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Review

Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance

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Abstract

Saccharomyces cerevisiae was the first eukaryotic organism whose complete genome sequence has been determined, uncovering the existence of numerous genes encoding proteins of the ATP-binding cassette (ABC) family. Fungal ABC proteins are implicated in a variety of cellular functions, ranging from clinical drug resistance development, pheromone secretion, mitochondrial function, peroxisome biogenesis, translation elongation, stress response to cellular detoxification. Moreover, some yeast ABC proteins are orthologues of human disease genes, which makes yeast an excellent model system to study the molecular mechanisms of ABC protein-mediated disease. This review provides a comprehensive discussion and update on the function and transcriptional regulation of all known ABC genes from yeasts, including those discovered in fungal pathogens.

Keywords: Fungus; ABC protein; PDR network; Gene regulation; Antifungal drug resistance; Pathogen; Pheromone secretion; Organelle; Stress response; Cellular detoxification

Contents

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1. Introduction

The ATP-binding cassette (ABC) protein family with currently more than 1000 members represents the largest protein family known to date [1]. These proteins operate in all living cells from bacteria to man [1,2] and fulfill a remarkable variety of cellular functions. While most ABC proteins are purely ATP-driven membrane translocators, some of them act as ion channels, channel regulators, receptors, proteases, and even sensing proteins [3]. As for the transport substrates, ABC transporters mediate membrane translocation of ions, heavy metals, carbohydrates, anticancer drugs, amino acids, phospholipids, steroids, glucocorticoids, bile acids, mycotoxins, antibiotics, pigments, peptides and even whole proteins [1,4–6]. However, the mechanism by which transport of such a substrate variety and size diversity can be achieved, while each ABC transporter maintains selectivity for its particular substrate, represents an intriguing and yet unsolved mystery.

All ABC proteins share a similar molecular architecture with a hallmark domain organization that includes the presence of at least one evolutionarily conserved ABC, also known as NBD (for nucleotide binding domain), as well as several predicted α-helical membrane-spanning segments (TMS). The TMSs and NBDs are normally arranged in a duplicated forward $(TMS_6\text{-NBD})_2$ or reverse $(NBD\text{-}TMS_6)_2$ configuration, but numerous half-size transporters with various topologies are also known [1,5,7]. Full-size ABC proteins usually have six predicted TMSs in each half, connected by a charged linker region. Additional TMSs proximal to the N-terminus also exist in some ABC proteins [8]. Finally, the hydrophilic NBDs encompass approximately 250 residues with five conserved protein motifs. The *Walker A* and *Walker B* motifs, also found in all other nucleotide binding proteins [9], and the *ABC signature* or *C motif* with the consensus sequence LSGGQ, are diagnostic hallmarks for all ABC proteins. In addition, there are two less conserved regions, the so-called *center region* between *Walker A* and *Walker B* and another one downstream of the *Walker B* motif [2].

Several mammalian ABC proteins are medically important, because mutations in the corresponding genes cause severe genetic diseases such as cystic fibrosis [10,11], adrenoleukodystrophy [12] and Zellweger syndrome [13,14], familial hyperinsulinemic hypoglycemia of infancy [15], Dubin-Johnson syndrome [16–18], hepatic cholestasis [19,20] and Stargardt's macular dystrophy [21]. Antigen presentation via class I MHC requires the action of dedicated peptide transporters of the ABC family [22]. Furthermore, overexpression of certain mammalian P-glycoproteins (P-gp) [23] and multidrug resistance-associated proteins (MRP) [8] renders both tumor and cultured cells highly resistant to many different chemotherapeutic drugs. The characteristics of the MDR phenomenon include an initial resistance to a single anticancer drug to which tumor cells are exposed, followed by the development of cross-resistance to many structurally and functionally unrelated drugs [24]. The same mechanism of multiple drug resistance development exists in pathogenic fungi such as *Candida* and *Aspergillus* [25], in parasites such as *Plasmodium* and *Leishmania* [26,27], as well as in many infectious bacteria [28].

The baker's yeast *Saccharomyces cerevisiae* is the first eukaryotic organism whose complete genome sequence has been determined, revealing the presence of numerous ABC genes [29,30]. Importantly, mammalian MDR is analogous to pleiotropic drug resistance (PDR) in yeast, which involves a highly complex network of transcriptional regulators that control expression of membrane ABC drug efflux pumps [31,32]. Moreover, yeast has several orthologues of mammalian disease genes, which makes yeast an invaluable model system for unraveling the molecular mechanisms of ABC protein function to better understand and treat ABC gene-mediated diseases. This review is devoted to a comprehensive discussion of yeast ABC proteins identified in pathogenic and non-pathogenic fungi.

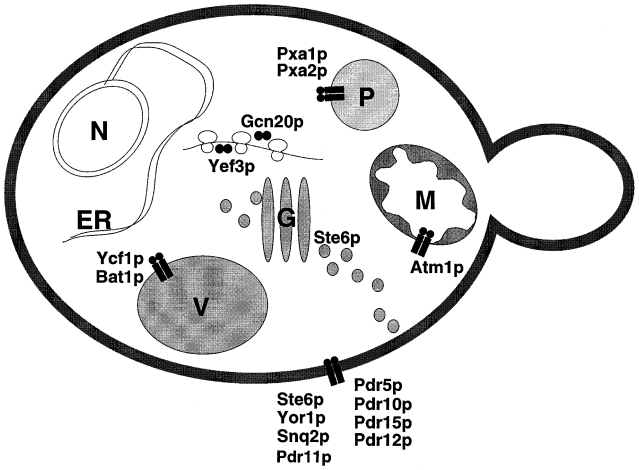

Fig. 1. Subcellular localization of *S. cerevisiae* ABC transporters. ER, endoplasmic reticulum; G, Golgi apparatus; M, mitochondrion; N, nucleus; P, peroxisomes; and V, vacuole. Ste6p is localized in Golgi vesicles, endosomes as well as in the plasma membrane.

2. The inventory of ABC proteins in *S. cerevisiae*

The genome sequencing project revealed the existence of 31 distinct ABC genes in this lower eukaryote [29,30,33]. Notably, each cellular compartment, except for the endoplasmic reticulum (ER) and nuclear membrane, appears to harbor at least one ABC transporter. Some membranes like the plasma membrane carry several different ABC transporters at any given moment (Fig. 1). Based on phylogenetic tree analysis, a classification of yeast ABC proteins into six distinct subfamilies has been suggested [33]. These families are defined as the MDR, the PDR, the MRP/CFTR, the ALDp, the YEF3 and the RLI subfamilies (Table 1).

2.1. The MDR subfamily – sex life and mitochondrial function

The MDR subfamily comprises four members, one of which is the Ste6p **a**-factor pheromone transporter, probably one of the best characterized yeast ABC transporters. The *STE6* gene was cloned some 10 years ago as a homologue of the mammalian P-gp [34,35]. Ste6p is a very short-lived [36], haploid-specific membrane transporter mediating *MAT***a** cell-specific export of the farnesylated **a**-factor sex pheromone whose precursor lacks a typical hydrophobic signal peptide [37,38]. Extracellular **a**-factor is essential for the sexual reproduction cycle of haploid yeast cells. Pheromone export occurs through a non-classical route, bypassing the vesicular secretory pathway [37,38]. While the steady-state localization of Ste6p appears to be in the Golgi complex and/or the endosomes [36,39,40], Ste6p functions at the plasma membrane providing the rate-limiting step in **a**-factor export [34,39]. After pheromone extrusion, Ste6p is rapidly endocytosed and delivered to the vacuole for terminal degradation [36,40]. In addition to *STE6*, several other genes are required for the production of **a**-factor [37,38]. These genes include those involved in the post-translational modifications and maturation of the pheromone precursor at the C-terminal CAAX box, as well as several proteases which are required to obtain a fully processed and biologically active sex pheromone [37,38].

Interestingly, severing experiments have shown that the two Ste6p halves, when coexpressed as individual proteins, can mediate pheromone export. This demonstrates that both Ste6p halves are required for function and that they can interact in vivo to form a functional **a**-factor transporter [38]. The substrate, **a**-factor, is extremely hydrophobic due to its C-terminal lipid modification and carboxymethylation. While mutations in the structural gene encoding **a**-factor do not dramatically affect its secretion, a lack of **a**-factor farnesylation or methylation debilitates its extrusion. Hence, the lipid moiety or its hydrophobicity may represent an essential recognition determinant for Ste6p. As with many other eukaryotic ABC transporters, Ste6p is fueled by ATP hydrolysis, because many NBD mutations destroy function [41], and because Ste6p binds photo-activateable ATP analogues [39]. Interestingly, Ste6p might also play an additional role in cell fusion, since *ste6* mutants were isolated that still mediate **a**-factor export, but fail to complete fusion of haploid mating partners [42]. Taken together, the precise mechanism by which Ste6p mediates the actual pheromone translocation across the plasma membrane is somewhat mysterious, but it appears as if intracellular **a**-factor precursor processing and translocation across the plasma membrane are tightly coupled.

The second member of the MDR family is the Atm1p half-size transporter [43], the only known mitochondrial ABC transporter in yeast. Atm1p is lo-

Table 1
S. cerevisiae ABC proteins

ABC protein	Substrate(s)	Length	Topology	Localization	Phenotype	Reference
MDR family						
Ste6p	**a**-factor	1290	$(TMS_6\text{-}ABC)_2$	PM, GV, ES	Sterile	[34,35]
Atm1p	Fe/S proteins	694	$TMS_6\text{-}ABC$	Mit	Slow-growth	[43]
Ssh1p/Mdl2p	?	820	$TMS_6\text{-}ABC$	?	Viable	[7,49]
Mdl1p	?	696	$TMS_6\text{-}ABC$	?	Viable	[49]
PDR family						
Pdr5/Sts1/Ydr1/ Lem1p	Drugs, steroids, antifungals	1511	$(ABC\text{-}TMS_6)_2$	PM	$Drug^{HS}$	[60–63]
Pdr10p	?	1564	$(ABC\text{-}TMS_6)_2$	PM	Viable	[77,178]
Pdr15p	?	1529	$(ABC\text{-}TMS_6)_2$	PM	Viable	[77,178]
Snq2p	Drugs, steroids, mutagens	1501	$(ABC\text{-}TMS_6)_2$	PM	$Drug^{HS}$	[59]
Pdr12p	Weak acids	1511	$(ABC\text{-}TMS_6)_2$	PM	Weak $acid^{HS}$	[78]
Pdr11p	?	1411	$(ABC\text{-}TMS_6)_2$	PM(?)	Viable	[72]
Adp1p	?	1049	$TMS_2\text{-}ABC\text{-}TMS_7$	?	Viable	[81]
YNR070w	?	1333	$(ABC\text{-}TMS_6)_2$	?	?	Z71685
YOR011w	?	1394	$(ABC\text{-}TMS_6)_2$	?	?	Z74919
YOL075c	?	1095	$(ABC\text{-}TMS_6)_2$	?	?	Z74817
MRP/CFTR family						
Yor1p	Oligomycin, reveromycin	1477	$(TMS_6\text{-}ABC)_2$	PM	$Drug^{HS}$	[97]
Ycf1p	GS-conj./Cd^{2+}	1515	$(TMS_6\text{-R-}ABC)_2$	Vac	Cd^{HS}	[87]
Bat1p	Bile acids	1661	$(TMS_6\text{-}ABC)_2$	Vac	Viable	[85]
YLL015w	?	1559	$(TMS_6\text{-}ABC)_2$	?	?	Z73120
YHL035c	?	1592	$(TMS_6\text{-}ABC)_2$	?	?	U11583
YKR103w	?	1218	$TMS_6\text{-}ABC\text{-}TMS_6$	?	?	Z28328
YKR104w	?	306	ABC	?	?	Z28329
ALDp family						
Ssh2/Pal1/Pxa1/Pat2p	Long-chain fatty acids	870	$TMS_6\text{-}ABC$	Px	Oleate−	[7,101,102,205]
YKL741/Pxa2/Pat1p	Long-chain fatty acids	853	$TMS_6\text{-}ABC$	Px	Oleate−	[100,101,206]
YEF3 family						
Yef3p	Drugs	1044	ABC_2	Ribo?, Cyto?	Essential	[111]
Gcn20p	?	752	ABC_2	Ps	Viable	[112]
YNL014w/Hef3p	?	1044	ABC_2	Cyto?	Viable	[116,117]
YPL226w	?	1196	ABC_2	?	?	Z73582
YER036c	?	610	ABC_2	?	?	U18796
RLI family						
YDR091c	?	608	ABC_2	?	?	Z50111
Others						
YDR061w	?	539	ABC	?	?	Z49209
YFL028c	?	289	ABC	?	?	D50617

Where no published references are available, the EMBL/GenBank sequence accession or the NCBI identification numbers are listed to allow for sequence retrieval. conj., conjugates; Cyto, cytoplasm; ES, endosome; GS, glutathione; GV, Golgi vesicle; HS, hypersensitivity; Mit, mitochondria; PM, plasma membrane; Ps, polysomes; Px, peroxisome; R, regulatory domain; Ribo, ribosome; TMS, transmembrane segment; Vac, vacuole.

calized in the inner mitochondrial membrane with the NBD facing the matrix [43]. An uncleaved targeting signal at the N-terminus of Atm1p, when fused to the N-terminus of otherwise cytoplasmic proteins, can properly target these proteins to the mitochondria. A Δ*atm1* deletion strain is unable to grow on non-fermentable carbon sources, lacks all major mitochondrial cytochromes and becomes *rho*$^-$ upon prolonged propagation [43]. Moreover, Δ*atm1* strains accumulate high levels of free iron, indicating a role for Atm1p in mitochondrial iron homeostasis [44]. Strikingly, a very recent study provides direct evidence that Atm1p is required for the export of Fe/S-containing proteins from the mitochondrial matrix [45]. Such a function of Atm1p seems plausible, since a close Atm1p homologue in humans, hABC7, has been linked to a heritable disease known as X-linked sideroblastic anemia with spinocerebellar ataxia [46–48].

Finally, two MDR family members of unknown function and subcellular localization were identified by PCR-based cloning using degenerate oligonucleotides. Both Mdl1p [49] and Mdl2p/Ssh1p [7,49] are typical half-size transporters. It is not known whether they form homo- or heterodimers or if they are functionally related at all. Deletion of either one or both genes does not result in obvious growth phenotypes [49].

Ste6p was the first yeast ABC transporter, closing a gap between the *Escherichia coli hlyB* hemolysin A export system [6] and the human P-glycoproteins mediating multidrug resistance [50]. Notably, functional expression of certain mammalian P-gps in yeast can partially complement loss of Ste6p, suggesting that P-gps are able to transport peptide substrates of various length [7,51]. Several other studies also demonstrated P-gp-mediated peptide transport [52–56]. On the other hand, Ste6p overexpression does not lead to a significant multidrug resistance phenotype, although some low resistance to the peptide ionophore valinomycin has been reported [34]. At any rate, the existence of inherent multidrug resistance phenotypes in yeast has prompted many laboratories to search for functional homologues of P-gps. These concerted efforts were rewarded by the discovery of PDR genes, which represent the second subfamily of yeast ABC transporters.

2.2. The PDR subfamily – pleiotropic drug resistance and cellular detoxification

The largest ABC subfamily in yeast has 10 members, all of which have a 'reverse' topological orientation, with the TMS following the NBD. Except for Adp1p, all PDR genes are full-size transporters with usually 12 predicted TMSs. Several PDR members share high homology with the *white*, *brown*, and *scarlet* transporters from *Drosophila melanogaster* and human ABC8 [57,58]. The best characterized PDR members, and by now perhaps the best characterized yeast ABC transporters overall, include the *PDR5*/*STS1*/*YDR1*/*LEM1* and *SNQ2* genes, both of which are functional homologues of mammalian P-gps, as their overexpression confers PDR in yeast [31,32].

Snq2p was the first identified PDR transporter conferring multidrug resistance, particularly resistance to mutagens such as 4-nitroquinoline-*N*-oxide (4-NQO) and methyl-nitro-nitrosoguanidine (MNNG) [59]. Shortly after the discovery of Snq2p, Pdr5p was independently identified by four different laboratories using various genetic approaches. *PDR5* was cloned as a cycloheximide resistance gene [60], as a gene mediating resistance to mycotoxins such as sporidesmin [61], and cross-resistance to cerulenin and cycloheximide [62], and, finally, as a selective transporter of glucocorticoids [63]. Although cells lacking Pdr5p and Snq2p are viable, they are hypersensitive to a vast number of xenobiotics, particularly antifungals and anticancer drugs [31,64]. In the mean time, Pdr5p and Snq2p were shown to transport hundreds (!) of structurally and functionally unrelated drugs with a considerable overlap in substrate specificity [59,64–67]. Notably, Snq2p also modulates resistance to metal ions such as Na^+, Li^+, and Mn^{2+} [68]. A Δ*snq2*Δ*pdr5* double deletion strain exhibits a more pronounced effect regarding intracellular metal ion accumulation and metal sensitivity, implying some functional overlap between Snq2p and Pdr5p. However, the formal possibility remains that expression of Pdr5p, Snq2p or both somehow modulates plasma membrane permeability rather than mediating active transport of metal ions.

The Pdr5p and Snq2p pumps are plasma membrane proteins [69–72], which undergo endocytosis and vacuolar delivery for terminal degradation

[69,70]. The cell surface localization of Pdr5p and Snq2p was also confirmed in living cells using functional Snq2p-GFP (Kuchler et al., unpublished) and Pdr5p-GFP variants [65]. Hence, ATP-dependent drug efflux is the most likely mechanism by which Pdr5p/Snq2p mediate PDR. This was confirmed for Pdr5p in vivo using rhodamine 6G accumulation and efflux assays in the presence and absence of inhibitors such as vanadate, vinblastine or the immunosuppressive agent FK506 [65,73]. Pdr5p and mammalian P-gps are therefore true functional homologues because they share several substrates, are inhibited by the same compounds, and because drug transport requires ATP hydrolysis. Like P-gp, Pdr5p can be photoaffinity labeled by a photo-activateable ATP analogue [74]. The NTPase activity of Pdr5p is not limited to ATP, as Pdr5p also hydrolyzes UTP and CTP and to a lower extent ITP and GTP, whereas the Snq2p ATPase seems restricted to ATP hydrolysis [72].

A detailed mutational analysis of Pdr5p enabled the identification of residues important for function, drug substrate specificity and inhibitor susceptibility [65]. Non-functional Pdr5p mutants can arise from misfolding in the ER membrane [75]. Notably, the C1427Y mutation in a predicted extracellular (lumenal) loop of Pdr5p causes its efficient retention in the ER membrane, followed by rapid polyubiquitin modification and degradation by the cytoplasmic proteasome [75]. The instability of C1427Y-Pdr5p is caused by a lack of disulfide bond formation in the ER lumen, which is a prerequisite for native Pdr5p folding and exit from the ER (Egner et al., in preparation). In contrast, a human P-gp lacking all cysteines is still functional in drug transport and is normally transported to the cell surface [76].

The random in vitro mutagenesis approach also allowed the isolation of several mutant Pdr5p pumps with altered substrate specificity [65]. The most interesting Pdr5p mutant has a S1360F exchange in the predicted α-helical TMS10. This mutation causes a restricted substrate specificity for the antifungal ketoconazole, with poor resistance to itraconazole and cycloheximide. Most remarkably, the inhibition of ketoconazole resistance by the immunosuppressive FK506 is completely absent in Pdr5p-S1360F, whereas wild type Pdr5p is unable to confer resistance to ketoconazole in the presence of FK506 [65]. The S1360F exchange is located on the hydrophilic side in the center of TMS10. Hence, this mutant allowed the genetic separation of drug substrate specificity and inhibitor susceptibility of Pdr5p. The S1360F exchange is most likely to cause a disturbance of general helix packing in Pdr5p due to the introduction of a large hydrophobic residue, thereby modulating drug specificity. Further mutational analysis of the Pdr5p TMS10 revealed that this helix is a primary determinant for substrate specificity, inhibitor susceptibility as well as the overall folding of Pdr5p (Egner et al., submitted).

The Pdr5p drug efflux pump has two additional homologues, Pdr10p and Pdr15p, which share more than 65% overall sequence identity with Pdr5p. *PDR10* and *PDR15* were identified earlier by Southern hybridization using a *PDR5*-specific probe [61] and later during the yeast genome sequencing [77]. Although the functions of Pdr10p and Pdr15p are unknown at present, both are localized at the cell surface (Wolfger et al., unpublished). Vegetative cells express very low amounts of both proteins, but their high expression under various stresses suggests that both genes might represent novel stress response genes (see Section 5.1).

The closest homologue of Snq2p is Pdr12p, sharing about 60% primary sequence identity. However, in contrast to Snq2p, expression of Pdr12p mediates pronounced resistance to weak organic acids such as sorbate, benzoate and propionate, compounds that are frequently used as food preservatives [78]. Thus, Pdr12p overexpression confers resistance to water-soluble carboxylate anions rather than to hydrophobic drugs [78]. The most recently identified substrates of Pdr12p are fluorescein-like compounds [79]. Moreover, acetate, which is produced through the normal metabolism, is also a Pdr12p substrate [78]. Expression of Pdr12p is dramatically induced under weak acid stress and low pH, although other stresses such as high osmolarity or ethanol also induce Pdr12p, albeit to a much lesser extent [78]. Pdr12p is also required for adaptation of cells to growth in the presence of metabolic stress. Consistent with these findings, Δ*pdr12* cells are highly sensitive to weak organic acids. Pdr12p-mediated weak acid resistance and its induction, however, are independent of transcriptional regulators normally involved in stress response, including Yap1p and Msn2p/Msn4p [80].

Furthermore, *PDR12* expression under stress is not influenced by the Pdr1p/Pdr3p master regulators of the PDR network, indicating that novel and as yet unidentified transcription factors control expression of certain PDR genes under normal and in response to adverse conditions [78].

The PDR family has additional homologues of Snq2p and Pdr12p, namely Pdr11p, YOR011w, and YNR070w, none of which has been studied so far and thus nothing is known about their function, drug substrates or regulation of expression. Pdr11p might be localized to the plasma membrane, since peptide sequencing of a 170 kDa protein from partially purified plasma membrane fractions also yielded Pdr11p-specific peptides [72]. Finally, although Adp1p was one of the first known PDR members originally discovered through yeast chromosome III sequencing [81], literally nothing is known as yet about the physiological function of Adp1p. Adp1p is closely related to YOL075c, another PDR member of unknown function. Notably, Adp1p has some interesting structural features such as only one central NBD linked to two and seven predicted TMSs at the N- and C-terminus, respectively, and the presence of EGF repeats in predicted extracellular domains. The non-essential Adp1p shows homology to human ABC8 [58,82] and *white*, *brown* and *scarlet* [57], fly ABC transporters presumably involved in pteridine pigment transport in the eye [83]. Interestingly, there is evidence for a placental-specific mammalian homologue of Adp1p, but its function is also unknown [84].

2.3. The MRP subfamily – vacuolar detoxification and heavy metal resistance

This subfamily harbors six ORFs that show significant homology to mammalian MRP and to a lesser extent to CFTR. At least two genes of this family, *YCF1* and *YOR1*, have been studied so far. A recent investigation characterized another related transporter, Bat1p, a bile acid transporter located in the yeast vacuolar membrane [85]. Bat1p mediates vacuolar uptake of bile acids such as taurocholate, suggesting that a Δ*bat1* deletion mutant might be a valuable host to analyze mammalian bile acid transporters associated with hepatic cholestasis [19]. The normal function of Bat1p is not well understood, but it might be responsible for vacuolar sequestration of heme-derived catabolites, which could be considered bile-like molecules in yeast.

The first evidence for active transporters in internal membranes was the discovery of a saturable, MgATP- and temperature-dependent transport of taurocholate and *S*-(2,4-dinitrophenyl)-glutathione (DNP-GS) into secretory vesicles isolated from a *sec1* strain [86]. Inhibition experiments implied that at least two dedicated ABC transporters could be involved [86]. Indeed, the first ABC transporter was identified in Ycf1p, a 1515-residue homologue of mammalian MRP [8]. There is also a weak homology to the human cystic fibrosis transmembrane conductance regulator [87], since Ycf1p has a rudimentary R-domain only present in CFTR [88]. Ycf1p overexpression allows yeast cells to grow in the presence of increased cadmium concentrations [87]. Moreover, both in vitro and in vivo experiments demonstrated that Ycf1p is a vacuolar glutathione S-conjugate pump [89]. In addition to Cd^{2+}, Ycf1p mediates vacuolar transport of oxidized glutathione (glutathione disulfide, GSSG), DNP-GS, and several other glutathione S-conjugates through an MgATP-dependent mechanism [89–91]. Another recently identified role of Ycf1p is to mediate resistance to arsenite, arsenate and antimony, because uptake of $As(GS)_3$ into vacuoles is completely abolished in Δ*ycf1* cells [92]. These metalloids are removed from the cytosol after their conjugation to GSH through Ycf1p-mediated transport into the vacuole. Taken together, Ycf1p appears to be a key player for the vacuolar detoxification via GSH conjugates. Interestingly, Ycf1p expression is regulated by stress response factors such as Yap1p [93], since *yap1* mutants exhibit cadmium hypersensitivity [94]. However, *YAP1* overexpression mediates Cd^{2+} resistance only in the presence of functional Ycf1p, whereas hyperresistance to Cd^{2+} through overexpressed Ycf1p was only partly dependent on the presence of functional Yap1p [94] (see also Section 5.2).

Mutational analysis of *YCF1* investigated the phenotypes of mutations that were constructed by analogy to the most prominent mutations found in human CFTR. The ΔF713 mutation in *YCF1* is the functional analogue of the ΔF508 deletion in human CFTR, the most frequent mutation in cystic fibrosis (CF) patients. This mutation leads to a Ycf1p mis-

localization to the ER membrane and loss of Cd^{2+} resistance [95]. Furthermore, wild type Ycf1p migrates as two distinct bands of approximately 120 kDa and 170 kDa when overexpressed, while the mutant ΔF713-Ycf1p appears only as a 120 kDa protein. Pulse-chase experiments showed that the 170 kDa band disappeared upon extended chase times, whereas the 120 kDa band is the only one comigrating with vacuolar marker proteins in fractionation experiments. Notably, both Ycf1p forms are functional. The 170 kDa might represent a functional Ycf1p precursor of the mature 120 kDa Ycf1p [95]. Hence, glycosylation is likely to play an important role in the trafficking and/or folding of Ycf1p [95] in the ER membrane, similar to the situation observed for Pdr5p [65].

The second well-characterized yeast MRP homologue is the *YOR1*/*YRS1* gene product Yor1p, another ABC protein of the plasma membrane [96]. Genetic screens for oligomycin and reveromycin A resistance lead to the independent cloning of *YOR1* [97] and *YRS1* [98]. A Δ*yor1* deletion mutant is viable, but hypersensitive to the respective drugs, as well as several other xenobiotics [97–99]. Like Pdr5p and Snq2p, Yor1p-dependent drug resistance is mediated by efflux across the plasma membrane, as shown by rhodamine B accumulation experiments [74,98]. In Δ*yor1* cells, a four-fold higher fluorescence was measured than in wild type cells. Furthermore, this efflux requires ATP hydrolysis but is H^+-ATPase-independent. Again, vanadate inhibits Yor1p-mediated drug transport [74].

Mutations were also introduced into *YOR1* based on prominent CFTR mutations. Although Ycf1p and Yor1p have a different cellular localization, the results from the mutational analysis were quite similar. The mutant ΔF670-Yor1p, the equivalent of the CFTR ΔF508, was also retained in the ER and failed to confer oligomycin resistance. Pulse-chase experiments using mutants defective in the secretory pathway and vacuolar degradation led to the following Yor1p degradation model [96]: Yor1p must exit the ER for proteolytic turnover, which most likely is independent of ubiquitination and takes place in the vacuole. However, the misfolded ΔF670-Yor1p mutant is degraded in the ER, although some contribution of vacuolar proteases cannot be ruled out. Ubiquitination stimulates the breakdown of the ΔF670-Yor1p mutant by the proteasome. Additional Yor1p mutants have been characterized, all of which are expressed at more or less wild type levels, but display reduced oligomycin resistance. While some of these mutations cause ER retention (e.g. insertion of an A at position 652), others (K715M, K715Q) are still normally delivered to the cell surface. Notably, the analogous mutation to K715M and K715Q in CFTR and Ycf1p resulted in hyperactive gain-of-function alleles [96].

Finally, the YKR103w and YKR104w ORFs were originally believed to represent two distinct genes separated by a single stop codon. However, these ORFs perhaps encode a non-essential full-size transporter, as the stop codon resulted from a sequencing error [33]. Likewise, the functions of the YHL035c, YLL015w genes have not been elucidated. Based on their homology to Ycf1p and Bat1p, however, it is tempting to speculate that they might have at least partially overlapping functions.

2.4. The yeast ALDp subfamily and human genetic diseases

This is a small subfamily of yeast ABC transporters with two peroxisomal membrane proteins, Pxa1p/Pat2p/Pal1p/Ssh2p and Pxa2p/Pat1p/Pal2p [7,100–102]. The half-size ABC protein Pxa1p is the closest homologue of the human peroxisomal ABC transporters Pmp70 [13,14,103] and ALDp [12] implicated in Zellweger syndrome and X-linked adrenoleukodystrophy (ALD), respectively. Both diseases are associated with apparent defects in peroxisome function and/or organelle assembly. Moreover, a demyelination of the nervous system, paralleled by the accumulation of very-long-chain fatty acids (VLCFA) in peroxisomes [14,104] is observed. Although these neurodegenerative diseases have sometimes different clinical phenotypes, most are characterized by a lack of mature peroxisomes and activities of peroxisomal enzymes [13,105,106].

Like their human counterparts, Pxa1p and Pxa2p are both half-size transporters of the peroxisomal membrane [100,102]. Since single deletions of each gene had the same effect as deletion of both genes, namely an inability to grow on oleic acid as the sole carbon source and a failure in β-oxidation, they are thought to heterodimerize to a functional transporter

[100,102]. The Pxa1p/Pxa2p transporter could mediate uptake of activated LCFA from the cytosol into the yeast peroxisomal matrix, were they are subjected to the β-oxidation machinery [100,101,107].

Both *PXA1* and *PXA2* are regulated by the transcription factors Oaf1p and Pip2p, which play a key role in activating genes in the presence of fatty acids such as oleate [108]. For *PXA2* this regulation was not unexpected, since its promoter contains an oleate response element (ORE). However, *PXA1* expression was also induced by oleate in an Oaf1p/Pip2p-dependent manner, despite an apparent lack of obvious OREs in its promoter region [108]. Thus, additional as yet unknown regulators might control peroxisomal function and proliferation in yeast.

Sequence comparisons of Pxa1p/Pxa2p with ALDp and PMP70 revealed two highly conserved motifs among Pxa1p and PMP70, ALDp and several P-gps [109]. Both motifs, designated loop1 between TMS4 and TMS5, and the EAA-like motif immediately distal of TMS1, are clearly important for Pxa1p function [109]. Discrete mutations in both regions of *PXA1* lead to a loss-of-function phenotype, although Pxa1p is still normally expressed and targeted to the peroxisome. Interestingly, mutations in the corresponding regions of ALDp have been linked to ALD, suggesting that the EAA-like motif and the loop1 motif are crucial for function throughout the ALDp subfamily and its human orthologues. In human cells, two additional half-size transporters with high homology to ALDp and PMP70 have been identified recently [105,106,110]. The suggestion arises that functional transporters could heterodimerize from different sets of half-size transporters to provide distinct functions in different tissues. This could explain the clinical differences of various ALD forms, as being the consequence of different ALD transporters [110]. The precise functions or substrates of PMP70 and ALDp have not been completely worked out, although it has been suggested that they may operate as transporters for VLCFA into the peroxisomal matrix. It will be interesting to determine if mammalian Pmp70 and ALDp, when functionally expressed in yeast, are able to restore growth of Δ*pxa1* cells on oleate, which would further strengthen the use of yeast as model system to study structure and function of the human ALD peroxisomal disease genes.

2.5. The YEF3 and RLI subfamilies and unclassified ORFs encoding ABC proteins

Two open reading frames, YFL028c and YDR061w, have not been classified, as their sequences are more distantly related to typical ABC proteins. Although they do contain at least one NBD with degenerate ABC signature motifs, they lack predicted membrane-spanning regions. Likewise, no TMSs are present in the RLI and YEF3 subfamilies, with a total of seven members, two of which, Yef3p [111] and Gcn20p [112], have been studied. While the precise subcellular localization is as yet undetermined, Yef3p appears to function as a translation elongation factor [113] that could be associated with the ribosome [114]. Notably, overexpression of Yef3p renders cells hypersensitive to paromomycin and hygromycin B, two translational inhibitors [115]. *YEF3* is the only essential yeast ABC gene, since its deletion is lethal in vegetative yeast cells. Interestingly, despite an 84% primary sequence identity, overexpression of a close Yef3p homologue, Hef3p, cannot rescue loss of Yef3p. The Hef3p function remains unknown, but like Yef3p, it exhibits ribosome-stimulated ATPase activity [116,117].

The role of the Gcn20p ABC protein is possibly in the regulation of amino acid utilization. Gcn20p coimmunoprecipitates with Gcn1p, indicating that Gcn20p and Gcn1p are components of a protein complex that couples a Gcn2p kinase activity to the availability of exogenous amino acids. A deletion of *GCN20* in otherwise wild type strains impairs derepression of *GCN4* translation and reduces the level of eIF-2α phosphorylation in vivo, indicating that Gcn20p is a positive effector of Gcn2p kinase function [112]. A recent study suggests that the Gcn1p/Gcn20p complex is a translational regulator of Gcn4p, which functions on elongating ribosomes by activation of the eIF2α kinase *Gcn2p* [118].

Other ABC proteins in this family have not been studied so far, but they are very closely related to either Yef3p or Gcn20p, implying a speculative role in translation elongation, regulation of carbon source utilization or amino acid starvation. Finally, the single RLI family member YDR091c exhibits homology to a human RNase L inhibitor, but its precise role in yeast has not been determined [33].

3. ABC proteins in the fission yeast *Schizosaccharomyces pombe*

Several ABC proteins have also been discovered in other fungal species including *Schizosaccharomyces pombe*. Six ABC proteins, all of which are membrane transporters, have been reported in fission yeast. The protein encoded by the *mam1* gene, a functional homologue of Ste6p, is responsible for the secretion of the mating pheromone M-factor [119]. Three ABC transporter genes were also isolated based on their ability to confer multidrug resistance, i.e. *pmd1* [120], *bfr1/hba2* [121,122], and the heavy metal tolerance gene *hmt1* [123]. The fourth member of the *S. pombe* MDR subfamily, the Abc1p protein [124], still awaits functional characterization. The *mam1* gene is only expressed in M-cells and its disruption leads to sterility. The fact that ectopic expression of Ste6p could restore mating ability in a *mam1* mutant strain [119] implies a functional conservation between these two pheromone transporters. Whether Mam1p is capable of exporting substrates other than M-factor remains to be determined.

The first fission yeast gene conferring multidrug resistance was *pmd1* [120]. Overexpression of the P-gp homologue Pmd1p confers resistance to the antifungal drug leptomycin B, as well as various other cytotoxic compounds. As expected, the null mutant is hypersensitive to a number of drugs [120]. However, despite its homology to Ste6p, *pmd1* mutants do not show decreased mating abilities [125]. Interestingly, actinomycin D hypersensitivity of a *pmd1* null strain can be rescued by functional expression of human P-gp, although human P-gp seems improperly glycosylated in *S. pombe* [56].

While the *S. cerevisiae* half-size transporter Atm1p exerts its function in the inner mitochondrial membrane [43,44], the *S. pombe* homologue Hmt1p is involved in the vacuolar sequestration of heavy metals [123,126]. Like plants and some other fungi, *S. pombe* synthesizes phytochelatins, which act as chelators in heavy metal detoxification [127]. Interestingly, the vacuolar membrane protein Hmt1p mediates ATP-dependent transport of both apophytochelatins and phytochelatin-Cd^{2+} complexes [126]. Since phytochelatins are synthesized from glutathione, it is tempting to speculate that Hmt1p plays a general role in the detoxification of GSH conjugates into the vacuole. It should be noted that the vacuole represents the prime organelle for detoxification in plants. Like in yeast, several plant ABC transporters have been identified, all of which are implicated in vacuolar sequestration of poisonous catabolites or environmental toxins [128–136].

It seems remarkable that the yeast PDR family, the largest subfamily in baker's yeast, has only one homologue as yet in fission yeast. The non-essential *bfr1/hba2* gene was isolated through its ability to confer brefeldin A resistance when overexpressed [121,122]. Notably, Bfr1p/Hba2p shares with its closest relatives, Pdr5p and Snq2p, a variation from the N-terminal consensus Walker A motif (GXXXXGK[S/T]), with the normally highly conserved lysine replaced by a cysteine [121,122].

4. ABC proteins in pathogenic fungi and antifungal resistance

It has been observed that long-term treatment of fungal infections with triazoles can result in severe antifungal resistance [137,138]. The discovery that Pdr5p mediates pronounced resistance to mycotoxins [61] and antifungal azoles [139] prompted the hunt for Pdr5p homologues in pathogenic fungi. The gene hunt was simplified by the fact that Δ*pdr5* mutants are hypersensitive to azole antifungals. Indeed, functional restoration of miconazole [140] or fluconazole/ketoconazole [139] resistance in Δ*pdr5* mutants allowed the identification of Cdr1p [139,140] and subsequently Cdr2p [141] from the opportunistic pathogen *Candida albicans*. Further studies on clinical strains revealed that there is a strong correlation between azole resistance and induced overexpression of Cdr1p and Cdr2p [139,142]. For instance, the azole minimal inhibitory concentration values of clinical *C. albicans* isolates increase dramatically during repeated antifungal treatment. In fact, clinical isolates exhibiting azole resistance frequently overexpress ABC transporters such as Cdr1p and Cdr2p [25], which share more than 60% primary sequence identity. Furthermore, deletion of both genes renders *C. albicans* strains highly susceptible to azole antifungals and many other drugs [143]. Surprisingly, a lack of Cdr2p did not lead to a significant hypersusceptibility to Cdr1p substrates, most likely be-

cause of the low Cdr2p expression, and because Cdr1p alone mediates efficient antifungal efflux. However, deletion of Cdr2p in a Δ*cdr1* background aggravated growth inhibition by various antifungals when compared to the single Δ*cdr1* deletion mutant [141]. Further, *CDR2* mRNA levels are elevated in clinical *Candida* isolates, suggesting that Cdr2p does play a role in antifungal resistance [141]. As previously reported for Pdr5p and Snq2p [67,73,144], expression of Cdr1p in a drug-hypersensitive *S. cerevisiae* strain mediates efflux of β-estradiol and corticosterone [145]. This transport process is energy-dependent and is inhibited to different extents by other Cdr1p substrates [145]. A recent report also suggested that Cdr1p acts as flippase for phosphatidylethanolamine [146], a hypothesis that was earlier proposed for mammalian P-gps [147] and yeast Pdr5p [148]. Consistent with a function in membrane lipid transport, changes in membrane fluidity also appear to affect Cdr1p function [149]. Like Cdr1p, mammalian P-gps such as Mdr1 and Mdr2 act as lipid flippases with distinct phospholipid specificity [150].

Taken together, active ABC pump efflux systems are a prime cause of antifungal resistance, although other resistance mechanisms such as changing membrane permeability, target alteration, and facilitated diffusion also contribute to clinical resistance [25,151]. Furthermore, ABC transporters implicated in antifungal resistance in other fungal pathogens have been identified. For instance, Pdh1p from *Candida glabrata* is the closest homologue of yeast Pdr5p with more than 72% primary sequence identity [152]. *PDH1* mRNA levels are upregulated in azole-resistant strains, although it cannot be excluded that other fluconazole resistance genes might also be induced [152]. *Candida dubliniensis*, another close relative of *C. albicans*, has also been reported to carry ABC drug transporters implicated in fluconazole resistance. The existence of Cdr1p and Cdr2p homologues in *C. dubliniensis* has been demonstrated by PCR [153]. Like the *C. albicans CDR* genes [139,141,154–156], several clinical isolates showed increased mRNA levels of *CdCDR1*. Finally, *C. albicans* has two additional ABC genes whose products do not appear to be involved in clinical azole resistance, despite their homology to the drug efflux pumps. Cdr3p seems to be an opaque-phase-specific transporter [157] of unknown function, and likewise no function is known for Cdr4p [158].

The only *Candida* homologue of the baker's yeast MDR subfamily is *HST6* [159], a functional homologue of Ste6p. *HST6* was isolated through its ability to suppress the mating defect of a Δ*ste6* mutant, thus mediating **a**-factor secretion. However, it is not clear what the physiological function or substrate of Hst6p might be, since *C. albicans* is a diploid, asexually reproducing fungus that is not known to secrete mating pheromones. Notably, *HST6* expression is repressed in diploid *S. cerevisiae* cells, while it is constitutively expressed in different cell types of *C. albicans* [159]. In a recent study, a putative Yor1p homologue from *C. albicans* was identified although functional data were not presented [99]. Finally, the Yef3p orthologue of the yeast Yef3p translation elongation factor (see Section 2.5) has also been identified in *C. albicans* [160].

The search for resistance transporters in other fungal pathogens (Table 2) has led to the discovery of ABC proteins in *Aspergillus nidulans* (*AtrC* [161], *AtrA* and *AtrB* [162]), *Aspergillus flavus* and *Aspergillus fumigatus* (*AflMDR1*, *AfuMDR1* and *AfuMDR2*, respectively [163]), in *Penicillium digitatum* (Pmr1p [164]) and in *Cryptococcus neoformans* (*CneMDR1* and *CneMDR2* [165]). Although few functional data are available on these fungal ABC transporters, their involvement in resistance development was proposed because of their high homology to yeast Pdr5p, Cdr1p and mammalian multidrug resistance genes. A very interesting novel ABC transporter was discovered in the plant fungus *Magnaporthe grisea*, a phytopathogen causing rice blast disease. Remarkably, deletion of *ABC1*, a close homologue of Pdr5p, in *M. grisea* causes a loss of virulence, indicating that Abc1p is an important pathogenicity factor [166]. Although the mechanism is unclear, it seems plausible that Abc1p mediates secretion of factors required for the invasion of plant cells or that *abc1* mutants are unable to detoxify toxic plant metabolites that counteract infection [166].

Finally, the regulatory mechanisms of CDR gene expression are largely unknown. However, such knowledge could be a key to the understanding of PDR gene function and help to combat antifungal resistance. By contrast, a large number of studies in

Table 2
ABC proteins identified in other fungi and fungal pathogens

ABC protein	Substrate(s)	Length	Topology	Localization	Phenotype	Reference
Candida albicans						
Hst6p	?	1323	$(TMS_6\text{-}ABC)_2$	?	?	[159]
Cdr1p	Antifungals	1501	$(ABC\text{-}TMS_6)_2$	PM	$Drug^{HS}$	[139,140,142]
Cdr2p	Antifungals	1499	$(ABC\text{-}TMS_6)_2$	?	$Drug^{HS}$	[143]
Cdr3p	?	1501	$(ABC\text{-}TMS_6)_2$	?	Viable	[157]
Cdr4p	?	1490	$(ABC\text{-}TMS_6)_2$	?	Viable	[158]
Elf1p	?	1191	ABC_2	?	Slow-growth	[207]
CaEf3p	?	1050	ABC_2	?	?	[160]
Candida glabrata						
Pdh1p	Drugs	1542	$(ABC\text{-}TMS_6)_2$	?	?	[152]
Schizosaccharomyces pombe						
Hba2p	Brefeldin A	1530	$(ABC\text{-}TMS_6)_2$	?	$Drug^{HS}$	[121,122]
Abc1p	?	1427	$(TMS_6\text{-}ABC)_2$	?	Viable	[124]
Pmd1p	Drugs	1362	$(TMS_6\text{-}ABC)_2$	?	$Drug^{HS}$	[120]
Mam1p	M-factor	1336	$(TMS_6\text{-}ABC)_2$	?	Sterile	[119]
Hmt1p	Phytochel./Cd^{2+}	830	$TMS_6\text{-}ABC$	Vac	Cd^{HS}	[123]
Aspergillus flavus						
AflMdr1p	?	1307	$(TMS_6\text{-}ABC)_2$	?	?	[163]
Aspergillus fumigatus						
AfuMdr1p	Drugs?	1349	$(TMS_6\text{-}ABC)_2$	?	?	[163]
AfuMdr2p	?	791	$TMS_4\text{-}ABC$	?	?	[163]
Aspergillus nidulans						
AtrAp	?	1466	$(ABC\text{-}TMS_6)_2$	?	?	[162]
AtrBp	Drugs?	1426	$(ABC\text{-}TMS_6)_2$	?	?	[162]
AtrCp	?	1293	$(TMS_6\text{-}ABC)_2$	?	?	[161]
Cryptococcus neoformans						
CneMdr1p	?	1408	$(TMS_6\text{-}ABC)_2$	?	?	[165]
Magnaporthe grisea						
Abc1p	?	1619	$(ABC\text{-}TMS_6)_2$	?	Reduced pathogenicity	[166]

HS, hypersensitivity; Phytochel., phytochelatins; PM, plasma membrane; TMS, transmembrane segment; Vac, vacuole.

baker's yeast have unraveled a complex regulatory network that controls PDR development. Hence, the next section will be devoted to a discussion of the yeast PDR network that regulates ABC gene expression under normal and adverse conditions.

5. Regulation of ABC gene expression – the yeast PDR network

As discussed above, PDR can arise from overexpression of ABC drug efflux pumps. In addition, mutations in genes encoding transcriptional regulators also lead to a complex PDR phenotype. The original discovery comes from observations showing that the *PDR5* gene is regulated by Pdr1p [167], and that *pdr5* mutants are hypersensitive to cycloheximide [168]. Two transcriptional regulators, Pdr1p [169] and Pdr3p [170,171], were identified, either one of which can mediate PDR. While Δ*pdr1* mutants are hypersensitive to many drugs [71,167], drug-resistant *pdr1* mutants, so-called gain-of-func-

tion mutants, overexpress not only Pdr5p [167] but also Snq2p [71,72]. Numerous gain-of-function alleles of *PDR1* [172] and *PDR3* [173] were identified, some of which cause various drug resistance phenotypes [66,71,167,174], while others also increase transmembrane flipping of phospholipid analogues [148]. However, it is not fully understood how point mutations in *PDR1* and *PDR3* can cause such diverse phenotypes, except that they must act through unknown target genes. Notably, neither the expression level nor the localization of the Pdr1p/Pdr3p regulators is influenced by these mutations (Pandjaitan et al., in preparation). Thus, it is tempting to speculate that an interaction with other as yet unidentified transcriptional regulators and the subsequent modulation of target genes cause the phenotype variety.

5.1. The master regulators – Pdr1p and Pdr3p

Pdr1p and Pdr3p are closely related and belong to the bi-nuclear Gal4p-like $Zn(II)_2Cys_6$ transcription factors, which share a similar domain organization. Deletion of both genes aggravates drug hypersensitivity, suggesting that they have overlapping functions [170]. The bi-nuclear Zn cluster at the N-terminus mediates DNA binding. In their middle region, several motifs are thought to be inhibitory domains, while the C-terminal region harbors an acid-rich activation domain (ARD) [172,173]. Furthermore, the ARD seems to be the site for phosphorylation of Pdr3p, since Pdr3p deletion mutants lacking the ARD are no longer phosphorylated (Pandjaitan et al., unpublished results). The functional importance of Pdr1p/Pdr3p phosphorylation is unclear, since the truncated non-phosphorylated form of Pdr3p is at least partially functional (Mahé et al., unpublished results). Most gain-of-function mutations in *PDR3* map to the first two motifs in the inhibitory domain, where they most likely impair inhibition, and to the ARD, where hyperactivation might be induced [170]. In contrast, *pdr1* mutations are scattered throughout the entire protein with some hot spots at the C-terminus [172]. Neither Pdr1p nor Pdr3p is subject to a reversible cytoplasmic-nuclear shuttling, as they constitutively localize to the nucleus (Pandjaitan et al., in preparation). However, the nuclear localization or the function of at least Pdr1p seems to require Pdr13p, a cytoplasmic Hsp70 homologue [175].

Pdr1p and Pdr3p act through *cis*-acting sites present in the promoters of target genes (Fig. 3). The consensus motif was named PDRE (for pleiotropic drug resistance element) and later identified as the perfect palindrome 5′-TCCGCGGA-3′ [176,177]. Notably, a Pdr3p-mediated autoregulation of the *PDR3* gene carrying two perfect PDREs exists [177], but the signals that activate this autoregulatory loop are not known at present. PDREs come in different flavors (Fig. 2) which nevertheless retain their ability to serve as Pdr1p/Pdr3p target sites. Hence, limited variations in the consensus PDRE still entertain Pdr1p/Pdr3p-mediated regulation (Fig. 2). Further, promoter PDREs are present in different numbers ranging from one to four as well as in different combinations. Whether or not this is important for Pdr1p/Pdr3p regulation is not clear. While in vivo studies are not available yet, in vitro studies using recombinant GST-Pdr3p and GST-Pdr1p indicate that each regulator can bind both perfect and degenerate PDREs of target genes [71,97,178–180]. More importantly, Pdr1p and Pdr3p can form homo- and heterodimers in vivo, indicating that target gene specificity could be brought about by Pdr1p/Pdr3p complexes recognizing distinct PDRE combinations, thereby exerting distinct regulatory functions. (Pandjaitan et al., in preparation). This might help to explain why *PDR5* transcription is grossly impaired in Δ*pdr1* cells [71,176], while *SNQ2* transcription is only abolished in Δ*pdr1*Δ*pdr3* mutants [71], and *YOR1* transcription is only slightly affected even in a Δ*pdr1*Δ*pdr3* double mutant [97]. The single Δ*pdr* mutants also exhibit striking effects. For instance, *PDR5* transcription is dramatically reduced in a Δ*pdr1* strain, whereas the *PDR15* mRNA, the closest homologue of *PDR5*, is increased [178]. The reason for this complementary regulation is not understood, but it clearly indicates that Pdr1p and Pdr3p can exert positive and negative effects on PDR target genes. The function of Pdr1p/Pdr3p is perhaps subject to regulation by other transcription factors. For instance, Ngg1p, a general transcription factor required for Gal4p-mediated glucose repression [181,182], might inhibit Pdr1p through a physical interaction with the Pdr1p/Pdr3p complex [183].

The first identified target genes for Pdr1p and

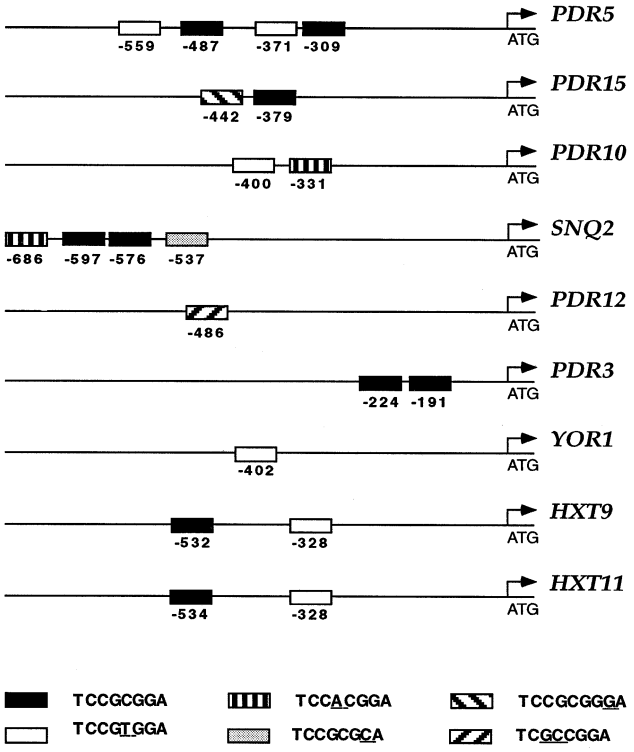

Fig. 2. Different PDREs are present in the promoter regions of Pdr1p/Pdr3p target genes. The underlined residues represent deviations from the perfect palindromic PDRE 5′-TCCGCGGA-3′.

Pdr3p were *PDR5* and *SNQ2* (Fig. 3). Both Pdr5p and Snq2p levels are increased by an order of magnitude in *pdr1–3* mutants, while their expression is virtually abolished in Δ*pdr1*Δ*pdr3* double mutants [71]. Thus, Pdr5p and Snq2p nicely describe the basic PDR network comprised by regulators and target pumps, which execute resistance development. *PDR5* and *SNQ2* have several PDREs in their promoters (Fig. 2), and both are controlled by Pdr1p/Pdr3p. However, *PDR5* and *SNQ2* are by no means the only genes of the PDR family with promoter PDREs. For instance, *PDR10* and *PDR15* transcription is strongly affected by Pdr1p and Pdr3p, showing that both Pdr10p and Pdr15p are subject to regulation within the PDR network. Likewise, the oligomycin transporter Yor1p is also regulated by Pdr1p/Pdr3p through a single degenerate PDRE [97]. Hence, at least one functional PDRE appears necessary and sufficient to confer Pdr1p/Pdr3p regulation [97,184].

Interestingly, two non-ABC genes with PDREs are also known. The *HXT9* and *HXT11* genes encode members of the large family of hexose transporters [180]. Basal expression of both *HXT9* and *HXT11* is indeed regulated by Pdr1p and Pdr3p, and Hxt9p/Hxt11p overexpression leads to drug hypersensitivity [180]. Although this is in contradiction to other Pdr1p/Pdr3p targets whose overproduction leads to drug resistance, it seems plausible that higher permease levels at the cell surface cause increased uptake of toxic compounds, particularly since hexose transporters are supposed to mediate uptake of molecules rather than efflux. Finally, the *PDR12* gene encoding a weak acid pump also contains one degenerate PDRE (Fig. 3). It might be that Pdr1p/Pdr3p exert a basal regulatory effect on *PDR12*, since the corresponding mRNA levels are decreased in *pdr1/pdr3* mutants (Pandjaitan et al., unpublished data). However, this PDRE does not contribute to *PDR12* regulation under stress [78].

5.2. Stress and ABC transporters – the Yap family

The second family of transcription factors regulating ABC protein expression is the bZip protein family, also known as the Yap family. They are related to the mammalian AP-1 transcription factor, a heterodimer of c-*jun* and c-*fos*, and to yeast Gcn4p. Eight related family members exist in yeast, and at least three of them are implicated in drug resistance

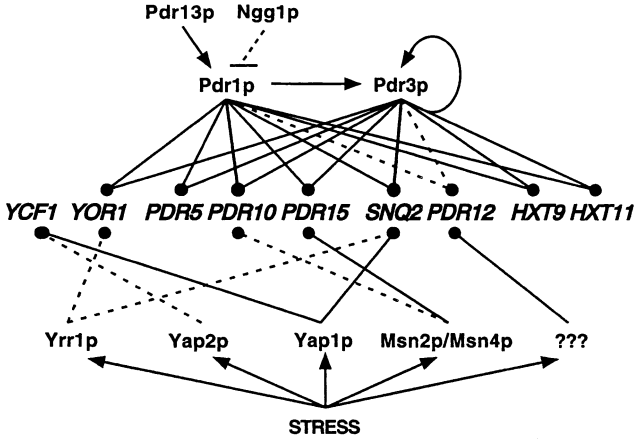

Fig. 3. The PDR network. The genes in the center represent ABC transporters and/or Pdr1p/Pdr3p target genes, which are currently known to be regulated by the transcriptional regulators above (Pdr1p/Pdr3p) and below (Yap1p, Yrr1p, Yap2p, Msn2p/Msn4p).

through the regulation of ABC transporters [185]. The best characterized member of this family is *YAP1/PAR1/SNQ3/PDR4* [186–188], whose function in stress response is also well documented [174,187–189]. Gene activation by Yap1p is mediated through ARE (AP-1 response element; 5′-TTAG/CTAA-3′) and the motif mediating Gcn4p binding, both of which are also recognized by other members of the Yap family [93,187,190].

Since *YAP1* overexpression causes Cd^{2+} resistance, a possible regulation of Ycf1p was investigated. Indeed, *YAP1*-mediated Cd^{2+} resistance requires functional Ycf1p. However, hyperresistance to Cd^{2+} as a consequence of *YCF1* overexpression is only partially dependent on the presence of functional Yap1p [94]. This hints at the existence of one or more additional regulators mediating stress response through *YCF1* regulation. Indeed, overexpression of Yap2p, a bZip protein with homology to Yap1p, mediates Cd^{2+} resistance only in the presence of Ycf1p [187]. Yap2p has also been identified as the *CAD1* gene in a genetic screen for cadmium resistance [187]. While Δ*cad1* deletion strains show elevated Cd^{2+} sensitivity, Cad1p overexpression causes resistance. Moreover, Δ*yap2* strains are also hypersensitive to oxidative stress [191]. Although Yap1p is much more important for oxidative stress response, Yap2p is clearly involved as well. Notably, a strain lacking Yap8p (Acr1p), another Yap member, is sensitive to arsenite and arsenate. However, the Yap8p target gene has not been identified, but the Ycf1p-mediated arsenite and arsenate resistance phenotype [92] implies a connection between Yap8p and Ycf1p [192]. Consistent with these findings, a functional YRE is present in the *YCF1* promoter [94].

Like Pdr1p and Pdr3p, Yap1p also contains an ARD at its C-terminus. In contrast to Pdr1p/Pdr3p, however, the Yap1p ARD confers negative regulation, since a mutant Yap1p lacking the ARD is constitutively active [95,193]. This activation is most likely mediated through a constitutive nuclear localization of mutant Yap1p. In unstressed cells, Yap1p is diffusely dispersed throughout the cytoplasm, but rapidly shuttled into the nucleus in response to stress, requiring the Ran1p shuttle [194]. The regulation of Yap1p is not only confined to its localization, but might also occur at the transcriptional level. Interestingly, both *YAP2* and *YAP1* have an upstream open reading frame in their 5′-untranslated region, which negatively regulates both *YAP1* and *YAP2*, since its deletion causes elevated transcription [195]. Yap1p also controls many other stress response genes, including *FLR1* [196], *TPS2* [189], *TRX2* [190], *GLR1* [197] or *GSH1* [198]. When activated by an oxidizing environment, Yap1p induces target genes that are important for maintaining cellular viability under stress. While the role of ABC genes in this process is unknown, it could be that ABC transporters are responsible for extracellular or vacuolar detoxification of toxic compounds produced under oxidative stress. The Ycf1p function in vacuolar sequestration of GSH conjugates supports this idea.

In addition to Pdr1p/Pdr3p and Yap1p, there is at least one additional regulator of the $Zn(II)_2Cys_6$ family, Yrr1p [199], involved in ABC transporter regulation. Control of Snq2p expression in response to stress requires Yrr1p, particularly for the 4-NQO-mediated induction of *SNQ2* mRNA levels. Furthermore, a *yrr1* gain-of-function mutation also induces *YOR1* mRNA levels [199], confirming that Yor1p is part of the PDR network. Moreover, expression of *SNQ2* in response to stress is also regulated by Yap1p, since induction of Snq2p biosynthesis requires Yap1p (Mahé et al., in preparation). However, there is no obvious YRE in the *SNQ2* promoter, indicating that yet unknown elements might mediate Yap1p regulation of *SNQ2*. Alternatively, Yap1p could act through Pdr1p or Pdr3p without directly binding to the *SNQ2* promoter region. Thus, regulation of *SNQ2* expression under normal and adverse conditions involves at least four distinct regulators: Pdr1p, Pdr3p, Yrr1p, and Yap1p [71,199]. Likewise, diazaborine resistance development is dependent on Pdr1p/Pdr3p and Yap1p [174], although in this case the actual target gene and mechanism is unknown. The fact that Yap1p-mediated diazaborine resistance requires functional Pdr1p/Pdr3p, suggests a functional cross-talk between the PDR network and stress response [174].

Finally, we have recently obtained evidence that transcription factors of the high osmolarity glycerol (HOG) pathway are involved in the regulation of the Pdr15p ABC transporter (Wolfger et al., in preparation). In addition to PDREs, *PDR15* contains several STREs (stress response elements) in its promoter

[178]. These motifs (5′-CCCCT-3′ or 5′-AGGGG-3′) mediate stress response through a heterodimeric Msn2p/Msn4p transcription factor complex [200–202]. STRE-mediated regulation is required for the HOG signaling pathway, which comprises a two-component osmo-sensing system, a mitogen-activated protein kinase cascade, and the downstream effectors Msn2p/Msn4p [80]. *PDR15* mRNA and protein levels are strongly induced in response to several stresses, including heat shock and osmotic stress (Wolfger et al., unpublished results). This induction requires functional Msn2p but surprisingly is independent of Hog1p and Pbs2p, the upstream regulatory kinases of the HOG pathway (Wolfger et al., in preparation). These data provide evidence for a novel and as yet unidentified signaling cascade operating in parallel to the HOG pathway but converging at the Msn2p/Msn4p downstream effectors for *PDR15* stress induction (Wolfger et al., unpublished results).

Still little is known about the regulation of drug efflux pumps in pathogenic fungi. It should be mentioned that the 5′-untranslated region of the *Candida glabrata PDH1* ABC transporter contains a sequence at position −200 to −190 that strongly resembles the yeast PDRE [152]. While the Pdr1p/Pdr3p homologues from *C. albicans* have been identified recently [203], it is not known if similar proteins exist in *C. glabrata* which would recognize these PDRE-like elements. Another indication for a functional cross-talk between drug resistance and stress response in pathogens comes from the identification of Cap1p [204], a homologue of Yap1p. Cap1p appears to be implicated in antifungal resistance as well as stress response in *C. albicans* [204].

To come to full circle, the complex connections within the yeast PDR network are depicted in Fig. 3. It seems reasonable to predict that the transcriptional regulators and target genes known to date comprise only a part of the whole regulatory network involved in the regulation of ABC transporters under physiological and adverse conditions. There is already evidence for novel regulators of ABC transporters such as the weak acid pump Pdr12p, since its induced expression under stress is independent of Pdr1p/Pdr3p, Yap1p, and Msn2p/Msn4p [78]. In addition, the case example of *PDR15* indicates that parallel stress signaling cascades may converge at downstream effectors such as Msn2p/Msn4p. The existing connections between PDR regulation and stress suggest physiological functions of ABC transporters in stress response, promising a tremendous experimental challenge for the years to come.

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