Multiple or pleiotropic drug resistance most often occurs in *Saccharomyces cerevisiae* due to substitution mutations within the Cys$_6$-Zn(II) transcription factors Pdr1p and Pdr3p. These dominant transcriptional regulatory proteins cause elevated drug resistance and overexpression of the ATP-binding cassette transporter-encoding gene, PDR5. We have carried out a genetic screen to identify negative regulators of PDR5 expression and found that loss of the mitochondrial genome (p$^r$ cells) causes up-regulation of Pdr3p but not Pdr1p function. Additionally, loss of the mitochondrial inner membrane protein Oxa1p generates a signal that results in increased Pdr3p activity. Both of these mitochondrial defects lead to increased expression of the PDR3 structural gene. Importantly, the signaling pathway used to enhance Pdr3p function in p$^r$ cells is not the same as in oxa1 cells. Loss of previously described nuclear-mitochondrial signaling genes like RTG1 reduce the level of PDR5 expression and drug resistance seen in p$^r$ cells but has no effect on oxa1-induced phenotypes. These data uncover a new regulatory pathway connecting expression of multidrug resistance genes with mitochondrial function.

The appearance of multidrug-resistant cells in human tumors is often associated with overproduction of ABC-binding cassette (ABC) transporter proteins, like Mdr1 or Mrp (1, 2). These plasma membrane-localized molecules act as ATP-dependent drug efflux pumps and eliminate chemotherapeutic agents from cells, allowing resistance to these cytotoxic drugs (3). In the yeast *Saccharomyces cerevisiae*, a similar multidrug resistant phenotype also typically involves overproduction of ABC transporter proteins and is referred to as pleiotropic drug resistance (Pdr) (reviewed in Ref. 4). Examples of the relevant ABC transporter genes that are overproduced in multiple drug resistance (Pdr) (reviewed in Ref. 4). Examples of the relevant ABC transporter genes that are overproduced in multiply drug-resistant cells include *PDR5* and *YOR1*, loci that confer drug resistance to cycloheximide and oligomycin, respectively (5–8).

Loss of normal transcriptional control of these *S. cerevisiae* ABC transporter protein-encoding genes is frequently associated with single amino acid substitutions within related Cys$_6$-Zn(II) transcription factors designated PDR1 (9) and PDR3 (10). Previous work has shown that these substitution mutant forms of Pdr1p (11) and Pdr3p (10) behave as dominant, hyperactive transcriptional regulatory proteins and elicit marked overproduction of target genes like *PDR5* and *YOR1* (8, 12). Both Pdr1p and Pdr3p bind to a sequence element referred to as the Pdr1p/Pdr3p response element (PDRE) that is found in the promoter region of all genes responsive to these transcription factors (13). Importantly, loss of *PDR1* or *PDR3* does not produce the same phenotypic effect on cells. Δpdr1 cells are extremely sensitive to cycloheximide or oligomycin while Δpdr3 cells are relatively normal in terms of tolerance to these drugs (10, 14). However, loss of both *PDR1* and *PDR3* causes a pronounced drug sensitivity, much greater than loss of either gene alone.

More recent work has demonstrated that Pdr1p but not Pdr3p is positively regulated by a Hsp70-related protein, Pdr13p (15). This finding indicated that, while Pdr1p and Pdr3p are 36% identical across their lengths, these proteins are regulated by different mechanisms. Since several different substitution mutations could be isolated that produced hyperactive forms of Pdr1p or Pdr3p, we set out to examine the possibility that these mutant proteins were able to escape negative regulation. To test this hypothesis, we utilized transposon mutagenesis to identify loci that served as negative regulators of *PDR5* gene expression through reduction of Pdr1p or Pdr3p function. From this screen, three different genes were identified that acted genetically as negative regulators of *PDR5* expression and resulting cycloheximide resistance: *FZO1*, encoding a mitochondrial GTPase required for normal organelle inheritance (16); *OXA1*, an inner mitochondrial membrane protein involved in biogenesis of cytochrome c oxidase (17); and *TIM17*, a gene encoding a component of machinery required to import proteins across the inner membrane (18). Similar induction of *PDR5* transcription was found when cells were induced to lose the mitochondrial genome (p$^r$). These data provide important new insight into the coordination of *PDR* gene expression with the functional status of the mitochondria and suggest that activation of ABC transporter gene expression may be required for cell viability upon compromise of mitochondrial function.

**MATERIALS AND METHODS**

**Yeast Strains and Media**—The genotypes of the yeast strains used in this study are listed in Table I. Yeast transformations were performed using the lithium acetate procedure (19) or a high-efficiency technique (20). StandardYPD (1% yeast extract, 2% peptone, 2% dextrose) and synthetic complete medium (21) were used for growth of cells and drug spot test assay (22). β-Galactosidase activity determinations using o-nitrophenyl-β-D-galactopyranoside hydrolysis was measured as described previously (23). Luminescent β-galactosidase assays were performed following the manufacturer’s specifications (CLONTECH). p$^r$ Derivatives of strains were generated using 25 μg/ml ethidium bromide as described in Ref. 24 and loss of mitochondrial DNA was assessed by 4,6-diamidino-2-phenylindole staining and visualizing by fluorescence.
microscopy. Gradient plate assays were carried out as described (8).

Selection for Cycloheximide-resistant Strains with Linked Transposon Insertions—A wild-type strain (SEY210) containing an integrated PDR5-lacZ gene was transformed with a transposon-mutagenized S. cerevisiae genomic library (25) using a high-efficiency protocol. Approximately 5000 transformants were plated on SC (21) plates containing 0.25 μg/ml cycloheximide. 14 transformants were recovered that exhibited elevated cycloheximide resistance. These transformants were then assayed for the level of expression of their integrated PDR5-lacZ reporter gene. Colonies with both elevated β-galactosidase expression and cycloheximide resistance were crossed to a wild-type strain and the resulting diploids subjected to standard genetic analysis. 5 colonies were found in which both the PDR5 overproduction and cycloheximide hyper-resistance phenotypes were linked to the transposon. The locations of transposon insertions in the genome were determined using plasmid rescue as described (25).

Plasmids—Low-copy number plasmids containing lacZ gene fusions with the TRP5 (tryptophan synthase), PDR5 (plasma membrane ABC transporter), CTT1 (cytosolic catalase), CUP1 (copper metallothionein), and HSP12 (small heat shock protein) (15) or GSH1 (γ-glutamylcysteine synthetase) (26) and TRX2 (thioredoxin) (27) genes were described

RESULTS

Genetic Screen for Negative Regulators of Pleiotropic Drug Resistance—Previous searches for mutants with an enhanced pleiotropic drug resistance phenotype have identified alterations in the genes encoding the zinc finger-containing transcription factors Pdr1p and Pdr3p (28). Briefly, PCR primers were designed with 50 bp from either the 5’ or 3’ end of the RTG2 gene and with 16 bp specific for amplification of the his5+ gene. The rtg2Δ::his5+ allele was generated using the 5’ primer GTGTCCTTTACTAAGGTGGTTGTGAAC-GTTTAAAAGTGTAGGCGTGCCACAACGGATCCCCGGGTTAATTAA and the 3’ primer GTGTCCTTTACTAAGGATTGTTTTGAACG-GTTTAAAAGTGTAGGCGTGCCACAACGGATCCCCGGGTTAATTAA.

TABLE I

| Strain description | Genotype | Source or ref. |
|--------------------|----------|---------------|
| SEY2610            | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | Scott Emr |
| TCH2               | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| oxax1Δ::Tn3        | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| fzo1Δ::Tn3         | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| tim17::Tn3         | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| W303               | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Artg2Δ             | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | Dieter Wolf |
| Δfo1Δ              | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | David Bedwell |
| Δfo1Δ, Artg1       | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, Artg2Δ      | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, Artg2Δ, Artg1Δ | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, Artg2Δ, Artg3Δ | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, Artg2Δ, [p+] | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, Artg3Δ, [p+] | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, [p+]        | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |
| Δfo1Δ, [p+]        | MATα leu2-3,112 ura3-52 lys2-801 trp1Δ::kanMX his3Δ200 sec6-2Δ9 Mel- | This study |

This study was carried out by transforming a wild-type strain containing an integrated PDR5-lacZ fusion gene with a yeast genomic library containing random transposon insertions (25). Approximately 5000 transformants were grown on selective media containing 0.25 μg/ml cycloheximide. Cycloheximide was included in the plates as an indicator of PDR5 expression, the key target gene for Pdr1p/Pdr3p-mediated cycloheximide resistance (12, 14). Cycloheximide hyper-resistant colonies were isolated and assayed for PDR5-lacZ-dependent β-galactosidase activity to ensure that PDR5 was also overproduced. The 14 transformants that exhibited both enhanced cycloheximide
Mitochondrial Regulation of PDR Gene Function

One concern underlying the isolation of these transposon insertion alleles is the possible production of truncated gene products that could possibly give rise to the observed phenotypes. To ensure that the effects on drug resistance and PDR5 expression seen in these transposon mutants were due to loss of gene function, we constructed two deletion derivatives in which either the entire coding sequence of FZO1 or a large segment of the OXA1 coding sequence was removed and replaced with *kanMX4* or *S. cerevisiae HIS3*, respectively. The essential role of TIM17 precluded a similar type of analysis for this locus and the transposon-generated allele was assayed for comparison to the gene deletion alleles of FZO1 and OXA1. These deletion mutants were then tested for drug resistance using medium containing a gradient of increasing concentration of cycloheximide (Fig. 2).

The yeast copperthionein-encoding gene, *GSH1,* and a large heat shock protein-encoding locus, *HSP12*, were also assayed for their response to these mitochondrial defects. *GSH1* showed little response to alterations in mitochondrial function, whereas *HSP12* responded to activation also independent of gene function, we constructed two deletion derivatives in order to facilitate strain construction. PDR5-dependent β-galactosidase was also assayed to quantify the effects of these mutants on expression of PDR5 (Fig. 2). Expression of a gene that is not a member of the Pdr1p/Pdr3p regulon, a gene involved in tryptophan biosynthesis (*TRP5* (37)), was also assayed as a control for the specificity of the effect of loss of FZO1, OXA1, or TIM17.

Wild-type cells carrying a PDR5-lacZ fusion gene produce approximately 72 units/OD of β-galactosidase activity. Expression of PDR5 increased 4-fold to 308 units/OD upon loss of OXA1. The Δfzo1 and tim17 mutations both led to a over a 10-fold increase in PDR5 expression generating 834 and 1116 units/OD, respectively. PDR5-lacZ expression in the Δfzo1 and tim17 mutant backgrounds is comparable to the highest observed PDR5 expression generated by gain-of-function alleles of Pdr1p and Pdr3p (11, 38). Expression of the TRP5-lacZ gene fusion showed little response to alterations in mitochondrial function, varying from 22 units/OD in the wild-type and *oxa1* strains to approximately 13 units/OD of β-galactosidase activity in the Δfzo1 and tim17 backgrounds.

Loss of FZO1, OXA1, or TIM17 Does Not Lead to Wide Scale Induction of Stress-responsive Gene Expression—While expression of the TRP5 gene is not significantly influenced in this collection of mitochondrial mutant strains, it is possible that compromising the mitochondrial function might elicit stress responses in the cell that would not be expected to alter TRP5 expression. As previous work has suggested that PDR5 may be a stress-responsive gene (39), we examined the possibility that the mutants we isolated that led to PDR5 activation also induced expression of other stress-related genes. We analyzed a variety of stress-responsive genes and found that none exhibited the same level of induction as seen at the PDR5 promoter.

The stress genes tested included the cytosolic catalase gene *CCT1* (40) and a small heat shock protein-encoding locus, *HSPI2* (41). Both of these genes are regulated by the stress-responsive transcription factors Msn2p and Msn4p (42, 43). The yeast copperthionein-encoding gene, *CUP1* (44), was also assayed for its response to these mitochondrial defects. *CUP1* is responsive to activation of the yeast heat shock transcription factor (45, 46). Finally, the oxidative stress-regulated genes *GSH1* and *TRX2* were examined in these different genetic backgrounds. Both *GSH1* and *TRX2* are regulated by the basic
Mitochondrial Regulation of PDR Gene Function

Fig. 2. Drug resistance and expression phenotypes of strains lacking normal mitochondrial function. A, wild-type (SEY6210) and isogenic derivatives carrying the indicated gene disruptions were grown to mid-log phase and assayed for cycloheximide resistance on YPD medium containing a gradient of drug. The concentration of cycloheximide increases from left to right as denoted by the bar of increasing width. The highest concentration of drug is 0.5 μg/ml cycloheximide. 1000 cells of each type were placed along the gradient and the plate allowed to develop at 30 °C. B, wild-type cells or isogenic mutant strains were transformed with low-copy number plasmids containing the indicated lacZ gene fusions. Transformants were grown in minimal medium to mid-log phase and assayed for plasmid-dependent β-galactosidase activity as described (23). Activities are expressed as units/μmol of cells.

region-leucine zipper-containing transcription factor Yap1p and are induced upon oxidative stress in cells (26, 47, 48). TRX2 expression is also controlled by the stress-responsive transcription factor Skn7p (49). Together, this collection of genes represents targets for most of the major stress-responsive transcription factors in the cell.

CUP1-lacZ expression increased 2-fold in an oxa1 background and 300% in the Δfzol and tim17 backgrounds (Fig. 2). HSP12-lacZ expression increased 2-fold in the Δfzol and tim17 strains but was unchanged in the presence of the oxa1 strain. CTT1-lacZ, GSH1-lacZ, and TRX2-lacZ expression was unaffected by any of the mutant backgrounds. The 2–3-fold increase in CUP1 and HSP12 expression was consistent with the generation of a mild stress response upon loss of the normal function of either FZO1 or TIM17. However, in these same genetic backgrounds, PDR5 expression was increased by 10-fold. We interpret these data to support the idea that the elevation in PDR5 expression represents one of the normal avenues used to respond to loss of mitochondrial function rather than a global activation of stress-responsive genes.

Loss of the Mitochondrial Genome Is Sufficient to Induce PDR5 Expression—An important feature of cells lacking the FZO1 gene is the concomitant loss of the mitochondrial genome (16). This absence of mitochondrial DNA is referred to as the ρ0 state (50) and importantly cannot be complemented through introduction of the wild-type FZO1 gene (16). To determine if the observed activation of PDR5 expression and drug resistance was due to loss of FZO1 or the cells becoming ρ0, we introduced a low-copy plasmid carrying FZO1 back into the Δfzol1 strain. Although these transformants contained wild-type FZO1 gene function, the high level PDR5 expression and drug resistance phenotype was not affected (data not shown). This suggested that loss of the mitochondrial genome was sufficient to trigger elevation of PDR5 expression. To evaluate this possibility, ρ0 derivatives of two different wild-type strains were generated corresponding to our standard wild-type strain (SEY6210) or a second commonly used wild-type strain (W303).

Loss of the mitochondrial DNA in either wild-type strain produced a marked increase in cycloheximide tolerance (Fig. 3). Similarly, PDR5 expression was increased by nearly 6-fold in the ρ0 derivative of SEY6210 compared with the ρ+ form. This analysis strongly suggests that loss of the mitochondrial genome results in a signal that acts to up-regulate PDR5 expression and corresponding drug resistance.

The finding that a chemically-induced ρ0 strain also exhibited high level PDR5 expression and cycloheximide resistance suggested that any mutant strain lacking normal mitochondrial function would potentially display these same resistance phenotypes. To address this issue, a mutant strain that lacked the β-subunit of the F1 mitochondrial ATPase was employed. This mutant strain is unable to grow on nonfermentable carbon sources due to an elimination of oxidative phosphorylation. The Δatp2 strain was transformed with a PDR5-lacZ reporter plasmid and with a low-copy number vector alone or carrying wild-type ATP2. Expression of PDR5 and the cycloheximide resistance phenotype of these transformants were then assayed (Fig. 4).

Loss of the ATP2 gene did not increase cycloheximide resistance and caused a modest 2-fold enhancement of PDR5 expression. Assay of TRP5-lacZ expression in the same genetic backgrounds demonstrated that TRP5-dependent β-galactosidase activity decreased from 85 units/OD in the ATP2 cells to 47 units/OD upon loss of this locus. While the Δatp2 strain is petite and lacks the ability to carry out oxidative phosphorylation, there is a minimal effect on PDR5 gene expression and no detectable effect on cycloheximide tolerance. Note that the oxa1 strain is also petite but exhibits a larger increase in PDR5 expression as well as cycloheximide resistance. These data
This plasmid contained a single PDRE from the PDR5 promoter. A selected r derivate was transformed with low-copy number plasmids carrying a TRP5- or PDR5-lacZ fusion gene. Transformants were grown to mid-log phase and assayed for β-galactosidase activity as described above. B, an ethidium bromide-treated r derivative of W303 was prepared. Our standard wild-type strain and its r derivative (SEY6210 and SEY6210 p) along with W303 and its r derivative were grown to an A500 of 1 and assayed for cycloheximide resistance using a gradient plate.

Support the idea that not all petite mutants will lead to the same PDR5-dependent phenotypes.

**Intact PDREs Are Necessary and Sufficient for Response to Mitochondrial Signals**—Previous analyses of the PDR5 promoter have indicated the central importance of three binding sites for the transcriptional regulatory proteins Pdr1p and Pdr3p in normal expression of this gene (13). These binding sites are referred to as PDREs and are found in the promoters of all genes regulated by Pdr1p or Pdr3p (13). To address the role of the three PDREs in the PDR5 promoter in the transcriptional response to these mutations lacking normal mitochondrial function, the expression of a wild-type PDR5-lacZ fusion gene was compared with that of a mutant variant lacking the three PDREs. The wild-type and PDRE-less PDR5-lacZ fusions were introduced into the various mutant backgrounds and PDR5-dependent β-galactosidase activity measured (Table II).

Removal of the PDREs from the PDR5 promoter (mPDR5-lacZ) abolished expression of the PDRS-lacZ fusion and eliminated any response to the mutant backgrounds. One concern with this result arose due to the potential presence of other transcriptional regulatory elements in the PDR5 promoter. Although this experiment demonstrates that the PDREs in the PDR5 5′ noncoding region are required for the up-regulation by mitochondrial mutants, it does not indicate that this effect is mediated through these specific binding sites.

To address this issue, a different reporter plasmid was used. This plasmid contained a single PDRE from the PDR5 promoter in place of the normal upstream activation sequences of a CYC1-lacZ gene fusion (PDRE-CYC1-lacZ) or an identical plasmid containing a mutant form of the PDRE to which neither Pdr1p nor Pdr3p could bind in vitro (mPDRE-CYC1-lacZ). These two plasmids were introduced into wild-type and Δzol1 strains, followed by measurement of CYC1-directed β-galactosidase levels (Table III).

The PDRE-CYC1-lacZ fusion plasmid produced 4.5-fold more β-galactosidase activity in the Δzol1 strain than when in a FZO1 background. Introduction of the mutant PDR5 into this same plasmid eliminated the Δzol1 induction of lacZ expression. These data strongly support the involvement of the PDREs in PDR5 as recipients of the response to mitochondrial dysfunction in these mutant backgrounds.

**Mitochondrial Signals Require Pdr3p but Not Pdr1p to Activate PDR5 Expression**—While the data above implicate the PDR5 PDREs as important participants in the response to mitochondrial defects, Pdr1p, Pdr3p, or both of these proteins are required for the transcriptional activity of cells lacking (Δatp2) or containing (ATP2) the ATP2 gene was assessed by spot test assay using a gradient plate as described above.

![Fig. 4. A mutant defective in oxidative phosphorylation has no effect on PDR5 gene function. A, a mutant strain lacking the gene encoding the β subunit of the mitochondrial ATPase was transformed with pRS314 (Δatp2) or with a plasmid containing the wild-type ATP2 gene (YCP1-ATP2: ATP2). Along with these plasmids, the TRP5-or PDR5-lacZ fusion plasmids were also introduced. Appropriate transformants were grown to mid-log phase and assayed for β-galactosidase activity. B, the relative cycloheximide resistance of cells lacking (Δatp2) or containing (ATP2) the ATP2 gene was assessed by spot test assay using a gradient plate as described above.](http://www.jbc.org/)

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**FIG. 3. Loss of the mitochondrial genome activates PDR5 expression and increases cycloheximide resistance. A, wild-type cells were treated with ethidium bromide to produce a derivative lacking mitochondrial DNA (24). A selected r derivate was transformed with low-copy number plasmids carrying a TRP5- or PDR5-lacZ fusion gene. Transformants were grown to mid-log phase and assayed for β-galactosidase activity as described above. B, an ethidium bromide-treated r derivative of W303 was prepared. Our standard wild-type strain and its r derivative (SEY6210 and SEY6210 p) along with W303 and its r derivative were grown to an A500 of 1 and assayed for cycloheximide resistance using a gradient plate.**

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**TABLE II**

| Wild-type       | β-Galactosidase activity (units/OD) |
|-----------------|------------------------------------|
|                 | oxal1                              | Δzol1 |
| TRP5-lacZ       | 31                                 | 38    |
| PDR5-lacZ       | 35                                 | 659   |
| mPDR5-lacZ      | 1                                  | 1     |

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**TABLE III**

| Wild-type       | β-Galactosidase activity (units/OD) |
|-----------------|------------------------------------|
|                 | oxal1                              | Δzol1 |
| TRP5-lacZ       | 31                                 | 38    |
| PDR5-lacZ       | 35                                 | 659   |
| mPDR5-lacZ      | 1                                  | 1     |
were constructed in Δfzo1 or oxa1 genetic backgrounds. All strains were assayed for their cycloheximide resistance phenotype. The Δfzo1 strains were also transformed with either PDR5- or TRP5-lacZ fusion plasmids and appropriate transformants then assayed for β-galactosidase activity.

Loss of the PDR3 gene completely ablated the induction of both PDR5 expression and cycloheximide resistance seen in the Δfzo1 strain (Fig. 5 and Table IV). In contrast, removal of the PDR1 gene had no significant effect on either phenotype. Loss of both genes further reduced expression of PDR5 and drug resistance. Expression of the TRP5-lacZ fusion was unaffected by these different strain backgrounds. Similarly, the increased cycloheximide resistance produced in the oxa1 background was eliminated upon disruption of PDR3, but was unaffected by loss of PDR1. These data indicate that the elevation of PDR5 expression and accompanying drug resistance requires the presence of PDR3 but not PDR1.

Components of the Retrograde Signaling Pathway Are Important for Propagating the Signal from Δfzo1/rho0 but Not from oxa1—The up-regulation of Pdr3p function in response to mitochondrial defects strongly resembles a previously described regulatory network termed retrograde regulation (51). Retrograde regulation refers to the nuclear response to reduction of mitochondrial function and involves activation of the expression of several different genes. Three genes have been identified as critical for this transcriptional up-regulation: RTG1 and RTG3 encoding two related basic helix-loop-helix transcription factors and RTG2, a gene encoding a potential ATPase (51, 52). Loss of any of these genes prevents ρ0 cells from increasing the expression of the citrate synthase-encoding locus, CIT2 (53). To determine if the retrograde signaling pathway and Pdr3p interacted, disruption mutations in RTG1 and RTG2 were constructed and assayed for drug resistance and PDR5 expression phenotypes.

Elimination of RTG1 or RTG2 from our wild-type background strain caused a modest decrease in both cycloheximide tolerance and PDR5-lacZ expression levels (Fig. 6). Similarly, introduction of the Δrtg1 or Δrtg2 mutation into an oxa1 background had no effect on either PDR5-dependent phenotype. Interestingly, TRP5-lacZ expression consistently increased, possibly due to the amino acid imbalance generated by the ρ0 alleles (51). In contrast, removal of either RTG locus from Δfzo1 cells caused a 50% reduction in PDR5-lacZ expression and a similar decline in cycloheximide resistance. These findings indicate that oxa1 activation of PDR5 expression is independent of the function of Rtg1p and Rtg2p, whereas a significant component of the Δfzo1 signal requires an intact retrograde signaling pathway.

PDR3 Requires Autoregulation and RTG1 for Induction in ρ0 Cells—Although Pdr1p and Pdr3p share 36% sequence identity across their length, a striking difference between the two structural genes encoding these proteins is the presence of two PDREs in the PDR3 promoter which are not found upstream of the PDR1 gene (54). Previous studies have provided evidence that PDR3 is subject to autoregulation that requires the presence of these PDREs (54). To determine if activation of Pdr3p function upon loss of normal mitochondrial function involved an increase in PDR3 expression, we constructed a PDR3-lacZ fusion gene and introduced this reporter construct into several different strains to evaluate PDR3 gene expression (Fig. 7).

Expression of PDR3 was very low in wild-type cells but was dramatically up-regulated in ρ0 cells. Introduction of a Δrtg1 allele into ρ0 cells decreased PDR3-lacZ expression to less than 40% the level seen in RTG1 ρ0 cells. Finally, the presence of the wild-type PDR3 structural gene was required for the induction of PDR3-lacZ expression in the ρ0 genetic background, strongly suggesting that autoregulation is the cause of the observed elevation of PDR3 expression.

These data indicate that the observed increase in Pdr3p
The function may come about through increased expression of PDR3. The \( r^o \)-induced increase in expression requires both the presence of PDR3 and RTG1 to occur. Since PDR3 expression is autoregulated, interpretation of this result was not straightforward and we examined the requirement for the PDR3 promoter in the mitochondrial regulation of Pdr3p function.

Positive Regulatory Signals from the Mitochondria and Rtg1p Influence Pdr3p Post-translationally and Not at the Level of the PDR3 Promoter—The finding that PDR3 expression is elevated in response to mitochondrial defects suggests that the promoter of this gene serves as the link between mitochondrial status and PDR5 expression. To explore this possibility, we constructed two different chimeric genes by exchanging the promoters of PDR1 and PDR3. In this fashion, we generated a PDR3 structural gene that responded to the transcriptional signals of PDR1 (PDR1:PDR3), as well as the reciprocal construct (PDR3:PDR1). The goal of this experiment was to evaluate if the observed elevation of drug resistance seen in \( r^o \) cells was linked to the presence of the PDR3 promoter only or if the PDR3 gene product was the target for response to mitochondrial defects. The chimeric genes were introduced into \( r^o \) or \( r^1 \) cells lacking both PDR1 and PDR3 as well as \( r^o \)Dpdr1,pdr3 cells carrying the \( D^D \)rtg1 allele. Appropriate transformants were then assayed for their relative cycloheximide resistance (Fig. 8).

Only the presence of the wild-type PDR3 gene or the PDR1: PDR3 chimeric gene supported the elevation in cycloheximide resistance seen in \( r^o \) cells. Neither the PDR1 gene nor the PDR3 promoter driving expression of the PDR1 structural gene (PDR3:PDR1) conferred increased cycloheximide resistance in the presence of a \( r^o \) lesion. These data provide important support for the view that the response to mitochondrial deficiency comes directly through the PDR3 gene product, which is both necessary and sufficient to enhance cycloheximide resistance (Fig. 8).

Unlike the clear dependence on the presence of the PDR3 coding sequence seen for response to the \( r^o \) state of the cell, loss of the RTG1 gene appeared to decrease drug resistance in all genetic backgrounds. We interpret this result to indicate that loss of Rtg1p reduces the overall fitness of cells in addition to the specific effect on PDR3 expression.

DISCUSSION

One of the challenges in the analysis of multidrug resistance protein is to understand the nature of the physiological role of...
the genes encoding these factors. The data reported here indicate that a physiological activator of Pdr3p and its target genes is the status of the mitochondria. Although the precise nature of the Pdr3p-inducing signal cannot be gleaned from this work, two common defects in all the mutants we have examined is the absence of a functional electron transport chain and lack of a normal F0 complex of the mitochondrial ATPase. Mutants lacking Oxa1p fail to normally assemble cytochrome \( c \) oxidase (55) while \( \rho^{o} \) cells (or \( D_fz_0 \) strains) lack mitochondrial genes that encode subunits of cytochrome \( c \) oxidase (50). Similarly, oxa1 and \( \rho^{o} \) cells do not properly produce the F0 complex of the mitochondrial ATPase, although the soluble F1 component is apparently normal (56). It is possible that the critical component that is under surveillance by Pdr3p are these integral membrane protein complexes. Alternatively, it may be the lack of normal electron transport chain function and/or F0 activity that causes activation of Pdr3p. Since we recovered only one oxa1 allele, we believe that the transposon screen is not saturated for mutations that could lead to activation of Pdr3p with subsequent elevation of cycloheximide resistance. Assessing drug resistance and PDR5 expression phenotypes of other mutations lacking wild-type electron transport chain function should shed light on the specific contribution of these proteins to Pdr3p regulation.

Irrespective of the specific signal being perceived by Pdr3p, the receptor of this signal is Pdr3p itself rather than another factor that up-regulates PDR3 expression. Our expectation is that the center region of Pdr3p is the ultimate target site for the mitochondrially-generated signal by analogy with other Cys\(_6\)-Zn(II) zinc cluster proteins like Leu3p (57, 58) and Gal4p (59). Both of these proteins are positively regulated through changes in function that are controlled by modulation of the center domain of these transcriptional regulators (60–62). Computer alignments of a large number of Cys\(_6\)-Zn(II) zinc cluster proteins suggest the presence of a conserved structural motif in this family of transcription factors (63). Interestingly, mutations that lead to high level activity of either Pdr1p or Pdr3p map to the center regions of these proteins (11, 38), supporting the idea that these domains of Pdr1p and Pdr3p will be critical in controlling their function.

The identification of Pdr3p as another member of the retrograde signaling response in \( S. \) \textit{cerevisiae} provides important insight into the complex nature of the physiological processes that are induced by mitochondrial dysfunction. Analyses of \( RTG \) genes have shown that loss of these factors produces important phenotypes even in \( \rho^{o} \) cells. For example, elimination of the \( RTG1 \) gene results in a glutamate auxotrophic strain (51). Loss of \( PDR3 \) does not have a similar effect as \( D_{pdr3} \) cells can grow in the absence of glutamate supplementation. Instead, loss of \( RTG1 \) has a relatively modest effect on drug resistance. Clearly, different loci are responsive to \( RTG \)- and \( PDR \)-encoded transcription factors.

While \( RTG \) and \( PDR \) genes appear to control distinct sets of

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In this work, we have observed that loss of the FZO1 locus produces a ρ₀ cell which has previously been shown to activate function of RTG genes. Since oxa1 induction of PDR5 expression is insensitive to loss of RTG genes, we suggest that oxa1 cells share a common pathway of Pdr3p activation with ρ₀ cells having an additional RTG-dependent component.

![Diagram](image)

**Fig. 9. Model for interaction of RTG and PDR loci.** A scheme outlining the genetic interactions between the RTG and PDR genes described in this work. RTG genes, there is strong evidence for interaction between these two pathways that both respond to mitochondrial defects. Cycloheximide resistance, PDR3 and PDR5 expression is depressed in cells that lack either RTG1 or RTG2, indicating that these genes play a role in PDR gene function. The mutants we have isolated suggest that two different signal pathways feed into modulation of Pdr3p. These pathways can be discriminated by the involvement of the RTG genes in the ultimate activity of Pdr3p. Loss of the mitochondrial genome (ρ₀, fzo1 mutants) requires RTG gene function for normal up-regulation of Pdr3p but oxa1 mutants do not. A diagram of the genetic interactions we have observed is shown in Fig. 9. While the interaction of Pdr3p with RTG genes is clear, more work is required to clarify at what level RTG genes impact on PDR gene function. Inspection of the PDR3 promoter for Rgt1p/Rtg3p-binding sites suggested that these factors are not likely to directly control expression of this locus (data not shown). Irrespective of the exact mechanisms at work here, PDR3 and the RTG genes appear to act together to elicit response to mitochondrial defects.

Finally, these studies provide valuable new insight into the physiological connections regulating PDR gene function, especially control of Pdr3p. Previously, we have observed distinct colony morphologies that appear on cycloheximide-containing medium upon loss of PDR1 or PDR3 alone (14). A small number of highly cycloheximide-resistant cells (≤5%) were found in wild-type or Δrho deletion cells but not in cells lacking the PDR3 gene. This small number of cells correlates well with the estimated occurrence of ρ₀ cells in a population of growing S. cerevisiae (50). An attractive explanation for this behavior is that in this small number of ρ₀ cells, Pdr3p is activated and PDR5 expression increases to very high levels. Understanding the nature of the Pdr3p-inducing signal elicted upon loss of normal mitochondrial activity and why activation of PDR5 gene expression is an important response to this physiological problem represent the next set of experimental goals.

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