The yeast ATP binding cassette (ABC) protein genes *PDR10* and *PDR15* are novel targets for the Pdr1 and Pdr3 transcriptional regulators

Hubert Wolfger[1,a], Yannick Mahé[1,2,a], Anne Parle-McDermott[3,a], Agnés Delahodde[b], Karl Kuchler[a,*]

[a] *Department of Molecular Genetics, University and Biocenter of Vienna, Dr. Bohr-Gasse 9/2, A-1030 Vienna, Austria*
[b] *Laboratoire de Génétique Moléculaire, CNRS, Ecole Normale Supérieure, 75230 Paris Cedex 05, France*

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Abstract The yeast transcription factors Pdr1 and Pdr3 control pleiotropic drug resistance (PDR) development, since they regulate expression of ATP-binding cassette (ABC) drug efflux pumps through binding to *cis*-acting sites known as PDREs (PDR responsive elements). In this report, we show by Northern blotting, gel shift mobility assays and DNase I footprinting that transcription of the ABC genes *PDR10* and *PDR15* is also controlled by Pdr1 and Pdr3. In addition, in vitro band shift assays demonstrate that a GST-Pdr1 fusion protein can bind to the PDREs of *PDR10* and *PDR15*. DNase I footprinting allowed the identification of the precise PDRE binding motifs, indicating the presence of a novel slightly degenerate PDRE motif in the *PDR15* promoter. Finally, *PDR10* and *PDR15* mRNA levels vary dramatically in abundance in isogenic yeast strains carrying either Δ*pdr1*, Δ*pdr3* and Δ*pdr1* Δ*pdr3* deletions or *pdr1-3* and *pdr3-2* gain-of-function mutations, demonstrating that both *PDR10* and *PDR15* are new members of the yeast PDR network.

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Key words: Transcriptional regulation; Pleiotropic drug resistance network; ABC transporter; *Saccharomyces cerevisiae*

1. Introduction

Multiple or pleiotropic drug resistance (PDR) in the yeast *Saccharomyces cerevisiae* can be controlled by the function of Pdr1 [1] and Pdr3 [2], both of which belong to the Zn_2Cys_6 family of transcriptional regulators. Although recent data also indicate that two hexose transporters can be controlled by Pdr1/Pdr3 [3], most known target genes of the yeast PDR network comprise membrane transporters of the ATP binding cassette (ABC) protein superfamily [4,5]. For instance, mRNA levels of *PDR5* [6], *SNQ2* [7,8] and possibly *YOR1* [9] are tightly regulated by Pdr1/Pdr3. Gain-of-function mutations such as *pdr1-3* or *pdr3-2* lead to pleiotropic drug resistance to a variety of different drugs due to overexpression of several drug efflux pumps [8,10]. By contrast, loss-of-function mutations such as Δ*pdr1* and Δ*pdr3* result in severe drug hypersensitivity phenotypes [2].

Transcriptional regulation within the PDR network was previously shown to require a PDRE (PDR responsive element) consensus motif, which is present in varying numbers in the promoters of PDR responsive genes [2,11–13]. Several functional *cis*-acting PDREs protected by Pdr1 in vitro have been identified in the *PDR5* promoter [12]. Likewise, binding sites for Pdr3 have been identified in the promoters of *PDR5* [11], *SNQ2* [8], *PDR3* [13] and most recently in the hexose transporter genes *HXT9* and *HXT11* [3]. Notably, a single nucleotide exchange in a PDRE motif of *PDR5* completely impaired the interaction of both Pdr1 and Pdr3 with the *PDR5* promoter [12]. This observation implies that the sequence motif for Pdr1/Pdr3 binding is quite restrictive. Nevertheless, in addition to the perfect palindromic PDRE motif, 5′-TCCGCGGA-3′, two other sequence elements, both of which contain one base change, 5′-TCCGTGGA-3′ and 5′-TCCGCGCA-3′, were identified as potential Pdr3 target sites in certain PDR genes [3,9,12]. This suggests that at least some sequence degeneration may be tolerated without abolishing Pdr1 and/or Pdr3 binding. Another potential target gene for transcriptional control by Pdr1 and Pdr3 was identified in the ABC transporter gene *YOR1*. Its overexpression confers increased resistance to oligomycin [9] and organic anions [14]. The *YOR1* promoter contains only one PDRE motif, 5′-TCCGTGGA-3′, but this PDRE has not been shown to bind either Pdr1 or Pdr3.

The yeast genome sequencing project revealed the existence of 31 genes encoding ABC proteins [5,15]. The well-described Pdr5 [16,17] and Snq2 [18] drug efflux pumps, both of which are controlled by Pdr1 and Pdr3 [6–8], have several homologues whose functions and (drug) substrates are currently unknown. For instance, two ABC protein genes, *PDR10* [19] and *PDR15*, are most closely related to *PDR5* [19]. Phylogenetic analysis suggests that Pdr5, Pdr10 and Pdr15 form a small subfamily of ABC proteins with more than 65% primary sequence identity to each other [5,15]. Interestingly enough, *PDR10* and *PDR15* contain two potential PDREs in their promoters. *PDR10* has two degenerate PDRE motifs, the first (5′-TCCGTGGA-3′) at position −407 and the second (5′-TCCACGGA-3′) at position −338 from the putative translational start. Likewise, the *PDR15* promoter has two PDRE motifs, a perfect element (5′-TCCGCGGA-3′) at position −379 and a degenerate element (5′-TCCGCGGGA-3′) at position −442 from the putative translational start site. In this report, we demonstrate that Pdr1/Pdr3-mediated regulation also includes the *PDR10* and *PDR15* genes. In addition, we

*Corresponding author. Fax: (43) (1) 79515-2900.
E-mail: kaku@mol.univie.ac.at

[1] The first two authors contributed equally to this work.

[2] Present address: Institut Curie, INSERM U-248, Section de Recherche 26, rue d'Ulm, 75248 Paris, Cedex 05, France.

[3] Present address: Department of Biochemistry, Molecular Biology Laboratory, Royal College of Surgeons, 123 St. Stephens Green, Dublin 2, Ireland.

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have identified a novel degenerate PDRE motif in the *PDR15* promoter.

2. Materials and methods

2.1. Media, yeast strains and culture conditions

Rich medium (YPD) and synthetic medium (SD), supplemented with auxotrophic components, were prepared essentially as described elsewhere [20]. The *S. cerevisiae* strains used in this study were YALF-A1 (*MAT***a** *ura3-52 leu2-3,112 his3-11,15 trp1-1 pdr1-3*) and its isogenic *PDR1* segregant YALF-I1. These strains were constructed by back-crossing strain DRI9-T8 [6] successively into strains VHM3 [21], CRY2 and CB002 (kindly provided by B. Fuller), all of which are isogenic to W303-1A [22]. Likewise, *pdr3-2* and its isogenic wild type strain, YYMI4-A4 (*MAT***a** *pdr3-2 ura3 leu2 his3 trp1 ade2*) and YYMI4-O3 (*MAT***a** *PDR3 ura3 leu2 his3 trp1 ade2*), were obtained by back-crossing a *pdr3-2* allele [10] into strain YALF-O2, an isogenic *PDR1* segregant of the above described strain YALF-A1, and then three times into CRY1 or CRY2. The strains, FY1679-28C (*MAT***a** *ura3-52 leu2Δ1 his3Δ200 trp1Δ63*), FY1679-28C/*pdr1::TRP1*, FY1679-28C/*pdr3::HIS3* and FY1679-28C/*pdr1::TRP1 pdr3::HIS3* have been described elsewhere [2]. All yeast strains were grown routinely at 30°C.

2.2. Plasmid construction

The plasmids used as templates for PCR were constructed as follows. A 2.1 kbp *Pst*I fragment from cosmid 9926 (ATCC 57093) containing the *PDR15* promoter was cloned into the *Pst*I site of pRS314 to obtain plasmid pHW1. Next, an 836 bp *Sma*I-*Eco*RV fragment of pHW1 was cloned into the *Sma*I site of YEp368 to obtain pHWZ15. A 4.07 kbp *Sau*3A fragment isolated from pUOA502 [19] containing the *PDR10* promoter was cloned into the *Bam*HI site of pBluescript SK$^-$ to obtain plasmid pAA05. Then, a 1.7 kbp *Hin*cII fragment from pAA05 was cloned into the *Sma*I site of YEp368 yielding plasmid pYM51.

2.3. RNA isolation, radiolabeling and Northern analysis

Total yeast RNA was isolated using a published procedure [23], and separated through a 1% agarose gel following the glyoxal procedure described elsewhere [24]. RNA was transferred onto nylon membranes by capillary blotting and hybridization of membranes was carried out by standard methods using the Church buffer (1% BSA fraction V, 7% SDS, 0.5 M Na-phosphate buffer pH 6.8, 1 mM EDTA) for both hybridization and washing, except for the *PDR10* probe, which was hybridized and washed using a buffer containing Denhardt's solution [24]. DNA restriction fragments to be used as probes were derived from *PDR5* [16], *STE6* [22], *SNQ2* [18], *PDR10* [19] and *PDR15* (K. Kuchler et al., unpublished data) and radiolabeled with [α^{32}P]dCTP using a Megaprime Labeling Kit (Amersham) under conditions recommended by the manufacturer. Autoradiographs of Northern blots were quantified by laser scanning densitometry using the Image Quant software.

2.4. GST fusion protein purification

GST-Pdr3 fusion protein was purified as described previously [8,13] using minor modifications. To construct a bacterial expression plasmid for a GST-Pdr1 fusion protein, a *PDR1*-specific PCR fragment was amplified from plasmid pSKPDR1 using an oligonucleotide from the N-terminal *PDR1* region (5′-G CGT GGA TCC CCG ATG CGA GGC TTG-3′) and the universal primer Pu, both containing a *Bam*HI site, and inserted into the *Bam*HI site of pGEX-2T [25]. Briefly, 400 ml LB medium containing 100 μg/ml ampicillin was inoculated with a 10 ml overnight *Escherichia coli* preculture carrying the GST-Pdr1 or the GST-Pdr3 fusion plasmid. Fusion protein expression was induced at an A_{600} of 0.5 by adding IPTG to a final concentration of 0.15 mM. After a 4 h incubation at 37°C, the cells were harvested and resuspended in 20 ml cold TpG (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1 mM $ZnCl_2$ or $ZnSO_4$, 0.5% NP-40; and PMSF 1 mM). Cells were disrupted by sonication and the insoluble material was removed by centrifugation at 15000 rpm for 15 min. After addition of 500 μl 50% slurry of glutathione Sepharose beads to the clear supernatant, binding was allowed at 4°C overnight with gentle shaking. The beads were collected by a brief centrifugation and washed twice with 10 ml TpG. Finally, the beads were resuspended in 250 μl TpG and the fusion protein was eluted by adding 250 μl of fresh elution buffer (50 mM Tris-HCl pH 8.0, 15 mM reduced glutathione). The fusion protein was eluted from the beads twice at room temperature for 10 min each. Eluates were combined and stored at −20°C [8,13].

2.5. DNA probe synthesis

PDR10 and *PDR15* promoter-specific probes PDR10-AB and PDR15-AB were synthesized by PCR using a 5′ labeled oligonucleotide A and an unlabeled oligonucleotide B in order to label only the coding strand. To do this, 2–3 pmol of oligonucleotide A was labeled with 5–10 μCi γ-ATP and 10 units T_4-polynucleotide kinase for 2 h at 37°C. The same molar amount of unlabeled oligonucleotide B was added to the mixture. The DNA was purified by phenol extraction and precipitated overnight at −20°C. After centrifugation (15000 rpm for 30 min at 4°C), the pellet was washed with 70% ethanol and dried under vacuum. The DNA was dissolved and the probe was synthesized by PCR under optimized conditions (0.2 mM dNTP, 2.5 units Taq, 1.5 mM $MgCl_2$). The amplified promoter probes PDR10-AB and PDR15-AB were purified by phenol extraction and precipitated in the presence of glycogen overnight at −20°C. The DNA probes were dissolved in H_2O at a final concentration of 100 fmol/μl. The labeled oligonucleotides for probe synthesis were: PDR10-A (5′-CGC TGC ACC ATT CGC GC-3′) and PDR15-A (5′-TGC CCT GGA AGG TGG CC-3′); the non-labeled oligonucleotides were PDR10-B (5′-TTG GTA CTG ATG ACA GCA GG-3′) and PDR15-B (5′-TCG CAC AGC AGT AGC AG-3′). Template DNA for PCR was derived from pYM51 (*PDR10*) and pHWZ15 (*PDR15*), respectively.

2.6. Mobility shift assay

Radiolabeled DNA fragments PDR10-AB and PDR15-AB harboring appropriate promoter regions of *PDR10* and *PDR15* were generated by PCR as described above. The purified fusion protein GST-Pdr1 was incubated for 30 min at 25°C with 5–20 fmol labeled probe in assay buffer (100 mM KCl, 20 mM Tris-HCl pH 8, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM $CaCl_2$, 0.1 mM EDTA with 5% glycerol, 0.05 μg/μl sonicated and non-denatured salmon sperm DNA). After incubation for another 20 min on ice, the mixture was loaded onto a native 4% polyacrylamide gel (acrylamide:bis-acrylamide 29.1:0.9 in 0.5×TBE buffer). After the gel was prerun at 200 V for 30 min, samples were applied and electrophoresis was carried out in 0.5×TBE buffer at 240 V. After electrophoresis, the gel was exposed to an autoradiography film at −70°C using intensifying screens.

2.7. DNase I footprinting

Binding conditions for the DNase I treatment were identical to those used in the mobility shift assay, but glycerol was excluded from the buffer. After a preincubation for 30 min at 25°C, the DNase I digestion was performed with increasing activities of DNase I (0.5 U, 1 U, 2 U; Boehringer Mannheim) and stopped after 1 min by phenol extraction. The DNA was precipitated in liquid N_2. After centrifugation, the pellet was washed once with 70% ethanol and resuspended twice in 100 μl H_2O with intermediate lyophilization and finally, after lyophilization, resuspended in formamide dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). After denaturation at 92°C for 5 min, half of the samples were loaded onto a denaturing gel (6%, acrylamide:bis-acrylamide = 19:1 in 0.5×TBE buffer containing 50% urea) along with the samples from a Maxam-Gilbert reaction performed on the same probes. Electrophoresis was carried out at 20 W. The gel was dried and exposed to an X-ray film with intensifying screen at −70°C.

2.8. Maxam-Gilbert sequencing reaction (A+G)

100 fmol 5′-end-labeled DNA fragments was dried under vacuum in the presence of 1 μg of salmon sperm carrier DNA. The DNA pellet was resuspended in 2 μl 2% formic acid and incubated for 10 min at 37°C. After drying, the pellet was dissolved in 100 μl 1 M piperidine and incubated for 30 min at 90°C. Piperidine was removed from the samples by lyophilization and the pellet was washed twice in distilled H_2O with intermediate lyophilization to remove residual piperidine. Finally, the pellet was resuspended in formamide dye at a concentration of 2 fmol/μl and loaded onto the DNase I footprint gel.

3. Results

3.1. Pdr1 binds to two distinct sites in the promoters of PDR10 and PDR15 in vitro

The completion of the sequencing project revealed the existence of 31 distinct ABC protein genes in yeast [5,15]. For instance, expression of *PDR5* and *SNQ2*, both of which encode ABC drug efflux pumps of broad substrate specificity, is controlled by the Zn_2Cys_6-type transcriptional regulators Pdr1 and Pdr3. This regulation requires so-called PDREs present in the promoters of PDR responsive genes, several of which have been identified so far (Fig. 1). The *PDR5* gene has two additional homologues, *PDR10* and *PDR15*, both of which are some 65% identical to Pdr5 over their entire length (data not shown). Interestingly, each promoter of these ABC genes contains two different putative PDREs in the 5′-untranslated region. Since only one PDRE is necessary and sufficient for conferring Pdr1/Pdr3-mediated regulation on genes, we wanted to test whether recombinant Pdr1 and Pdr3 can bind to the promoters of *PDR10* and *PDR15*. To accomplish this, a GST-Pdr1 fusion protein was expressed in *E. coli*. After its purification, the fusion protein was used in gel retardation assays. As shown in Fig. 2, recombinant GST-Pdr1 protein could indeed bind to the promoters of both *PDR10* and *PDR15* in vitro, because the mobility of the free DNA probes was retarded in the gel only in the presence of the GST-Pdr1 protein but not in the control lanes. Increasing the amount of GST-Pdr1 led in each case (*PDR10* and *PDR15*) to a supershift, suggesting that Pdr1 binds to both sites of each promoter (Fig. 2). Similar results were obtained using a recombinant GST-Pdr3 fusion protein, which also bound twice to both promoters (data not shown). These results demonstrate that the *PDR10* and *PDR15* promoters contain functional PDREs specifically recognized by recombinant GST-Pdr1 and GST-Pdr3 in vitro.

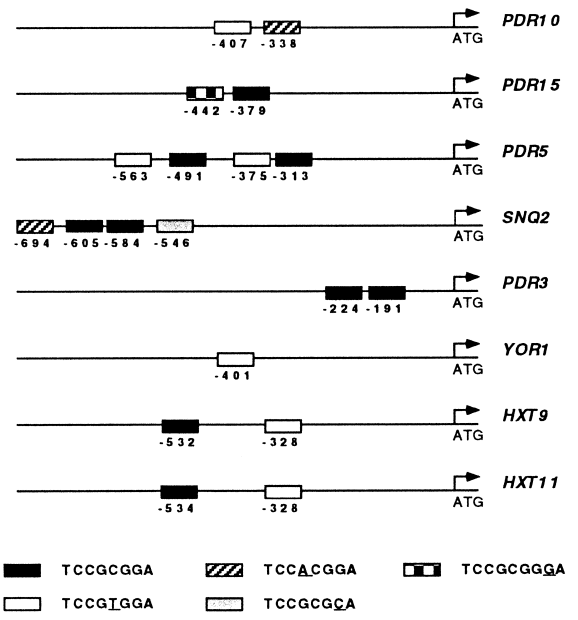

Fig. 1. Different PDREs are present in the promoters of Pdr1/Pdr3 target genes. Boxes represent the PDRE motifs. Numbers below indicate the position of the first PDRE nucleotide relative to the putative translational start site. The exact PDRE sequences are given in the legend.

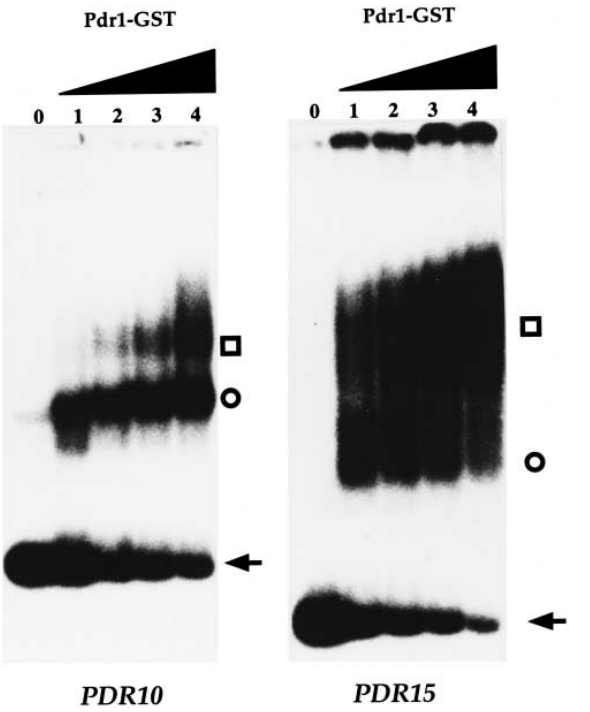

Fig. 2. GST-Pdr1 can bind to both PDREs in the promoters of *PDR10* and *PDR15*. In the gel retardation assay, the band of the free DNA probe (arrows) is partially shifted (circles) upon addition of recombinant Pdr1-GST fusion protein. Increasing the amount of Pdr1-GST from about 0.1 nM (lane 1) to 16 nM (lane 4) leads to a supershift (boxes). In the control (lane 0) no fusion protein was added.

3.2. Identification of two Pdr1 binding sites in the PDR15 promoter

To precisely identify the actual sequence elements recognized by Pdr1 in the *PDR15* promoter, a DNase I footprint assay was performed using the specific DNA probe PDR15-AB in the presence or absence of the GST-Pdr1 protein. The results shown in Fig. 3 confirmed that the above suspected PDREs (Fig. 1) in the *PDR15* promoter can indeed be protected by recombinant GST-Pdr1. The Maxam-Gilbert reaction on the same DNA fragment (A+G) enabled the identification of the actual sequence of the Pdr1-protected sites. As shown in Fig. 3, the sequences protected by GST-Pdr1 contain the consensus PDRE motif (5′-TCCGCGGA-3′) and the degenerate 5′-TCCGCGGGA-3′ PDRE site. Similarly, footprint analysis on the *PDR10* promoter also confirmed the two different potential PDRE motifs to be protected by GST-Pdr1 in vitro (data not shown).

3.3. mRNA levels of PDR10 and PDR15 fluctuate in pdr1 and pdr3 mutants

Transcription of the *PDR5* gene is under the control of Pdr1/Pdr3, since *PDR5* mRNA was found to be of higher abundance in *pdr1-3* gain-of-function mutants [6]. To test if this is also the case for *PDR10* and *PDR15*, we performed Northern analysis on total RNA isolated from isogenic *PDR1* and *pdr1-3* strains as well as *PDR3* and *pdr3-2* gain-of-function mutants (Fig. 4). Quantification of the results by laser scanning densitometry indicated that the *PDR10* mRNA was 10-fold increased in a *pdr1-3* mutant when compared to the isogenic *PDR1* wild type strain (Fig. 4) However, *PDR10*

mRNA levels were almost unchanged in the *pdr3-2* mutant. The mRNA for *PDR15* was about 5-fold more abundant in *pdr1-3* and at least 20-fold increased in *pdr3-2* when compared to each of the corresponding isogenic wild type strains WT-2 and WT-3 (Fig. 4). Hybridization of the same Northern blot with *PDR5*- and *STE6*-specific probes served as positive and negative controls for target genes of the yeast PDR network, respectively, and allowed the verification of equal RNA loading in each lane. While *PDR5* mRNA was massively induced in both *pdr1-3* and *pdr3-2* strains, *STE6* mRNA levels remained constant in all strains tested as described previously [8].

Previous studies indicated that both Pdr1 and Pdr3 can recognize the PDRE consensus motif with the sequence 5′-TCCGCGGA-3′ [11–13]. Consistent with the observed upregulation of *PDR10* and *PDR15* in gain-of-function mutants, one would predict and expect reduced mRNA levels of the genes in question in yeast strains lacking Pdr1 and/or Pdr3. Thus, we performed Northern analysis on total RNA isolated from a set of isogenic yeast strains harboring Δ*pdr1*, Δ*pdr3* and Δ*pdr1* Δ*pdr3* deletion mutations. Although the amount of *PDR10* mRNA was not affected in the Δ*pdr1* strain, it was severely affected in the Δ*pdr3* and in the Δ*pdr1* Δ*pdr3* double mutant. Surprisingly, we consistently found increased mRNA levels of *PDR15* in a Δ*pdr1* strain when compared to the corresponding isogenic wild type WT-1 (Fig. 4). By contrast, *PDR15* transcription was almost completely obliterated in the Δ*pdr3* and the Δ*pdr1* Δ*pdr3* double mutant. Again, Pdr3 appears to have a more pronounced effect on the control of *PDR15* than Pdr1. As expected, the levels of *STE6* mRNA were completely unaffected in any of the strains tested, confirming equal RNA loading in each lane.

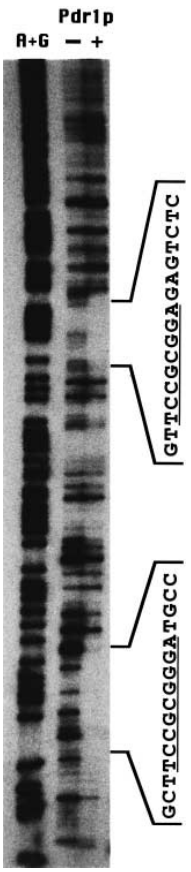

Fig. 3. DNase I footprint analysis identifies PDREs protected by a Pdr1-GST fusion protein in the *PDR15* promoter. The 5′-labeled DNA fragment PDR15-AB was treated with DNase I with (+) or without (−) prior incubation with Pdr1-GST fusion protein. Samples from a Maxam-Gilbert reaction (A+G) on the same DNA fragment were also loaded. Both sequences protected by Pdr1-GST contain a PDRE motif (underlined).

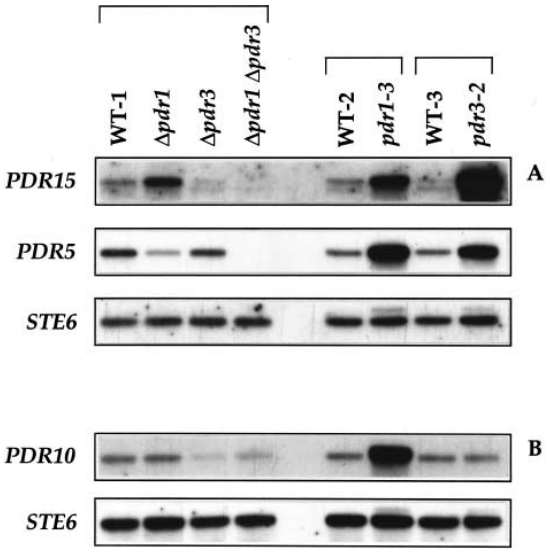

Fig. 4. Northern blot analysis on RNA from isogenic Δ*pdr1*/Δ*pdr3* and *pdr1-3* and *pdr3-2* mutant strains. Total yeast RNA was isolated from strains FY1679-28C (WT-1), FY1679-28C/*pdr1*::*TRP1* (Δ*pdr1*), FY1679-28C/*pdr3*::*HIS3* (Δ*pdr3*), FY1679-28C/*pdr1*::*TRP1* *pdr3*::*HIS3* (Δ*pdr1* Δ*pdr3*), YALF-I1 (WT-2), YALF-A1 (*pdr1-3*), YYMI4-O3 (WT-3) and YYMI4-A4 (*pdr3-2*). About 18 μg RNA were fractionated as described in Section 2. The radiolabeled DNA fragments used for hybridization were derived from *PDR10* (nt +93 to +1109; exposure time: 190 h), *PDR15* (nt +13 to +721; exposure time: 120 h), *PDR5* (nt +14 to +977; exposure time: 45 min) and *STE6* (nt −1 to +981; exposure time: 20 h on blot A, 70 h on blot B).

Taken together, our results demonstrate that both *PDR10* and *PDR15* are novel targets for regulation by the Pdr1 and Pdr3 transcription factors. In addition, our results suggest a novel PDRE motif with the sequence 5′-TCCGCGGGA-3′ in the *PDR15* promoter to be recognized by the Pdr1/Pdr3 regulators.

4. Discussion

In this report we have shown that *PDR10* and *PDR15* are subject to transcriptional control by the yeast PDR network. Hence, the yeast PDR network now comprises all of the yeast Pdr5 subfamily members, as well as Snq2 [8], Yor1 [9], Hxt9 and Hxt11 [3]. Moreover, we show that the collection of PDREs mediating Pdr1/Pdr3 control also include the novel 5′-TCCGCGGGA-3′ element, in addition to the PDREs known so far (5′-TCCGCGGA-3′, 5′-TCCGTGGA-3′, 5′-TCCGCGCA-3′, and 5′-TCCACGGA-3′) found in the promoters of PDR responsive genes (Fig. 1). These results suggest that Pdr1 and Pdr3 tolerate at least some sequence degeneration in the PDRE without abolishing their binding to target genes. The functional significance of various PDRE motifs present in different numbers and relative position to the translational start site is not entirely clear. However, we propose that different combinations of PDREs could contribute to a

differential control of target genes by Pdr1, Pdr3 or both transcription factors under certain physiological conditions. For example, the 5′-TCCACGGA-3′ PDRE has been shown to bind both Pdr1 and Pdr3 in the *PDR5* promoter [12], whereas no binding of Pdr3 to the same element was observed in the promoter of *HXT11* [3]. In contrast, our results from gel retardation assays and DNase I footprints (Fig. 1, open boxes) on the *PDR10* promoter indicate binding of both Pdr1 (Fig. 2) and Pdr3 (data not shown) to the 5′-TCCACGGA-3′ PDRE.

Finally, one can argue that Pdr1 and Pdr3 might have different affinities for different combinations of PDREs. One could speculate that different target genes can be differentially controlled by either Pdr1 or Pdr3 depending on the combinations of PDREs present in their promoters. This would provide a possible explanation for the drastic difference in the expression levels of *PDR5*, *PDR10* and *PDR15* in normal cells, all of which possess quite different PDREs. Indeed, evidence from Northern blot analysis suggests that *PDR5* is most highly abundant, while *PDR10* and *PDR15* are expressed at much lower levels. It will therefore be important in future studies to evaluate the binding affinities of authentic Pdr1 and Pdr3 for the different PDRE combinations of individual PDR genes.

Additional transcription factors such as Yap1 [26], which is otherwise required for the general stress response [26,27], might also contribute to transcriptional control within the PDR network. For instance, induced expression of Snq2, which is also regulated by Pdr1 and Pdr3 [8], in cells responding to various external stress conditions requires a functional *YAP1* gene (Y. Mahé et al., in preparation). Furthermore, we have recently shown that a Yap1-mediated diazaborine resistance phenotype requires the presence of functional Pdr1 and Pdr3 [28], demonstrating a link between the general stress response pathway and the PDR network. Interestingly, the transcription factor Ngg1, a dual function regulator required for glucose repression [29], is thought to directly inhibit Pdr1 function in wild type cells [30], thereby also contributing to this process of specific gene regulation within the PDR network.

Surprisingly, our studies also revealed that a Δ*pdr1* deletion mutation not always leads to reduced expression of target genes. Our data clearly and consistently demonstrate that *PDR15* mRNA is up-regulated several-fold in a Δ*pdr1* strain, but almost abolished in a Δ*pdr1* Δ*pdr3* double deletion (Fig. 4). While we consider the possibility of an increased *PDR15* mRNA stability unlikely, we speculate that this upregulation could be due to a lack of interaction with other yet unknown transcription factors, which together with Pdr1 could negatively control *PDR15* in wild type cells. For instance, Pdr3 might become more important for *PDR15* control and exhibit higher affinities to the *PDR15* promoter when Pdr1 is absent, which is exactly what our results indicate. This is also consistent with the 20-fold higher abundance of *PDR15* mRNA in *pdr3-2* gain-of-function mutants (Fig. 4).

A similar discrepancy between the effects of Δ*pdr1*/Δ*pdr3* deletions and *pdr1-3*/*pdr3-2* gain-of-function mutations is observed in the regulation of *PDR10*. While the Δ*pdr1* strain shows only a slight decrease in *PDR10* mRNA levels, it is highly up-regulated in the *pdr1-3* mutant. By contrast, the Δ*pdr3* deletion severely reduces *PDR10* mRNA levels, whereas the *pdr3-2* gain-of-function mutant seems to have hardly any effect at all. Taken together, we hypothesize that these results imply a functional cross-talk between Pdr1 and Pdr3 and perhaps other yet unknown transcriptional regulators in the regulation of *PDR10* and *PDR15*. While the physiological function of Pdr10 and Pdr15 remains obscure at the moment, it will certainly be interesting to determine whether or not Pdr10 and Pdr15, like Pdr5, represent functional ABC drug efflux pumps.

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