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Multiple Pdr1p/Pdr3p Binding Sites Are Essential for Normal Expression of the ATP Binding Cassette Transporter Protein-encoding Gene *PDR5**

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***Saccharomyces cerevisiae* has large number of genes that can be genetically altered to produce a multiple or pleiotropic drug resistance phenotype. The homologous zinc finger transcription factors Pdr1p and Pdr3p both elevate resistance to many drugs, including cycloheximide. This elevation in cycloheximide tolerance only occurs in the presence of an intact copy of the *PDR5* gene that encodes a plasma membrane-localized ATP binding cassette transporter protein. Previously, we have found that a single binding site for Pdr3p present in the *PDR5* promoter is sufficient to provide Pdr3p-responsive gene expression. In this study, we have found that there are three sites in the *PDR5* 5′-noncoding region that are closely related to one another and are bound by both Pdr1p and Pdr3p. These elements have been designated Pdr1p/Pdr3p response elements (PDREs), and their role in the maintenance of normal *PDR5* expression has been analyzed. Mutations have been constructed in each PDRE and shown to eliminate Pdr1p/Pdr3p binding *in vitro*. Analysis of the effect of these mutant PDREs on normal *PDR5* promoter function indicates that each element is required for wild-type expression and drug resistance. A single PDRE placed upstream of a yeast gene lacking its normal upstream activation sequence is sufficient to confer Pdr1p responsiveness to this heterologous promoter.**

Multidrug resistance has been defined as broad range resistance to chemotherapeutic agents associated with human tumors (1). Although there are several different mechanisms that can contribute to multidrug resistance, one of the best understood involves overexpression of certain members of the ATP binding cassette transporter family of proteins: MDR1 (2–4) and the multidrug resistance-associated protein (5). Elevated levels of these transporter proteins lead to an enhanced rate of drug efflux from tumor cells with subsequent multidrug resistance (6–8). The resulting cross-resistance to varied cytotoxic agents represents a major impediment to chemotherapy.

Saccharomyces cerevisiae displays an analogous phenotype to mammalian multidrug resistance, termed pleiotropic drug resistance (Pdr).[1] The genes involved in the Pdr phenotype have thus far fallen into two general categories: membrane transporter proteins and their cognate transcriptional regulatory proteins (9). As in higher eukaryotes, several of these membrane transporter proteins are members of the ATP binding cassette transporter superfamily of proteins, including *PDR5*, *SNQ2*, and *YOR1*. *PDR5* has been shown to play a role in resistance to a number of cytotoxic agents, including cycloheximide (10–12). Loss of function *PDR5* mutants are sensitive to drugs due to an inability to efficiently efflux drugs, suggesting that *PDR5* is directly involved in the efflux of these substances (13).

Two homologous zinc finger transcription factors, encoded by *PDR1* and *PDR3*, have previously been demonstrated to be key effectors of pleiotropic drug resistance, including cycloheximide tolerance (14, 15). Epistasis analyses have demonstrated that cycloheximide resistance mediated by Pdr1p or Pdr3p requires the presence of *PDR5* (14, 16). Direct measurement of *PDR5* gene expression indicates that both Pdr1p and Pdr3p can modulate transcription of *PDR5*. Deletion analysis of the *PDR5* promoter indicates that the presence of a single DNA element, located at −187 in the 5′-noncoding region of the gene, is sufficient to maintain Pdr3p-responsive gene expression (14).

We have assessed the action of Pdr1p on the *PDR5* promoter. *PDR1* is the major contributor to drug resistance of the *PDR1*/*PDR3* gene pair, presumably due to its importance as a transactivator (14). We show here that the same segment of the *PDR5* promoter required for Pdr3p-responsive expression is also required for Pdr1p-responsive expression. Analysis of the *PDR5* sequence has shown the presence of two other elements related by primary sequence to the Pdr1p/Pdr3p response element (PDRE) at −187. We have shown by DNase I footprinting analysis that both Pdr1p and Pdr3p are able to bind to these PDREs *in vitro*. Using site-directed mutagenesis, we have introduced mutations into the *PDR5* PDREs that block the ability of both Pdr1p and Pdr3p to bind to these sequence elements. The various combinations of mutant PDREs were placed back in the context of a *PDR5-lacZ* fusion gene or the native *PDR5* locus and analyzed in *S. cerevisiae* cells. These experiments revealed that all three sites are required for wild-type expression of *PDR5*, with each site contributing approximately equally. Furthermore, an oligonucleotide corresponding to one of these sites is capable of conferring Pdr1p responsiveness on a heterologous reporter system. From these experiments, we

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[1] The abbreviations used are: Pdr, pleiotropic drug resistance; bp, base pairs; PCR, polymerase chain reaction; UAS, upstream activation sequence.

conclude that the PDRE is both necessary and sufficient to function as the *in vivo* site of action of Pdr1p and is likely to fulfill this role for Pdr3p as well.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—The isogenic set of *pdr* mutant strains used in the present study has been described elsewhere (14) and consists of: SEY6210 (*MAT*α, *leu2-3,−112*, *ura3-52*, *his3-Δ200*, *trp1-Δ901*, *lys2-801*, *suc2-Δ9*, *Mel*$^-$), PB2 (SEY6210 *pdr3-Δ1::hisG*), PB4 (SEY6210 *pdr1-Δ2::hisG*, *pdr3-Δ1::hisG*), and DKY1 (SEY6210 *pdr5-Δ1::hisG*). Yeast transformations were performed by the lithium acetate procedure of Ito *et al.* (17). Standard yeast media were used for growth of cells and drug resistance assays (18). Drug resistance assays were performed by spot test (19). β-galactosidase activity was determined as described in Ref. 20.

Plasmids—The translational fusion constructs between the *PDR5* 5′-promoter deletions and *Escherichia coli lacZ* have been described (14). The high copy *PDR1* plasmid pRS425-PDR1 was constructed by insertion of a *Bam*HI fragment carrying *PDR1* into the unique *Bam*HI site of pRS425 (21). The low-copy plasmid carrying the *PDR5* gene was constructed in two steps. First, a *Bsp*106/*Sal*I fragment of *PDR5* was cloned into the *Cla*I/*Sal*I sites of pRS314 to form pSM86. The *PDR5* gene was reformed by inserting a *Sal*I/blunt-ended *Hin*dIII fragment containing the 3′-end of the *PDR5* gene into pSM86 that was cleaved with *Xho*I, treated with dNTPs and the Klenow fragment, and then cut with *Sal*I. This recombinant was designated pSM89. 1148 bp upstream of the *PDR5* transcription start site are contained in pSM89. To produce an appropriate control plasmid for analysis of the mutant PDRE-containing *PDR5* promoters, a *Not*I/*Stu*I fragment containing 360 bp upstream of the transcription start site was transferred into pSM89, producing pDK17. The template used for PCR mutagenesis was the p2-5 subclone that contained a *PDR5* promoter fragment extending from an *Eco*RI linker placed at position −360 to a *Hin*dIII site at position 187 cloned into *Eco*RI/*Hin*dIII-digested pBluescript KSII$^+$. Oligonucleotides corresponding to either the wild-type PDRE site 3 (GAT CTC CGC GGA ACT CTT CTA CGC CGT G) or mutant PDRE site 3 (GAT CTC CtC Gag ACT CTT CTA CGC CGT G) were annealed and cloned into a unique *Bgl*II site in pLGΔBS (22). The nucleotide positions that were altered from the wild type are indicated in lowercase. Derivatives that contained single insertions of the site 3 PDRE either as the wild-type or mutant version were then transferred into a low-copy plasmid as *Sal*I/*Sac*I fragments to form pDK52 and pDK53, respectively. These two plasmids contain either the wild-type or the mutant version of the site 3 PDRE, placed as the upstream activation sequence (UAS) for a *CYC1-lacZ* fusion gene in a centromere-containing vector.

Site-directed Mutagenesis—Clustered base substitution mutations were introduced into the three PDREs in the *PDR5* promoter using a PCR-based strategy (23). Briefly, a mutagenic primer was annealed to p2-5 template DNA along with the M13 reverse primer. The mutagenic primers were: site 1, TCT CTT TCC TCG AGA TCG CTC ATG CC; site 2, CGT GAT TCC GTC GAC AGG TCA GAT CTG; site 3, TGT CTC CTC GAG ACT CTT CTA CGC CG. PCR was performed and the resulting product was purified from an agarose gel. This PCR product was then mixed with a PCR product prepared from amplification of the same template using the T7 primer and a *PDR5*-promoter specific primer (AGC GAA CAG CCA TAA GGT TAT GCA C). These two PCR products overlapped by at least 100 bp and were annealed and subjected to PCR in the presence of the T7 and M13 reverse primers. Each PCR product was gel purified, cleaved with *Eco*RI and *Eco*RV, and cloned into pBluescript. Clones containing mutated PDREs were identified by restriction mapping, and the sequence of the entire amplified region was determined to check for PCR errors. Each single PDRE mutant was then introduced as an *Eco*RI/*Eco*RV fragment back into the context of the *PDR5* promoter carried on p2-5. The double and triple PDRE mutant promoters were constructed by using the unique *Bgl*II and *Bcl*I sites that separate the PDREs to combine the single mutants and form the multiply mutant *PDR5* promoters. The PDRE mutant promoters were transferred back into the *PDR5-lacZ* fusion gene as *Eco*RI/*Hin*dIII fragments and back into the *PDR5* gene as *Not*I/*Stu*I fragments.

DNA Binding Methods—The DNase I protection assay was performed as described previously (24). Two different pOTS-*Nco*I2 (25) subclones expressing the N-terminal 248 amino acids of Pdr1p and the N-terminal 213 residues of Pdr3p were used to produce these proteins in bacteria as before (14).

RESULTS

Pdr1p Activates PDR5 Gene Expression through the Same Region of the PDR5 Promoter as Pdr3p—We have previously demonstrated that wild-type expression of the *PDR5* gene required the presence of at least one of the homologous zinc finger-containing transcription factors Pdr1p or Pdr3p (14). Mutagenesis of a *PDR5-lacZ* fusion gene indicated that a DNA element located at position −140 in the *PDR5* promoter was required for Pdr3p responsiveness of this locus (14). Several studies implicate *PDR1* as the major contributor to drug resistance of the *PDR1/PDR3* gene pair (14, 15). Since Pdr1p and Pdr3p are homologous transcriptional regulators, it is reasonable to suggest that their sites of action on the *PDR5* promoter would also be related. To directly test this suggestion, we assessed the ability of a series of *PDR5* promoter mutations to respond to the presence of *PDR1* on a high copy plasmid.

Low-copy plasmids containing *PDR5-lacZ* fusion genes with variable amounts of *PDR5* 5′-noncoding DNA were introduced into wild-type *S. cerevisiae* cells along with a high copy plasmid containing the *PDR1* gene or the vector plasmid alone (Fig. 1). The levels of *PDR5*-dependent β-galactosidase were then determined in the presence of these two different gene dosages of *PDR1*. A *PDR5* 5′-flanking region containing 1093 bp upstream of transcription start produced 29 units/A_{600} of β-galactosidase in the presence of single-copy *PDR1*. Introduction of the high copy *PDR1* plasmid increased *PDR5*-dependent enzyme activity to 72 units/A_{600}. Truncation of the *PDR5* 5′-flanking DNA present in the *PDR5-lacZ* plasmid to 360 bp upstream of the transcription start site had no effect on the expression or the response of the fusion gene to high copy *PDR1*. Further removal of *PDR5* 5′-flanking DNA to −316 caused a large reduction in enzyme activity produced in the presence of single-copy *PDR1* but maintained the response to high copy *PDR1*. A truncation of the *PDR5* 5′-flanking sequence to −187 produced a fusion gene that was essentially inactive except in the presence of high copy *PDR1*. Either a site-directed mutation in the previously described Pdr3p binding site or truncation to −112 abolished the ability of the resulting *PDR5-lacZ* fusion gene to respond to high copy *PDR1*.

From this analysis, we concluded that the same DNA element required to maintain the Pdr3p responsiveness of *PDR5* was also necessary for Pdr1p control of expression. The dramatic loss of function exhibited upon deletion of the −316 to −187 region indicated that an important element for control of *PDR5* expression had been lost in the −187 construct. Previous experiments have shown that either *PDR1* or *PDR3* must be present for significant expression of *PDR5* (14). We next investigated the possible existence and relative importance of other Pdr1p/Pdr3p binding sites in the *PDR5* promoter.

The PDR5 Promoter Contains Three Sites That Are Bound by Both Bacterially Expressed Pdr1p and Pdr3p—Both Pdr1p and Pdr3p are members of the C6 zinc cluster family of DNA-binding proteins (26). These factors have been found to recognize rotationally symmetric, GC-rich DNA elements related to $CGGN_xCCG$, where x can vary from 0 to 11 (27). We inspected the DNA sequence of the *PDR5* promoter from −360 to −112 and found two sites in addition to the previously described element (−143 to −125) that were candidates for PDREs. The sequence of each site, along with its position in the *PDR5* promoter, is shown in Fig. 2. We have designated these PDREs as sites 1, 2, and 3, with site 1 being the 5′-most of the three. Comparison of these three sequences led to the prediction that TTCCGCGGAA might be the consensus sequence for binding of Pdr1p and Pdr3p.

To determine whether these related DNA sequences were able to be recognized by Pdr1p and/or Pdr3p, we carried out

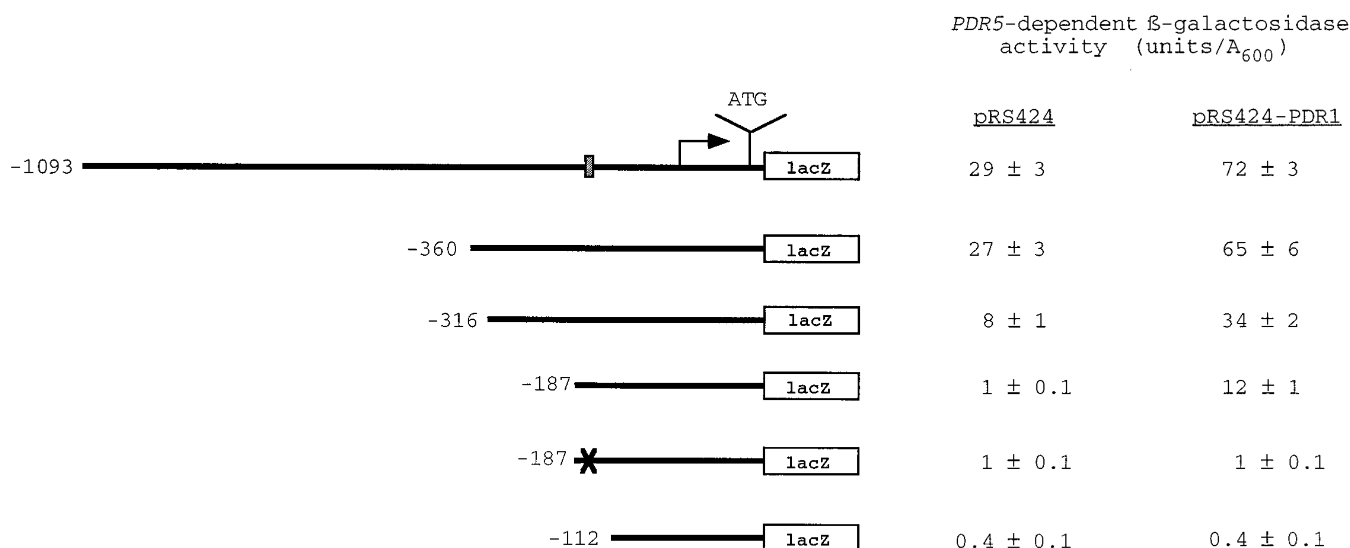

FIG. 1. **Mapping the sequence requirements for Pdr1p-responsive *PDR5* gene expression.** The indicated *PDR5-lacZ* fusion plasmids were introduced into a wild-type strain (SEY6210) along with a high copy vector plasmid containing the *PDR1* gene (pRS425-PDR1) or the high copy vector alone (pRS425). The numbers on the left-hand end of the lines represent the amount of *PDR5* 5′-flanking DNA present in each fusion plasmid. *Box*, binding site for Pdr3p; *arrow*, transcription start site for *PDR5* mRNA; *ATG*, translation start site. Values reported are the average of six independent determinations ± S.D.

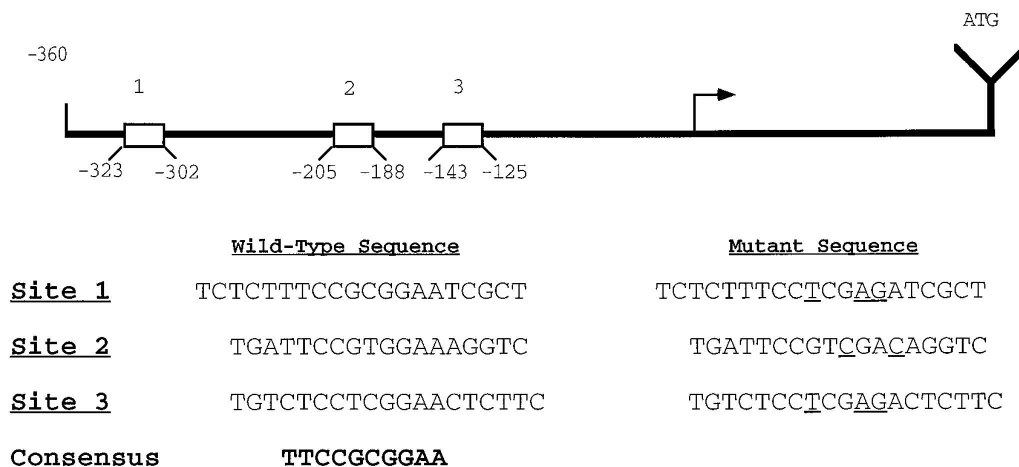

FIG. 2. **Location and sequence of Pdr1p/Pdr3p response elements in the *PDR5* promoter.** A graphical depiction of the *PDR5* promoter is shown. The numbers refer to the position of a given sequence element relative to the transcription start site. The three PDREs are referred to as sites 1, 2, and 3, with site 1 located the farthest upstream. The symbols are defined in the legend to Fig. 1. The wild-type sequence of each PDRE is shown in the left column and the sequence of the clustered substitution mutant is shown in the right, with the altered residues underlined. The consensus PDRE suggested from this comparison is presented below the sequences of the wild-type PDREs.

DNase I protection assays with bacterially produced Pdr1p and Pdr3p (Fig. 3). Each of these three sites can be specifically recognized by bacterially produced Pdr1p and Pdr3p. The DNase I protection pattern on each wild-type template is identical, irrespective of whether Pdr1p or Pdr3p is used.

Having established that three PDREs exist in the −360 to −112 interval of the PDR5 promoter, we prepared mutant versions of each binding site (Fig. 2). This was done in preparation for a functional analysis of the role of each PDRE in *PDR5* promoter action. Clustered base substitution mutations were introduced in each PDRE, changing two or three positions of each binding site. The ability of bacterially generated Pdr1p and Pdr3p to bind to each mutant PDRE was next assayed by DNase I protection analysis (Fig. 3). This assay demonstrated that none of the three mutant PDREs could be recognized *in vitro* by either Pdr1p or Pdr3p. These mutant *PDR5* promoters were then used to analyze the effect of loss of a given PDRE on *PDR5* expression and drug resistance.

The Three PDREs in the PDR5 Promoter Are Required for Wild-type Expression of PDR5—To assess the quantitative contribution of each PDRE to *PDR5* expression, each mutated PDRE was introduced into a *PDR5-lacZ* fusion plasmid containing −360 bp of 5′-noncoding DNA. The *PDR5-lacZ* fusion gene was used to simplify measurement of *PDR5* expression. Additionally, each mutated PDRE was placed back into the context of the wild-type *PDR5* gene. These constructs allowed the physiological consequences of loss of a given PDRE to be evaluated in terms of Pdr5p-mediated cycloheximide resistance. All seven possible combinations of the three mutant PDREs were constructed upstream of both the *PDR5-lacZ* and wild-type *PDR5* genes. This resulted in the generation of two different plasmids for each type of promoter mutation. The *PDR5-lacZ* and *PDR5* versions of each type of promoter mutation were then transformed together into a Δ*pdr5* strain and assayed for β-galactosidase activity as well as cycloheximide resistance (Figs. 4 and 5).

As described above, a *PDR5-lacZ* fusion gene with 360 bp upstream of the transcription start site produces wild-type

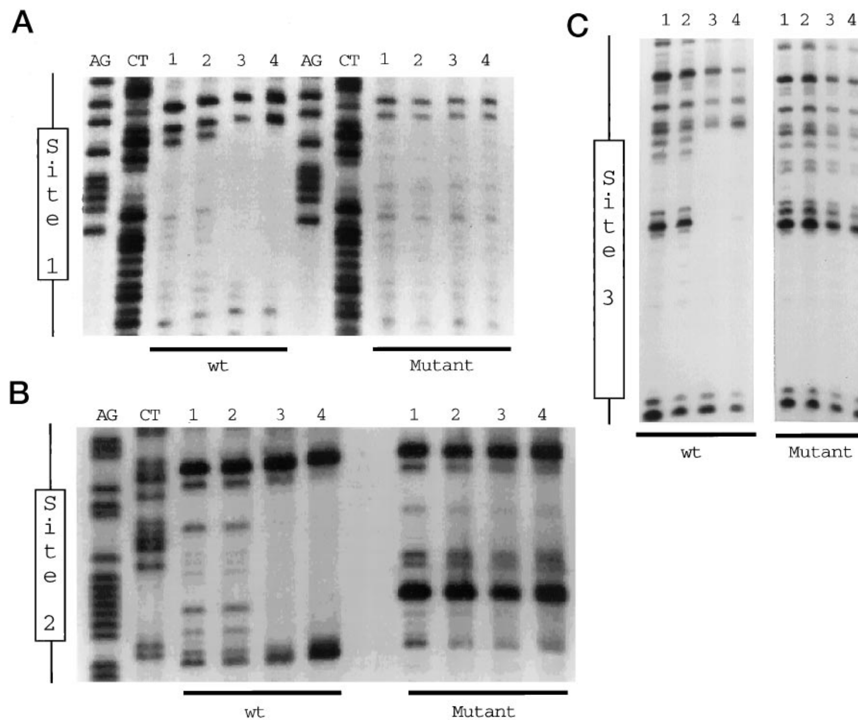

FIG. 3. **DNase I footprinting analysis of Pdr1p and Pdr3p binding to the wild-type and mutant PDREs in the *PDR5* promoter.** A DNase I protection assay was performed on several different *PDR5* templates to evaluate the ability of bacterially produced Pdr1p and Pdr3p to bind to the wild-type and mutant PDREs. The purine-specific (*AG*) and pyrimidine-specific (*CT*) Maxam-Gilbert chemical sequencing reactions were carried out on the wild-type DNA template corresponding to sites 1 and 2 to precisely localize the protected region of DNA. This has previously been done for site 3 (14). In each panel, *lane 1* refers to a DNase I protection reaction carried out in the absence of protein. *Lane 2* is identical to *lane 1*, with the exception that 10 μl of a bacterial protein extract prepared from a cell carrying the empty bacterial expression vector is used in the binding reaction prior to incubation with DNase I. *Lane 3* is identical to *lane 2* except that 10 μl of a bacterial protein extract prepared from cells expressing Pdr3p is used in the binding reaction, and *lane 4* is identical to *lane 3* except that the bacterial protein extract is prepared from cells expressing Pdr1p. The location of the protected region is indicated on the *left* side of each panel and the use of either the wild-type or the mutant version of each PDRE is indicated at the *bottom* of the panel. Each template was radiolabeled with T4 polynucleotide kinase and [γ-^{32}P]-ATP. *A*, binding of Pdr1p and Pdr3p to PDRE site 1. An *Eco*RI/*Bgl*II template labeled at *Eco*RI was used for this DNase I protection experiment. *B*, binding of Pdr1p and Pdr3p to PDRE site 2. A *Hin*dIII/*Eco*RI template labeled at *Hin*dIII was used for this DNase I protection assay. *C*, binding of Pdr1p and Pdr3p to PDRE site 3. A *Bgl*II/*Hin*dIII template labeled at *Bgl*II was used for this DNase I footprinting experiment.

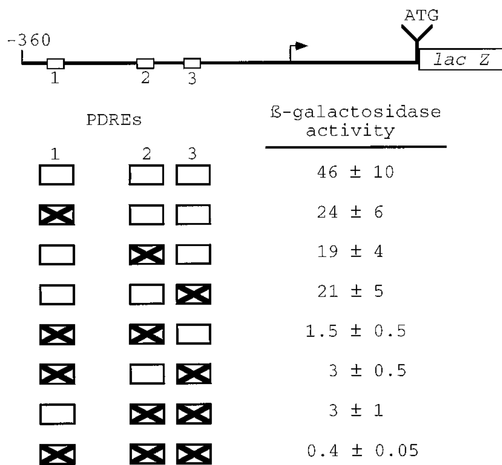

FIG. 4. **Analysis of *PDR5-lacZ* fusion genes with mutant PDREs.** The various mutant forms of the three PDREs were returned to the *PDR5-lacZ* fusion gene containing 360 bp upstream of the transcription start site. The basic structure of each *PDR5-lacZ* construct is depicted by the drawing at the top of the figure with symbols as defined in the legend to Fig. 1. Wild-type PDREs are denoted by an *open box* and a mutant PDRE is indicated by a box with an X. Each *PDR5-lacZ* plasmid was transformed into a Δ*pdr5* strain and assayed for *PDR5*-dependent β-galactosidase as before (20). The values presented are the average of four independent determinations ± S.D.

levels of *PDR5*-dependent β-galactosidase activity. Removal of any one of the PDREs from this fusion gene led to an approximately 50% reduction in the level of expression. Loss of any two of the three PDREs resulted in a much more dramatic loss of *PDR5* expression. The three double PDRE mutant *PDR5* promoters were only able to drive 3–6% of wild-type *PDR5* expression. The mutant promoter lacking all three PDREs was essentially inactive.

Along with the β-galactosidase assays, cycloheximide resistance assays were performed. Approximately 5000 cells transformed with one of the *PDR5* promoter mutants driving the expression of *PDR5* were spotted on media containing various concentrations of the drug cycloheximide. The Δ*pdr5* strain transformed with vector only (pRS314) is unable to grow in the presence of 0.1 μg/ml cycloheximide (Fig. 5). Transformants containing the *PDR5* gene lacking all three of the PDREs were also unable to tolerate this low level of cycloheximide. The mutant *PDR5* promoters lacking only a single PDRE were able to support growth on 0.2 μg/ml cycloheximide nearly as well as the wild-type promoter. However, the three double PDRE mutant promoters all exhibited a strong reduction in growth relative to either the single mutants or the wild-type at 0.2 μg/ml cycloheximide.

These experiments demonstrate the importance of the PDREs in the function of the *PDR5* promoter. The ability of one of the site 3 PDREs to confer Pdr1p-responsive expression to a heterologous promoter was evaluated next.

An Oligonucleotide Corresponding to Site 3 Can Function as a UAS—The decrease in *PDR5* expression upon removal of each PDRE established that these DNA elements are necessary for normal *PDR5* promoter function. Furthermore, the removal of the site 3 PDRE from the *PDR5-lacZ* fusion gene containing

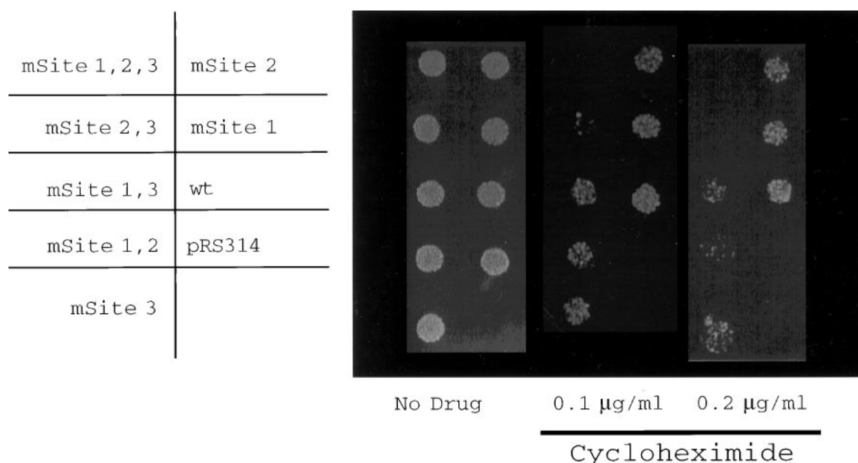

FIG. 5. **Wild-type cycloheximide resistance requires the presence of three intact PDREs in *PDR5*.** Each PDRE mutant form of the *PDR5* promoter was placed upstream of the wild-type *PDR5* structural gene. These mutant forms of the *PDR5* locus were introduced into a Δ*pdr5* strain and assessed for their ability to complement the cycloheximide hypersensitivity of this strain. Appropriate transformants were grown to an A_{600} of 1, and then aliquots of each culture were spotted onto minimal medium containing the indicated concentrations of cycloheximide. The plates were incubated at 30 °C for the desired length of time. The position of each type of *PDR5* transformant is shown on the *left*. Transformants containing the empty low-copy vector plasmid are denoted as *pRS314*, and cells carrying the wild-type *PDR5* gene are indicated as *wt*. The PDRE sites that have been mutated are listed as *mSite*.

187 bp of 5′-flanking DNA abolished the Pdr1p (and Pdr3p) responsiveness of this promoter. To determine whether a single PDRE was sufficient to serve as a Pdr1p-dependent UAS, an oligonucleotide corresponding this DNA element was synthesized. This site 3 PDRE oligonucleotide was cloned upstream of a *CYC1-lacZ* fusion gene that lacked intrinsic UAS function. The mutant form of this site 3 PDRE was also synthesized as an oligonucleotide and was likewise cloned upstream of *CYC1-lacZ*. Each of these *PDRE-CYC1-lacZ* fusion plasmids, along with *PDR5-lacZ* control plasmids, was introduced into a Δ*pdr1,pdr3* strain containing different alleles of the *PDR1* gene. The three different groups of transformants contained either no functional *PDR1* gene, the wild-type *PDR1* locus, or a mutant version of *PDR1* (*PDR1-6*) that causes overproduction of *PDR5* transcript and enhanced drug resistance (28, 29).[2] β-galactosidase activity was determined from appropriate transformants.

The wild-type site 3 PDRE cloned upstream of *CYC1-lacZ* (*PDRE-CYC1-lacZ*) was strongly responsive to the *PDR1* allele present (Table I). The *PDRE-CYC1-lacZ*-dependent β-galactosidase activity increased from 0.5 units/A_{600} in the absence of *PDR1* to 2 units/A_{600} when *PDR1* was restored. The presence of the *PDR1-6* allele elevated site 3 PDRE-driven enzyme activity to 21 units/A_{600}. By comparison, the mutant version of the site 3 PDRE (*mPDRE-CYC1-lacZ*) was unable to respond to the changes in the *PDR1* allele and produced from 0.3 to 0.5 units/A_{600} in all genetic backgrounds assayed. The *PDR5-lacZ* fusion gene with −360 bp of 5′ upstream DNA was assayed as a control for the behavior of the differing *PDR1* backgrounds. This fusion plasmid showed the previously described strong response of *PDR5* expression to these different *PDR1* alleles (28, 29).[2] A derivative of this *PDR5-lacZ* fusion containing only a functional site 3 PDRE was assayed in parallel as a control for the function of site 3 PDRE in its native *PDR5* environment. The site 1, 2 PDRE mutant *PDR5-lacZ* fusion gene produced extremely low levels of β-galactosidase activity that were not detectably influenced by the presence or absence of the wild-type *PDR1* gene. However, this mutant form of the *PDR5* promoter still maintained its Pdr1p responsiveness since the *PDR5*-dependent β-galactosidase activity could be strongly enhanced by introduction of the *PDR1-6* allele. Additionally,

TABLE I
Analysis of the upstream activation sequence function of PDR5 site 3 PDRE

Fusion plasmid present[b]	β-Galactosidase activity, PDR1 allele[a]		
	Δpdr1	PDR1	PDR1–6
		units/A₆₀₀[c]	
−360 *PDR5-lacZ*	1 ± 0.3	35 ± 9	1260 ± 80
mSite 1, 2 *PDR5-lacZ*	0.6 ± 0.2	1 ± 0.3	35 ± 6
PDRE-CYC1-lacZ	0.5 ± 0.2	2 ± 0.2	21 ± 4
mPDRE-CYC1-lacZ	0.3 ± 0.2	0.5 ± 0.1	0.5 ± 0.2

[a] The allele of *PDR1* present in each transformant is listed below.
[b] The indicated *lacZ* fusion plasmids were introduced into strains differing in the allele of *PDR1* present. Expression of each fusion gene was then assessed by β-galactosidase enzyme assay as described (20). −360 *PDR5-lacZ* refers to the wild-type *PDR5-lacZ* gene fusion containing 360 bp of 5′ noncoding DNA. mSite 1, 2 *PDR5-lacZ* is identical to −360 *PDR5-lacZ* except that only PDRE site 3 remains intact. *PDRE-CYC1-lacZ* refers to a *CYC1-lacZ* plasmid containing an oligonucleotide corresponding to PDRE site 3 replacing the normal *CYC1* UAS elements. *mPDRE-CYC1-lacZ* is identical to *PDRE-CYC1-lacZ* except that a mutant form of PDRE site 3 has been used to replace the *CYC1* UAS elements.
[c] β-Galactosidase values represent the average of three independent determinations ± S.D.

when the same site 1, 2 PDRE mutant promoter was placed upstream of the *PDR5* structural gene, the resulting mutant *PDR5* gene was capable of complementing the cycloheximide sensitivity of a Δ*pdr5* mutant strain (Fig. 5). This was not true of a *PDR5* gene lacking all three PDREs (Fig. 4), confirming the small but significant functional role of the site 3 PDRE in the absence of the other two elements.

DISCUSSION

Both *PDR1* and *PDR5* are major contributors to the ability of wild-type *S. cerevisiae* cells to tolerate a large variety of cytotoxic agents. Loss of function mutations in either of these loci elicit a severe drug hypersensitive phenotype of the resulting strains. Control of *PDR5* expression is a key determinant in setting the level of drug tolerance of cells. In the current study, we have identified *cis*-acting elements that are required for wild-type *PDR5* expression and drug resistance. These elements are bound *in vitro* by both Pdr1p and Pdr3p and have been designated PDREs. The PDREs in the *PDR5* promoter show the typical sequence composition of recognition sequences for C6 zinc cluster proteins with sequence comparison suggest-

[2] E. Carvajal, A. Goffeau, and E. Balzi, submitted for publication.

ing that the consensus PDRE consists of TTCCGCGGAA. The same element has been found in other Pdr1p target genes, such as *PDR3* (30), *SNQ2* (28, 31), *D4405*,[3] and *YOR1* (29). In the case of *PDR3* (30) and now *PDR5* (this study), the PDREs have been shown to be required for Pdr1p transcriptional control of the gene in question. We conclude that the PDRE is the *in vivo* site of action for Pdr1p and likely for Pdr3p.

Maintenance of wild-type *PDR5* transcription requires the presence of either the *PDR1* or *PDR3* gene (14). This finding indicated that *PDR5* transcription was strictly *PDR1*/*PDR3*-dependent. We have extended this observation by producing a *PDR5* promoter that is unable to be bound by bacterially produced Pdr1p or Pdr3p. This triple PDRE mutant promoter is not able to drive *PDR5* expression as measured by either *PDR5*-dependent β-galactosidase activity or drug resistance. These data further support the view that the main, if not the only, source of activation of *PDR5* transcription is supplied by the action of Pdr1p/Pdr3p at the PDREs. This is not true for all Pdr1p/Pdr3p regulated genes since both *YOR1* (29) and *SNQ2* (28) have significant Pdr1p/Pdr3p independent components of expression.

This study of PDRE function in the *PDR5* promoter also indicates that each of these regulatory elements appears to contribute roughly equally to overall *PDR5* expression levels. As more PDREs are mutated, expression of the *PDR5-lacZ* gene is progressively diminished until all three sites are removed. The same trend is seen when the mutant *PDR5* promoters are placed upstream of the *PDR5* structural gene, with the possible exception of the site 2, 3 PDRE double mutant promoter. This mutant version of the *PDR5* promoter was found to be reproducibly weaker, in terms of conferring cycloheximide tolerance, than the other two double PDRE mutant promoters, even though comparison of these three double mutant PDRE mutant promoters using the *PDR5-lacZ* fusion gene did not reveal any significant differences. One potential explanation for this observation is provided by the possible cycloheximide inducibility of *PDR5* that has been described (12, 30). Since our β-galactosidase assays were performed on cells grown in the absence of cycloheximide, a defect in inducibility would not have been detected. We were unable to demonstrate induction of our wild-type *PDR5-lacZ* fusion by cycloheximide (data not shown), but this finding is complicated by the need for increased synthesis of β-galactosidase in the presence of the translation inhibitor cycloheximide. Direct RNA measurements must be carried out to examine the possible role of cycloheximide inducibility in the function of the *PDR5* promoter.

Mutant forms of either *PDR1* or *PDR3* have been identified that lead to overproduction of *PDR5* transcript (16, 32). The basis for the alteration of function in these dominant, drug resistant mutant transcription factors is not yet well defined. In this study, we show that a single PDRE placed upstream of a *CYC1-lacZ* fusion gene confers Pdr1p responsive expression on this heterologous promoter and is strongly stimulated by the presence of the *PDR1-6* dominant allele of *PDR1*. These data demonstrate two important facts about Pdr1p-responsive transcription. First, a single PDRE is sufficient to confer transcriptional regulation by Pdr1p. Secondly, the enhanced ability to stimulate transcription exhibited by the Pdr1p encoded by the *PDR1-6* allele is promoter independent. The high degree of transactivation afforded by the *PDR1-6* allele can be conferred on a heterologous promoter by placing 28 bp of *PDR5* DNA upstream of this unrelated promoter. This observation is consistent with the belief that the Pdr1-6p is a stronger transactivator and that this enhanced transactivation does not require cooperation with other transcriptional regulators bound to nearby sites.

[3] L. Lambert, J. L. Jonniaux, W. S. Moye-Rowley, S. Goffeau, and E. Balzi, submitted for publication.

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