Physiological functions of multidrug transporters in yeast

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Overexpression of drug extrusion pumps belonging to the ABC (ATP-binding cassette) super family of proteins is one of the most common mechanisms of multidrug resistance in various organisms. Both pathogenic and non-pathogenic yeast cells also become resistant to a variety of drugs by overexpressing genes encoding ABC drug efflux pumps. Recent evidences reveal that not only the well-characterized human drug extrusion pump (MDR1/P-gp), but its close homologues in yeast also mediate several cellular functions. Keeping in view the importance of ABC drug transporters in yeasts, this review particularly focuses on their physiological roles.

THE rapidly growing ATP-Binding Cassette (ABC) superfamily, also known as 'traffic ATPases', comprises an extremely diverse class of membrane-transport proteins[1–5]. These proteins, which were discovered almost two decades ago in bacteria as high-affinity nutrient transporters, shot to prominence when their ability to confer multidrug resistance (MDR) in cancer cells was realized[6]. Among several mechanisms that seem to contribute to the MDR phenomenon, overexpression of drug extrusion pumps belonging to the ABC superfamily is the most frequent cause of resistance to antifungals, herbicides, anticancer and cytotoxic drugs. To date, the most documented and well-characterized ABC drug extrusion pump has been the P-glycoprotein (human MDR1/P-gp) of tumour cells[6]. The presence of proteins homologous to human MDR1/P-gp in all organisms, ranging from the prokaryotes to eukaryotes, including yeasts and plants, portrays drug extrusion as a general mechanism of MDR.

ABC proteins can transport a variety of structurally diverse hydrophobic substrates[2]. The functional diversity of the ABC proteins is also reflected in their ever-emerging physiological roles in nutrient, peptide, lipid and cholesterol transport, the biosynthesis of molecules like heme, cell development in plants, apoptosis, and translational regulation[7–13]. Interestingly, human diseases such as cystic fibrosis, adrenoleukodystrophy, Dubin–Johnson syndrome, Tangier disease are associated with mutations in human genes encoding ABC transporters, which again reflects their relevance in cellular physiology[14,15]. In view of the above, a general question pertaining to the physiological importance of ABC drug-transporter proteins always comes up. It is expected that such a large family of transporters cannot be dedicated merely to drug efflux. This view is supported by the fact that very close homologues of the ABC proteins are not drug extrusion pumps, and mediate dedicated physiological functions. Studies dealing with physiological signals that control the expression of the drug extrusion pumps have also provided new insights not only into the complexities of regulatory circuits but also into the requirement of these pumps in normal cell functioning[16].

When challenged with antifungals and other drugs, both pathogenic and non-pathogenic yeasts have the capacity to overcome their inhibitory action through specific resistance mechanisms[17–23]. One of the most prominent resistance mechanisms includes overexpression of genes encoding drug extrusion pumps belonging to the ABC superfamily. In view of the limited scope of the article, we have not attempted to discuss the physiological functions mediated by all the ABC proteins of yeasts; rather we have focused on the cellular functions of only the drug extrusion pumps of this superfamily. How these pumps are involved in conferring multidrug resistance in yeasts is a widely reviewed subject and hence this aspect has not been discussed in this article[18–23].

Drug transporters of the ABC superfamily of yeasts

Yeast ABC transporters, like their mammalian homologues, possess specific domains for membrane association and ATP-binding and hydrolysis. A typical yeast ABC protein comprises of two homologous halves, each made up of a hydrophilic, cytoplasmic, nucleotide-binding domain (NBD) and a hydrophobic domain represented by six transmembrane stretches (TMS). In addition, the NBD consists of one Walker A, Walker B and a signature or C-motif. The completion of the *Saccharomyces cerevisiae* genome-sequence project led to the identification of 30 putative ABC proteins which are divided into six clusters, viz. the PDR, MDR, MRP/CFTR, RL1, YEF3 and ALDP subfamilies[14,24,25]. The PDR subfamily is the largest among these clusters and most of the drug transporters of *S. cerevisiae* belong to this subfamily of ABC proteins (discussed below). Table 1 lists only those ABC

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Table 1. ABC transporters of yeast

Organism	Gene name	Subfamily[a]	Size[b]	Function	Topology[c]
Saccharomyces cerevisiae[d]	*PDR5*	PDR	1511	Drug efflux pump, phospholipid translocator[41,86,106]	
	PDR10	PDR	1564	Drug efflux pump[107]	
	PDR12	PDR	1511	Resistance to water-soluble, monocarboxylic acids with chain lengths from C-1 to C-7 (ref. 59)	
	PDR15	PDR	1529	Potential drug transporter[24,108]	
	PDR11	PDR	1411	Sterol transporter in anaerobic yeast[75]	
	SNQ2	PDR	1501	Drug efflux pump[47]	
	YCF1	MRP/CFTR	1515	Cd^{2+} and glutathione-S conjugate pump[48]	
	YOR1/YRS1	MRP/CFTR	1477	Oligomycin and multidrug resistance, phospholipid translocator[46,86,109]	
	STE6	MDR	1290	a-factor export[31]	
	BAT1		1559	Bile acid transporter[110]	
	ATM1	MDR	690	Mitochondrial DNA maintenance, essential protein[111]	
	MDL1	MDR	695	Peptide transporter[112]	
	MDL2/SSH1	MDR	812	Peptide transporter[112]	
	PXA1/SSH2/PAL1	ALDP	870	*b*-oxidation of fatty acids[113]	
	PXA2/PAT1	ALDP	853	Interaction with PXA1, small-molecule transport[113]	
Schizosaccharomyces pombe	*BFR1/HBA2*	PDR	1530	Brefeldin A transport[114]	
	Mam1	MDR	1336	M-factor transport[115]	
	pmd1	MDR	1362	Drug efflux pump[116]	
	Abc1	PDR	1427	Unknown[117]	
	Hmt1	ALDP	830	Vacuolar transporter of phytochelatins (the metal chelating peptide)[118]	
Candida albicans	*CaCDR1*	PDR	1501	Drug efflux pump, phospholipid translocator[52,84,85]	
	CaCDR2	PDR	1499	Drug efflux pump, phospholipid translocator[52,84,85]	
	CaCDR3	PDR	1501	Opaque-phase specific, phospholipid translocator[29,85]	
	CaCDR4	PDR	1490	Phospholipid translocator?[30,56]	
	HST6	MDR	1323	Transport of a-factor, drugs?[93]	
	CaYOR1	MRP/CFTR	ND	Drug efflux pump?[119]	
	CaCDR5	PDR	–	Drug efflux pump?[44]	nd
	CaYCF1	MRP/CFTR	1606	Drug efflux pump?[120]	
Candida glabrata	*CgCDR1*	PDR	1499	Drug efflux pump[121]	
	PDH1	PDR	1542	Drug efflux pump[122]	
	CgCDR2	PDR	–	Drug efflux pump[121,123]	nd
Candida dubliniensis	*CdCDR1*	PDR	–	Drug efflux pump?[124]	nd
	CdCDR2	PDR	–		
Candida krusei	*ABC1*	PDR	–	Drug efflux pump?[125]	nd
	ABC2	PDR	–	Drug efflux pump?[125]	

[a]Names of subfamilies based on sequence similarity with the subfamilies characterized in *S. cerevisiae.*
[b]Number of amino acid residues.
[c]Topology of proteins is shown schematically. Spheres depict the nucleotide binding domains while the grey curves depict the transmembrane segments.
[d]For *S. cerevisiae* and *C. albicans* only those genes are listed that have been identified with a function.
nd, Topology not determined or predicted.

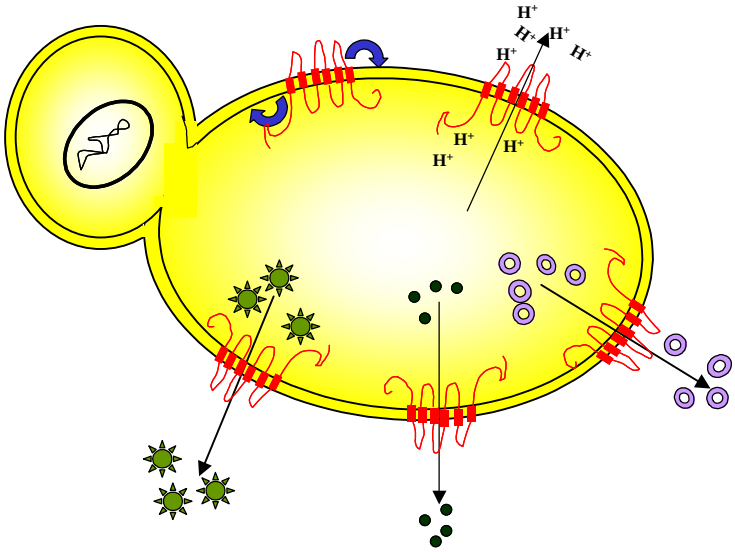
transporters of yeasts that have been assigned functions with their predicted topology.

Among various pathogenic fungi, most of the MDR-related studies have predominantly been focused on *Candida* and particularly *C. albicans*, as it accounts for a majority of systemic infections in immunocompromised patients. It is the most common form of fungemia in Western hospitals and more than 80% of the HIV-infected population develops oropharyngeal and clinical thrush[26]. Infections caused by non-*albicans* species, such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* have also been increasing, especially in neutropenic patients and neonates[22,27]. A large number of drug transporters of the ABC superfamily, viz. the CDRs (*Candida* Drug Resistance), have already been identified in *C. albicans* as well as in other non-*albicans* species (Table 1).

Physiological role of the drug extrusion pumps

While the role of ABC transporters in expelling xenobiotics from the cells is highly conserved, there is ample evidence to suggest that such a large family of proteins performs many other physiological functions. Several studies have demonstrated that not only the human MDR1/P-gp, but also the yeast ABC drug transporters can mediate a variety of cellular functions (Figure 1)[13,28]. The obvious question emerges as to how these ABC proteins, in spite of bearing close sequence similarity, are able to mediate diverse physiological functions. Of note is the fact that all the homologous ABC transporters are not drug transporters. For example, Ste6p of *S. cerevisiae* and Cdr3p and Cdr4p of *C. albicans* are similar to yeast drug-transporters proteins like Cdr1p, Cdr2p, Pdr5p and human MDR1/P-gp, but are incapable of extruding drugs[29–32] (Table 1). Therefore, there is a concerted effort to identify the molecular determinants within these proteins, which permit this diversity in function. Studies involving human MDR1/P-gp have already identified protein segments and amino acid residues implicated in drug binding, drug transport and ATP hydrolysis[33,34].

In comparison, studies pertaining to the identification of the molecular determinants of yeast ABC drug transporters have only been initiated recently. These studies have already revealed the importance of some of the amino acid residues, and stretches of yeast ABC drug transporters are crucial in protein folding, membrane localization and in drug transport. In a study employing Cdr1p, the ABC drug extrusion pump of *C. albicans*, it has been shown that the deletion of a 79 amino acid stretch from the C-terminal, which encompasses the transmembrane segment 12 (TMS 12) of this transporter, did not result in the total loss of its ability to efflux cytotoxic agents[35]. The expression of this truncated *CDR1* (ΔCdr1p) in *S. cerevisiae* resulted in impaired sensitivity to selected drugs like cycloheximide, anisomycin, sulphomethuron methyl and nystatin, while its ability to confer resistance to drugs like *o*-phenanthroline, 4-nitroquinoline-*N*-oxide, cerulenin, azoles, oligomycin, erythromycin, chloramphenicol and benomyl remained unaltered. Of note is the finding that the TMS 12 deletion neither led to any significant impairment in NTPase (ATPase and UTPase) activities nor in its ability to efflux rhodamine123 and *b*-estradiol. The deletion of TMS 12 also did not affect the targetting of ΔCdr1p to the plasma membrane, when overexpressed in baculovirus–insect cell expression system[35]. In order to identify the important residues and domains in the *S. cerevisiae* Pdr5p, the entire *PDR5* gene was subjected to



FUNCTION	*Saccharomyces cerevisiae*	*Candida albicans*
Transport of drugs/inorganic ions	***PDR5***, *SNQ2* *YOR1*, *YCF1*	***CDR1***,*CDR2*, *CaYOR1*
Efflux of steroids	***PDR5*** *SNQ2*	***CDR1***
Transport of sex pheromones	**STE6*	**HST6*
Phospholipid translocation	***PDR5*** *YOR1 ?*	***CDR1*** *CDR2*,**CDR3* **CDR4*
Ion transport regulator (H^+)	-	***CDR1***

Figure 1. Multifunctional roles attributed to ABC drug transporters in yeast. The other ABC transporters, which are not involved in MDR such as *CDR3*, *CDR4*, *STE6* and *HST6* (marked with ∗) are also included to highlight a particular function[42,46–48,52,68–70,85,93,94,104].

random *in vitro* mutagenesis and screened for mutants, which conferred altered drug resistance[36]. The sequencing of selected mutants revealed that mutations were predominantly found to be localized in each nucleotide-binding domain, the transmembrane (TMS) domain 10, and, even in the predicted extracellular hydrophilic loops (Table 2)[36,37]. Interestingly, some point mutations also affected folding of Pdr5p, suggesting that a proper folding of this protein is a major determinant of substrate specificity.

While random screening for MDR inhibitors represents the most common approach to look for effective drugs, a clear understanding of the structure and function of ABC proteins involved in drug resistance represents another approach which could lead to rational designing of inhibitors to block the drug-efflux-pump proteins[38]. To date, most of our knowledge of ABC protein structure comes from the secondary structure predictions based upon the primary protein sequences. Hence, there is a great need for a biochemically, biophysically and genetically tested 3D structure of these proteins. In this regard it is pertinent to mention that an initial structure of purified detergent-solubilized and liposome-reconstituted human MDR1/P-gp has been determined to 2.5 nm resolution by electron microscopy and single-particle image analysis[39]. This structural model confirmed the existence of cytoplasmic NBDs and twelve TMS. These studies predicted the presence of a large aqueous pore at the extracellular face of the membrane as well as the existence of a pore opening to the lipid phase. The presence of a large pore is suggested to be consistent with the broad substrate specificity of this pump. Recently, the determination of a high-resolution crystal structure of MsbA (lipid A transporter of *Escherichia coli*), which is closely related to mammalian P-gps, further confirms the general architecture of the MDR–ABC transporters[40]. These crystal structures are beginning to confirm the biochemical data obtained earlier with these proteins. Such structural data of yeast ABC drug transporters, particularly of pathogenic yeasts, would certainly set the stage for studies related to the mechanistic

Table 2. Phenotypic analyses of PDR5p mutants[a]

Mutant	Amino acid substitution[b]	Domain	Subcellular localization	Drug resistance profile	Rhodamine 6G efflux	Steroid transport	FK506
Pdr5-14	C199Y A676V **T1460I** **V1467I**	NBD1, Walker A TMD5 Extracellular loop 6 Extracellular loop 6	Plasma membrane	Reduced resistance to cycloheximide, itraconazole and ketoconazol than wild type Pdr5p.	Reduced efflux than wild type Pdr5p.	Severely reduced transport of dexamethasone and estradiol than wild type Pdr5p.	Sensitive
PDR5-26	G557D A1398T P1421S **C1427Y**	Extracellular loop 1 TMD 11 Extracellular loop 6 Extracellular loop 6	Endoplasmic reticulum	Sensitive to cycloheximide, itraconazole and ketoconazol in comparison to wild type Pdr5p.	No efflux	Not determined	Sensitive
PDR5-46	V149M **G905S** G908S	N-terminal cytoplasmic domain NBD2, Walker A NBD2, Walker A	Plasma membrane	Sensitive to cycloheximide, itraconazole and ketoconazol in comparison to wild type Pdr5p.	No efflux	Does not transport either estradiol or dexamethasone.	Sensitive
PDR5-57	G138D **G1009C**	N-terminal cytoplasmic domain NBD2, C-motif	Plasma membrane	More sensitive to cycloheximide than the wild type Pdr5p. Resistance to itraconazole and ketoconazole similar to wild type Pdr5p.	Reduced efflux than wild type Pdr5p.	Not determined	Sensitive
PDR5-71	**G302D**	NBD1, C-motif	Plasma membrane	More sensitive to cycloheximide than wild type Pdr5p. Resistance to itraconazole and ketoconazole similar to wild type Pdr5p.	No efflux	Transports both dexamethasone and estradiol but at a lower level than wild type Pdr5p.	Sensitive
PDR5-127	S140N V150L T360I V782I V783I **S1360F**	N-terminal cytoplasmic domain N-terminal cytoplasmic domain N-terminal cytoplasmic domain TMD 6 TMD 6 TMD 10	Plasma membrane	More sensitive to cycloheximide and itraconazole than wild type Pdr5p. Resistance to ketoconazole similar to wild type Pdr5p.	Reduced efflux than wild type Pdr5p.	Does not transport dexamethasone. Transports estradiol, but at a lower level the wild type Pdr5p.	Resistant

[a]Compiled from ref. 36.
[b]Amino acid substitution(s) responsible for the phenotype is marked in bold.

aspects of these proteins. The following sections examine the physiological functions mediated by yeast ABC drug transporters.

Protection against natural toxins/metabolites

PDR5/STS1/LEM1 (Pleiotropic Drug Resistance, Sporidesmin Toxicity Suppressor, Ligand Effect Modulator), the well-characterized drug transporter of *S. cerevisiae*, was identified on the basis that it could confer resistance to a number of unrelated drugs when overexpressed[41–43]. Thereafter, several yeast ABC transporters were characterized whose overexpression led to resistance to one or more drugs. However, the drug resistance phenotype in *S. cerevisiae* is not always the result of overexpression of a single gene, but is rather a consequence of the upregulation of numerous other genes that encode ABC drug extrusion pumps[44]. For example, there are *SNQ2* (Sensitivity to 4-Nitroquinoline N-oxide), *YOR1* (Yeast Oligomycin Resistance) and *YCF1* (Yeast Cadmium Factor) whose overexpression is known to result in resistance to specific compounds[45–48]. While *SNQ2* is a close homologue of *PDR5* (40% amino acid identity to *PDR5*), *YOR1* and *YCF1* are similar to their human homologue MRP1 and MDR1/P-gp. Unlike Pdr5p and Snq2p that are localized in the plasma membrane, Ycf1p is localized in the vacuolar membrane and is capable of expelling drugs only as glutathione conjugates[49]. The homology of Ycf1p to human CFTR has fuelled studies on its role as a drug transporter[48,50]. A comprehensive study on the toxicity of 349 compounds on the *pdr5*, *snq2* and *yor1* deleted strains demonstrated that these drug transporters have a substrate profile that is overlapping to a large extent and yet different and specific[51].

C. albicans and other pathogenic yeasts have also recruited a battery of genes to render common antifungals ineffective. *CDR1*, the ABC transporter of *C. albicans* and a homologue of *PDR5* of *S. cerevisiae*, expels a variety of drugs, including antifungals like azoles[52]. A search for other homologues of *CDR1* in *C. albicans* has led to the identification of *CDR2*, *CDR3* and *CDR4*, of which only *CDR2* – which is 84% identical to *CDR1* – is found to be involved in antifungal resistance[29,30,53]. The deletion of *CDR2* alone did not render *C. albicans* cells (CAF4-2) hypersensitive to tested drugs; however, its deletion in a mutant background where *CDR1* was deleted elicited hypersensitivity to many antifungals. The lack of hypersensitivity of the single Δ*cdr2* mutant to drugs was attributed to the absence of its mRNA in azole-susceptible isolates[53]. The azole-resistant clinical isolates of *C. albicans*, isolated from AIDS patients show among other genes (*ERG11* and *CaMDR1*), an overexpression of the mRNA encoding the ABC drug extrusion pumps *CDR1* and *CDR2* (refs 54 and 55), thus confirming their role in antifungal resistance. In contrast, *CDR3* is a phase-specific gene[29], which is upregulated in the opaque phase, while *CDR4* is a putative phospholipid translocator[56]. In addition to the above genes, partial sequences of fourteen new genes bearing homology to the NBDs of human MDR1 have been identified in *C. albicans*[57]. These new sequences show no significant homology to known *CDRs*, but nonetheless some of them could have an effect on drug susceptibilities of *C. albicans*.

Several properties of ABC drug transporters, particularly their wider specificity and occurrence do point to their role in the protection of cells against cytotoxic agents. A physiological role for human MDR1/P-gp in effluxing compounds has been confirmed on the basis of its expression on the apical membranes of gut epithelia, liver cells, kidney tubules and at the blood–tissue barrier[28]. The pattern of MDR1/P-gp expression in tissues and studies on MDR1/P-gp knockout mice indicate that it may protect the organism from toxic compounds in our diet. The ability of ABC drug transporters of yeast to extrude unrelated drugs should be seen as part of their ability to expel the cytotoxic agents. Interestingly, the expression of *PDR5* and *CDR1* is growth-phase-dependent, being highest in the stationary growth phase and possibly linked to their role in effluxing intracellular cytotoxic metabolites accumulating during growth[45,54,55,58]. Dicottignies *et al.*[45] observed that the disruption of both *PDR5* and *SNQ2* genes in *S. cerevisiae* cells reduced the exponential cell growth rate, suggesting that the presence of either *PDR5* or *SNQ2* is important for cell growth. These transporters may expel intracellular toxic products accumulated during cell growth.

In spite of the fact that ABC transporter Pdr12p of *S. cerevisiae* shares a > 37% identity with *PDR5* and *SNQ2*, it neither confers resistance to NQO, a substrate specific for *SNQ2* nor to cycloheximide, a substrate specific to *PDR5* (ref. 59). Pdr12p confers resistance to weak organic acids like sorbate, benzoate, acetate and propionate that are used as food preservatives. Weak acids induce the expression of Pdr12p and as a result, it becomes one of the most abundant membrane proteins in acid-adapted cells. Interestingly, wild-type *S. cerevisiae* cells, if cultured at low pH (4.5) in the absence of weak acids, do not display active efflux of fluorescein (a flourescent substrate used to monitor Pdr12p-mediated efflux)[60]. Thus, it appears that Pdr12p is not an active transporter in the absence of weak acid stress. Further, experiments have shown that Pdr12p activity may be negatively regulated by the Cmk1p Ca^{2+}/calmodulin-dependent protein kinase[61]. Interestingly, the activity of Pdr12p homologues in *Klyveromyces lactis* is regulated by a Sit4p phosphatase, suggesting that activities of certain ABC pumps are subject to post-translation modification cycles[62]. That ABC transporters may be subjected to post-translation modification was further demonstrated in a recent study, where it is shown that the drug transporters of *C. glabrata*, Cdr1p and Pdh1p, are phosphorylated in a glucose-dependent manner[63]. Taken

together, it appears that the physiological function of *PDR12* is to protect cells against potential toxicity of weak organic acids secreted by competitor organisms (that share the same niche with yeasts), wherein it extrudes acid anions and releases them into aqueous phase of periplasm. Such energy-dependent efflux may be able to lower the intracellular level of weak acids. The involvement of an active extrusion pump for weak acids also indicates why some species of yeast are capable of causing food spoilage in spite of the addition of weak organic acids as food preservatives[64].

Sterol transport and homeostasis

Earlier studies showed that human MDR1/P-gp when overexpressed could export dexamethasone, corticosterone and aldosterone[65]. The relative abundance of human MDR1/P-gp in the mouse pregnant uterus and adrenal glands favours their role in steroid hormone secretion[66]. Taken together, this led to the question if the yeast ABC drug transporters could also extrude steroids. Subsequent studies indeed confirmed that yeast drug transporters could expel human steroid hormones. While investigating for interactive non-receptor proteins that could potentiate the human glucocorticoid receptor (GR) and ligand interaction in yeast, a ligand effect modulator (LEM1) protein was identified. Further, analysis of LEM1 showed that it was an interactive non-receptor protein identical to previously characterized PDR5 (refs 43 and 67). Two studies later demonstrated that steroids indeed were the substrates for Pdr5p and Snq2p (refs 68 and 69).

Cdr1p can also specifically transport human steroid hormones, namely β-estradiol and corticosterone[70]. The *CDR1*-mediated steroid transport activity was demonstrated by using a *pdr5* null mutant strain of *S. cerevisiae*. This *S. cerevisiae* transformant harbouring the *CDR1* gene, accumulated less (two-to-three-fold) β-estradiol and corticosterone than the non-transformed counterpart[70]. Furthermore, another steroid hormone, progesterone that also induces the overexpression of *CDR1*, was not transported and also did not affect the accumulation of either β-estradiol or corticosterone[54]. Interestingly, progesterone is also not a substrate of human MDR1/P-gp transporter, although it can bind to it[65]. This shows the functional conservation in terms of substrate specificity between the drug extrusion pumps.

In a recent study it was shown that *PDR5*-mediated fluconazole resistance could be altered due to mutations that affect sterol homeostasis. *Pdr1-100*, a gain of function allele of the transcription regulator, *PDR1* is known to upregulate *PDR5*, thus leading to high level of drug resistance[71]. In this study *erg3* single mutation, which is defective in converting toxic episterol to ergosta-5,7,24 (28)-tetraenol, was found to be resistant to fluconazole. Interestingly, the resistance to fluconazole decreased in a *S. cerevisiae erg3 pdr1-100* double mutant strain, which was attributed to a competition between the endogenous sterols and azoles (both being substrates of Pdr5p)[71]. Additional genetic evidence supporting this concept came from another study by Kaur and Bachhawat[72], who observed that Pdr5p functions less efficiently in *erg* mutant strains of *S. cerevisiae* defective in ergosterol metabolism. Additionally, it was observed that the loss of function of *CPR1* gene, which codes for the NADPH-dependent cytochrome P-450 oxidoreductase and of *YMR034c*, which codes for a putative sterol transporter results in azole hypersensitivity[71]. That there is a genetic interaction between sterol homeostasis and *Pdr1-100* was further evident from the mutants, where resistance to fluconazole was decreased in ymr034 and *cpr1* background. The importance of human MDR1/P-gp in cholesterol trafficking has already been demonstrated, where MDR1/P-gp transports cholesterol and its precursors from the plasma membrane to the ER[73,74]. A role of the ABC drug transporters of yeast in sterol homeostasis still remains an open area. Recently, *PDR11* has been shown to be responsible for ergosterol entry into anaerobic *S. cerevisiae* cells, which is suggestive of their role in sterol homeostasis[75].

Any fluctuation in sterol composition which in turn affects membrane fluidity, also alters functioning of ABC drug transporters of yeasts[72,76,77]. In order to ascertain the functioning of the drug extrusion pumps *CDR1* (ABC family) and *CaMDR1* (Major Facilitator Superfamily) of *Candida albicans* in different lipid environments, they were independently expressed in *S. cerevisiae erg* mutants background. While the fold change in drug resistance mediated by *CaMDR1* remained same or increased in *erg* mutants, susceptibility to fluconazole and cycloheximide mediated by *CDR1* was increased (decrease in fold resistance). These recent results demonstrate that between the two drug extrusion pumps, Cdr1p appeared to be more adversely affected by the fluctuations in membrane lipid environment (particularly to ergosterol). Taken together, it appears that the functioning of yeast ABC pump is closely linked to the status of membrane lipids, wherein the overall drug susceptibility phenotype of a cell appears to be an interplay between drug diffusion, extrusion pumps and membrane lipid environment[77].

Phospholipid translocation

Asymmetric distribution of phospholipids is well known across the plasma membrane of numerous cell types[78,79]. In the majority of cell types, phosphatidylethanolamine (PtdEtn) and phosphatidylserine (PtdSer) are located in the inner monolayer, whereas phosphatidylcholine (PtdCho), sphingomyelin and glycolipids are located in the outer monolayer of the plasma membrane[78,79]. The asymmetrical distribution of membrane lipids is specific and its loss has been linked to various pathophysiological consequences[78,80–82].

Interestingly, human MDR1/P-gp has been shown to be involved in maintaining the membrane lipid asymmetry, where it acts as a general phospholipid translocator for different phospholipids and sphingomyelins, while MDR2 (or MDR3) appears to be rather specific for translocating PtdCho between the two lipid monolayers of the plasma membrane[83]. Recently, some ABC proteins of *S. cerevisiae* and *C. albicans* have also been shown to function as phospholipid translocators. Cdr1p, the drug extrusion pump of *C. albicans*, elicits energy-dependent in-to-out translocation (floppase) of phospholipids[84]. The decrease in the availability of PtdEtn in the exoplasmic leaflet of the plasma membrane (PM) of a homozygous *CDR1* disruptant confirmed its involvement in phospholipid translocation. Of note, a double disruption of *CDR1* and *CDR2* drug transporters encoding genes resulted in even lesser PtdEtn in the outer monolayer compared to the single *CDR1* disruptant, thereby implicating that *CDR2* could also contribute to phospholipid translocation. Interestingly, a *S. cerevisiae* transformant expressing *CaMDR1* (a drug transporter of the major facilitator superfamily) of *C. albicans* does not affect PtdEtn distribution pattern between the two leaflets, thus suggesting that phospholipid translocation activity is specific to and a feature of the ABC drug transporters[84].

Additional experiments using fluorescent-tagged phospholipid analogues revealed that Cdr1p and Cdr2p elicit outwardly-directed phospholipid transbilayer exchange (floppases), while Cdr3p, which is not a drug extrusion pump and does not confer MDR, is involved in inwardly (out-to-in) directed translocation of phospholipids (flippase)[85]. In addition to the difference in the directionality of phospholipid translocation, the floppase activities of Cdr1p and Cdr2p and the flippase activity of Cdr3p are further distinguishable. For example, flippase and floppase activities of these ABC transporters have distinct sensitivities to mercurials like *N*-ethylmalemide (NEM) which specifically block –SH groups and Cytochalasin E which induces alterations in cytoskeleton, by particularly disrupting the actin organization in a variety of eukaryotic cells (Table 3). Interestingly, drugs like fluconazole, cycloheximide and miconazole can affect transbilayer movement of phospholipids mediated by Cdr1p and Cdr2p, but have no effect on Cdr3p-mediated transbilayer exchange[85]. These studies suggest that Cdr1p and Cdr2p presumably have common binding sites for drugs and phospholipids, while the flippase activity of Cdr3p is independent of drug binding. The difference in the directionality of phospholipid transfer between Cdrps could be linked to their ability to efflux cytotoxic drugs. It is thus presumed that since Cdr3p pump is inwardly directed (flippase) it is unable to participate in drug efflux. However, comprehension of the molecular basis of functional differences between these transporters will have to wait for further experimentation. The *S. cerevisiae* ABC drug extrusion pumps are also involved in phospholipid translocation across the plasma membrane[86,87]. Decottignies *et al*.[86] have demonstrated that the absence of ABC transporter *YOR1* or *PDR5* resulted in increased accumulation of a fluorescent PtdEtn, thus suggesting that Pdr5p and Yor1p are PtdEtn translocators. The fluorescent intensity of the double deleted strain Δ*yor1*Δ*pdr5* was even more pronounced, indicating that the transporters may act independently. None of the other tested ABC transporters *SNQ2*, *PDR10*, *PDR11*, *YCF1*, *PDR15* exhibited phospholipid translocase activity[86].

Peptide transport/secretion

Localization of mouse mdr2, a homologue of human MDR3, in the canalicular membrane, suggests its function in biliary secretion. Disruption of *mdr2* in murine cells leads to the formation of abnormal bile, with a specific deficiency in PtdCho (refs 88 and 89). PtdCho-deficient bile causes extensive liver damage, thereby implying the importance of mdr2 in phospholipid secretion. The secretory

Table 3. Yeast ABC transporters as phospholipid translocators

Yeast	Drug transporter	Translocator			Reference
		Substrate	Direction	Inhibitor	
S. cerevisiae	*PDR5*	Phosphatidylethanolamine	Not determined	Not determined	86
	YOR1	Phosphatidylethanolamine	Not determined	Not determined	86
C. albicans	*CDR1*	Phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine	In-to-out (floppase)	Cytochalasin E and NEM sensitive	84, 85
	CDR2	Phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine	In-to-out (floppase)	Cytochalasin E and NEM sensitive	84, 85
	CDR3	Phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine	Out-to-in (flippase)	Cytochalasin E and NEM insensitive	85
	CDR4	Phosphatidylethanolamine	In-to-out (floppase)?	Not determined	56

role of ABC transporters is not restricted to mammals, as these proteins also play an important role in the plant–fungal pathogen interaction[90]. These transporters in the plant pathogenic fungi are required for ensuring the infection process, probably by secreting compounds which protect them from plant defence mechanisms[90]. Nonetheless, such secretory roles for the yeast ABC drug transporters, if any, have not been identified, except for the demonstration of a well-defined secretory function for *STE6* of *S. cerevisiae* (secretion of mating pheromone). Ste6p secretes or transports the 'a' pheromone peptide in *S. cerevisiae*, an essential component of the mating pathway of this budding yeast. Although Ste6p displays high homology to the human MDR1/P-gp and other drug transporters of yeasts, it is unable to confer drug resistance[31]. Interestingly, although the human *MDR1/P-gp* gene can functionally complement the yeast *ste6* mutant, it is also capable of conferring resistance to the immunosuppressive agent FK520 (refs 91 and 92) (Table 1). The Ste6p-mediated export of a-factor is dependent on ATP hydrolysis as the energy source. This has been confirmed by mutational analyses, where a mutation in the NBD led to impairment in the transport of a-factor[42]. In addition, it has been demonstrated that both NBDs are required for the transport of pheromone, since the two duplicate halves of this transporter are incapable of functioning independent of each other. They can function only when co-expressed in a cell where they tightly interact with each other to form a functional pore for facilitating the transport of the a-factor[42].

A homologue of *STE6* designated as *HST6* (Homologue *STE6*) has been characterized in *C. albicans*, which can complement the mating defect of *ste6* mutant strain of *S. cerevisiae*, implying that a-factor can be recognized as its substrate[93]. *HST6* is expressed constitutively and its expression levels do not change between different morphological forms, thereby suggesting an important biological role for *HST6* in *C. albicans*. Although the relevance of *HST6* in *C. albicans* remains to be elucidated, its role in a cryptic sexual cycle in *C. albicans* can be anticipated. It would not be out of context to mention here that the genome-sequencing project of *C. albicans* revealed the presence of a *MTL* (*MAT*-like) locus[94]. The possibility of 'forced mating' in *C. albicans* has recently been demonstrated simultaneously by two groups[95,96]. Taken together, the existence of *HST6* and the possibility of existence of a sexual cycle in *C. albicans* may suggest an important secretory role of this ABC transporter.

Ion transport

The overexpression of human MDR1/P-gp protein, which presumably is directly responsible for the extrusion of drugs, is the most commonly accepted mechanism of drug resistance. However, the pump model contains a number of unsettled aspects: for example, the wider specificity of MDR protein violates the enzyme specificity, coupling principle and appears to be inconsistent with the kinetics of passive diffusion and energetics of partitioning for many drugs[97]. An altered partitioning model, where MDR1/P-gp protein is envisaged to act as complex ion transporter, is proposed for settling some of the above-mentioned inconsistencies[97]. The altered partitioning model proposes that the overexpression of MDR proteins alters intracellular concentration of drugs rather indirectly by affecting intracellular pH and membrane potential, and does not directly translocate drugs[2,98–100]. In accordance with the proposed role, human Mdr1p/P-gp has been shown to inhibit Cl^-/HCO_3^- exchange, regulate Cl^- conductance, and if overexpressed in *S. cerevisiae*, act as an H^+/K^+ pump[101,102]. It has also been suggested that MDR1/P-gp is associated with volume-regulated chloride channels[103]. Recently, it has been proposed that the yeast drug extrusion pumps could also participate in H^+ transport across the plasma membrane. The antifungal FMDP-conjugate peptides [N^3-(4-methoxyfumaroyl)-l-2,3-diaminopropanoicacid], are transported into *C. albicans* cells through peptide permeases[104]. The accumulated conjugate is cleaved intracellularly by peptidases and as a consequence, the released FMDP inhibits the activity of the glucosamine-6-phosphate synthase. The enzyme glucosamine-6-phosphate synthase is an important enzyme for cell-wall synthesis and hence its inhibition is crucial for the survival of *C. albicans* cells. It was observed that the carrier-mediated entry of FMDP-conjugates into yeast cells was pH-dependent, since its antifungal activity was more pronounced at low external pH[104]. Interestingly, it was observed that *S. cerevisiae* cells expressing *CDR1* were hypersensitive to this peptide conjugate. Furthermore, *CDR1* transformants were found to elicit a threefold faster efflux of protons compared to the parent cell type. Subsequently, it was shown that lowering of external pH due to ejection of protons by Cdr1p stimulated the uptake of FMDP-conjugate and thus potentiates its antifungal activity[104]. Therefore, it can be inferred that Cdr1p can also act as a proton pump, particularly affecting the accumulation of those antifungals whose entry is pH-dependent and carrier-mediated. Recently, a K^+-dependent sensitivity of fluconazole has been demonstrated in *S. cerevisiae* strain S228c. It was observed that the addition of 150 mM KCl rendered yeast cells more sensitive to fluconazole and this effect was due to of K^+-ion rather than of anion or osmolarity of the medium. The presence of KCl did not affect the intra and extracellular pH. These results suggest that the ionic movements linked to MDR proteins could be an important determinant in eliciting drug susceptibility[105].

Concluding remarks

The finding that the multidrug transporters have not exclusively been 'evolved' for extrusion of drugs is becom-

ing increasingly apparent. It has already been established that human MDR1/P-gp, a drug transporter of malignant cells might have other cellular functions. This functional aspect of human MDR1/P-gp seems to be conserved, as is evident that yeast drug transporters also mediate several physiological functions. In this review we have attempted to analyse a few of the known physiological functions that are associated with yeast ABC drug extrusion pumps. Clearly, the interest in physiological relevance of multidrug transporters is now expanding and it would be worthwhile to examine in greater detail as to how ABC drug transporters achieve this multifunctional feat. The molecular dissection of these proteins would be the first step wherein identification of the responsible residues and domains could help elucidate the molecular mechanism of their diverse functions. Determination of the crystal structure of the yeast ABC drug extrusion pump protein would be of considerable medical importance, since it would not only explain the basis of proposed additional functions but will also lead to a rational design of inhibitors for blocking drug extrusion from resistant cells.

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