Expression regulation of the yeast *PDR5* ATP-binding cassette (ABC) transporter suggests a role in cellular detoxification during the exponential growth phase

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Abstract The yeast ATP-binding cassette transporter Pdr5p mediates pleiotropic drug resistance (PDR) by effluxing a variety of xenobiotics. Immunoblotting demonstrates that Pdr5p levels are high in the logarithmic growth phase, while its levels decrease sharply when cells exit exponential growth. Here, we show that *PDR5* promoter activity is dramatically reduced when cells stop growing due to a limitation of glucose or nitrogen or when they approach stationary phase. Interestingly, Pdr3p, a major transcriptional regulator of *PDR5*, shows the same regulatory pattern. Feeding glucose to starved cells rapidly re-induces both *PDR5* and *PDR3* transcription. Importantly, diminished Pdr5p levels, as present after starvation, are rapidly restored in response to xenobiotic challenges that activate the transcription factors Pdr1p and Pdr3p. Our data indicate a role for yeast Pdr5p in cellular detoxification during exponential growth.

Key words: ABC transporter; Pdr5p; Detoxification; Exponential growth; Yeast

1. Introduction

In *Saccharomyces cerevisiae*, ATP-binding cassette (ABC) transporters are involved in a phenomenon known as pleiotropic drug resistance (PDR), which is similar to multidrug resistance observed in mammalian cells. One of the best-characterized proteins involved in PDR is the yeast Pdr5p ABC efflux pump, a polytopic plasma membrane protein consisting of 12 predicted transmembrane-spanning α-helices and two ATP-binding domains [1–4]. Like its functional homologues Snq2p [5] and Yor1p [6], Pdr5p extrudes hundreds of structurally and functionally unrelated xenobiotics across the plasma membrane [5,7–10]. Despite Pdr5p being one of the best-characterized ABC drug efflux pumps in yeast, its physiological function is still unclear, although it is generally believed to play a role in cellular detoxification processes.

Transcription of yeast ABC genes such as *PDR5* [11,12], *SNQ2* [13], *PDR10*, *PDR15* [14], *YOR1* [6], is controlled by the $Zn(II)_2Cys_6$ zinc finger regulators Pdr1p [15], Pdr3p [16] and Yrr1p [17]. The Pdr1p/Pdr3p transcription factors share partially overlapping function and control their target promoters by binding to the pleiotropic drug resistance elements (PDREs). Both regulators can form homo- or heterodimers to modulate target promoter activities [18].

PDR5 transcription control by Pdr1p and Pdr3p occurs through four PDREs [14,19]. Recent findings indicate that *PDR5* can be induced by some of its drug substrates such as 2,4-dichlorophenol (DCP), as well as by membrane detergents [18,20]. Furthermore, a high expression of both Pdr5p and Pdr3p is observed upon loss of the mitochondrial function or lack of functional F_0-ATPase [21,22]. This Pdr5p induction through as yet unknown signals requires both PDREs in the *PDR5* promoter and Pdr3p. Interestingly, *PDR3* is also controlled by two perfect PDREs in its promoter, which perhaps involves an autoregulatory loop [23]. Different regulatory effects of Pdr1p/Pdr3p, including both positive and negative control of individual targets, have been observed when comparing the expression levels of *PDR5*, *PDR10* and *PDR15* in *pdr1*Δ or *pdr3*Δ strains [14]. Despite entertaining the same PDREs, deletion of *PDR1* reduces *PDR5* expression, but increases *PDR15* mRNA levels [14]. Another zinc finger transcription factor, Rdr1p, has a negative role in controlling *PDR5* and *PDR15* in a PDRE-dependent manner [24]. Hence, Pdr1p/Pdr3p-dependent gene regulation varies considerably between different promoters, probably due to other factors present in the respective context [14].

In this study we analyzed the regulation of the *PDR5* promoter throughout the growth phase of yeast cells. Pdr5p levels decrease rapidly when cells stop growth by exiting the exponential growth phase, growth on non-fermentable carbon sources or by limitation of nutrients such as glucose or nitrogen. This regulatory pattern was also observed for the dedicated regulator Pdr3p, but not for Pdr15p, another Pdr1p/Pdr3p target gene. Nevertheless, low levels of *PDR5* expression on non-fermentable carbon sources still allow induction by xenobiotic compounds activating Pdr1p/Pdr3p, indicating that the PDREs are functional under these conditions. These results suggest that Pdr5p functions as a cellular detoxification determinant under logarithmic growth conditions.

2. Materials and methods

2.1. Yeast strains, plasmids and growth conditions

Rich medium (YPD) and synthetic medium (SC), supplemented with appropriate auxotrophic components, were prepared as described

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Table 1
Yeast strains used in this study

Yeast strain	Relevant genotype	Source
YPH499	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 lys2-801a trp1-Δ1 ade2-Δ101*^{oc}	[34]
YKKA7	*pdr5*Δ::*TRP1*; otherwise isogenic to YPH499	[2]
YJH1	*pdr1*Δ::*HIS3Mx6*; otherwise isogenic to YPH499	This study
YYA18	*pdr3*Δ::*KanMx6*; otherwise isogenic to YPH499	This study
YYA20	*ura3-52::PDR5-lacZ-URA3*; otherwise isogenic to YPH499	This study
YCS-15HA	*PDR15-3HA-KanMx6*; otherwise isogenic to YPH499	This study
W303-1A	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 lys2-801a trp1-Δ1 ade2-Δ101*^{oc}	[13]
FY1679-28c	*MAT***a** *ura3-52 his3-Δ200 leu2-Δ1 trp1-Δ63*	[18]
naΔ1	*pdr1*Δ::*TRP1*; otherwise isogenic to FY1679-28c	[16]
naΔ3	*pdr3*Δ::*HIS3*; otherwise isogenic to FY1679-28c	[16]
naΔ1Δ3	*pdr1*Δ::*TRP1 pdr3*Δ::*HIS3*; otherwise isogenic to FY1679-28c	[16]
YRP11	HA-*PDR3-LEU2*; otherwise isogenic to FY1679-28c	[18]

elsewhere [25]. Glucose starvation was carried out in YPGE medium containing 1% ethanol and 2% glycerol instead of glucose. For nitrogen starvation, cells were grown on SC medium and then shifted to SC medium without amino acids and ammonium sulfate containing only 10% of the usual concentrations of essential amino acids.

Yeast strains and plasmids used in this study are listed in Table 1. Yeast strains YJH1 (*pdr1*Δ::*HIS3Mx6*) and YYA18 (*pdr3*Δ::*KanMx6*) were obtained by genomic integration of appropriate polymerase chain reaction (PCR) products synthesized from the plasmids pFA6aHIS3Mx6 and pFA6aKanMx6 [26]. For strain YYA20, the integrating plasmid pCS5zi, carrying the *lacZ* gene under the control of the *PDR5* promoter, was linearized with *Nco*I and integrated into the *URA3* locus of YPH499 cells. Plasmid pRE104 was obtained by recombination of a PCR product containing GFP-HIS3Mx6, which was amplified from pCKSF1 [27] containing GFP-HIS3Mx6 fused to the C-terminus of *PDR5*. Correct genomic integration of all constructs was verified by PCR analysis.

2.2. Preparation of extracts and immunoblotting

For immunoblot analysis, 3 OD_{600} equivalents of cells were harvested, diluted in 1 ml water and incubated on ice with 150 μl YEX lysis buffer (1.85 M NaOH, 7.5% β-mercaptoethanol) for 10 min. Proteins were precipitated by adding 150 μl of 50% trichloroacetic acid. Samples were centrifuged at 13 000 × *g* and the pellet was resuspended in sample buffer (40 mM Tris–HCl pH 6.8, 8 M urea, 5% sodium dodecyl sulfate (SDS), 0.1 mM EDTA, 0.1 g/l bromophenol blue, 1% β-mercaptoethanol). Proteins were separated through SDS–polyacrylamide gel electrophoresis gels and blotted onto nitrocellulose membranes. Pdr5p was immunodetected using polyclonal anti-Pdr5p serum 'Gaston 6' [28]. HA-Pdr3p was detected with monoclonal anti-HA antibody 16B12 (BabCo, Berkeley, CA, USA).

2.3. β-Galactosidase assays and fluorescence microscopy

β-Galactosidase measurements were carried out exactly as described before [29]. All experiments were done at least in triplicate. Fluorescence studies were done in living cells grown in YPGE for 6 h, or centrifuged and again transferred into YPD medium. Green fluorescent protein (GFP) was visualized using a Zeiss Axiovert microscope, images were obtained using a Quantix CCD camera (Roper Scientific) with IP LAB software (Spectra Services) and processed in Photoshop 5.5 (Adobe).

2.4. Northern analysis

RNA preparation and separation was done exactly as described [14]. The membrane was prehybridized in 10×Denhardt's buffer (1 g Ficoll 400, 1 g polyvinylpyrrolidone, 1% (w/v) bovine serum albumin fraction V), 2×SSC, 1% SDS for 3 h at 65°C. Probes (*PDR5*: 960 bp, *GAP1*: the first 495 bp) were labeled using Megaprime labeling kit and added to the prehybridization solution after purification of the radiolabeled probe through a NICK column and subsequent heat denaturation. After an overnight incubation at 65°C, the membrane was washed with 2×SSC, 1% SDS and 1×SSC, 1% SDS, both at 65°C, and analyzed with a Phosphor Imager (Storm 1840, Molecular Dynamics).

2.5. In vivo footprint analysis

Cells were grown in exponential growth phase in SC medium for four generations and then shifted to YPD or YPGE for 2 h. Cells

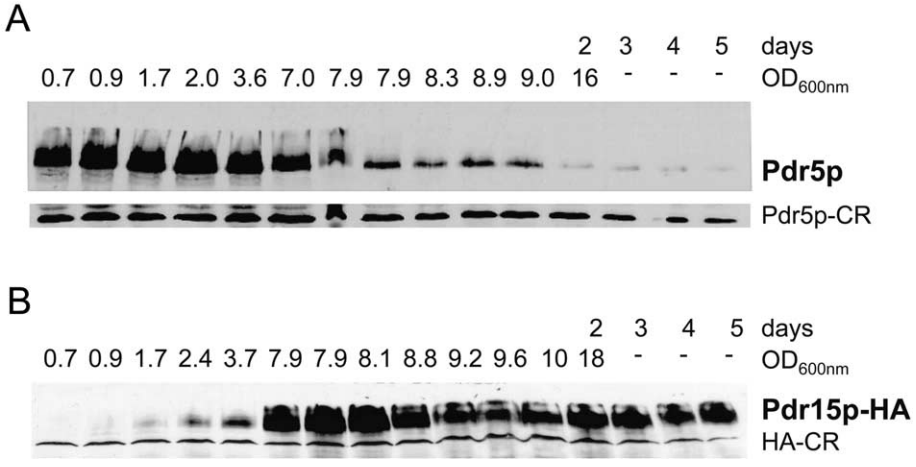

Fig. 1. Pdr5p levels decrease when cells exit exponential growth phase. YPH499 (A) and YCS-15HA (B) cells were inoculated in YPD medium to an OD_{600} of 0.2 and then cultured for 5 days. Samples were initially taken every 90 min (OD_{600} 0.5–3.7), then every 2 h (until OD_{600} 9.0 (A) or 10 (B)), and then every 24 h. The OD_{600} was measured, protein extracts were prepared and Pdr5p and Pdr15-HA levels were detected by immunoblotting using polyclonal anti-Pdr5p antibodies or the anti-HA antibodies 16B12, respectively. Cross-reactions against the anti-Pdr5p (Pdr5p-CR) and the anti-HA antibodies (HA-CR) were used as loading controls to confirm equal sample loading.

were harvested and in vivo footprints were carried out exactly as previously described [18] using the primers (Pdr5-220as 5′-ACATCACAATTATATATGTATAGGAGAGGCC-3′ and Pdr5-530s 5′-AGAAAACGTCCGTGGAGAACCATTCGGTCG-3′). Gels were scanned using a Phosphor Imager (Storm 1840, Molecular Dynamics) and quantified (Image Quant 5.0, Molecular Dynamics).

3. Results

3.1. Pdr5p levels decrease when cells enter stationary phase

Pdr5p is highly expressed in logarithmically growing cells. We therefore investigated Pdr5p expression levels under different growth conditions (Fig. 1). YPH499 (Fig. 1A) and YCS-15HA (Fig. 1B) cells were grown in YPD for 5 days, samples were taken first every 90 min and later once per day and subjected to immunoblotting. From OD_{600} 0.4 to 7.0, Pdr5p appeared as a strong band on immunoblots (Fig. 1). However, in cultures exceeding an OD_{600} of 7, which coincides with the end of exponential growth, cellular Pdr5p levels decreased sharply and rapidly. After the culture passed an OD_{600} of 9.0, Pdr5p levels remained very low albeit detectable. To prove that this expression pattern is specific to Pdr5p, we tested the levels of another yeast ABC transporter, Pdr15p, one of the closest homologues of Pdr5p. Interestingly, and in contrast to Pdr5p, Pdr15p-HA levels increased strongly during later growth stages (Wolfger et al., submitted for publication) and remained constant over the 4 days following the post-diauxic shift. Hence, these results demonstrate that Pdr5p is strongly expressed during exponential growth but its levels decrease rapidly at the end of logarithmic growth.

3.2. PDR5 levels are diminished in cells limited for growth or nutrients

Upon exit of the exponential growth phase, yeast cells encounter various adverse conditions, including glucose, nitrogen or nutrient limitation, but also suffer from accumulation of catabolites, some of which are toxic and thus exert growth-inhibitory effects. To test whether some of these conditions influence Pdr5p expression, we first starved cells for glucose. Logarithmically growing YPH499 cells were shifted from YPD to YPGE medium containing glycerol/ethanol as non-fermentable carbon sources. Samples were taken from YPD-grown cells and after 2 h of growth in YPGE. Then, YPGE was either replaced by YPD, or 2% of glucose was added to the YPGE culture. Northern blotting showed high *PDR5* mRNA levels in YPD-grown cells (Fig. 2A). However, after only 2 h in YPGE, *PDR5* mRNA levels were nearly abolished. Replacing the non-fermentable carbon sources by glucose again restored *PDR5* expression within 20 min. Addition of 2% glucose to YPGE medium also increased *PDR5* after 40 min, demonstrating that addition of glucose elicits a signal for *PDR5* induction.

To analyze whether this regulation is dependent on the *PDR5* promoter, YYA20 cells carrying a *PDR5-lacZ* reporter gene were grown in YPD to an OD_{600} of 1.0 and shifted to YPGE medium for 16 h. The YPGE medium was then replaced by YPD for 1 h. The high β-galactosidase activities in the exponential growth phase were reduced to about 10% after 16 h of glucose limitation (Fig. 2B). Addition of YPD restored about 40% of the original β-galactosidase activity within 1 h.

The induction of Pdr5p following glucose starvation was also directly visualized by fluorescence microscopy using YKKA7 cells expressing *PDR5*-GFP from plasmid pRE104 (Fig. 1C). The typical rim staining of Pdr5p was completely absent after 12 h of glucose limitation. However, 20 min after shifting from YPGE to YPD, a faint staining of the plasma membrane appeared, which was more pronounced after 1 h (Fig. 2C), indicating restored Pdr5p biogenesis and its proper plasma membrane targeting.

To test whether a lack of glucose is the only signal triggering Pdr5p repression, we also performed nitrogen starvation experiments (Fig. 3). YPH499 cells were grown to an OD_{600} of 1.0 and SC medium replaced by nitrogen starvation medium. Northern blot analysis revealed that *PDR5* mRNA levels were severely diminished after 4 h of nitrogen starvation and remained low during a 24 h period (Fig. 3A). Nitrogen starvation was confirmed by the sharp increase of the *GAP1*

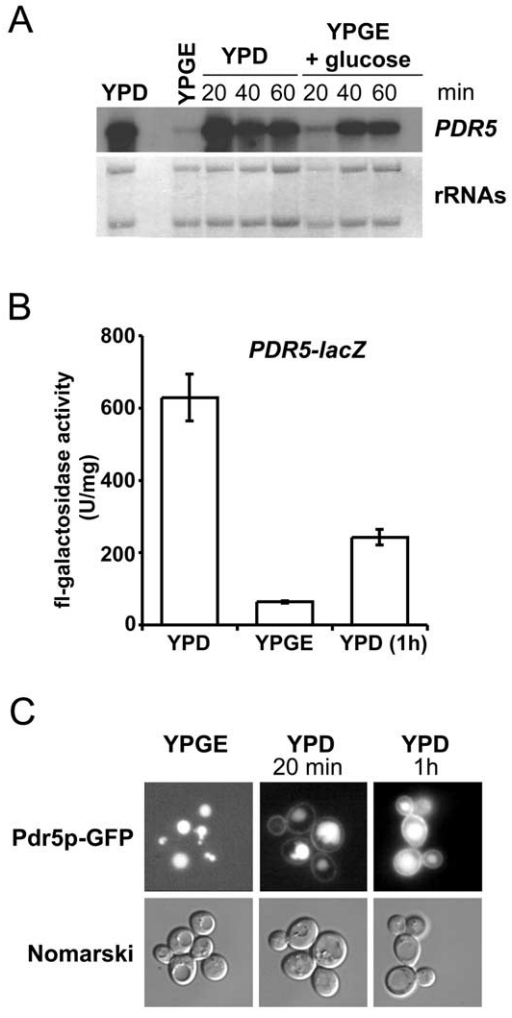

Fig. 2. Pdr5p is repressed during growth on non-fermentable carbon sources. A: YPH499 cells were grown in YPD to an OD_{600} of 1.0 (YPD), supplied with YPGE for 2 h (YPGE), before replacing YPGE by YPD (YPD, 20′, 40′, 60′) or glucose addition to YPGE (YPGE, 20′, 40′, 60′). Samples were taken and *PDR5* mRNA levels were detected by Northern blotting. B: Strain YYA20 was grown in YPD to an OD_{600} of 1.0, shifted to YPGE for 16 h (YPGE), before replacing YPGE by YPD for another 1 h (YPD). Cell extracts were prepared and β-galactosidase enzyme activities were assayed in triplicate. C: YKKA7 strain carrying plasmid pRE104 was grown overnight in YPGE (YPGE). Cells were then placed in YPD (YPD 20′, YPD 1 h) and inspected by microscopy in a Zeiss Axiovert microscope using fluoresceine isothiocyanate filters.

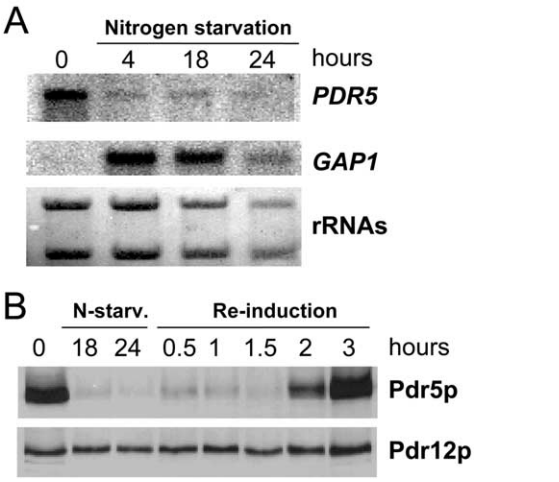

Fig. 3. Nitrogen starvation strongly decreases *PDR5* expression. A: YPH499 cells were grown in SC medium to an OD_{600} of 1.0 (0). Nitrogen depletion was carried out in N-starvation medium for 24 h. Samples were taken and *PDR5* and *GAP1* mRNA levels were detected by Northern blotting. B: YPH499 cells were grown in SC medium to an OD_{600} of 1.0 (0), depleted for nitrogen over 24 h, before addition of ammonium sulfate as well as amino acids to terminate nitrogen starvation. Samples were taken at the indicated time points and Pdr5p and Pdr12p levels were detected by immunoblotting as described above.

mRNA for the general amino acid permease within the first 4 h. Gap1p is known to be strongly up-regulated during nitrogen starvation [30]. However, Pdr5p levels were rapidly replenished when nitrogen sources became available again. YPH499 cells were grown in SC medium, shifted to nitrogen starvation medium and incubated for 24 h. Starvation was terminated by addition of ammonium sulfate as well as amino acids to levels present in normal SC medium (Fig. 3B). The high Pdr5p levels present in SC medium were severely diminished after a 18 or 24 h period of nitrogen limitation. Addition of nitrogen sources to the starvation medium swiftly restored normal Pdr5p levels within 2 h (Fig. 3B). This was also confirmed by β-galactosidase measurements in YYA20 cells, where growth under nitrogen limitation for 18 h reduced the β-galactosidase activity to about 30% of normal. However, within 2 h after refeeding cells with SC medium, 60% of the normal activity was recovered (data not shown). This demonstrates that not only glucose starvation, but also lack of nitrogen strongly reduces *PDR5* promoter activity.

3.3. *Influence of Pdr1p and Pdr3p on expression of Pdr5p under starvation conditions*

PDR5 and several other yeast ABC efflux pumps are controlled by the Pdr1p and Pdr3p regulators. The *PDR3* promoter also contains two perfect PDREs, and its regulation proceeds through an autoregulatory loop. We thus tested whether *PDR3* is also repressed by glucose limitation. Strain YRP11 expressing HA-Pdr3p under the control of its own promoter was grown in YPD to an OD_{600} of 1.0, shifted to YPGE for 6 h, and again transferred to YPD. As for Pdr5p, HA-Pdr3p levels were strongly reduced upon onset of starvation, but rapidly increased in the presence of glucose (Fig. 4A). Therefore, not only Pdr5p, but also one of its main transcriptional regulators is repressed during growth on non-fermentable carbon sources.

Next, we investigated the role of Pdr1p/Pdr3p in regulation of Pdr5p under starvation conditions. YPH499, YJH1 (*pdr1*Δ) and YYA18 (*pdr3*Δ) cells growing exponentially in YPD medium were put on YPGE for 4 h and shifted back to YPD (Fig. 4B). In Northern blot analysis, all three strains showed

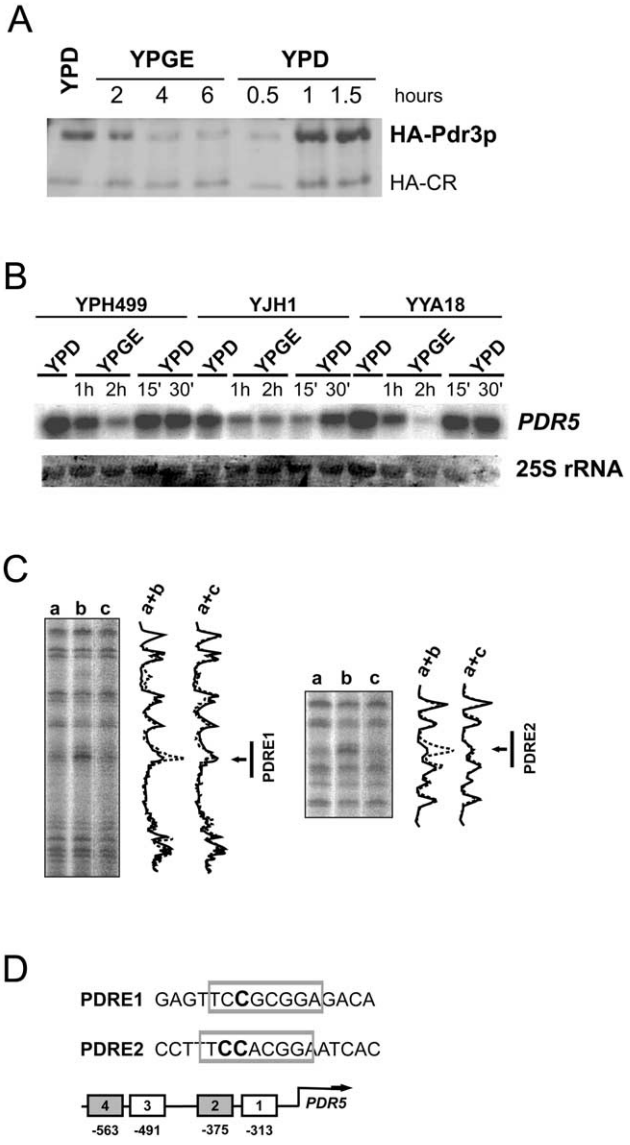

Fig. 4. Influence of Pdr1p and Pdr3p on *PDR5* repression. A: YRP11 cells were grown in YPD to an OD_{600} of 1.0 (YPD), shifted to YPGE for 6 h (2, 4, 6 h) and back-transferred into YPD for 90 min (0.5, 1, 1.5 h). Samples were taken and HA-Pdr3p levels were detected by immunoblotting using monoclonal anti-HA antiserum 16B12. Cross-reactions against the anti-HA antibodies (HA-CR) were used as loading controls to confirm equal sample loading. B: Strains YPH499, YJH1 (*pdr1*Δ) and YYA18 (*pdr3*Δ) were grown in YPD to an OD_{600} of 1.0 (YPD), YPGE was added for 2 h (YPGE) and again replaced by YPD. *PDR5* mRNA was detected by Northern blotting. C: FY1679-28c (a,c) and naΔ1Δ3 cells (b) were grown in SC medium for 4 h and shifted to YPD (a,b) or to YPGE (c) for 2 h each. Cells were harvested and treated with DMS. DNA was prepared and the methylation status of the perfect PDRE1 and PDRE2 was detected by primer extension analysis. D: Topology and position relative to the initiating methionine of all PDRE *cis*-acting motifs in the *PDR5* promoter. White boxes, perfect PDRE; gray boxes, degenerate PDRE; bold letters in the sequence of the PDRE1 and PDRE2 consensus motifs indicate protected nucleotide bases.

almost identical changes in *PDR5* mRNA levels at all time points. Reduced levels of *PDR5* mRNA were evident after 1 and 2 h of starvation, while *PDR5* was rapidly re-induced within 15–30 min after the shift to YPD medium (Fig. 4B). Therefore, neither Pdr1p nor Pdr3p alone can be responsible for the repression of *PDR5*.

To test whether Pdr1p and Pdr3p decorate the *PDR5* promoter during glucose starvation, we performed in vivo footprint experiments of FY1679-28c wild type cells and cells lacking both regulators. Cells were grown in SC medium to the exponential growth phase for 4 h. One aliquot of each culture was shifted to YPGE, while another was grown in YPD. After 2 h, cells were harvested, treated with DMS, and the methylation status of the PDRE1 and PDRE2 *cis*-acting sites was analyzed by primer extension (Fig. 4C). As shown previously, primer extension analysis detects modified nucleotide bases, thus revealing a direct promoter binding of Pdr1p or Pdr3p to its cognate PDRE motifs [18]. In wild type cells, methylation of PDRE1 and PDRE2 in the *PDR5* promoter was clearly reduced when compared to the deletion strain (Fig. 4C, a+b), indicating binding of both transcription factors. In the control strain lacking both Pdr1p and Pdr3p, the methylation of PDREs was not reduced. However, we did not detect a difference in the methylation protection in wild type cells harvested from YPD or YPGE, (Fig. 4C, a+c), suggesting that various PDRE motifs attract Pdr1p, Pdr3p and/or Pdr1p/Pdr3p binding even in the absence of glucose, as already shown earlier for logarithmically growing cells [18].

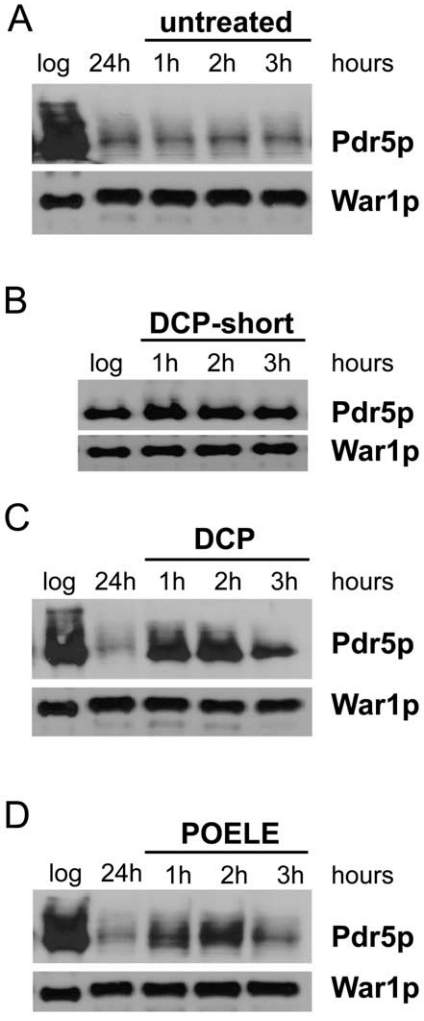

Fig. 5. Pdr5p is induced by its substrates in stationary phase. W303-1A cells were inoculated into YPD to an OD_{600} of 0.2 and grown for 24 h, or until reaching an OD_{600} of 10. Culture aliquots were left untreated (A) or stressed with 0.3 mM DCP (C) or 100 μM POELE (D) for the indicated time periods. Another YPD culture aliquot was grown for 24 h without drugs; next, cells were re-inoculated into fresh YPD, grown to the exponential phase for 4 h, followed by treatment with 0.3 mM DCP (DCP-short) for various times as indicated (B). The panels refer to the untreated control (A), DCP-short for logarithmic cells (B), DCP treatment of stationary phase cells (C), and POELE treatment (D). Samples were taken and cell free extracts subjected to immunoblotting for Pdr5p levels (A–D). Immunoblotting for War1p levels served as loading control to confirm equal sample loading.

3.4. *Repressed Pdr5p can be induced by drug treatment in stationary cells*

Recently, Pdr5p was shown to be induced by some of its drug substrates, including the herbicide DCP [20] (Mamnun et al., in preparation). To test the re-induction of Pdr5p by DCP under starvation conditions, W303-1A cells were grown to an OD_{600} of about 10.0. Samples were taken when cells were in exponential growth phase, as well as prior to DCP stress treatments. Then, 0.3 mM DCP (Fig. 5C) or 100 μM polyoxyethylene-9-laurylether (POELE) (Fig. 5D) was added to the cultures, while one control aliquot remained untreated (Fig. 5A). Another culture aliquot was re-grown in YPD to the exponential growth phase, before imposing DCP stress (Fig. 5B). Samples were taken after 1, 2 and 3 h and Pdr5p levels were detected by immunoblotting. As observed before, DCP did not further increase the already high Pdr5p levels in exponentially growing cells. However, cells at an OD_{600} of 10, which had severely reduced Pdr5p levels, showed a massive increase of expression in response to DCP treatment. POELE also increased Pdr5p expression, albeit to a much lower level. Untreated control cells (Fig. 5A) showed that Pdr5p levels remained low in the absence of these stresses. Notably, other known Pdr5p substrates such as ketoconazole failed to restore levels of Pdr5p expression (data not shown). Taken together, these data suggest a role for Pdr5p in cellular detoxification during logarithmic growth of cells or when cells can rely upon optimal growth conditions.

4. Discussion

Although the physiological role of the Pdr5p efflux pump is not fully understood, it is generally believed that Pdr5p might play a role in cellular detoxification. Pdr5p, which is highly abundant in exponentially growing cells, effluxes hundreds of cytotoxic compounds [1–4,7–9], many of which may actually resemble toxic metabolites. During periods of their life cycle, yeast cells also have to cope with limiting or suboptimal growth conditions. Therefore, we analyzed whether *PDR5* expression is modulated by suboptimal conditions such as non-fermentable carbon sources or nutrient starvation. Interestingly, Pdr5p levels are indeed dramatically reduced when cells encounter these growth-limiting conditions because of a reduced transcription or repression of the *PDR5* gene.

The transcription factors Pdr1p/Pdr3p are considered the main regulators of Pdr5p expression. Indeed, these master regulators are responsible for high levels of *PDR5* transcrip-

tion in exponentially growing cells *PDR15* [6,14]. Interestingly, a close inspection of published microarray datasets [31] (see also http://www-genome.stanford.edu/yeast_stress) for expression levels of other Pdr1p/Pdr3p target genes during stationary phase, diauxic shift and nitrogen depletion showed that only *PDR5*, but not *SNQ2*, *YOR1*, *PDR10* or *PDR15* are strongly repressed under these conditions. By contrast, expression of other ABC genes such as *YOR1*, *PDR15* and *PDR10* is induced during prolonged stationary phase [31], suggesting that *PDR5* repression does not merely reflect a general response of Pdr1p/Pdr3p targets. However, our data also show that at least one PDRE-regulated gene, *PDR3*, is co-regulated with Pdr5p under glucose limitation conditions. A similar phenomenon was found in cells with impaired mitochondrial activities [21]. In ρ^0 cells, transcription of *PDR5*, *PDR15* and *PDR3* is increased [21,22,32], while *SNQ2* and *YOR1* were only mildly affected [22]. Hence, regulation of Pdr1p and Pdr3p is distinct and specific for every target gene, implying that additional regulatory mechanisms must exist [33], perhaps depending on the actual growth conditions or the cellular energy status. One possibility of specific control of Pdr1p and Pdr3p targets is the formation of various Pdr1p/Pdr3p homo- and/or heterodimers [18] depending on the promoter context. However, this appears unlikely at least for Pdr5p from these data, since single deletions of Pdr1p or Pdr3p do not influence *PDR5* repression during glucose starvation. In vivo footprint analysis demonstrates that binding of Pdr1p and Pdr3p to at least two *PDR5* promoter PDREs is not inhibited by glucose limitation, indicating constitutive occupancy of the *PDR5* promoter by Pdr1p/Pdr3p. Nevertheless, since Pdr1p and Pdr3p are the main regulators of *PDR5* expression, they are likely to have a pivotal role in the growth stage-specific regulation described here. Our results therefore imply that Pdr1p and Pdr3p might recruit additional and as yet unidentified transcription factors that lead either to *PDR5* activation under optimal growth conditions or to repression when cells have to cope with growth-limiting conditions.

The identity of the regulators acting upstream of Pdr1p and Pdr3p remains an open question. We excluded several candidate transcription factors or glucose sensors (data not shown). The zinc finger regulator Rdr1p was identified as a repressor of *PDR5*, which displays its inhibitory effects via the PDREs [24]. However, deletion of *RDR1* increased Pdr5p levels during exponential growth, which is consistent with its purported negative effect on Pdr5p expression regulation. Lack of Rdr1p, however, does not prevent repression during nitrogen starvation (data not shown). Other known regulators required for the transition from fermentative to respiratory growth, like the *SNF1* kinase or protein kinase A, are also not required for the transcriptional regulation of *PDR5* under these conditions (data not shown). Therefore, the signals as well as the pathways that regulate *PDR5* repression under growth-limiting conditions are unclear at the moment.

The tight regulation of Pdr5p levels may be interpreted in several ways. High expression during exponential growth might point to a role in detoxification in this growth stage. Further, Pdr5p levels might serve as a precaution to fight xenobiotic attacks, as they mount due to competition with other competitor organisms when nutrients are not limiting. By contrast, high expression levels of Pdr5p might be detrimental under conditions when energy supplies or nutrients become limiting. Alternatively, Pdr5p could directly interfere with membrane features such as selective permeability for molecules that are required to re-enter growth from stationary phase. Removal of Pdr5p in non-growing cells would prevent pumping and loss of such molecules, and may thus lead to reduced ability to resume growth. In this respect it is interesting to note that Pdr5p can be re-induced even in starved cells by some of its drug substrates, including DCP or the detergent POELE. DCP is a highly toxic bacterial degradation product of the plant herbicide 2,4-dichlorophenoxy acetic acid (2,4-D). Indeed, Pdr5p is induced by 2,4-D and it has been implicated in the extrusion of 2,4-D [20]. However, this induction was only observed at the mRNA level, and Pdr5p protein levels were only slightly elevated (Mamnun et al., in preparation), which might be explained by the high levels of basal Pdr5p expression in exponentially growing cells. Nevertheless, these data are consistent with results from our work, which allow for the conclusion that Pdr5p is a stress-responsive gene present mainly during phases of rapid growth where constant detoxification is needed. The rapid re-induction of Pdr5p in quiescent cells challenged by specific stress signals suggests that Pdr5p serves as a major detoxification safeguard for yeast cells.

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