The Yeast Pdr15p ATP-binding Cassette (ABC) Protein Is a General Stress Response Factor Implicated in Cellular Detoxification*

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ATP-binding cassette (ABC) transporters play important roles in drug efflux, but some may also function in cellular detoxification. The Pdr15p ABC protein is the closest homologue of the multidrug efflux transporter Pdr5p, which mediates pleiotropic drug resistance to hundreds of unrelated compounds. In this study, we show that the plasma membrane protein Pdr15p displays limited drug transport capacity, mediating chloramphenicol and detergent tolerance. Interestingly, Pdr15p becomes most abundant when cells exit the exponential growth phase, whereas its closest homologue, Pdr5p, disappears after exponential growth. Furthermore, in contrast to Pdr5p, Pdr15p is strongly induced by various stress conditions including heat shock, low pH, weak acids, or high osmolarity. PDR15 induction by the Pdr1p/Pdr3p regulators but requires the general stress regulator Msn2p, which directly decorates the stress response elements in the PDR15 promoter. Remarkably, however, Pdr15p induction bypasses upstream components of the high osmolarity glycerol (HOG) pathway including the Hog1p and Pbs2p kinases as well as the dedicated HOG cell surface sensors. Our data provide evidence for a novel upstream branch of the general stress response pathway activating Msn2p. In addition, the results demonstrate a crosstalk between stress response and the pleiotropic drug resistance network.

Yeast cells in the environment have to cope with various adverse conditions including hyper- or hypo-osmotic, oxidative stress, heat shock, acidic or alkaline challenge, toxins from plants, fungi or bacteria, and heavy metals as well as herbicides. Therefore, yeasts developed a variety of protective mechanisms ranging from general environmental stress response to highly specific regulatory pathways devoted to stress adaptation. For instance, certain membrane proteins of the ATP-binding cassette (ABC) transporter family are implicated in cellular detoxification and also mediate pleiotropic drug resistance (PDR). Furthermore, overexpression of Pdr5p (1, 2), Snq2p (3), and Yor1p (4) causes enhanced efflux of hundreds of xenobiotics (5). Pdr5p is a highly abundant plasma membrane pump of extraordinarily broad substrate specificity (5), which may detoxify cells by extruding substrates in an ATP-dependent manner (6, 7). The Pdr10p and Pdr15p proteins display the closest primary sequence identity with Pdr5p, sharing 66 and 74% identical residues, respectively (8). However, Pdr10p and Pdr15p have never been isolated in any genetic screen for drug resistance and no other physiological roles have been discovered to date. Notably, several genomics approaches hint PDR15 as a gene modulated by many adverse stress conditions (transcriptome.ens.fr/mgmv) but a correlation of microarray data with the expression of Pdr15p has not yet been possible.

Similar to PDR5 (7, 9), SNQ2 (10), YOR1 (11), and some other drug resistance genes (12), PDR15 is regulated by the homologous transcription factors Pdr1p and Pdr3p, the master regulators of the yeast PDR network (8), requiring the same regulatory elements, the so-called pleiotropic drug resistance elements in target promoters (13). Interestingly, Pdr1p/Pdr3p can exert opposing regulatory effects upon target genes as deletion of PDR1 reduces PDR5 expression but increases PDR15 mRNA levels. In turn, Pdr3p is essential for basal PDR15 expression but only a minor regulator of PDR5 expression (14).

Several stress regulators are also involved in the regulation of ABC gene expression. Yap1p (15), a regulator of oxidative stress response, controls the expression of the vacuolar cadmium ABC pump Ycf1p (16). Yrr1p (17) is involved in the control of Yor1p and Snq2p (18). Finally, the Zn(II)2Cys6 regulator War1p induces the Pdr12p ABC pump upon weak acid stress (19). The related C3H2-zinc finger proteins Msn2p and Msn4p are the master regulators of general stress, because they modulate ~180 genes (20) in response to different environmental stresses including nutrient limitation, heat shock, high osmolarity, or salt treatment. Interestingly, the gene expression pattern of Msn2p/Msn4p targets is distinct for every stress condition (20) and targets include heat shock genes such as HSP12 or HSP104 (21), catalase CTTL1 (22), and those involved in glycerol synthesis as well as other stress-related genes.

Msn2p/Msn4p regulate target genes through so-called stress response elements (STREs) with the core consensus sequence 5'-CCCTCCT-3' in either orientation (23, 24). Msn2p/Msn4p function demands increased intracellular cAMP levels, protein kinase A (25), and the high osmolarity glycerol (HOG) MAPK signaling pathway (26). HOG signaling transduces stress signals from surface osmosensors via intracellular MAPKs such as the MAPKK Pbs2p and MAPK Hog1p (27), triggering a rapid activation of Msn2p (25). Hog1p may also control the activity of several other transcription factors including Msn1p,
Hot1p, and Sko1p (28–30). The lack of Hog1p renders cells hypersensitive to high osmolarity (26) and other adverse conditions. The direct binding of Hog1p to other osmостress regulators such as Hot1p mediates their binding to certain STRE promoters (30). However, the precise mechanisms of Hog1p-regulated response via Man2p/Msn4p activation remain unknown.

In this study, we show that PDR15 is a target of Msn2p, thus a general stress response gene. Pdr15p is highly induced by various adverse conditions such as high osmolarity, heat shock, low pH, weak acids, and starvation. The induction requires Msn2p, which directly decorates STREs in the PDR15 promoter in vivo. Furthermore, Pdr15p is also controlled by endogenous metabolic stress because it is strongly induced in vivo when yeast cells exit the exponential growth phase. Interestingly, Pdr15p and its closest homologue Pdr5p are differently regulated during various growth phases. Hence, our results describe for the first time a link between ABC efflux pumps and the general stress response and suggest a role for Pdr15p in cellular detoxification during metabolic stress or in non-growing cells.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions—All of the Saccharomyces cerevisiae strains used in this study are listed in Table I. Rich medium (YPD) and synthetic complete medium (SC) were prepared essentially as described elsewhere (31). Unless otherwise indicated, all of the yeast strains were routinely grown at 30 °C.

Gene Disruptions and Strain Constructions—The deletion of PDR15 was performed by a PCR-based method (short-flanking homology) using the disruption cassette of the plasmid pUG6 (32). The deletion of PDR5 was performed essentially as described elsewhere (2). The deletion of PDR10 was performed essentially as described previously (6) using a yeast Shuttle vector containing the hisG gene (33) and on the downstream side by regions homologous to nt 442 to 4739. For epitope tagging of Pdr5p serum (34) and Pdr15p-HA with monoclonal anti-HA-antibody (35), yeast strains were grown at 30 °C or analyzed with a PhosphorImager (Storm 840, Amersham Biosciences). Cells were harvested, resuspended in 5-ml culture medium, and treated with 5 μl of dimethyl sulfate in the presence of potassium sorbate for 5 min to methylate DNA. The reaction was stopped by rinsing cells with ice-cold 1× TNEβ (10 mM Tris-HCl, pH 7.5, 40 mM NaCl, 1 mM EDTA, 100 mM β-mercaptoethanol). DNA was isolated after spherolysing cells in SCEβ (1 ss fiber, 100 mM trisodium citrate, 10 mM EDTA, 100 mM β-mercaptoethanol, pH 7.0–7.5) and following lysis (2% SDS, 100 mM Tris-HCl, pH 9.0, 50 mM EDTA) at 65 °C for 5 min. Proteins were precipitated in 0.63 M potassium acetate overnight at 4 °C. DNA from the supernatant was purified by several cycles of ethanol precipitation and RNA digestion followed by digestion with EcoRI. Primer extension was carried out with α-32P-end-labeled oligonucleotides (PDR15-FTP-551S, 5’-AAGAACATATTCTTCCAGACAAAGCC-3’) for 40 cycles (1 min at 94 °C, 2 min at 58 °C, and 3 min at 72 °C). Oligonucleotides used for this reaction specifically bind to the PDR15 promoter. The products were precipitated twice with ethanol for further purification, dissolved in denaturing loading buffer (50 mM NaOH, 0.5 mM EDTA, 4 μl urea, 0.1% bromophen blue, 0.1% xylene cyanol), and resolved in 8% denaturing sequencing gels. Dried sequencing gels were scanned in a STORM PhosphorImager and quantified using the standard software ImageQuant 5.0.

RESULTS

Pdr15p Is a Plasma Membrane Protein Conferring Resistance to a Limited Number of Substances—Because Pdr15p is the closest homologue of Pdr5p, we were interested to identify common functional features of these proteins. First, we wanted to deter-
mine the intracellular localization of Pdr15p. Strain YHW15GFP expressing Pdr15p with a C-terminally fused GFP tag and the corresponding wild type YPH499 were grown in YPD to early or late exponential growth phases. Interestingly, no Pdr15p-GFP signals were detectable in cells growing in early exponential phase (data not shown). However, a distinct fluorescence rim staining, typically indicating plasma membrane localization, was observed in YHW15GFP cells in the late exponential growth phase (Fig. 1). This signal was specific for Pdr15p-GFP because it was absent in wild type control cells. Hence, Pdr15p, similar to its homologue Pdr5p, is a plasma membrane protein suggesting an overlapping function and may be the capability of Pdr15p to export various cytotoxic compounds.

To test this idea, we constructed a set of seven isogenic strains containing all possible combinations of deletions of the Pdr5p subfamily genes, PDR5, PDR10, and PDR15. These strains were grown to the exponential growth phase, and identical cell numbers were spotted onto YPD plates containing a wide variety of typical drug substrates of Pdr5p and other ABC transporters (Fig. 2). However, in most cases, we found that only a loss of Pdr5p but not that of Pdr10p or Pdr15p caused drug hypersensitivity (data not shown). Nevertheless, deletion of PDR15 in a pdr5Δ strain increased hypersensitivity to chloramphenicol as well as to the detergent polyoxyethylene-9-lauryl ether as well as to the detergent polyoxyethylene-9-lauryl ether (Fig. 2). Although the absence of PDR15 alone did not cause any obvious growth phenotypes when compared with the wild type, the pdr5Δ pdr15Δ double deletion exhibited clearly reduced growth on plates containing chloramphenicol or polyoxyethylene-9-lauryl ether, respectively, when compared with the pdr5Δ single deletion (Fig. 2). Deletion of PDR10 on the other hand did not result in any detectable growth phenotype in the presence of these substances. These data demonstrate a possible drug transport capacity of Pdr15p and suggest that the physiological roles or substrates of Pdr5p, Pdr10p, and Pdr15p may be largely distinct despite a high degree of primary sequence identity.

Pdr15p Is Induced Upon Various Stresses in a Pdr1p/Pdr3p-independent Way—Besides the obvious similarities between PDR5 and PDR15, previous studies already suggested that PDR15 mRNA levels are extremely low in exponentially growing cells, contrasting the high basal expression level of PDR5 (14). To learn more about the cellular role of Pdr15p, we investigated the expression pattern of this transporter under various stress conditions. YPH499 cells were grown in YPD to A_600 of 1.0 and stressed with 0.95 M sorbitol, 0.4 M NaCl for osmotic stress, or 5 mM potassium sorbate for weak acid stress. Furthermore, cells growing at room temperature were shifted to 37 °C to impose a heat shock. Before stress treatment and after the indicated time points, samples were taken and total RNA was prepared. Analysis of PDR15 mRNA levels showed that all of the stress conditions caused a dramatic increase of PDR15 mRNA levels (Fig. 3A). Low basal levels of PDR15 increased within 10–20 min following stress impact. However, the PDR15 induction appeared transient, peaking after 20 min under all of the stress conditions investigated. After ~60 min of stress treatment, PDR15 mRNA levels dropped to levels similar to the uninduced state. Notably, PDR5 mRNA levels were not affected by any of the stress conditions tested (Fig. 3A and data not shown).

To see whether the transient induction of PDR15 mRNA leads to an increase of Pdr15p protein, we performed immunoblotting to detect Pdr15p-HA under different adverse conditions. YCS15-HA cells were grown in YPD to A_600 of 1.0 and stressed either with high salt conditions (0.4 M NaCl), low pH (pH 4.5), or weak acid stress (data not shown). Samples were harvested before and after stress treatment at the indicated time points, and cell extracts were subjected to immunoblotting with the monoclonal HA antibody 16B12 to detect Pdr15p-HA (Fig. 3B). Equal loading was confirmed by a cross-reaction of the anti-HA antibody. In unstressed cells, Pdr15p levels were very low and the protein migrated as a single band with an approximate molecular mass of 180 kDa. ~10 min after NaCl addition, protein levels started to increase sharply (Fig. 3B, upper panel). A second, faster migrating band specific for Pdr15p-HA appeared. Because Pdr15p-HA is a glycosylated protein (data not shown), we concluded that this faster migrating band perhaps represents an unmodified Pdr15p-HA precursor or the intermediate form. After 20 min, both immunoreactive bands corresponding to Pdr15p were clearly induced and overall Pdr15p levels remained highly elevated during the 120-min stress duration (Fig. 3B) Similar results as in the high salt stress experiment were obtained for all other adverse conditions tested (Fig. 3B). Hence, Pdr15p protein levels remained high throughout the stress treatments even though the mRNA induction was only transient. Interestingly, a deletion of PDR15 did not result in increased sensitivity to any of the
stress conditions applied in our study (data not shown).

The Pdr1p and Pdr3p regulators control the basal expression of Pdr15p (14). Therefore, we tested the influence of these transcription factors on Pdr15p stress induction. Cells lacking Pdr1p, Pdr3p, or both transcription factors as well as the corresponding isogenic wild type cells were grown to an A600 of 1.0, and 5 mM potassium sorbate was added. Samples were taken at various time points, and PDR15 mRNA was detected by Northern analysis (Fig. 4). As observed previously, PDR15 mRNA levels rapidly but transiently increased after the addition of potassium sorbate in wild type cells. Notably, cells lacking Pdr1p displayed a higher PDR15 expression than wild type cells, which is in agreement with earlier results indicating a negative control by Pdr1p in wild type cells (14). However, the fast induction upon stress treatment was unaffected by the loss of Pdr1p. The same was true for Pdr3p, because its loss reduced basal PDR15 mRNA levels but did not affect PDR15 induction in response to potassium sorbate. In cells lacking both regulators, PDR15 induction by potassium sorbate was also not affected despite severely reduced basal expression levels (Fig. 4). These data demonstrate that Pdr1p and Pdr3p are not required for the stress regulation of Pdr15p, although they modulate basal expression of PDR15.

Msn2p, but Not Hog1p, Is Required for Pdr15p Induction—In a search for potential stress regulatory elements, a close inspection of the PDR15 promoter revealed the existence of five potential STREs at positions −756 (STRE1), −711 (STRE2), −550 (STRE3), −544 (STRE4), and −351 (STRE5) upstream of the putative translational start site. To test whether Pdr15p is regulated by transcription factors recognizing STREs, we performed Northern analysis of cells lacking Msn2p and Msn4p and other components of the HOG pathway (Fig. 5, A and B). The yeast strains YM24 (msn2Δ msn4Δ) and the corresponding wild type W303-1A were grown in YPD medium and treated with 5 mM potassium sorbate as described previously. Northern analysis of the total RNA showed that wild type cells again displayed a transient induction of PDR15. However, loss of Msn2p and Msn4p completely abolished the stress-dependent induction of PDR15 (Fig. 5A). The same was true for cells stressed with 0.5 mM NaCl. The dramatic increase in PDR15 mRNA upon stress required the presence of Msn2p/Msn4p (Fig. 5B). The basal PDR15 mRNA levels in unstressed cells were unaffected in the deletion strain, but high salt or weak acid stress no longer resulted in PDR15 induction, demonstrating that PDR15 is indeed regulated by Msn2p/Msn4p. These results were also confirmed at the protein level (Fig. 5C). YHW5 cells (msn2Δ msn4Δ), as well as YHW4 cells (WT) growing in YPD, were treated with 5 mM potassium sorbate for the indicated time, and PDR15 mRNA was detected by immunoblotting using anti-HA antibody 16B12. The induction of Pdr15p-HA after addition of potassium sorbate in wild type cells was completely absent in the msn2Δ msn4Δ double mutant, confirming that these regulators are required for Pdr15p stress induction (Fig. 5C).

To test the influence on PDR15 expression by HOG pathway components acting upstream of Msn2p/4p, cells lacking Pbs2p, Hog1p, or Msn2p/4p as well as the corresponding wild type strain were grown to an A600 of 1.0 and 0.5 mM NaCl was added. Samples were taken at various time points, and PDR15 mRNA was detected by Northern analysis (Fig. 5B). Interestingly, although the stress-dependent induction of PDR15 mRNA was abolished by the absence of Msn2p/4p, it was only slightly diminished in cells lacking either Hog1p or Pbs2p (Fig. 5B). ACT1 mRNA levels were used to verify equal loading. Deletion of Hot1p and Msn1p (28, 35), other known stress regulators, failed to influence the induction of Pdr15p in response to salt stress (data not shown), which excludes a contribution of these transcription factors to the regulation of the Pdr15p transporter.

To verify the specific role of Msn2p and Msn4p in the PDR15 stress induction, we reintroduced the genes into msn2Δ msn4Δ cells using centromeric plasmids carrying functional Msn2p-GFP and Msn4p-GFP variants, respectively, under the control of the ADH promoter. Expression of Msn2p-GFP and Msn4p-GFP was verified by fluorescence microscopy (data not shown). The functionality of the fusion proteins was verified by their
ability to induce catalase T expression under salt stress (data not shown). The resulting strains as well as a wild type control and the YHW5 double deletion strain were grown in the appropriate selective medium to an $A_{600\text{nm}}$ of 1.0. Before and after a 90-min potassium sorbate stress, protein extracts were used for immunodetection of Pdr15p-HA. Pdr15p-HA levels were significantly higher in wild type cells (YHW4) than in the corresponding $msn2\Delta$ $msn4\Delta$ (YHW5) deletion strain (Fig. 5D, lanes 1 and 2). In cells expressing Msn2p alone, levels of Pdr15p were even higher than in wild type cells, perhaps of the overexpression of the regulator (Fig. 5D, lane 3), whereas the presence of Msn4p failed to restore Pdr15p induction (Fig. 5D, lane 4). This result demonstrated that Msn2p, but not Msn4p, mediates stress regulation of Pdr15p.

**Fig. 5.** PDR15 stress regulation requires Msn2p but not other members of the HOG pathway. A and B, total RNA was prepared from strains W303-1A (WT), YM24 ($msn2\Delta$ $msn4\Delta$), VRT2010 ($pbs2\Delta$), and YHS18 ($hog1\Delta$) grown to the logarithmic growth phase in YPD. Cells were stressed for the indicated time with 5 mM potassium sorbate (A) or 0.5 M NaCl (B). PDR15 and ACT1 mRNAs were detected using appropriate probes homologous to the 5' ends of the respective open reading frames. Equal loading was confirmed with methylene staining of the 18 and 28 S rRNA (data not shown). C, whole cell extracts were prepared from strains YHW4 (WT) and YHW5 ($msn2\Delta$ $msn4\Delta$) grown to logarithmic growth phase in YPD and stressed for the indicated time with 5 mM potassium sorbate. Immunodetection of HA-tagged Pdr15p was performed using monoclonal anti-HA antibody 16B12 diluted 1:2500. An unspecific cross-reaction against the 16B12 antibody confirms equal loading.

**Fig. 6.** Msn2p decorates two STREs in the PDR15 promoter in vivo. In vivo footprint analysis was performed as described under "Experimental Procedures" on the strains W303-1A (WT), YM24 ($msn2\Delta$ $msn4\Delta$), and YM24 containing a plasmid overexpressing Msn2p driven by the ADH1 promoter (pAdh-Msn2-GFP). The middle panel shows a scheme of the PDR15 promoter region from nt −555 to −390 upstream of the start codon. The boxes indicate the positions of putative pleiotropic drug resistance elements (PDRE) and STREs. The left panel contains a sequencing reaction of the PDR15 promoter with the same primers used for the in vivo footprint. G, A, T, and C depict the lanes with the reactions containing the respective deoxy variants of the bases. The nucleotide sequence from nt −555 to −333 from the putative start site containing two of a total of five putative STREs (5'-CCCTT-3') present in the PDR15 promoter is given below. Protected nucleotides are capitalized, and recognized STREs are boxed in gray. On the right panel, the in vivo footprint reactions of the three strains are aligned to the sequencing panel along with a computer analysis of all three in vivo footprint reactions, showing the relative intensities of the respective bands. Black line, wild type; dotted line, $msn2\Delta$ $msn4\Delta$; gray line, Adh-Msn2.

**Msn2p Binds Directly to the PDR15 Promoter.**—As indicated above, the PDR15 promoter contains five putative STREs with the sequence 5'-CCCTT-3' or 5'-AGGGG-3', respectively. To investigate, whether Msn2p directly recognizes and decorates these STREs in vivo, we performed in vivo footprint analysis of the PDR15 promoter. Experiments were carried out in wild type cells (W303-1A) and the $msn2\Delta$ $msn4\Delta$ (YM24) strain as well as in YM24 cells overexpressing Msn2p-GFP from a centromeric plasmid under the control of the ADH promoter (Adh-MSN2). All of the strains were grown to the exponential growth phase stressed by the addition of 5 mM potassium sorbate for 10 min and then treated with dimethyl sulfate to methylate guanine and to a lesser extent adenine in the chromosomal DNA. After DNA purification, the methylation status was determined by primer extension analysis (Fig. 6). Comparing the methylation patterns of wild type cells (black line) with that of $msn2\Delta$ $msn4\Delta$ cells (dotted line), we detected a strong protection of nucleotides in the region from −550 to −536, which was absent in the deletion strain. These nucleotides corresponded precisely to STRE3 and STRE4 as well as to nucleotides immediately downstream of STRE4. The specificity of this protection was confirmed by reintroduction of MSN2 into the double deletion strain from a plasmid, fully restoring protection to levels seen in the wild type strain (gray line). These results confirm that Msn2p decorates at least two STREs in the PDR15 promoter in vivo and confirm a transcriptional control of PDR15 by Msn2p. The presence of functional STREs in its promoter implies that PDR15 may play important roles in the protection of cells during adverse growth conditions.
Pdr15p induction. Previous observations already suggested that Msn2p/Msn4p function is only partly redundant. Msn2p also contributes more to the stress induction of CTTI and HSP26 than Msn4p (22). However, distinct functions of both transcription factors in HOG-mediated stress response have not yet been studied systematically. The regulation of PDR15 implies a stress-related phenotype of cells lacking Pdr15p. Nevertheless, pdr15Δ cells do not show obvious viability or growth phenotypes (data not shown). However, this is not unexpected because even the absence of Msn2p/Msn4p, both of which control more than 180 STRE genes, does not lead to severe stress-induced growth phenotypes (22).

The induction kinetics of PDR15 mRNA is transient, but high protein levels persist over the time monitored. This transient induction might explain why PDR15 has not been identified in global genomic approaches addressing general stress response, because most experiments were carried out after prolonged stress treatments. Nevertheless, evidence from some microarray approaches detects PDR15 as a stress gene responding to various adverse conditions (transcriptome.ens.fr/ymgv) including weak acid stress. A transient mRNA induction is quite typical for other stress-induced genes such as CTTI or HSP12 that are induced upon osmotic stress or salt stress conditions (22).

With pleiotropic drug resistance elements and STREs in its promoter, PDR15 is not only target of Msn2p but also controlled by Pdr1p/Pdr3p (14). Likewise, certain other yeast ABC genes are not only regulated by Pdr1p/Pdr3p but also by stress regulators, adding an additional layer of complexity to the PDR network. For instance, Sng2p and Yor1p are under the control of both Pdr1p/Pdr3p and Yrr1p (17, 18). Ycf1p, although not controlled by Pdr1p/Pdr3p, is induced by oxidative stress through Yap1p (36). Furthermore, the weak acid efflux pump Pdr12p is tightly controlled by War1p in the presence of its potential substrates (19). Therefore, Pdr15p is the first PDR

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**Fig. 7. Expression of Pdr15p sharply increases when cells exit the exponential growth phase.** Immunoblotting was used to detect expression of Pdr15p-HA and Pdr5p during growth of yeast cells in different growth phases. Whole cell extracts were prepared from strain YCS15-HA grown in YPD to the indicated A_{600 nm} (OD_{600 nm}). Immunodetection of HA-tagged Pdr15p was performed using monoclonal anti-HA antibody 16B12 diluted 1:2500. Pdr5p was detected with polyclonal antibodies diluted 1:200,000. An unspecific cross-reaction against the 16B12 antibody confirmed equal sample loading.

**Pdr5p and Pdr15p Levels Are Differentially Regulated Depending on the Growth Phase**—Notably, the Msn2p/Msn4p stress response is also activated when cells exit the exponential growth phase where they have to cope with various nutrient limitations and catabolite accumulation (20). To investigate expression levels of Pdr15p-HA, YCS15-HA cells were inoculated to an A_{600 nm} of 0.01 in YPD grown at 30 °C and samples were taken every 90 min. Cellular extracts were prepared and subjected to immunoblotting to detect both Pdr15p-HA and Pdr5p (Fig. 7). Equal loading of extracts was confirmed by a cross-reaction to the 16B12 anti-HA antibody. From A_{600 nm} 0.4 to 2.0, Pdr15p-HA was hardly detectable by immunoblotting (Fig. 7). However, in cultures exceeding an A_{600 nm} of ~3 reaching the end of the exponential growth phase, cellular Pdr15p-HA levels rapidly increased. Conversely, Pdr5p was only highly expressed in exponentially growing cells but its level rapidly decreased at higher cell densities to become virtually undetectable after 16 h of growth at an A_{600 nm} of 9 (Fig. 7). We confirmed these results in a long-time starvation experiment in which cells were growing through the diauxic shift until an A_{600 nm} of 16.0 was reached. Pdr15p levels remained highly induced throughout the post-diauxic phase, whereas Pdr5p protein was no longer detectable at this high cell density (data not shown). Taken together, these data suggest a function of Pdr15p in stationary phase cells, which might be related to cellular detoxification. Furthermore, our results provide compelling evidence for a novel upstream branch of the general stress response pathway. The signal of this new branch merges at the level of Msn2p activation, and the subsequent transcriptional induction of PDR15 demonstrates a cross-talk between PDR and general stress response.

**DISCUSSION**

The Pdr15p ABC protein is the closest homologue of the well described Pdr5p efflux pump, which mediates extrusion of hundreds of different cytotoxic compounds, thereby causing pleiotropic drug resistance. Pdr15p not only shares 74% primary sequence identity with Pdr5p, previous studies also show that both proteins, like other PDR or non-PDR genes, are regulated by Pdr1p and Pdr3p (12–14). This common regulation implies similar cellular roles of both ABC transporters, although screens for increased drug resistance or related mutant strains never resulted in the isolation of the PDR15 gene. Therefore, we studied Pdr15p properties and its expression regulation to close in on a possible function of this ABC pump. In this work, we demonstrate a connection between general stress response and the transcriptional activation of a PDR network gene under adverse conditions or upon endogenous metabolic stress. Although we show that Msn2p is necessary for the stress induction of PDR15 via cis-acting STRE elements, our data also suggested a novel hitherto unknown upstream branch of the HOG pathway (44) or at least a novel activator of Msn2p. Although Msn2p and Msn4p do recognize STREs in vivo (26), previous microarray studies on the HOG response (35) or general stress response (20) failed to identify PDR15 as a target for Msn2p/Msn4p. Msn2p/Msn4p activity is normally tightly regulated by the HOG pathway, which transduces extracellular stress signals, ultimately resulting in the phosphorylation, activation, and rapid nuclear translocation of both Msn2p and Hog1p to elicit stress response (30, 35, 47). Surprisingly, Pdr15p is the first Msn2p-dependent stress target showing a strong HOG-independent induction, which is completely abolished in a msn2Δ msn4Δ deletion strain. Furthermore, this induction bypasses Hog1p as well as Pbs2p kinases and known cell surface sensors. As Msn2p-dependent response can also be triggered in the absence of known upstream HOG components including the cell surface sensors Sho1p and Sln1p (data not shown), we propose a new branch of the STRE-driven stress pathway-activating Msn2p. Our results are in fact entirely consistent with previous observations where induction of the Msn2p/Msn4p target gene CTTI requires the presence of these regulators (22) but is independent of either Hog1p or Pbs2p, at least in the case of sorbate stress (26). Whether this putative signaling mechanism overlaps with the HOG pathway (44) concerning stress specificity or whether it is also triggered by other signals normally not activating the HOG pathway will have to be elucidated in future experiments. Notably, a low stress-dependent induction in the absence of HOG1 has been observed before for genes such HSP104, GAC1, or CTTI (26, 35, 36). It is also noteworthy that Msn4p does not contribute to Pdr15p stress regulation, because only Msn2p is required for Pdr15p induction. Previous observations already suggested that Msn2p/Msn4p function is only partly redundant. Msn2p also contributes more to the stress induction of CTTI and HSP26 than Msn4p (22). However, distinct functions of both transcription factors in HOG-mediated stress response have not yet been studied systematically. The regulation of PDR15 implies a stress-related phenotype of cells lacking Pdr15p. Nevertheless, pdr15Δ cells do not show obvious viability or growth phenotypes (data not shown). However, this is not unexpected because even the absence of Msn2p/Msn4p, both of which control more than 180 STRE genes, does not lead to severe stress-induced growth phenotypes (22).

The induction kinetics of PDR15 mRNA is transient, but high protein levels persist over the time monitored. This transient induction might explain why PDR15 has not been identified in global genomic approaches addressing general stress response, because most experiments were carried out after prolonged stress treatments. Nevertheless, evidence from some microarray approaches detects PDR15 as a stress gene responding to various adverse conditions (transcriptome.ens.fr/ymgv) including weak acid stress. A transient mRNA induction is quite typical for other stress-induced genes such as CTT1 or HSP12 that are induced upon osmotic stress or salt stress conditions (22).
family member regulated by the general stress response, suggesting that this transporter can play an important role in the protection of cells during suboptimal metabolic conditions such as those mounting when cells exit exponential growth. At this cell density, glucose becomes a limiting factor and such growth conditions also activate Msn2p (37), perhaps because catabolites and toxic metabolites start to accumulate. This may cause cellular damage or enhanced membrane stress. Indeed, Pdr15p levels are dramatically induced when cells exit the exponential growth phase; thus, we propose a role for Pdr15p in cellular detoxification in this growth phase. Although the question for the physiological substrates of Pdr15p remains unanswered at this point, our work clearly shows a contribution of Pdr15p to chloramphenicol and detergent resistance. It will be a challenge to identify endogenous substrates, but if they are to be found among catabolites, a metabolome analysis may provide clues as to possible Pdr15p substrates. The tight regulation of PDR15 by Msn2p in response to metabolic stress may lead to new insights into possible ABC transporter functions, because Pdr15p is the first ABC transporter involved in the general stress response. The tantalizing question regarding upstream activating signals remains unanswered, but one may speculate that membrane stress exerted by short-lived yet toxic lipid breakdown products that accumulate in cells exiting exponential growth or toxic catabolites could constitute a signal for Msn2p activation and subsequent Pdr15p induction.

Most interestingly, the levels of the closest Pdr15p homologue Pdr5p sharply decrease at higher cell densities. As for Pdr15p, a physiological Pdr5p substrate has not been identified. Nevertheless, it is generally accepted that Pdr5p is implicated in cellular detoxification, whereas it can also mediate extrusion of hundreds of functionally and structurally different xenobiotics. The substrate specificity of Pdr5p and Pdr5p seems different, although the complementary regulation may also hint similar physiological substrates exported by the respective transporter depending on growth phases. Furthermore, the possible role of Pdr1p/Pdr3p target genes in phospholipid flipping (38) upon starvation or nutrient limitation (39) implies a function of certain ABC transporters in the phospholipid flipping (38) upon starvation or nutrient limitation more, the possible role of Pdr1p/Pdr3p target genes in phospholipid flipping (38) upon starvation or nutrient limitation more, the possible role of Pdr1p/Pdr3p target genes in phospholipid flipping (38) upon starvation or nutrient limitation.
The Yeast Pdr15p ATP-binding Cassette (ABC) Protein Is a General Stress Response Factor Implicated in Cellular Detoxification

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