IP)2C increased in ρ⁰ cells compared with a wild-type strain. This increase was reversed by nearly 60% to near-normal levels upon introduction of a Δpdr3 allele into the ρ⁰ background. Note that although the increase in M(IP)2C was always seen, the magnitude of the change was modest in these phosphate labeling experiments. Possible reasons for this are discussed below.

**IPT1 Is Required for Normal Drug Tolerance Phenotypes**—As the above data demonstrate that IPT1 expression responds to both Pdr1p and Pdr3p and that sphingolipid metabolism is altered in response to activation of Pdr3p, the role of IPT1 in drug resistance was analyzed. Isogenic strains carrying either the wild-type or deleted forms of the IPT1 gene were assayed by testing the ability to grow on medium containing various drugs (Fig. 5).

Loss of the IPT1 gene had differential effects on the various resistance phenotypes tested. Introduction of the ipt1-Δ1:URA3 allele into strains carrying either the PDR1-3 or PDR1-2 mutant form of PDR1 caused resistance to the translation inhibitor hygromycin to increase. Interestingly, deletion of the hyperactive allele of the PDR1 gene also caused a similar increase in hygromycin tolerance. Similarly, loss of IPT1 from the PDR1-3 strain led to an increase in resistance to cycloheximide.

A different pattern of phenotypic behavior was seen for the mitochondrial ATPase inhibitor oligomycin and the antifungal drug t-buconazole. Removal of IPT1 from the PDR1-3 strain led to a pronounced decrease in tolerance to these two compounds. The role of Pdr1p and levels of M(IP)2C in drug resistance phenotypes is complex and required for normal drug resistance.

**DISCUSSION**

Sphingolipids are important components of eukaryotic membranes and participate as signal transduction intermediates in regulatory pathways from humans to yeast. Rapid progress has been made in *S. cerevisiae* in the establishment of gene-enzyme relationships, but the understanding of the functional roles of these lipids is limited (1). Analysis of the IPT1 gene indicated that loss of this gene leads to a modest increase in calcium tolerance (3) and resistance to syringomycin E (31). Changes in calcium tolerance have been seen for many mutants lacking the normal complement of sphingolipid biosynthetic loci (32), and syringomycin E is believed to directly interact with mature sphingolipids and sterols (33, 34). Here we extend the functional roles of M(IP)2C in the cell by showing a requirement for synthesis of this lipid to ensure normal drug resistance phenotypes. The effect of M(IP)2C varies in relation to the different drugs assayed, suggesting that this lipid may play different roles in determining the activity of various membrane proteins or the permeability properties of membranes.

The differential resistance phenotypes observed in cells lacking IPT1 suggest that lowered levels of M(IP)2C or altered levels of other sphingolipids may act to inhibit some transporters, like Pdr5p, yet stimulate others, like Yor1p. It is interesting to compare the resistance phenotypes of IPT1 or PDR1 disruption mutations in terms of the ability to grow on hygromycin- or oligomycin-containing medium. Loss of either IPT1 or PDR1 seems to have the same relative effect on these two drugs, suggesting that Pdr1p-dependent activation of IPT1 expression might be the cause of the sensitivity or resistance seen for hygromycin or oligomycin. Although the locus modulating hygromycin resistance remains unknown, it is not likely to be PDR5 (35). Activation of IPT1 by Pdr1p leads to a reduction in the ability to tolerate hygromycin, but an increase in oligomycin resistance. We have already demonstrated that Pdr1p-dependent activation of YOR1 expression elevates resistance to oligomycin (36) and have now shown that only in the presence of a wild-type IPT1 locus can this increased oligomycin resistance be seen (Fig. 5). In opposition to the effect on oligomycin tolerance, elimination of IPT1 leads to an increase...
in cycloheximide resistance, a phenotype significantly dependent on PDR5 function (37). Further experiments are required to determine if the changes in membrane sphingolipid content influence the activity, trafficking, or synthesis of these transporters.

Identification of IPT1 as a target gene for PDR gene regulation has important implications for the understanding of the physiology defined by the PDR regulon. Previous experiments from a variety of laboratories have determined that a number of different membrane transporter proteins are controlled by Pdr1p and/or Pdr3p at the transcriptional level (9, 36, 38, 39). Although the drug resistance effects of these membrane transporters are clear, their normal functions are not, with the possible exception of Pdr5p and Yor1p. Experiments performed using a fluorescent phosphatidylethanolamine derivative have indicated that these two ABC transporters may be involved in controlling the phospholipid content of the plasma membrane (40, 41). Further implication of the PDR system in control of membrane content has come from the finding that Pdr17p, a putative phospholipid transfer protein, is regulated by Pdr1p/Pdr3p and is involved in phosphatidylserine metabolism (42, 43). Coupled with the definition of IPT1 as a Pdr1p/Pdr3p-regulated gene, these data strongly suggest that a physiological role of PDR genes may be to modulate the composition of membranes in S. cerevisiae.

The relatively small but highly reproducible change in \( \text{MIP}_2 \text{C} \) levels seen in \( \rho^- \) cells may be explained in several different ways. First, the rate-limiting step in biosynthesis of \( \text{MIP}_2 \text{C} \) is likely to occur upstream of Ipt1p at the first step in the pathway, serine palmitoyltransferase (1). Second, turnover of \( \text{MIP}_2 \text{C} \) may also be elevated in these cells to maintain a relatively constant level of this sphingolipid. It is notable that the elevated levels of IPC-IV seen in a \( \rho^- \) cell are not restored to normal upon the loss of PDR3 (Fig. 4). This indicates the presence of at least one additional regulatory pathway acting on sphingolipid biosynthetic loci that could also influence \( \text{MIP}_2 \text{C} \) synthesis. Third, the 300% increase in \( \text{MIP}_2 \text{C} \) synthesis detected by pulse labeling with serine compared with the smaller change in steady-state levels may reflect the contribution from salvage pathways that enter above the initial serine palmitoyltransferase reaction. Finally, measurement of \( \text{MIP}_2 \text{C} \) was accomplished using total lipids. Since \( \text{MIP}_2 \text{C} \) is highly enriched in lipid raft domains (44), the actual increase in the effective concentration might be much greater in terms of localized effects. Experiments are underway to distinguish between these possibilities.

Along with their role in maintaining the normal structure of membranes, sphingolipid derivatives have been found to potent second messengers. Ceramide is a key modulator of the stress response, often serving an antiproliferative role in control of growth during an environmental challenge (see Ref. 45 for a review). In S. cerevisiae, heat shock of cells was observed to cause an elevation of ceramide levels and related metabolites like DHS, phytosphingosine, and their phosphorylated products (46, 47). Ablation of the genes encoding proteins responsible for DHS 1-phosphate and phytosphingosine 1-phosphate breakdown caused large enhancements of heat resistance and supports the notion that these sphingolipid intermediates are involved in the heat stress response (27, 48).

We have consistently observed a small but reproducible increase in ceramide production in \( \rho^- \) cells compared with the \( \rho^+ \) parental strain, which is unaffected by loss of PDR3. The availability of strains bearing different genetic blocks in ceramide metabolism will allow testing of the possibility that ceramide or some other sphingolipid metabolite might be the signal leading to Pdr3p activation in response to defects in the mitochondrion. In larger eukaryotes, ceramide signaling is an important modulator of cell viability through control of entry into apoptosis (reviewed in Ref. 45). Our finding that Pdr3p both controls sphingolipid biosynthesis and in turn is responsive to mitochondrial status (13) suggests the possibility that this regulatory circuit also exists in S. cerevisiae. Further analysis of the relationship between PDR loci, mitochondrial function, and sphingolipid biosynthesis will allow this hypothesis to be tested.

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