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Fungal PDR transporters: Phylogeny, topology, motifs and function

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ABSTRACT

The overexpression of pleiotropic drug resistance (PDR) efflux pumps of the ATP-binding cassette (ABC) transporter superfamily frequently correlates with multidrug resistance. Phylogenetic analysis of 349 full-size (~160 kDa) PDR proteins (Pdrps) from 55 fungal species, including major fungal pathogens, identified nine separate protein clusters (A–G, H1a/H1b and H2). Fungal, plant and human ABCG-family Pdrps possess a nucleotide-binding domain [NBD] and a transmembrane domain [TMD] in a family-defining 'reverse' ABC transporter topology [NBD–TMD] that is duplicated $[NBD–TMD]_2$ in full-size fungal and plant Pdrps. Although full-size Pdrps have similar halves indicating early gene duplication/fusion, they show asymmetry of their NBDs and extracellular loops (ELs). Members of cluster F are most symmetric and may be closely related to the evolutionary ancestor of Pdrps. Unique structural elements are predicted, new PDR-specific motifs identified, and the significance of these and other structural features discussed.

1. Introduction

Fungi cause significant morbidity and mortality in humans, particularly in the immunocompromised (Pfaller and Diekema, 2007). They cause superficial infections of skin or mucosal surfaces and they can disseminate hematogenously. The azole antifungals are generally well tolerated and the second generation triazoles such as voriconazole (VCZ) are effective against a wider range of fungi than their predecessors (Odds et al., 2003; Shao et al., 2007; Pasqualotto and Denning, 2008). Some fungi, such as *Candida glabrata* and *Candida krusei*, often show reduced susceptibility to azoles and resistance can be induced in *Candida albicans*, *C. glabrata* and *Cryptococcus neoformans* (Kanafani and Perfect, 2008). An important mechanism causing azole resistance is energy dependent drug efflux catalyzed by membrane-located efflux pumps (Sanglard and Bille, 2002) that usually belong to the PDR sub-family of ABC transporters. While the expression of Pdrps strongly correlates with azole resistance, the mechanisms of substrate binding and translocation, and the identity of the 'natural' substrates of these pumps are not known. Recent advances in whole genome sequencing have provided open reading frame sequences for entire repertoires of Pdrps from increasing numbers of fungi of industrial, agricultural and medical importance. This information permits the use of sequence comparison to identify residues and motifs conserved across species, and establishes evolutionary relationships among Pdrps. This review uses these approaches to identify features of Pdrps that may be important for function, including antifungal drug resistance.

2. Clinical context

Candida species cause superficial infections of the mucous membranes in a significant proportion of the population (Odds, 1979; Cannon et al., 1995; Sobel, 2007). Denture wearers often suffer from *Candida*-associated denture stomatitis (Wilson, 1998), and many women have recurrent vulvovaginal candidiasis (Sobel, 2007) while their premature babies often contract thrush during birth. Severe oropharyngeal candidiasis (OPC) is common in AIDS patients who do not have access to highly active anti-retroviral therapy (HAART) (de Repentigny et al., 2004), while oral candidiasis often affects cancer patients undergoing chemotherapy and/or radiotherapy (Davies et al., 2006). When fungi penetrate the epithelial surfaces of immunocompromised hosts they can cause invasive fungal infections (IFIs) that are associated with high morbidity and mortality. The fungal genera most often associated with IFIs are *Candida, Aspergillus* and *Cryptococcus* (Pfaller et al., 2006).

Candida species are the most common cause of opportunistic mycoses worldwide, with 72–228 infections per million population (Pfaller et al., 2006). They are also the fourth leading cause of nosocomial bloodstream infections in the USA (Wisplinghoff et al., 2004). In Europe and the USA the majority of invasive infections are caused by *C. albicans*, followed by *C. glabrata*, *Candida*

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tropicalis and then *Candida parapsilosis* (Pfaller and Diekema, 2007). *C. tropicalis* is often associated with fungaemia and invasive candidiasis in patients with cancer, especially leukemics and hematopoietic stem cell (bone marrow) transplant recipients (Wingard, 1995; Kontoyiannis et al., 2001). *C. parapsilosis* is frequently found on the hands of health care workers (Strausbaugh et al., 1994), forms biofilms on catheters (Kuhn et al., 2002), and has been implicated in nosocomial outbreaks of catheter-associated fungemia (Sarvikivi et al., 2005). *C. krusei* is the fifth most common cause of candidiasis (Pfaller et al., 2008) and accounts for 2–4% of all *Candida* blood stream infections (Pfaller and Diekema, 2007).

Aspergillus species are commonly encountered in the environment, often in rotting vegetation. Clinical aspergillosis includes both an allergic reaction to the fungus and an invasive lung infection that can disseminate to other organs in the severely immunocompromised (Pfaller et al., 2006). Invasive aspergillosis (IA) accounts for 60–80% of IFIs and has an associated mortality rate of more than 50% (Kontoyiannis et al., 2005; Lai et al., 2008). The most common cause of IA is *Aspergillus fumigatus* (85%) followed by *Aspergillus flavus* (5–10%) and *Aspergillus terreus* (2–10%) (Kontoyiannis and Bodey, 2002; Singh and Paterson, 2005; Pfaller et al., 2006). *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus ustus* are isolated rarely. IA affects a narrower range of patients than invasive candidiasis, with severe neutropenia a major risk factor (Pfaller et al., 2006; Shao et al., 2007).

Cryptococcosis is an invasive fungal infection caused predominantly by the yeasts *C. neoformans* and *Cryptococcus gattii*. Pulmonary cryptococcosis results from inhalation of the yeast. Hematogenous dissemination leads to cryptococcal meningitis (Lin and Heitman, 2006) in severely immunocompromised patients. In AIDS patients without HAART, cryptococcosis has an attributable mortality of up to 44% (Corbett et al., 2002). Cryptococcosis is a significant opportunistic infection in solid organ transplant recipients, with a mortality of 42% (Silveira and Husain, 2007). The standard treatment for cryptococcal meningitis is a combination therapy; two weeks with amphotericin B (AMB) and 5-fluorocytosine (5-FC) followed by fluconazole (FLC) for a minimum of ten weeks. Lifetime treatment with FLC is recommended to prevent relapse should the patient become immunocompromised (Saag et al., 2000).

There are few classes of antifungal agents with which to treat patients with IFIs. The target of the widely used azole antifungals is the cytochrome P_{450} enzyme 14α-lanosterol demethylase, which is an essential enzyme of ergosterol biosynthesis (Sanglard and Bille, 2002; Odds et al., 2003). The enzyme, encoded by *ERG11* (also referred to as *CYP51*), is anchored in the endoplasmic reticulum by a single transmembrane segment. Inhibition of Erg11p depletes membranes of ergosterol and results in the accumulation of toxic sterols that inhibit growth (Sanglard and Bille, 2002; Akins, 2005). The fact that azole antifungals are often fungistatic rather than fungicidal facilitates the development of drug resistance.

3. Azole drug resistance

The mechanisms responsible for azole antifungal drug resistance in fungi have been reviewed extensively elsewhere (Kontoyiannis and Lewis, 2002; Sanglard and Bille, 2002; Akins, 2005) and will be noted briefly. The drug target Erg11p can be overexpressed or develop point mutations that reduce azole binding (White et al., 1998; Sanglard and Bille, 2002). Some filamentous fungi possess two *CYP51* genes (*CYP51A* or *CYP51B*) that are found in two distinct gene clusters (Mellado et al., 2001; Ferreira et al., 2005). *A. fumigatus* is innately resistant to FLC and ketoconazole (KTC). Gene knock-out experiments show that AfuCyp51Ap, but not AfuCyp51Bp, is responsible for the innate resistance (Mellado et al., 2005). Although the prevalence of itraconazole (ITC)- or VCZ-resistant *A. fumigatus* isolates is low, resistance is usually due to point mutations in AfuCyp51Ap (Nascimento et al., 2003; da Silva Ferreira et al., 2004; Chen et al., 2005).

A common cause of high-level azole resistance in fungi is overexpression of plasma membrane transport proteins that pump the azoles out of cells, thus reducing intracellular azole concentrations below the levels at which Erg11p is inhibited (White et al., 1998; Perea et al., 2001). The expression of *C. albicans* genes encoding drug efflux pumps often correlates with the increased azole resistance of clinical isolates (White, 1997; Maebashi et al., 2001; Rogers and Barker, 2003). This is also the case for *C. glabrata* (Sanglard et al., 1999; Bennett et al., 2004), and *C. neoformans* (Posteraro et al., 2003; Sanguinetti et al., 2006). In *C. krusei* an insensitivity of Erg11p to azoles combined with the expression of PDR efflux pumps such as CkAbc1p is thought to be responsible for its innate azole resistance phenotype (Katiyar and Edlind, 2001; Lamping et al., 2009).

4. Efflux-mediated antifungal resistance

There are two main classes of efflux pumps responsible for fungal drug resistance, each with a different pumping mechanism and source of energy. ATP-binding cassette (ABC) proteins are primary transporters that use energy from the hydrolysis of ATP. Major facilitator superfamily (MFS) pumps are secondary transporters that utilize the electrochemical gradient across the plasma membrane to translocate substrates. Both classes of pumps are integral membrane proteins with distinctive functional domains: ABC pumps contain nucleotide-binding domains (NBDs) while both ABC and MFS pumps contain transmembrane domains (TMDs) that confer substrate specificity.

In *C. albicans*, the expression of two ABC pumps Cdr1p and Cdr2p and the MFS transporter Mdr1p (Ben^rp) has been implicated in the resistance of clinical isolates to azoles (Sanglard et al., 1995; White, 1997; Maebashi et al., 2001; Perea et al., 2001). Expression of the ABC pumps is more often associated with resistance than expression of Mdr1p, and the expression of Cdr1p appears to be the predominant contributor to clinically significant azole resistance (Holmes et al., 2008; Tsao et al., 2009). In *C. glabrata* azole resistance can be caused by expression of the ABC proteins Cdr1p and Cdr2p (also called Pdh1p) (Miyazaki et al., 1998; Sanglard et al., 2001; Izumikawa et al., 2003). In *C. neoformans* the ABC proteins CneAfr1p and CneMdr1p are the only efflux pumps that have been linked to antifungal drug resistance (Thornewell et al., 1997; Sanguinetti et al., 2006). There is no convincing evidence for a contribution by MFS transporters to azole resistance in *C. glabrata* or *C. neoformans* although the *A. fumigatus* Mdr3p MFS pump may be involved in resistance (Nascimento et al., 2003).

5. Fungal ABC transporters

ABC transporters are found in all living organisms. Most ABC proteins are membrane proteins and many are thought to transport a variety of substances across membranes. The functional unit of ABC transporters consists of a cytoplasmic NBD and a membrane associated TMD (Taglicht and Michaelis, 1998; Dassa and Bouige, 2001; Higgins, 2001; Bouige et al., 2002; Dean, 2002; Rees et al., 2009). There is one such unit in half-size transporters and two units in full-size transporters (Fig. 1). The NBDs (NBD1 and NBD2) in full-size transporters are involved in cooperative ATP binding and hydrolysis. The TMDs (TMD1 and TMD2) usually operate in pairs and each usually includes six putative α-helical transmembrane segments (TMS1–12). The number and arrangement of the NBDs and TMDs within the pump polypeptide varies according

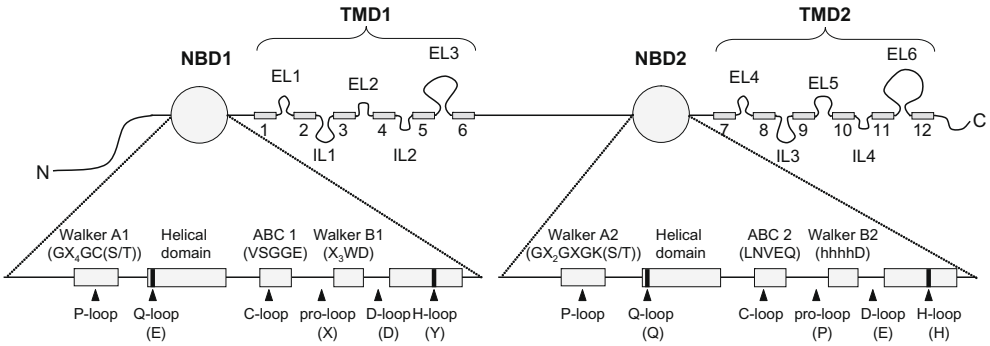

Fig. 1. Predicted topology of a typical full-size fungal PDR transporter showing the major topological features and highlighting the asymmetry of consensus motifs in the NBDs (see text for details; N = N-terminus; C = C-terminus; NBD = nucleotide-binding domain; TMD = transmembrane domain; EL = extracellular loop; IL = intracellular loop). The transmembrane segments are numbered 1–12. Conserved amino acids in brackets are given in the one letter code with X representing any amino acid, and h any aliphatic amino acid.

to the type of ABC protein (Table 1). The four domain fungal ABC proteins typically have a molecular mass of approximately ~160 kDa. The high level of homology usually found between the amino-terminal and carboxy-terminal halves of the protein suggests gene duplication and fusion, but subtle differences between each half of the protein may have functional implications. Many organisms, including fungi, have half-size transporters consisting of one NBD and one TMD (Gbelska et al., 2006). Biochemical and crystallographic evidence indicates that the half-size transporters are likely to function as either hetero- or homodimers (Higgins, 2007).

Sequencing of entire fungal genomes has revealed the repertoire of ABC proteins in a range of species (Dassa and Bouige, 2001; Bouige et al., 2002; Verrier et al., 2008). The first fungal genome to be sequenced was that of *Saccharomyces cerevisiae* (Goffeau et al., 1996), and it was found to encode 29–30 ABC proteins (Decottignies and Goffeau, 1997; Taglicht and Michaelis, 1998). Each ABC protein contains at least one NBD but a subset lacks a predicted TMD. This subset comprises proteins involved in DNA repair or other ATP-requiring functions. *C. albicans* is predicted to have a similar number (27) of ABC proteins as *S. cerevisiae* (Gaur et al., 2005) and *C. glabrata* approximately two-thirds that number (18) (Dujon et al., 2004). Much larger numbers of ABC proteins are found in *A. fumigatus* (49) and *C. neoformans* (54) (Loftus et al., 2005; Nierman et al., 2005).

ABC proteins can be divided into sub-classes according to sequence homology and domain topology (Dassa and Bouige, 2001; Bouige et al., 2002; Dean, 2002; Verrier et al., 2008). Fungal ABC proteins have been most extensively studied in the model yeast *S. cerevisiae* (Decottignies and Goffeau, 1997; Taglicht and Michaelis, 1998; Bauer et al., 1999; Gbelska et al., 2006). The ABC proteins of *S. cerevisiae* have been classified into five classes: PDR, MDR (multidrug resistance), MRP (multidrug resistance-associated protein), RLI (RNase L inhibitor)/ALDP (adrenoleukodystrophy protein), and YEF3 (yeast elongation factor EF-3) (Taglicht and Michaelis, 1998) (Table 1). Of these, the PDR, MDR, and MRP transporters are most often associated with antifungal resistance. The major pumps involved in the azole resistance of *C. albicans, C. glabrata, C. krusei* and *C. neoformans* are Pdrps.

Table 1
Classes of fungal ABC transporters.

Transporter class	Topology	Example	Putative function
Half-size PDR	$[NBD–TMD]_1$	*S. cerevisiae* Adp1p	Membrane transport
PDR	$[NBD–TMD]_2$	*S. cerevisiae* Pdr5p	Multidrug resistance
		C. albicans Cdr1p	Azole drug resistance
		C. neoformans Afr1p	Azole drug resistance
MDR	$[TMD–NBD]_1$	*A. fumigatus* Mdr1p	Cilofungin resistance
		C. neoformans Mdr1p	Azole drug resistance
	$[TMD–NBD]_2$	*S. cerevisiae* Ste6p	α-factor export
MRP	$[TMD–NBD]_2$	*S. cerevisiae* Yor1p	Multidrug resistance
Others			
RLI/ALDP	$[TMD–NBD]_1$	*S. cerevisiae* Pxa1p	Fatty acid transport
YEF3	$[NBD]_2$	*S. cerevisiae* Yef3p	Translation
	[NBD]	*S. cerevisiae* Caf16p	Transcription

6. Domain arrangements of ABC transporters

The domain arrangement of most eukaryotic ABC transporters is $[TMD–NBD]_2$ (Bauer et al., 1999; Bouige et al., 2002; Dean, 2002; Gaur et al., 2005; Tusnady et al., 2006; Verrier et al., 2008). This is achieved either by the formation of homo- or heterodimers of two half-size transporters (e.g. human TAP1/2 also known as ABCB2/3 according to the nomenclature of the Human Genome Organization (HUGO)) or, as is the case for most eukaryotic ABC transporters, by a single polypeptide encoding a full-size transporter (e.g. human ABCB1 and ABCC1). Fungal PDR transporters have a reverse topology $[NBD–TMD]_2$ (Fig. 1 and Table 1). The plant PDR transporters are the only other class of full-size ABC transporters with the same reverse $[NBD–TMD]_2$ arrangement (van den Brule and Smart, 2002; Jasinski et al., 2003; Crouzet et al., 2006; Verrier et al., 2008). Although full-size PDR transporters have yet to be found in bacteria or any animal species, numerous half-size ABC transporters with a [NBD–TMD] topology have been identified in plants, fungi, and animals. For example, the white, brown and scarlet (WBC) transporters of *Drosophila melanogaster* are believed to transport the red and brown eye color pigment precursors guanine and tryptophan (Bouige et al., 2002). WBC homologs are found in many species and are involved in both the trafficking of lipids and in multidrug resistance (e.g. human ABCG2; (Dean, 2002)). Plants such as *Arabidopsis thaliana*, rice or tobacco possess numerous WBC homologs, but their functions are poorly understood (Verrier et al., 2008). Homologs of these half-size PDR or WBC transporters have also been detected in some fungi (e.g. *ADP1* in *S. cerevisiae* (Purnelle et al., 1991)). Verrier et al. (2008), proposed a unified nomenclature for plant ABC proteins that groups both half-size WBC and full-size PDR transporters into the ABCG family, consistent with the HUGO nomenclature for human and mouse ABC transporters (Dean, 2002).

The NBDs of ABC proteins usually contain seven conserved motifs: (1) The Walker A motif or P-loop $GX_4GK[S/T]$ (X is any amino acid) connects a conserved β-sheet with an adjacent α-helix; (2) The Walker B motif hhhhD (h is any aliphatic amino acid) that forms a β-sheet; (3) The ABC signature motif or C-loop LSGGQ that connects two α-helices just upstream of the Walker B motif; (4) The Q-loop located between the Walker A and ABC signature motif that is N-terminal of a helical structure that makes close contact

with the intracellular loop (IL) regions of MDR transporters (Dawson and Locher, 2006; Aller et al., 2009); (5) The pro-loop that connects the C-terminal α-helix of the ABC signature motif with the Walker B β-sheet; (6) The H-loop (or switch region) located ~30 amino acids C-terminal of the Walker B motif that connects a conserved β-sheet with an adjacent α-helix (Decottignies and Goffeau, 1997; Linton and Higgins, 1998); and (7) The D-loop that connects the Walker B β-sheet with a structurally conserved α-helix. In fully functional PDR transporters the H-, P-, Q-, and D-loops, together with the ABC signature motif from the opposite NBD, form one of two 'composite' nucleotide-binding pockets (CNBP1 and CNBP2) (Taglicht and Michaelis, 1998; Jones and George, 2004). A useful illustration of the essential motifs within the NBD structure is given by Jones and George (2004).

7. Domain interactions and substrate transport in ABC transporters

It is widely accepted that substrate transport by ABC transporters involves interactions between the membrane bound and cytosolic domains. These interactions are normally tightly coupled with substrate binding, ATP binding and ATP hydrolysis. Bacterial ABC transporters usually fully couple substrate transport with ATP hydrolysis despite being multiunit entities made up of separate NBDs and TMDs. The 'ATP switch model' proposed by Higgins and Linton (Higgins and Linton, 2004) reflects current thinking on the efflux mechanism of ABC transporters. The first step is the binding of substrate to a high-affinity binding site in the TMD that is usually considered to be open to the cytosol or the inner leaflet of the membrane. Binding of efflux substrate triggers a conformational change in the NBDs via formation of new contacts between the IL regions and the 'helical domain' of the NBDs. These allow NBD dimerization in the presence of ATP. The resultant conformational change opens the substrate-binding pocket to the extracellular space and the substrate is released. After substrate release, hydrolysis of the bound ATP returns the transporter to its internally open conformation and the transport cycle can be repeated. Thus, binding of ATP, and not its hydrolysis, is considered the power stroke of substrate transport. Despite significant biochemical efforts, the molecular details of this model remain to be determined (Jones and George, 2004; Jones et al., 2009).

Both human ABCB1 and ABCC1, the prototype MDR- and MRP-type transporters, respectively, are associated with multidrug resistance of cancer cells (Dean, 2002). Their innate basal level of ATPase activity is stimulated several-fold in the presence of substrate. Fungal ABC transporters are not stimulated significantly by the presence of xenobiotic substrates and a fully uncoupled transport mechanism for the archetypal fungal PDR transporter Pdr5p has been suggested (Ernst et al., 2008). In contrast, Sauna and colleagues (Sauna et al., 2008) proposed coupling between TMS2 and NBD1 of Pdr5p via contact between the Q-loop of NBD1 and IL1 (Fig. 1). They reported restoration of substrate transport in a transport-deficient, but 'ATPase active', S558Y mutant of Pdr5p by a N242K mutation located two amino acids N-terminal to the invariant glutamate of the Q-loop in NBD1 (Sauna et al., 2008). As discussed below, S558 is close to the extracellular boundary in TMS2 and part of a stretch of amino acids conserved in fungal Pdrps.

The importance of contact between the Q-loop and IL regions in coupling substrate transport to ATP hydrolysis is illustrated by the human peptide transporters TAP1 and TAP2 (Herget et al., 2007). These two half-size MDR-type transporters are members of the human ABCB family of ABC transporters. TAP1 and TAP2 form a functional heterodimer that is involved in the adaptive immune response. TAP1/2 transports small peptide antigens into the ER lumen for presentation on MHC class I molecules. The binding of small peptides by TAP1/2 involves IL1 and leads to a conformational change and new contacts between IL1 and the Q-loop of TAP1. This triggers dimerization of the two NBDs in the presence of ATP, followed by substrate transport and ATP hydrolysis (Herget et al., 2007). The amino acid sequence of the IL1 peptide sensor and transmission interface of TAP1 (Herget et al., 2007) is conserved in the IL regions of human ABCB1 (Aller et al., 2009), the *Staphylococcus aureus* ABCB1 homolog Sav1866 (Dawson and Locher, 2006), the bacterial lipid A exporter MsbA (Chang, 2003) and even the *E. coli* vitamin B12 importer BtuCD (Locher et al., 2002). Despite obvious differences between the MDR, MRP, and PDR families of ABC transporters, the use of contact points between the 'helical domain' of NBDs and IL regions that couple ATP hydrolysis to substrate transport in both directions appears conserved (Schneider and Hunke, 1998; Holland and Blight, 1999; Dawson and Locher, 2006; Higgins, 2007; Hollenstein et al., 2007; Rees et al., 2009).

Although fungal Pdrps such as *S. cerevisiae* Pdr5p and *C. albicans* Cdr1p are obviously associated with multidrug resistance, their biological functions are probably related to membrane biogenesis and lipid homeostasis (Shahi and Moye-Rowley, 2009). This could mean that the 'uncoupled' ATPase activity claimed for Pdr5p (Ernst et al., 2008) may actually reflect ATPase activity that is coupled to the transport of endogenous pump substrate(s) such as phospholipids, sphingolipids or sterols.

Crystal structures of the *S. aureus* multidrug transporter Sav1866 (Dawson and Locher, 2006) and the mouse homolog of human ABCB1 (Aller et al., 2009) confirm features of existing models for the transport mechanism. Both transporters have a central binding cavity large enough to bind many different substrates, and sometimes more than one substrate simultaneously. An intriguing question is how can multidrug transporters sequester, with sufficient affinity, a diversity of chemically unrelated hydrophobic and charged compounds from the cytoplasm or lipid bilayer? Useful insight comes from the crystal structures of the *S. aureus* multidrug binding transcription regulator QacR with six different structurally diverse drugs bound (reviewed by (Schumacher and Brennan, 2003)). Similar to ABCB1 (Aller et al., 2009) these structures reveal a sizeable drug-binding pocket that includes multiple drug-binding mini-pockets. The drug-binding pocket is exceptionally rich in aromatic residues and contains negatively charged amino acids that can neutralize positively charged substrates such as rhodamine 6G.

8. Phylogenetic characterization of fungal PDR transporters

Phylogenetic and biochemical studies suggest that ABC transporters are evolutionarily ancient proteins (Dassa and Bouige, 2001). Individual families are thought to have emerged before the separation of the three kingdoms of living organisms and functional constraints are probably responsible for the conservation of individual families (Saurin et al., 1999; Dassa and Bouige, 2001; Bouige et al., 2002).

Despite their importance in the response of fungi to their environment, little is known about the structure and transport mechanism of Pdrps. We have recently data-mined the available fungal genome sequences to identify full-size PDR-type open reading frames (ORF) in fungal pathogens of humans. Alignments of the predicted protein sequences and phylogenetic analysis allowed the subdivision of fungal PDR transporters from 14 fungal species into eight distinct clusters (clusters A–H), (Cannon et al., 2009). This review extends that analysis by considering Pdrps from 55 fungal species belonging to the Ascomycota and the Basidiomycota

Table 2
Number of PDR genes present in fungal species[a] and their cluster distribution (A-H2).

Ascomycota

Pezizomycotina

Dothideomycetes	N/A[b]	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Alternaria brassicicola (Ab)											1	1
Leptosphaeria maculans (Lm)									1		1	2
Mycosphaerella graminicola (Mycg)	1		1	2					1		1	6
Phaeosphaeria nodorum (Pn)			1	1					4		1	7
Pyrenophora tritici-repentis (Pt)			2	1					3		2	8
Venturia inaequalis (Vi)				1					1			2
Total	1		4	5					10		6	26

Eurotiomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Aspergillus clavatus (Ac)			4	2			1	1	4		2	14
Aspergillus flavus (Af)			3	2			1	1	2		3	12
Aspergillus fumigatus (Afu)			4	2			1		4		2	13
Aspergillus niger (Anig; CBS 513.88/FGSC A1513)			5	6					2		2	15
Aspergillus oryzae (Ao)			4	3			1	1	3		6	18
Aspergillus terreus (At)			8	2			1		5		4	20
Coccidioides immitis (Ci)			2	1			1		2			6
Emericella nidulans (An; Aspergillus nidulans[c])			5	2					4		3	14
Neosartorya fischeri (Nf)			7	2			1		4		3	17
Penicillium chrysogenum (Pc)			5	2			1	2	3		3	16
Penicillium digitatum (Pd)			3								1	4
Penicillium expansum (Pe)			2	1			1	1	3		2	10
Talaromyces stipitatus (Ts)			2	1			1	2	4		1	11
Trichophyton rubrum (Tr)				1								1
Total			54	27			10	8	40		32	171

Leotiomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Botryotinia fuckeliana (Bf)			1	1					2		2	6
Monilinia fructicola (Mf)											1	1
Sclerotinia sclerotiorum (Ss)			1	1					2		1	5
Total			2	2					4		4	12

Sordariomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Chaetomium globosum (Cglo)				1					1		2	4
Gibberella moniliformis (Gm; Fusarium verticillioides[c])			1									1
Gibberella pulicaris (Gp)			1									1
Magnaporthe grisea (Mg)			4				1		2			7
Neurospora crassa (Nc)									2			2
Podospora anserina (Pa)			1	1					2		1	5
Trichoderma atroviride (Ta; Hypocrea atroviridis[c])											1	1
Total			7	2			1		7		4	21

	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Total (Pezizomycotina)	1		67	36			11	8	61		46	230

Saccharomycotina

Saccharomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Ashbya gossypii (Eg; Eremothecium gossypii[d])		2			2							4
Candida albicans (Ca)		4			1		1					6
Candida dubliniensis (Cd)		3			1							4
Candida glabrata (Cg)		2			2	1	1					6
Candida guilliermondii (Cgu)		1					1					2
Candida lusitaniae (Cl)		3					1					4
Candida tropicalis (Ct)		6			2		1					9
Debaryomyces hansenii (Dh)		4			1							5
Candida krusei (Ck)		1										1
Kluyveromyces lactis (Kl)		2			2		1					5
Kluyveromyces thermotolerans (Kth)		1			1		1					3
Lodderomyces elongisporus (Le)		3			1							4
Pichia stipitis (Ps)		4			1		2					7
Saccharomyces cerevisiae (Sc)		3			3	2	1					9
Saccharomyces kluyveri (Sk)		3			5		1					9
Vanderwaltozyma polyspora (Vp)					3							3
Yarrowia lipolytica (Yl)			4		1		1					6
Zygosaccharomyces rouxii (Zr)		4			4		1					9
Total		46	4		30	3	13					96

Total (Saccharomycotina)		46	4		30	3	13					96

Taphrinomycotina

Schizosaccharomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Schizosaccharomyces japonicus (Sj)										1		1
Schizosaccharomyces pombe (Sp)	1									1		2
Total	1									2		3

Total (Taphrinomycotina)	1									2		3

Total (**Ascomycota**)	2	46	71	36	30	3	24	8	61	2	46	329

Basidiomycota

Agaricomycotina

Tremellomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Cryptococcus neoformans (Cne)			2				1	3	1			7
Total			2				1	3	1			7

Agaricomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Coprinopsis cinerea (Cc)									1	2		3
Laccaria bicolor (Lb)									1	4		5
Total									2	6		8

Total (Agaricomycotina)			2				1	3	3	6		15

Ustilaginomycotina

Ustilaginomycetes	N/A	A	B	C	D	E	F	G	H1a	H1b	H2	Total
Ustilago maydis (Usm)									2	1		3
Malassezia globosa (Malg)										2		2
Total									2	3		5

Total (Ustilaginomycotina)									2	3		5

Total (**Basidiomycota**)			2				1	3	5	9		20

Total PDRs for all species	2	46	73	36	30	3	25	11	66	11	46	349

[a]Abbreviations of species names used for Supplementary Figs. 1–12 are shown in brackets.
[b]N/A: not assigned to cluster.
[c]Anamorph/Teleomorph (MycoBank, http://www.mycobank.org).
[d]Synonym (MycoBank, http://www.mycobank.org).

phyla (the Dikarya) that include pathogens of both humans and plants (Table 2, Figs. 2 and 3).

The following criteria were used to identify Pdrps from these species: (1) Access to the protein sequence from a reputable database (where possible we used the UniProt sequences because of their superior annotation); (2) An e-value $<10^{-35}$ when compared by BlastP with the *S. cerevisiae* Pdr5p query (this high level of similarity is identified only in full-size ABC transporters of the typical $[NBD–TMD]_2$ Pdrp topology (Balzi et al., 1994)); (3) A polypeptide length >1000 amino acid residues to eliminate partial or half-size sequences; and (4) Sequences suspected to originate from redundant submissions to databases were eliminated manually. The protein sequences and other information for the 349 Pdrps identified are compiled in Supplementary Table 1. The number of Pdrps per species varies greatly. For example, only two Pdrps were identified in *Schizosaccharomyces pombe* compared with 20 in *A. terreus* (Table 2). This differential gene multiplication may reflect the environmental niches occupied by each species and the range of xenobiotics they produce or encounter.

A phylogenetic tree of 78 Pdrps from 12 representative species was generated (Fig. 3). These species comprise the two reference-species *S. cerevisiae* and *C. albicans*, plus one species from each of the Ascomycota and Basidiomycota classes (Table 2 and Fig. 2 black dots). Clustering of the resultant 78 full-size Pdrps confirmed

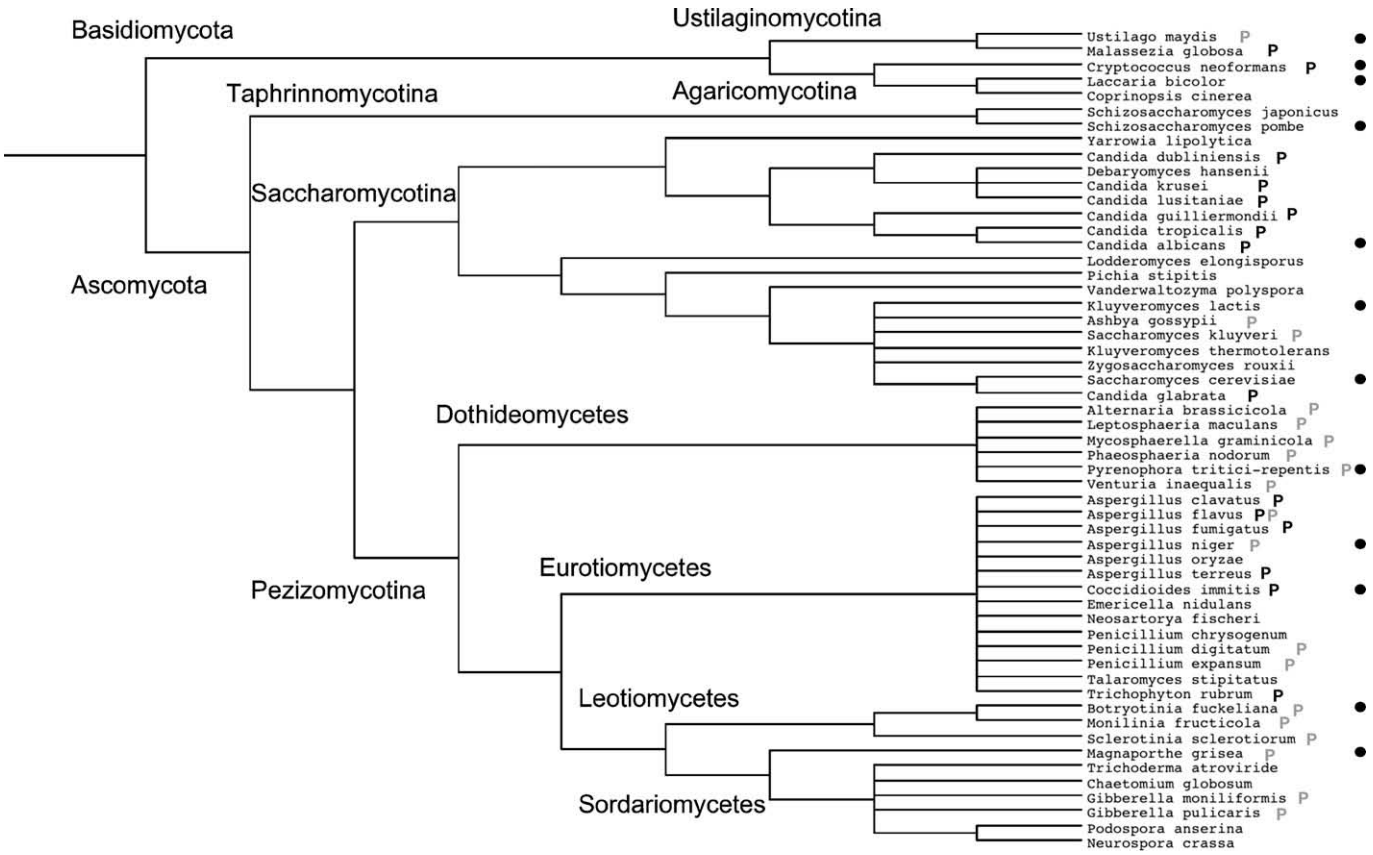

Fig. 2. Phylogenetic tree of the 55 fungal species used for the analysis of fungal PDR transporters based on (James et al., 2006). Black dots indicate the fungal species that were used for the cluster analysis of fungal PDR transporters presented in Fig. 3. P indicates a pathogenic species (animal pathogens are shown in black and plant pathogens in gray). The Ascomycota species used in this analysis belong to three different subphyla: (1) The Pezizomycotina from four classes (Dothiomycetes including *Pyrenophora tritici-repentis*, Eurotiomycetes such as *Aspergillus niger*, Leotiomycetes such as *Botryotinia fuckeliana*, and Sordariomycetes including *Neurospora crassa* and *Magnaporthe grisea*); (2) The Saccharomycotina (including *S. cerevisiae* and *C. albicans*); and (3) The Taphrinomycotina (such as *Schizosaccharomyces pombe*). The Basidiomycotina species analyzed here belong to two subphyla: the Agaricomycotina (including *C. neoformans*, *Coprinopsis cinerea* and *Laccaria bicolor*) and the Ustilaginomycotina (such as *Ustilago maydis*).

the identification of the A–H clusters described previously (Cannon et al., 2009) and split the H cluster into clusters H1 and H2 (Fig. 3). As previously reported, clusters A (Pdr5p/10p/15p-like), D (Snq2p/Pdr12p-like) and E (Aus1p/Pdr11p-like) contain only Saccharomycotina members (Cannon et al., 2009). The relative position of cluster E is variable and it associates with cluster D in some tree configurations. It is striking that the *S. cerevisiae* Pdr5p-like cluster A shares a common ancestor with cluster B that comprises members from the Pezizomycotina and Basidiomycota species only. Similarly, the *S. cerevisiae* Snq2p-like cluster D shares a common ancestor with cluster H1 that contains Pezizomycotina and Basidiomycota members. In some phylogenetic reconstructions, cluster H1 is split into two by the presence of some cluster D Pdrps. As a consequence, we distinguish two sub-clusters H1a and H1b. The H1b Pdrps share a more recent common ancestor with cluster D than cluster H1a Pdrps (Table 2 and Fig. 3). *S. cerevisiae* Pdr5p and Snq2p are multidrug efflux pumps with partially overlapping substrate specificities (Kolaczkowski et al., 1998). Our analysis indicates that their differentiation may have occurred during or after speciation of the Basidiomycota.

Cluster F (YOL075c-like) is the only cluster with members from each of the three phyla analyzed: Saccharomycotina, Pezizomycotina and Basidiomycota (Cannon et al., 2009). Multiple cluster F Pdrps within individual species are rare and F is the only cluster where the Walker A1 motif contains the GXGK sequence found in most other ABC transporters instead of the GXGC motif that characterizes almost all other Pdrps (Table 3 and Supplementary Table 1) (Balzi et al., 1994). Furthermore, in contrast to all other fungal Pdrps, cluster F members exhibit symmetric NBDs more typical of mammalian ABC transporters (Table 3). Members of the F cluster are therefore considered, in a broad sense, to be Pdrps and may be close living relatives of a common ancestor. Cluster E (Aus1p/Pdr11p-like) Pdrps contain neither the GXGC nor the GXGK core motif in NBD1 and lack an ABC signature motif in NBD2, but are otherwise similar to other fungal Pdrps (Table 3 and Supplementary Table 1). Notably, the cysteine of the core Walker A1 motif is replaced by a valine or serine in five Pdrps (Table 3 and Supplementary Table 1). This suggests that the consensus fungal Pdrp Walker A1 motif lacks the lysine found in most other ABC transporters rather than contains a cysteine. Members of clusters E and F are in a strict sense not Pdrps as they do not possess this typical GXGC core sequence in Walker A1.

9. Characterization of PDR clusters

After classifying the 349 Pdrps into the nine clusters A-H2 (Table 2, Fig. 3), individual clusters were investigated in greater detail. This analysis used a subset of 263 Pdrps remaining after removing 85 (~25%) gene sequences (marked with an asterisk in Supplementary Table 1) that appeared to have incorrect intron/exon boundary predictions or possible sequencing errors (these minor nucleotide changes are unlikely to have affected the phylogenetic cluster analysis). The Pdrps within the nine clusters were aligned with the ClustalW(accurate) program (Supplementary Figs. 1–12). The PredictProtein server (Rost et al., 2004) was then used to predict

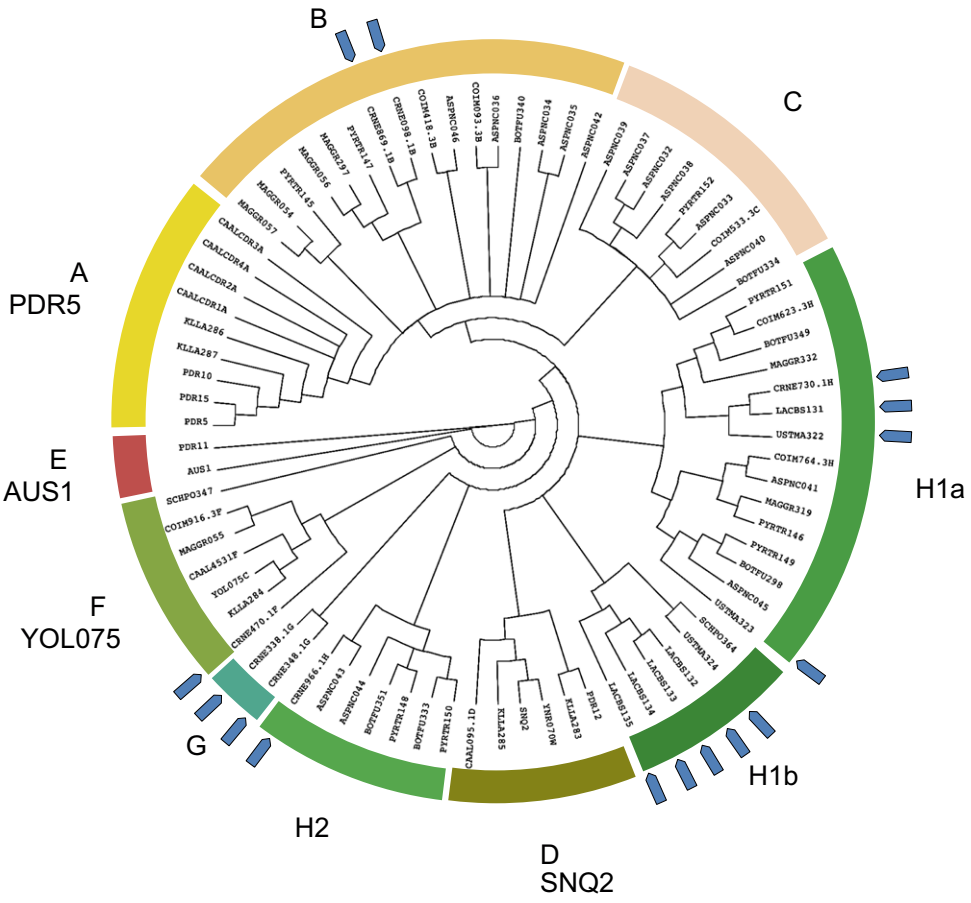

Fig. 3. Phylogenetic tree of 78 PDR transporters from 12 fungal species representing the different phylogenetic classes of Dykarya. The tree is based on a Parsimony method with 100 bootstraps. Only branches with at least 50% support are presented (extended Majority rule in Phylip PROTPARS). Blue arrows indicate PDR transporters from fungal species belonging to the Basidiomycetae. The relative phylogenetic position of the SCHPO347 sequence from *Schizosaccharomyces pombe* at the border of clusters F and E is very unstable from one bootstrap run to the next and thus difficult to assign unequivocally to cluster F,E or H2.

TMSs and the secondary structures of 5–10 selected Pdrps within each cluster. Important motifs (highly conserved primary sequences in the NBDs and TMDs) plus the predicted topology of each PDR cluster are summarized in Table 3. ClustalW alignments for each PDR cluster are presented in Supplementary Figs. 1–10 with subcluster H1a and H1b split into two (Supplementary Figs. 8 and 9). The topology predictions of the 5–10 Pdrps selected for each cluster correlated well with the TMS boundaries predicted for ScPdr5p, CaCdr1p and CkAbc1p (Saini et al., 2005; Tutulan-Cunita et al., 2005; Holmes et al., 2006; Prasad et al., 2006; Lamping et al., 2009). They consistently identified 12 TMSs for each Pdrp with a maximum 2-residue variation in the localization of individually predicted TMSs in the Pdrp alignments shown in Supplementary Figs. 1–12. Some PDR cluster G and F proteins were exceptions. Their TMS numbers and boundaries, especially for TMS1, 2, 7 and 8, were not recognized easily because their primary sequences were less hydrophobic. It is emphasized that although the predicted sub-domains of the Pdrps appear consistent with available biochemical data, PDR transporter topology awaits experimental verification, especially in the TMDs. Gaps appeared in the alignments within the TMDs. These usually occurred within predicted ELs (e.g. EL1 of cluster D shown in Supplementary Figs. 4 and 11). Our observations are consistent with significant TMS and IL size and primary sequence conservation but greater variation in EL size.

Closer inspection of individual PDR clusters indicated that cluster B PDR transporters can be divided into six orthologous families (Supplementary Fig. 2), cluster D proteins into two orthologous families (SNQ2- and PDR12-type; Supplementary Fig. 4), cluster F proteins into three different protein families (Supplementary Fig. 6), cluster H1a into four (Supplementary Fig. 8), and cluster H2 also into four protein families (Supplementary Fig. 10), while no division into sub-families or orthologs was obvious for PDR cluster A, C, E, G and H1b transporters (Supplementary Figs. 1, 3, 5, 7 and 9).

10. Fungal PDR transporters have a PDR-specific 'composite' nucleotide-binding pocket 1 (CNBP1) and a typical CNBP2

A characteristic feature of fungal PDR transporters is asymmetry between their NBDs (Gauthier et al., 2003; Prasad et al., 2006; Ernst et al., 2008).

10.1. NBD1s of fungal PDR clusters

The NBD1s of most fungal PDR transporters (members of PDR clusters A–D, G, H1 and H2) contain a typical ABC signature motif (ABC1) [V/I/L/C]SGGE but have degenerate, fungal PDR-specific Walker A1 and B1 motifs (Fig. 1 and Table 3). Walker A1 contains a C instead of the K required for ATP hydrolysis in other ABC transporters i.e. $GX_2GXGC[S/T]$ vs. $GX_4GK[S/T]$ (Jha et al., 2003b,a). The fungal PDR Walker B1 X_3WD shares only its last two amino acids with the consensus Walker B motif hhhhD (Rai et al., 2005). Furthermore, the essential E at the beginning of the D-loop is replaced with a D, the highly conserved Q of a typical ABC transporter NBD1

Table 3
Conserved motifs and dimensions of the N-terminal (top) and C-terminal halves (bottom) of individual clusters of full-size fungal PDR transporters.

N-ter	N-extension	Walker A1 (P-loop)	↔	Q-loop	Helical domain	ABC1 (C-loop)	↔	Walker B1	D-loop	↔	H-loop	H-TMS1	EL1	IL1	EL2	IL2	EL3
A	143–222	GRPG[S/A]**GC**[S/T]	43	E	63–64	[V/I]SGGE	15	[I/F/V/L][Q/H]CWD	N	32	Y	152–156	12–14	34	10	12	88–91
B	139–233	G[R/P]PGS**G[C/V]**[S/T]	43-(45)	E	64	VSGGE	15	[L/I/F]Q[C/A]WD	N	32	Y	153–156	12	34	10	12	89–92
C	148–219	G[R/Q]PG[S/A]**GC**S	(42)-43	E	64	V[S/P]GGE	15	[L/I/M/V/F][A/G/C/Q] [A/S/C]WD	N	32	Y	155–157	12	34	10	12	87–91
D	139–164	GRPG[S/A]**G[C/S]**[S/T]	42–43	E	64	VSGGE	15	[I/V/L/F][Y/F][C/S/A]WD	N	32	Y	154–157	12–16	32–34	10	12	93–95
E	65–74	G[A/Y/N]P - - - - T	44	E	57	VSGGE	15	VYLWD	N	32	S	155–165	12	34	10	12	92
F	47–115	G[G/S/A]SG[S/A]**GK**[T/S]	37–58	Q	57–61	[L/C]SGGE	15	[V/I/L/F][L/M]F[C/L]D	E	31–33	H	128–145	15–16	37–38	7–16	8	66–82
G	95–139	G[R/K]PG[S/A]**GC**[T/S]	42	E	59–64	VSGG[E/Q]	15	[L/V][I/T/F/A/Q][C/S/M][W/L/F/Y]D	N	32	Y	139–156	12	34	10	12	91–92
H1a	153–313	G[R/K/Q]P[G/E/S][S/A]**GC**[S/T]	41–42	E	58–66	[V/I]SGGE	15	[V/I/L/T][V/L/Q/M/C/T/A/S/I][C/A/S/G]WD	N	32	Y	145–166	12	34	10	12	88–99
H1b	175–283	G[R/Q]PG[S/A]**GC**[S/T]	42	E	63–72	VSGGE	15	[V/I][A/G/V/L][C/A/L/M][W/F/Y]D	N	32	Y	154–175	12	34	10	12	87–92
H2	83–162	G[R/N]PG[S/A]**GC**[T/S]	41–42	E	65	VSGGE	15	[V/I][F/Q/Y/V/M/A][C/F/T/V]WD	N	32	Y	150–154	12	34	10	12	90–93
C-ter	TMS6-A2	Walker A2 (P-loop)	↔	Q-loop	Helical domain	ABC2 (C-loop)	↔	Walker B2	D-loop	↔	H-loop	H-TMS7	EL4	IL3	EL5	IL4	EL6
A	93–145	G[A/S/Y][S/T]GA**GK**T	38–39	Q	58	LN[V/I]EQ	16	L[L/V/I]FLD	E	31	H	132–140	12	31	22	9	104–110
B	90–114	G[V/A/S][S/T]GA**GK**T	38-(39)	Q	58	LNVE[Q/R]	16	L[L/C/F/I]F[L/F/V/I]D	E	31	H	138–148	12	31	22	9	106–119
C	88–110	GVSGA**GK**T	38	Q	58	LNV[E/C]Q	16	L[L//I/V]FLD	E	31	H	136–144	12	31	15-(22)	9	107–111
D	94–110	GESGA**GK**T	37–38	Q	58	LNVEQ	16	LLF[L/V]D	E	31	H	138–146	12	(30)-31	(15)-16	9	107–108
E	112–127	GESGA**GK**T	40	Q	48	- - - - Q[c]	16	LLFLD	E	31	H	146–158	12	31	16	8	107
F	77–107	GPSG[S/G]**GK[**T/S]	43–44	Q	59–60	ISGGE	15	[V/I]L[L/F]LD	E	31–32	H	129–142	11	34	8	11	64–72
G	88–104	G[V/A/M/S]SGA**GK[**T/S]	38	Q	58–59	L[N/G][L/V]E[Q/E][c]	15–16	[L/I/V]L[F/L]LD	E	31	H	131–148	12	30–31	16	9	107–110
H1a	86–122	G[A/S/P]SG[A/S]**GK**[T/S]	38	Q	54–58	L[N/S/A/G/D/T][V/Q/I/P/T/E][E/G][Q/E/K/D/A]	16	[L/V][L/M]FLD	E	31	H	132–144	12	30–31	14–18	9	107–111
H1b	95–108	GESGA**GK**T	38	Q	(54)-58	LNVEQ[c]	16	LLFLD	E	31	H	138–142	12–14	31	14–16	9	96–110
H2	78–114	G[S/C/A]SGA**GK**T	38	Q	58-(59)	L[S/N][V/I]EQ	15–16	L[I/L]FLD	E	31	H	133–137	12	31	15-(16)	9	108–114

N-extension = the size of amino-terminal extensions of PDR transporters; H-TMS1 = the region between the H-loop and TMS1; TMS6-A2 = the region between TMS6 and Walker A2; H-TMS7 = the region between the H-loop of NBD2 and TMS7.

[a]Amino acids are shown in single letter code. Amino acids in brackets are ordered from left to right in order of abundance.

[b]The numbers indicate the primary sequence distance between bordering conserved amino acids or motifs (↔) or found within the indicated structures.

[c] The ABC signature motif in NBD2 is absent in two cluster H1b, two cluster G, and all cluster E PDR transporters.

Q-loop is replaced with an E, the pro-loop P with any amino acid X, and the H-loop H with a Y (Fig. 1 and Table 3).

In contrast, NBD1 of cluster F proteins is comparable to the typical NBD of ABC transporters i.e. Walker A1 is GX_2GXGK, ABC1 is (L/C)SGGE, Walker B1 is hhhhD, Q is conserved in the Q-loop, P in the pro-loop, E is found at the beginning of the D-loop and H is conserved in the H-loop (Table 3 and Supplementary Fig. 6).

The three cluster E proteins are thought to be cholesterol importers and may be the only fungal Pdrps that import, rather than export, substrates (Li and Prinz, 2004; Nakayama et al., 2007). They have typical fungal PDR-specific ABC1 and Walker B1 motifs, an E in the Q-loop and no conserved P in the pro-loop, but they lack the Walker A1 motif. Unlike all other PDR transporters, except cluster F Pdrps that have the typical H, cluster E proteins have S instead of Y in the H-loop of NBD1 (Table 3 and Supplementary Fig. 5).

10.2. NBD2s of fungal PDR clusters

The fungal PDR transporters, except those in clusters E and F, have typical ABC transporter Walker A2, Walker B2, pro-, Q-, and H-loop motifs in NBD2 (Fig. 1 and Table 3). However, the ABC signature motif (ABC2) of the full-size Pdrps in NBD2 (except clusters E and F) is a degenerate consensus LNVEQ sequence (Fig. 1 and Table 3). The NBD2s of cluster E and F Pdrps also have the typical Walker A2 and Walker B2 motifs and the typical pro-, Q-, and H-loops (Table 3). However, there are detectable differences in their 'helical domain' organization and in ABC2. Cluster E proteins have insertions and deletions in the 'helical domain' and, most significantly, lack five amino acids in ABC2 (Table 3 and Supplementary Figs. 5 and 11). Cluster F proteins have a slightly larger 'helical domain' with six amino acids inserted near Walker A2, but their typical ABC2 signature motif (ISGGE) is consistent with the symmetry of their NBDs (Table 3 and Supplementary Figs. 6 and 11). A summary of these features is given in Fig. 1 and Table 3 and individual features are highlighted in Supplementary Figs. 1–12.

10.3. Fungal PDR transporters form fungal PDR-specific composite NBD1/NBD2 ATP-binding pockets

Crystal structures of bacterial and human ABC transporter NBDs show two nucleotide-binding pockets mediate functional NBD dimerization. These are located at the NBD1 and NBD2 interface, with each NBD monomer contributing to both binding pockets. If the catalytic activity of fungal Pdrps involves similar intra-molecular interactions between the NBDs, their transport activity will require the contribution from a composite nucleotide-binding pocket 2 (CNBP2; comprising Walker A2, Walker B2 and ABC1) with the hallmarks of a typical ABC transporter ATP-binding pocket and a composite nucleotide-binding pocket 1 (CNBP1; comprising Walker A1, Walker B1 and ABC2) specific for Pdrps (see Fig. 4 and (Ernst et al., 2008)). It is not clear whether the atypical CNBP1 of PDRs can bind and hydrolyze ATP or perhaps performs a regulatory function unique to PDRs (Wada et al., 2005). Although such functions may be possible in PDR monomers the isolation of *S. cerevisiae* Pdr5p as dimers (Ferreira-Pereira et al., 2003) may indicate that intermolecular interactions are also important for regulation and function of Pdrps.

11. Additional characteristics of the cytosolic domains of fungal Pdrps

PDR cluster A is the best-studied family of medically important Pdrps. It includes the prototypic multidrug efflux pumps *S. cerevisiae* Pdr5p and *C. albicans* Cdr1p, *C. glabrata* Cdr1p and Cdr2p (also known as Pdh1p), and *C. krusei* Abc1p. In PDR cluster A the 'helical domain' of NBD1 is ~6 amino acids larger than in NBD2 (63-4 compared to 58; Table 3). This size difference between the 'helical-domains' of NBD1 and NBD2 is conserved in all PDR clusters except cluster F proteins that have 'helical-domains' of similar size (Table 3). In human ABCC1 a change of 'helical domain' symmetry affects substrate transport and ATP hydrolysis in a fashion similar to the K684M mutation of the essential K of Walker A1 (Gao et al., 2000). Furthermore, the linker region between the H-loop of NBD1 and TMS1 in fungal PDRs is ~155 amino acids while the equivalent linker region of NBD2 is ~15 amino acids shorter (Table 3). Finally, all fungal Pdrps apart from E and F members have N-terminal extensions of ~100–200 amino acids (Table 3 and Fig. 4). The size and amino acid sequences of these extensions are variable but seem cluster specific. These yet to be explored cytosolic N-terminal extensions are rich in polar (S/T) and charged amino acids that could provide contact points for accessory proteins or post-translational modifications that modulate the function, localization, and/or turnover of Pdrps.

12. PDR-specific topology of TMDs including ELs and ILs

12.1. Extracellular loops

The small EL1 (11–16 amino acids with most Pdrps containing 12 amino acids; Figs. 1 and 4 and Table 3) appears quite conserved within groups of Pdrps i.e. distinct sub-clusters or families of orthologous proteins of possibly similar function. The size of EL2 is also quite conserved i.e. 7–16 amino acids, with most comprising 10 amino acids (Table 3). Consistent with the apparent twofold symmetry of ABC transporters, EL4 (usually 12 amino acids) is of identical size to EL1. The size of EL5 is less conserved and ranges from eight (cluster F) to 23 (clusters A and B) amino acids (Table 3). All PDR transporters contain two large, partially homologous, extracellular loops, EL3 and EL6 (Fig. 4 and Table 3).

12.2. ELs may contribute to substrate transport

Overexpression of PDR transporters often confers resistance to xenobiotics, and this resistance can be ablated (chemosensitization) with pump inhibitors such as FK506 (Nakamura et al., 1993; Kralli and Yamamoto, 1996) and milbemycins (Lamping et al., 2007, 2009). Fungi expressing Pdrps can also overcome the chemosensitization of pump inhibitors, by acquiring suppressor mutations in the pump protein. We have identified suppressor mutations in *S. cerevisiae* Pdr5p and *C. albicans* Cdr1p that overcome pump inhibition by FK506, milbemycins and an in-house D-octapeptide derivative (B.C. Monk et al., and K. Tanabe et al., NIID Tokyo; unpublished results). The mutations were located predominantly in EL3 and EL6 but also in EL1, EL4 and occasionally within individual TMSs. Further evidence for a role of ELs in substrate transport comes from random mutagenesis of *S. cerevisiae* Pdr5p (Tutulan-Cunita et al., 2005). Two amino acid mutations in EL5 and EL6 were found to affect the transport of a subset of efflux pump substrates. Other mutations affecting substrate specificity were located in TMS4 and TMS9, the 'helical domain' close to the Q-loop of NBD1, and near the D-loop. Thus drug interactions within Pdrps depend on structural elements located in different parts of the protein, but often in the ELs. The functional importance of ELs in other ABC transporters is illustrated by the uncharacteristically large EL1 of human ABCA1 that is essential for ApoA-I mediated cholesterol transport (Wang et al., 2000). Mutations in NBD1, NBD2, EL1, the similarly large EL4 and the much smaller EL6 of ABCA1 cause Tangier disease and familial HDL deficiency but few

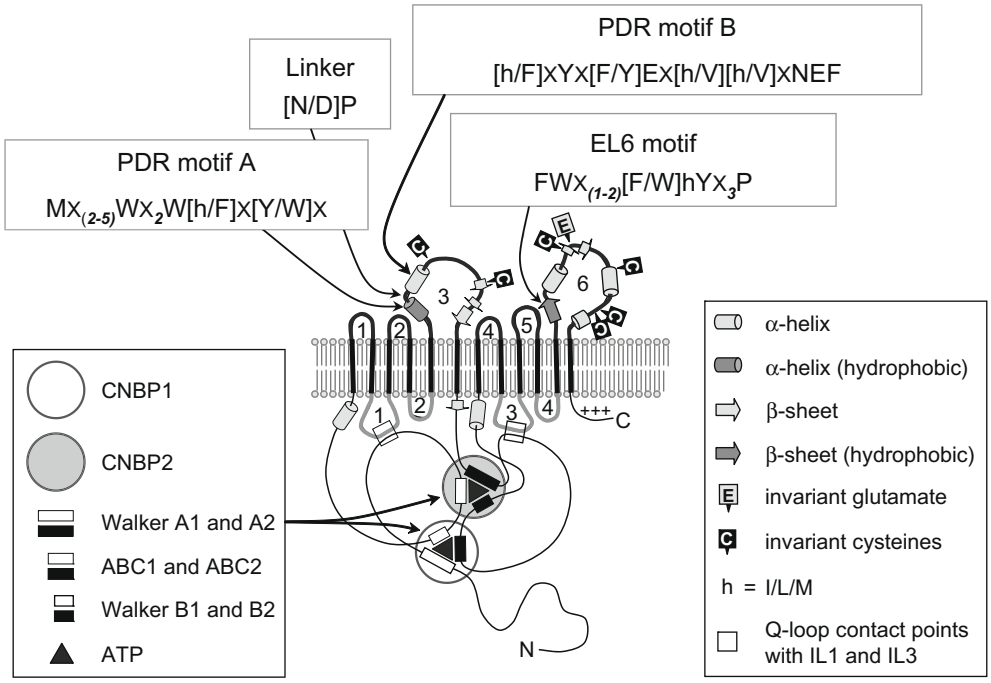

Fig. 4. Analysis of the primary and secondary structure predictions of fungal PDR transporters revealed highly conserved ELs, particularly EL3 and EL6. These have unique structural features and highly conserved motifs indicating possible involvement in the substrate transport mechanism (the hydrophobic PDR motif A and PDR motif B in EL3 separated by a conserved linker and a highly conserved hydrophobic EL6 motif). EL3 and EL6 possess six invariant Cs, two in EL3 and four in EL6. They also contain two invariant Es, one each in EL3 and EL6. Predicted interactions between essential components of the NBDs and the intracellular loop regions are also shown i.e. the 'composite' nucleotide-binding pockets 1 (CNBP1) and 2 (CNBP2) that consist of conserved motifs from each of the two NBDs are circled and the predicted interactions between the Q-loops and IL1 and IL3 are highlighted with open squares. Also highlighted are predicted positively charged α-helices close to TMS1 and TMS7 that may be involved in membrane insertion/protein folding, and a predicted short, intracellular β-sheet close to TMS6 that is conserved between individual clusters of PDR transporters and possibly involved in aspects of the transport mechanism.3

mutations associated with Tangier disease are located in any of the 12 TMS of ABCA1 (Tanaka et al., 2001).

13. PDR-specific motifs in EL3 and EL6

Alignment of the EL3 and EL6 sequences of 263 fungal Pdrps reveals four major characteristics: (1) EL3 and EL6 are similar but have distinct features e.g. EL3 is shorter (~90 amino acids) than EL6 (~110 amino acids). Only PDR cluster F proteins have similar sized but smaller (~70 amino acids) EL3 and EL6 (Table 3); (2) The Pdrps contain two invariant Cs and one invariant E in EL3. They also contain four invariant Cs and a highly conserved E located four amino acids C-terminal of the first invariant C in EL6 (only ZYRF08888 of cluster D and CNAG-06338, CNAG-06348 and B6H8M2 of cluster G have a D instead; Fig. 4, Table 4 and Supplementary Tables 2 and 3). Cluster F proteins are the exception. They have only two conserved Cs in each of EL3 and EL6; (3) The N-terminal portions of EL3 and EL6 reveal new conserved hydrophobic, PDR-specific motifs named PDR motif A and PDR motif B in EL3 (Fig. 4 and Table 4), and a hydrophobic motif equivalent to PDR motif A in EL6 (Fig. 4 and Table 5); and (4) Predicted secondary structures in EL3 and EL6 appear strongly conserved (Fig. 4).

13.1. EL3

Four highly conserved amino acids G[Y/F]X[I/L/V] are predicted to form a short β-sheet after the α-helix of TMS5 leading into EL3 (Fig. 5). This is followed by a short loop of four variably charged hydrophilic amino acids, then two highly conserved α-helices linked by two conserved amino acids [N/D]P (Fig. 4). While some residues in these two α-helices are specific for individual orthologs (e.g. the *SNQ2*, *PDR12* or *AFR* family) other residues, mostly on one face of a helical wheel projection, are strongly conserved between all clusters. Both helices contain conserved aliphatic and aromatic amino acids: α-helix 1 = MX_2WX_2W[h/F]X[Y/W]; α-helix 2 = [h/F]XYX[F/Y]EX[h/V][h/V]XNEF. The only invariant amino acid is the E at the C-terminal end of helix 2 (Table 4). These two motifs are also conserved in other PDRs (full-size and half-size plant, fungal and human PDRs; data not shown). We have therefore denoted these two conserved α-helices PDR motif A and PDR motif B (Fig. 4 and Table 4). They are not to be confused with the plant PDR-specific motifs PDR sig1, PDR sig2 and PDR sig3 described by van den Brule and Smart (2002).

The C-terminus of EL3 is predicted to contain three short β-sheets with a few highly conserved amino acids that may be important for its structure (Fig. 4) i.e. two invariant Cs, three highly conserved Gs and three aromatic amino acids $CX_{\sim 20}CX_3GX_3GX_5GX_2$[Y/F]$X_4$[Y/F]XY (Supplementary Table 2). Located five amino acids downstream is the conserved sequence WRNFG[I/V] that connects the C-terminus of EL3 with TMS6 (Fig. 5). The ~150 amino acid 'PDR_CDR' protein family motif that is recognized in Pdrps by Blastp searches (pfam06422 (Gauthier et al., 2003; Finn et al., 2008)) includes this C-terminal half of EL3 from the second conserved C, TMS6, the conserved β-sheet mentioned in the next section, and the linker region of Pdrps (Fig. 4).

13.2. EL6

Three conserved amino acids G[I/V][M/L/V] begin EL6 as part of a short predicted β-sheet at the end of the TMS11 α-helix (Fig. 5). This conserved β-sheet is followed by eight variably charged

Table 4
Conservation of amino acids in the PDR motifs A and B of full-size fungal PDR transporters.

	PDR motif A											Linker		PDR motif B											
aa[a]	M	X	X	W	X	X	W	h F	X	Y W	X	N D	P	h	X	Y	X	F	E	X	h V	h V	X	N	E	F
%[b]	81	–	–	99	–	–	98	100	–	96	–	94	95	95	–	89	–	97	87	–	94	89	–	88	100	94

[a] Amino acids are shown in single letter code with h = I, L or M, and X = any amino acid. Amino acids are ordered, from the top, in descending order of abundance.
[b] Numbers represent the percent conservation of the primary sequence positions in 244 full-size fungal PDR transporters (PDR cluster F proteins excluded).

Table 5
Conservation of the hydrophobic motif of EL6[a] in full-size fungal PDR transporters.

	EL6 motif										
aa[b,c]	F	W	X	(X)	F W	h	Y	X	X	X	P
%[d]	95	98	–	(–)	95	96	87	–	–	–	100

[a] This motif is equivalent to the PDR motif A and the linker of EL3 shown in Table 4.
[b] Amino acids are shown in single letter code with h = I, L or M and X = any amino acid. Amino acids are ordered, from the top, in descending order of abundance.
[c] (X) indicates that this amino acid is present in some, but not other, Pdrps (see text for detail).
[d] Numbers represent the percent conservation of the primary sequence positions in 244 full-size fungal PDR transporters (PDR cluster F proteins excluded).

hydrophilic amino acids and then a conserved stretch of 10–11 amino acids rich in aromatic residues (FWX$_{(1-2)}$[F/W]hYX$_3$P). This arrangement and the hydrophobic stretch of conserved amino acids is similar to EL3, however, unlike PDR motif A, the conserved hydrophobic motif of EL6 is often predicted to form a β-sheet rather than an α-helix (Fig. 4). Similar to the highly conserved P that separates PDR motifs A and B in EL3, an invariant P separates the hydrophobic motif of EL6 from an adjacent α-helix (Fig. 4, Table 5 and Supplementary Figs. 1–12). However, unlike PDR motif B, the primary sequence of this α-helix is only conserved within Pdrp orthologs (e.g. compare the four protein families of cluster H1a shown in Supplementary Fig. 8). Cluster F proteins also have two predicted α-helices separated by an invariant P. However, their primary sequences are less well conserved and can be readily divided into three distinct groups, or perhaps orthologs (marked with red, green and purple sidebars in Supplementary Fig. 6). Like EL3, the remainder of EL6 CX$_3$[E/D]X$_3$[F/L/V/I]X$_{6-9}$CX$_2$[Y/F]X$_3$[Y/F]X$_{4-16}$GX$_{10-12}$CX[F/Y/V]C (Supplementary Table 3) contains a few conserved amino acids, including four invariant Cs, an invariant acidic E/D and a few aromatic amino acids. There are 20 amino acids between the most C-terminal of the invariant Cs and a conserved exit motif WR[N/D][F/Y/W/V/L/I]G[I/L/F/V] that is very similar to the C-terminal portion of EL3 (Fig. 5).

14. EL3 and EL6 of Pdrps have conserved structural features likely to be important for their transport mechanism

We hypothesize that the Pdrps have evolved conserved structural features in their large ectodomains to fulfill unique functions that differentiate them from other ABC transporter families. Human half-size PDR transporters ABCG1, 2, 4, 5 and 8 (Dean, 2002) share their TMD topology with fungal and plant Pdrps (small EL1 and EL2 and large EL3; Supplementary Tables 4 and 5). Their large EL3 (~70 amino acids) also contains a predicted hydrophobic α-helix rich in aromatic amino acids and a second conserved α-helix, very similar to fungal PDR motifs A and B, with the caveat that the five ABCG proteins fall into two sub-groups. The PDR motifs A and B of ABCG2 and ABCG5 are very similar to those of fungal PDRs but they are more degenerate in ABCG1, 4 and 8 (data not shown).

The six highly conserved Cs of EL3 and EL6 of fungal Pdrps seem likely to have structural, and possibly functional, importance. The second conserved C in EL6 was mutated in a functional characterization of *C. albicans* Cdr1p (C1418Y). The mutation increased the susceptibility of the Cdr1p-expressing host to some xenobiotics (cycloheximide and miconazole) but not others (e.g. FLC) (Shukla

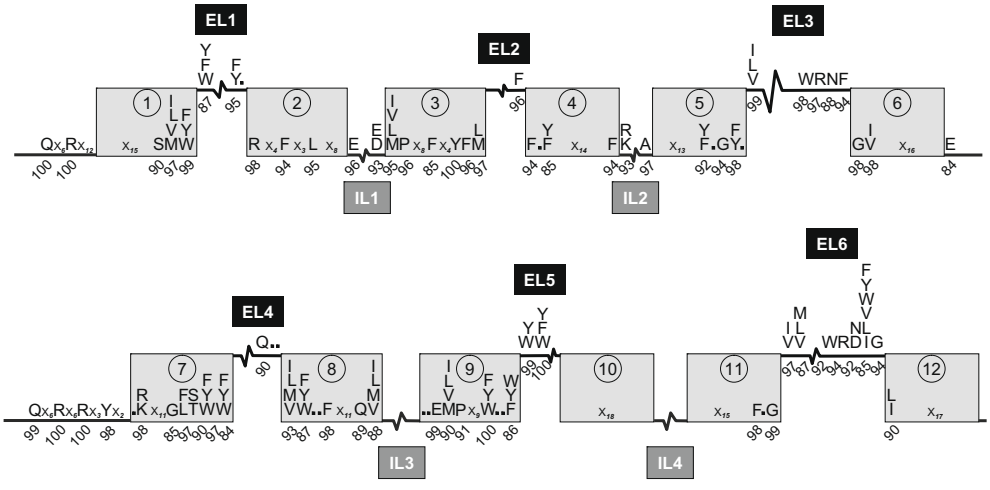

Fig. 5. Conservation of amino acids in the TMDs of fungal PDR transporters. 244 PDR transporters were used for this analysis (all cluster A–D, E, G, H1a, H1b and H2 proteins but without cluster F transporters). Only conserved amino acids of TMS1–12 and some highly conserved amino acids adjacent to these TMSs are shown. The conserved amino acids are shown using the one letter code with the percent conservation given underneath each individual residue. Non-conserved amino acids are depicted as X and the number of these amino acids are indicated as subscript in italics. Individual TMS (1–12) are shown as gray boxes. Each TMS is 18 amino acids long. A dot represents a non-conserved amino acid between conserved residues. EL1–6 are presented as thick black lines connecting individual TMSs and IL1-4 are shown as thick gray lines. The conserved amino acids N-terminal to TMS1 and TMS7 are part of the two predicted positively charged α-helices (~20 amino acids in size) shown in Fig. 4.

et al., 2003). Studies with ABCG2 also highlight the significance of EL Cs (Henriksen et al., 2005). C592 and C608 of ABCG2 EL3 form intra-molecular disulfide bonds that appear important for substrate specificity for *C. albicans* Cdr1p and consistent with the hypothesis that ELs play important roles in substrate transport. Furthermore, C603 contributes to the homodimer formation required for ABCG2 function by forming intermolecular disulfide bonds (Henriksen et al., 2005).

Although there were no conserved Cs in EL3 or EL6 of the plant PDR transporters that we examined, each EL3 and EL6 contains an invariant negatively charged amino acid like all fungal PDR transporters (i.e. the E of PDR motif B in EL3 and a D in a position similar to the invariant E in EL6 of fungal PDRs). The importance of conserved negatively charged amino acids in TMSs for the binding and transport of positively charged hydrophobic drugs has been demonstrated in bacterial multidrug transport systems such as EmrE (Muth and Schuldiner, 2000) or MdfA (Edgar and Bibi, 1999). We postulate that these conserved E and D residues in EL3 and EL6 of Pdrps may be important for the binding/transport of hydrophobic and/or positively charged efflux pump substrates and that EL3 and EL6 contribute to substrate binding during the transport cycle.

15. IL1 and IL3 are highly conserved in fungal PDR transporters

Pdrps appear to possess unique IL arrangements. Crystal structures show that MDR-type ABC transporters have two large ILs in each TMD that are required for the cross-talk between the NBDs and TMDs (Dawson and Locher, 2006; Aller et al., 2009). In contrast, plant, fungal, and human PDR transporters are predicted to possess one large and one small IL in each TMD (Figs. 1 and 4, Table 3 and Supplementary Tables 4 and 5). The TMD topology of Pdrps may indicate a unique TMD arrangement that is distinct from MDR transporters. According to Rees et al. (2009), other yet to be discovered TMD folds for ABC transporters almost certainly exist in addition to the three TMD folds identified so far (type I and II importers and type III exporters such as Sav1866 or ABCB1; (Rees et al., 2009)). While the Pdrp IL2 and IL4 are very short and have no obvious conserved motifs, IL1 (34 amino acids) and IL3 (31 amino acids) are highly conserved within Pdrp clusters and/or families and are similar in size to the large ILs of MDR-type transporters. IL1 and IL3 are rich in conserved charged and hydrophobic amino acids, especially near the center of each loop (Tables 6 and 7). Secondary structure predictions indicate that the TMS2 α-helix extends ~6 amino acids into IL1 while the equivalent TMS8 α-helix of TMD2 breaks at the beginning of IL3 followed by a short (~5 amino acid) β-sheet. Both IL1 and IL3 then share a predicted centrally located ~6 amino acid α-helix reminiscent of the 'coupling helices' of IL1 and IL3 of MDRs (Dawson and Locher, 2006; Aller et al., 2009). The C-terminal half of IL1 is invariably predicted to be an α-helix that is broken at the entrance to TMS3 by three highly conserved amino acids [E/D]hP (Table 6; h = I, V, L or M). The equivalent α-helix in the C-terminal half of IL3 reaches three amino acids into the predicted TMS9 region and is broken by a comparable amino acid triplet EhP (Table 7). IL1 has a negatively charged amino acid at each end with a core of highly conserved amino acids that includes two invariable positively charged amino acids centered around the predicted 'coupling helix' (Table 6). The C-terminal α-helix of IL1 (X_{10} between P and [E/D] in Table 6) is highly conserved within individual PDR clusters, perhaps reflecting a selective functionality (Supplementary Figs. 1–12). IL3 possesses highly conserved amino acids across the whole region including the C-terminal α-helix (Table 7).

16. TMDs of fungal PDR transporters have positively charged N-terminal α-helices and a highly conserved β-sheet just after TMS6

Individual TMS are thought to determine the broad substrate specificity of ABC transporters by contributing to a large, centrally located, binding pocket that is open to either the cytosolic or the extracellular space during the reaction cycle (Egner et al., 2000; Shukla et al., 2003; Saini et al., 2005; Tutulan-Cunita et al., 2005). There are conserved features flanking the TMDs (Fig. 4). All Pdrps have a ~15 amino acid α-helix located ~4 amino acids N-terminal of the TMS1 and TMS7 α-helices (Fig. 4). Both α-helices are rich in positively charged amino acids (Fig. 5 and Supplementary Figs. 1–12) and these may be important for determining the insertion of the first TMS of each half of the transporter in the membrane (Hartmann et al., 1989; von Heijne, 1995; Gafvelin et al., 1997). As a general rule, the net charge difference between the 15 amino acids N-terminal and the 15 residues C-terminal of the first hydrophobic TMS of an integral membrane protein determines its orientation, with the more positively charged part remaining in the cytosol (Gafvelin et al., 1997). The orientation of the first TMS then automatically determines the orientation of the following TMSs. The topology of fungal Pdrps proposed in Figs. 1 and 4 complies with this rule. Both helices prior to TMS1 and TMS7 have a similar core of conserved amino acids. They include an invariant Q (except one PDR G cluster protein) and one or two invariant Rs. The consensus for the α-helix N-terminal of TMS1 is $QX_{6}RX_{12}$ and the consensus for the α-helix N-terminal to TMS7 is $QX_{6}RX_{5-6}RX_{3}YX_{2}$ (Fig. 5).

A predicted ~6 amino acid β-sheet located ~9 amino acids C-terminal to TMS6 (Fig. 4) is conserved in PDR cluster A–D proteins (Supplementary Figs. 1–4, 11 and 12) while cluster H proteins have a unique β-sheet motif (Supplementary Figs. 8–12). The ~9 amino acids separating TMS6 and this β-sheet are highly conserved within Pdrp orthologs (Supplementary Figs. 11 and 12). These sequences may contribute to the transport mechanism or the substrate specificity within groups of PDR transporters.

17. TMS boundaries of fungal PDR transporters are highly conserved

Conservation of primary sequence within any given TMS is higher within, than between, clusters. Highly conserved amino

Table 6
Conservation of amino acids in the IL1 of full-size fungal PDR transporters.

	IL1																			TMS3	
aa [a,b]	E	I L	X_3	Y F W	X_2	R	X	I V L M	X_2	R K	X_3	Y F	A S G	X	Y F	X	P	X_{10}	E D	I V L M	P
%[c]	96	91	–	84	–	99	–	100	–	100	–	95	91	–	85	–	94	–	93	95	96

[a] Amino acids are shown in single letter code with X = any amino acid. Amino acids are ordered, from the top, in descending order of abundance.
[b] Subscripts next to X denote the number of amino acids.
[c] Numbers represent the percent conservation of the primary sequence positions in 244 full-size fungal PDR transporters (PDR cluster F proteins excluded).

Table 7
Conservation of amino acids in the IL3 of full-size fungal PDR transporters.

	IL3																									TMS9			
aa[a,b]	X	P	X	F Y	X_3	R	X	I V L M	Y F W	E D	X	R	E	X_2	S	K R N	X	Y F	X	W	X_2	F	I L V M	X_3	I V	X_2	E	I L V M	P
%[c]	–	86	–	90	–	97	–	98	97	84	–	100	99	–	92	96	–	99	–	93	–	85	84	–	81	–	99	90	91

[a] Amino acids are shown in single letter code with X = any amino acid. Amino acids are ordered, from the top, in descending order of abundance.
[b] Subscripts next to X denote the number of amino acids.
[c] Numbers represent the percent conservation of the primary sequence positions in 244 full-size fungal PDR transporters (PDR cluster F proteins excluded).

acids present in all Pdrps are summarized in Fig. 5 (the percent conservation of individual amino acids is also shown) and circled in blue in the 90% consensus sequence lines shown at the bottom of Supplementary Figs. 1–12. These conserved sequences are likely to be important for the overall structure and function of Pdrps but are less likely to be involved in their substrate specificity. Overall it appears that most TMS boundaries are highly conserved containing either hydrophobic or charged amino acids (for details see Fig. 5). Each TMS is predicted to form a long (~18–30 amino acids) α-helix except TMS3 and TMS9 that consist of two shorter α-helices broken by the highly conserved amino acid triplet EhP at the end of IL1 and IL3, as discussed above. Similar to the invariant negatively charged amino acids in EL3 and EL6 described above, the highly conserved Es in TMS3 and TMS9 may also be involved in the binding and/or transport of positively charged, hydrophobic amino acids. It is striking that full-size plant PDR transporters also contain conserved E or D residues in similar positions in their respective TMS3 and TMS9 (data not shown). Interestingly, both TMS1 and TMS7 have conserved polar amino acids S (TMS1) or [S/T] (TMS7) close to EL1 and EL4, respectively. The conserved TMS1 S residue is predicted to be located near the S558 of ScPdr5p TMS2 that has been implicated in coupling ATP hydrolysis and substrate transport (Sauna et al., 2008). TMS10 and the cytosolic side of TMS11, including IL4, show no conservation of primary sequence. Finally, most Pdrps are predicted to have a very short C-terminal tail (~10–20 amino acids) located in the cytosol. This tail is extremely rich in positively charged amino acids with an average net positive charge of ~5–6 (Fig. 4). This may be important for its association with the inner leaflet of the plasma membrane lipid bilayer.

18. Concluding remarks

Fungal Pdrps are a large family of ABC transporters involved in the import and export of a diversity of compounds across biological membranes. Although the expression of some members has been correlated with clinically significant antifungal drug resistance, the physiological functions of these and other family members are poorly understood. The availability of Pdrp sequences in well-curated databases of whole fungal genomes permits phylogenetic analysis, searches for Pdrp-specific primary sequence motifs and the ability to ascribe some putative functions. We have categorized Pdrps from 55 fungal species into nine separate clusters with one group (H1b) overlapping two clusters (D and H1a). Pdrps possess a unique set of features that may have evolved from an ancient common ancestor that appears to be most closely related to PDR cluster F proteins. It is also possible that Pdrps arose independently of PDR cluster F proteins through gene fusion of two different half-size Pdrps (Bouige et al., 2002; Crouzet et al., 2006). Typical fungal Pdrps have uniquely conserved 'asymmetric' NBDs. They have PDR-specific arrangements of ILs and ELs, they contain PDR-specific motifs in EL3 (the hydrophobic PDR motif A and PDR motif B), and they possess a highly conserved PDR-specific hydrophobic motif in EL6. PDR motif A and PDR motif B are separated by a conserved [N/D]P sequence and have an invariant E at the C-terminus of PDR motif B. EL6 is slightly larger. It also contains an invariant P separating its hydrophobic motif from an adjacent, structurally conserved, α-helix and an invariant E three amino acids C-terminal of the first invariant C (Fig. 4).

Although we have focused on fungal Pdrps, plants possess a large number of Pdrps and the human ABCG family of transporters can be categorized as Pdrps as well. Unlike plants and fungi, humans appear to have only half-size PDR transporters (Dean, 2002). All half-size PDR transporters have NBDs that resemble NBD2s of full-size fungal and plant PDRs, but their EL3 size can vary significantly (~51–101 amino acids) (Supplementary Table 5). We queried databases for the PDR-specific motifs, PDR motif A and PDR motif B, and found these two motifs are present in all Pdrps including those from plants and humans but not in any other ABC transporters. Despite obvious similarities between fungal and plant PDRs (all half-size and full-size Pdrps from *Arabidopsis thaliana*, rice and tobacco were tested) the following differences were found: (1) Plant Pdrps have a plant-specific ABC signature motif in NBD2 (van den Brule and Smart, 2002); (2) Plant Pdrps have a ~30 amino acids larger NBD1 'helical domain' than fungal Pdrps (compare Tables 3 with Supplementary Table 4); and (3) Plant Pdrps lack the invariant Cs found in EL3 and EL6 of fungal Pdrps. We suggest that while fungal and plant Pdrps share a common ancestor and have important PDR-specific features in common and are therefore likely to share a similar transport mechanism, adaptive evolution has minimized their functional homology. In short, plant and fungal Pdrps do not appear to share any orthologs (Crouzet et al., 2006).

Our analysis of the literature also indicates that a universal nomenclature of half-size and full-size PDR transporters is required. We recommend that the HUGO nomenclature for ABC transporters be adopted but in a way that both the relatedness of genes (e.g. SNQ2, PDR12 or AFR orthologs of different fungal species) and their classification as half-size or full-size PDR transporters can still be recognized.

While the function of most PDR transporters discussed in this review remains to be elucidated it seems likely that different Pdrp orthologs share different subsets of substrates. The phylogenetic analysis presented here permits this hypothesis to be tested by comparing the properties of individual cluster members heterologously expressed in a suitable host such as *S. cerevisiae*. This approach could help elucidate how cluster members within this important family of ABC proteins bind and translocate their range of substrates. Another important outcome of the present study has been the discovery of orthologs with possibly similar function, in different fungal species. Thus, the analysis of the function of a Pdrp in one species may give important clues to the function of orthologs in other species. Since ABC pump function depends upon coordinated interaction between different parts of the molecule, the discovery of motifs specific to individual Pdrp clusters or sub-clusters should provide information about their biochemical properties. In the light of our growing understanding of possible structure–function relationships, the use of site-directed mutagen-

esis to modify individual motifs, the analysis of single nucleotide polymorphisms and the creation of chimeric transporters that contain different combinations of domains, sub-domains and motifs can all provide practical methods to explore PDR transporter structure and function.

However, most critical will be the validation of the topological map of Pdrps proposed in this study and the structural resolution of at least one member of this important protein family. The ability to express affinity tagged versions of many of these pumps in *S. cerevisiae* and the availability of inhibitors of these enzymes will be a major advantage in obtaining structural information at high resolution. Greater understanding of Pdrp structure and function will ultimately help elucidate Pdrp-mediated drug resistance and facilitate structure directed design of 'smart' drugs that inhibit multiple targets simultaneously and thus reduce the likelihood of drug resistance (Monk and Goffeau, 2008).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fgb.2009.10.007.

References

Akins, R.A., 2005. An update on antifungal targets and mechanisms of resistance in *Candida albicans*. Med. Mycol. 43 (4), 285–318.
Aller, S.G., Yu, J., Ward, A., Weng, Y., Chittaboina, S., et al., 2009. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. Science 323 (5922), 1718–1722.
Balzi, E., Wang, M., Leterme, S., Van Dyck, L., Goffeau, A., 1994. *PDR5*, a novel yeast multidrug resistance conferring transporter controlled by the transcription regulator *PDR1*. J. Biol. Chem. 269 (3), 2206–2214.
Bauer, B.E., Wolfger, H., Kuchler, K., 1999. Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. Biochim. Biophys. Acta 1461 (2), 217–236.
Bennett, J.E., Izumikawa, K., Marr, K.A., 2004. Mechanism of increased fluconazole resistance in *Candida glabrata* during prophylaxis. Antimicrob. Agents Chemother. 48 (5), 1773–1777.
Bouige, P., Laurent, D., Piloyan, L., Dassa, E., 2002. Phylogenetic and functional classification of ATP-binding cassette (ABC) systems. Curr. Protein Pept. Sci. 3 (5), 541–559.
Cannon, R.D., Holmes, A.R., Mason, A.B., Monk, B.C., 1995. Oral *Candida*: clearance, colonization, or candidiasis? J. Dent. Res. 74 (5), 1152–1161.
Cannon, R.D., Lamping, E., Holmes, A.R., Niimi, K., Baret, P.V., et al., 2009. Efflux-mediated antifungal drug resistance. Clin. Microbiol. Rev. 22 (2), 291–321.
Chang, G., 2003. Structure of MsbA from *Vibrio cholera*: a multidrug resistance ABC transporter homolog in a closed conformation. J. Mol. Biol. 330 (2), 419–430.
Chen, J., Li, H., Li, R., Bu, D., Wan, Z., 2005. Mutations in the *cyp51A* gene and susceptibility to itraconazole in *Aspergillus fumigatus* serially isolated from a patient with lung aspergilloma. J. Antimicrob. Chemother. 55 (1), 31–37.
Corbett, E.L., Churchyard, G.J., Charalambos, S., Samb, B., Moloi, V., et al., 2002. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. Clin. Infect. Dis. 34 (9), 1251–1258.
Crouzet, J., Trombik, T., Fraysse, A.S., Boutry, M., 2006. Organization and function of the plant pleiotropic drug resistance ABC transporter family. FEBS Lett. 580 (4), 1123–1130.
da Silva Ferreira, M.E., Capellaro, J.L., dos Reis Marques, E., Malavazi, I., Perlin, D., et al., 2004. In vitro evolution of itraconazole resistance in *Aspergillus fumigatus* involves multiple mechanisms of resistance. Antimicrob. Agents Chemother. 48 (11), 4405–4413.
Dassa, E., Bouige, P., 2001. The ABC of ABCS: a phylogenetic and functional classification of ABC systems in living organisms. Res. Microbiol. 152 (3–4), 211–229.
Davies, A.N., Brailsford, S.R., Beighton, D., 2006. Oral candidosis in patients with advanced cancer. Oral Oncol. 42 (7), 698–702.
Dawson, R.J., Locher, K.P., 2006. Structure of a bacterial multidrug ABC transporter. Nature 443 (7108), 180–185.
de Repentigny, L., Lewandowski, D., Jolicoeur, P., 2004. Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. Clin. Microbiol. Rev. 17 (4), 729–759 (table of contents).
Dean, M., 2002. The Human ATP-Binding Cassette (ABC) Transporter Superfamily: NCBI; Available Online at the NCBI Bookshelf: <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mono_001&part=A137>, 50 p.
Decottignies, A., Goffeau, A., 1997. Complete inventory of the yeast ABC proteins. Nat. Genet. 15 (2), 137–145.
Dujon, B., Sherman, D., Fischer, G., Durrens, P., Casaregola, S., et al., 2004. Genome evolution in yeasts. Nature 430 (6995), 35–44.
Edgar, R., Bibi, E., 1999. A single membrane-embedded negative charge is critical for recognizing positively charged drugs by the *Escherichia coli* multidrug resistance protein MdfA. Embo. J. 18 (4), 822–832.
Egner, R., Bauer, B.E., Kuchler, K., 2000. The transmembrane domain 10 of the yeast Pdr5p ABC antifungal efflux pump determines both substrate specificity and inhibitor susceptibility. Mol. Microbiol. 35 (5), 1255–1263.
Ernst, R., Kueppers, P., Klein, C.M., Schwarzmueller, T., Kuchler, K., et al., 2008. A mutation of the H-loop selectively affects rhodamine transport by the yeast multidrug ABC transporter Pdr5. Proc. Natl. Acad. Sci. USA 105 (13), 5069–5074.
Ferreira, M.E., Colombo, A.L., Paulsen, I., Ren, Q., Wortman, J., et al., 2005. The ergosterol biosynthesis pathway, transporter genes, and azole resistance in *Aspergillus fumigatus*. Med. Mycol. 43 (Suppl. 1), S313–319.
Ferreira-Pereira, A., Marco, S., Decottignies, A., Nader, J., Goffeau, A., et al., 2003. Three-dimensional reconstruction of the *Saccharomyces cerevisiae* multidrug resistance protein Pdr5p. J. Biol. Chem. 278 (14), 11995–11999.
Finn, R.D., Tate, J., Mistry, J., Coggill, P.C., Sammut, S.J., et al., 2008. The Pfam protein families database. Nucleic Acids Res. 36 (Database issue), D281–D288.
Gafvelin, G., Sakaguchi, M., Andersson, H., von Heijne, G., 1997. Topological rules for membrane protein assembly in eukaryotic cells. J. Biol. Chem. 272 (10), 6119–6127.
Gao, M., Cui, H.R., Loe, D.W., Grant, C.E., Almquist, K.C., et al., 2000. Comparison of the functional characteristics of the nucleotide binding domains of multidrug resistance protein 1. J. Biol. Chem. 275 (17), 13098–13108.
Gaur, M., Choudhury, D., Prasad, R., 2005. Complete inventory of ABC proteins in human pathogenic yeast, *Candida albicans*. J. Mol. Microbiol. Biotechnol. 9 (1), 3–15.
Gauthier, C., Weber, S., Alarco, A.M., Alqawi, O., Daoud, R., et al., 2003. Functional similarities and differences between *Candida albicans* Cdr1p and Cdr2p transporters. Antimicrob. Agents Chemother. 47 (5), 1543–1554.
Gbelska, Y., Krijger, J.J., Breunig, K.D., 2006. Evolution of gene families: the multidrug resistance transporter genes in five related yeast species. FEMS Yeast Res. 6 (3), 345–355.
Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., et al., 1996. Life with 6000 genes. Science 274 (5287), 546–563. 547.
Hartmann, E., Rapoport, T.A., Lodish, H.F., 1989. Predicting the orientation of eukaryotic membrane-spanning proteins. Proc. Natl. Acad. Sci. USA 86 (15), 5786–5790.
Henriksen, U., Fog, J.U., Litman, T., Gether, U., 2005. Identification of intra- and intermolecular disulfide bridges in the multidrug resistance transporter *ABCG2*. J. Biol. Chem. 280 (44), 36926–36934.
Herget, M., Oancea, G., Schrodt, S., Karas, M., Tampe, R., et al., 2007. Mechanism of substrate sensing and signal transmission within an ABC transporter: use of a Trojan horse strategy. J. Biol. Chem. 282 (6), 3871–3880.
Higgins, C.F., 2001. ABC transporters: physiology, structure and mechanism – an overview. Res. Microbiol. 152 (3–4), 205–210.
Higgins, C.F., 2007. Multiple molecular mechanisms for multidrug resistance transporters. Nature 446 (7137), 749–757.
Higgins, C.F., Linton, K.J., 2004. The ATP switch model for ABC transporters. Nat. Struct. Mol. Biol. 11 (10), 918–926.
Holland, I.B., Blight, M.A., 1999. ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. J. Mol. Biol. 293 (2), 381–399.
Hollenstein, K., Dawson, R.J., Locher, K.P., 2007. Structure and mechanism of ABC transporter proteins. Curr. Opin. Struct. Biol. 17 (4), 412–418.
Holmes, A.R., Lin, Y.H., Niimi, K., Lamping, E., Keniya, M., et al., 2008. ABC transporter Cdr1p contributes more than Cdr2p does to fluconazole efflux in fluconazole-resistant *Candida albicans* clinical isolates. Antimicrob. Agents Chemother. 52 (11), 3851–3862.
Holmes, A.R., Tsao, S., Ong, S.W., Lamping, E., Niimi, K., et al., 2006. Heterozygosity and functional allelic variation in the *Candida albicans* efflux pump genes *CDR1* and *CDR2*. Mol. Microbiol. 62 (1), 170–186.
Izumikawa, K., Kakeya, H., Tsai, H.F., Grimberg, B., Bennett, J.E., 2003. Function of *Candida glabrata* ABC transporter gene, *PDH1*. Yeast (Chichester, England) 20 (3), 249–261.
James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., et al., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443 (7113), 818–822.
Jasinski, M., Ducos, E., Martinoia, E., Boutry, M., 2003. The ATP-binding cassette transporters: structure, function, and gene family comparison between rice and *Arabidopsis*. Plant Physiol. 131 (3), 1169–1177.
Jha, S., Karnani, N., Lynn, A.M., Prasad, R., 2003a. Covalent modification of cysteine 193 impairs ATPase function of nucleotide-binding domain of a *Candida* drug efflux pump. Biochem. Biophys. Res. Commun. 310 (3), 869–875.

Jha, S., Karnani, N., Dhar, S.K., Mukhopadhayay, K., Shukla, S., et al., 2003b. Purification and characterization of the N-terminal nucleotide binding domain of an ABC drug transporter of *Candida albicans*: uncommon cysteine 193 of Walker A is critical for ATP hydrolysis. Biochemistry 42 (36), 10822–10832.

Jones, P.M., George, A.M., 2004. The ABC transporter structure and mechanism: perspectives on recent research. Cell. Mol. Life Sci. 61 (6), 682–699.

Jones, P.M., O'Mara, M.L., George, A.M., 2009. ABC transporters: a riddle wrapped in a mystery inside an enigma. Trends Biochem. Sci. 34 (10), 520–531.

Kanafani, Z.A., Perfect, J.R., 2008. Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact. Clin. Infect. Dis. 46 (1), 120–128.

Katiyar, S.K., Edlind, T.D., 2001. Identification and expression of multidrug resistance-related ABC transporter genes in *Candida krusei*. Med. Mycol. 39 (1), 109–116.

Kolaczkowski, M., Kolaczowska, A., Luczynski, J., Witek, S., Goffeau, A., 1998. In vivo characterization of the drug resistance profile of the major ABC transporters and other components of the yeast pleiotropic drug resistance network. Microb. Drug Resist. 4 (3), 143–158.

Kontoyiannis, D.P., Lewis, R.E., 2002. Antifungal drug resistance of pathogenic fungi. Lancet 359 (9312), 1135–1144.

Kontoyiannis, D.P., Bodey, G.P., 2002. Invasive aspergillosis in 2002: an update. Eur. J. Clin. Microbiol. Infect. Dis. 21 (3), 161–172.

Kontoyiannis, D.P., Vaziri, I., Hanna, H.A., Boktour, M., Thornby, J., et al., 2001. Risk factors for *Candida tropicalis* fungemia in patients with cancer. Clin. Infect. Dis. 33 (10), 1676–1681.

Kontoyiannis, D.P., Lionakis, M.S., Lewis, R.E., Chamilos, G., Healy, M., et al., 2005. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. J. Infect. Dis. 191 (8), 1350–1360.

Kralli, A., Yamamoto, K.R., 1996. An FK506-sensitive transporter selectively decreases intracellular levels and potency of steroid hormones. J. Biol. Chem. 271 (29), 17152–17156.

Kuhn, D.M., Chandra, J., Mukherjee, P.K., Ghannoum, M.A., 2002. Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. Infect. Immun. 70 (2), 878–888.

Lai, C.C., Tan, C.K., Huang, Y.T., Shao, P.L., Hsueh, P.R., 2008. Current challenges in the management of invasive fungal infections. J. Infect. Chemother. 14 (2), 77–85.

Lamping, E., Ranchod, A., Nakamura, K., Tyndall, J.D., Niimi, K., et al., 2009. Abc1p is a multidrug efflux transporter that tips the balance in favor of innate azole resistance in *Candida krusei*. Antimicrob. Agents Chemother. 53 (2), 354–369.

Lamping, E., Monk, B.C., Niimi, K., Holmes, A.R., Tsao, S., et al., 2007. Characterization of three classes of membrane proteins involved in fungal azole resistance by functional hyperexpression in *Saccharomyces cerevisiae*. Eukaryot. Cell 6 (7), 1150–1165.

Li, Y., Prinz, W.A., 2004. ATP-binding cassette (ABC) transporters mediate nonvesicular, raft-modulated sterol movement from the plasma membrane to the endoplasmic reticulum. J. Biol. Chem. 279 (43), 45226–45234.

Lin, X., Heitman, J., 2006. The biology of the *Cryptococcus neoformans* species complex. Annu. Rev. Microbiol. 60, 69–105.

Linton, K.J., Higgins, C.F., 1998. The *Escherichia coli* ATP-binding cassette (ABC) proteins. Mol. Microbiol. 28 (1), 5–13.

Locher, K.P., Lee, A.T., Rees, D.C., 2002. The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. Science 296 (5570), 1091–1098.

Loftus, B.J., Fung, E., Roncaglia, P., Rowley, D., Amedeo, P., et al., 2005. The genome of the basidiomycetous yeast and human pathogen *Cryptococcus neoformans*. Science 307 (5713), 1321–1324.

Maebashi, K., Niimi, M., Kudoh, M., Fischer, F.J., Makimura, K., et al., 2001. Mechanisms of fluconazole resistance in *Candida albicans* isolates from Japanese AIDS patients. J. Antimicrob. Chemother. 47 (5), 527–536.

Mellado, E., Diaz-Guerra, T.M., Cuenca-Estrella, M., Rodriguez-Tudela, J.L., 2001. Identification of two different 14-alpha sterol demethylase-related genes (*cyp51A* and *cyp51B*) in *Aspergillus fumigatus* and other *Aspergillus* species. J. Clin. Microbiol. 39 (7), 2431–2438.

Mellado, E., Garcia-Effron, G., Buitrago, M.J., Alcazar-Fuoli, L., Cuenca-Estrella, M., et al., 2005. Targeted gene disruption of the 14-alpha sterol demethylase (*cyp51A*) in *Aspergillus fumigatus* and its role in azole drug susceptibility. Antimicrob. Agents Chemother. 49 (6), 2536–2538.

Miyazaki, H., Miyazaki, Y., Geber, A., Parkinson, T., Hitchcock, C., et al., 1998. Fluconazole resistance associated with drug efflux and increased transcription of a drug transporter gene, *PDH1*, in *Candida glabrata*. Antimicrob. Agents Chemother. 42 (7), 1695–1701.

Monk, B.C., Goffeau, A., 2008. Outwitting multidrug resistance to antifungals. Science 321 (5887), 367–369.

Muth, T.R., Schuldiner, S., 2000. A membrane-embedded glutamate is required for ligand binding to the multidrug transporter EmrE. EMBO J. 19 (2), 234–240.

Nakamura, T., Liu, Y., Hirata, D., Namba, H., Harada, S., et al., 1993. Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. EMBO J. 12 (11), 4063–4071.

Nakayama, H., Tanabe, K., Bard, M., Hodgson, W., Wu, S., et al., 2007. The *Candida glabrata* putative sterol transporter gene *CgAUS1* protects cells against azoles in the presence of serum. J. Antimicrob. Chemother. 60 (6), 1264–1272.

Nascimento, A.M., Goldman, G.H., Park, S., Marras, S.A., Delmas, G., et al., 2003. Multiple resistance mechanisms among *Aspergillus fumigatus* mutants with high-level resistance to itraconazole. Antimicrob. Agents Chemother. 47 (5), 1719–1726.

Nierman, W.C., Pain, A., Anderson, M.J., Wortman, J.R., Kim, H.S., et al., 2005. Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*. Nature 438 (7071), 1151–1156.

Odds, F.C., 1979. *Candida* and Candidosis. Leicester University Press, Leicester. 382 p.

Odds, F.C., Brown, A.J., Gow, N.A., 2003. Antifungal agents: mechanisms of action. Trends Microbiol. 11 (6), 272–279.

Pasqualotto, A.C., Denning, D.W., 2008. New and emerging treatments for fungal infections. J. Antimicrob. Chemother. 61 (Suppl. 1), i19–i30.

Perea, S., Lopez-Ribot, J.L., Kirkpatrick, W.R., McAtee, R.K., Santillan, R.A., et al., 2001. Prevalence of molecular mechanisms of resistance to azole antifungal agents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. 45 (10), 2676–2684.

Pfaller, M.A., Diekema, D.J., 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. 20 (1), 133–163.

Pfaller, M.A., Pappas, P.G., Wingard, J.R., 2006. Invasive fungal pathogens: current epidemiological trends. Clin. Infect. Dis. 43, S3–14.

Pfaller, M.A., Diekema, D.J., Gibbs, D.L., Newell, V.A., Nagy, E., et al., 2008. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. J. Clin. Microbiol. 46 (2), : 515–521.

Posteraro, B., Sanguinetti, M., Sanglard, D., La Sorda, M., Boccia, S., et al., 2003. Identification and characterization of a *Cryptococcus neoformans* ATP binding cassette (ABC) transporter-encoding gene, *CnAFR1*, involved in the resistance to fluconazole. Mol. Microbiol. 47 (2), 357–371.

Prasad, R., Gaur, N.A., Gaur, M., Komath, S.S., 2006. Efflux pumps in drug resistance of *Candida*. Infect. Disord. Drug Targets 6 (2), 69–83.

Purnelle, B., Skala, J., Goffeau, A., 1991. The product of the *YCR105* gene located on the chromosome III from *Saccharomyces cerevisiae* presents homologies to ATP-dependent permeases. Yeast 7 (8), 867–872.

Rai, V., Shukla, S., Jha, S., Komath, S.S., Prasad, R., 2005. Functional characterization of N-terminal nucleotide binding domain (NBD-1) of a major ABC drug transporter Cdr1p of *Candida albicans*: uncommon but conserved Trp326 of Walker B is important for ATP binding. Biochemistry 44 (17), 6650–6661.

Rees, D.C., Johnson, E., Lewinson, O., 2009. ABC transporters: the power to change. Nat. Rev. Mol. Cell Biol. 10 (3), 218–227.

Rogers, P.D., Barker, K.S., 2003. Genome-wide expression profile analysis reveals coordinately regulated genes associated with stepwise acquisition of azole resistance in *Candida albicans* clinical isolates. Antimicrob. Agents Chemother. 47 (4), 1220–1227.

Rost, B., Yachdav, G., Liu, J., 2004. The predictprotein server. Nucleic Acids Res. 32 (Web Server issue), W321–W326.

Saag, M.S., Graybill, R.J., Larsen, R.A., Pappas, P.G., Perfect, J.R., et al., 2000. Practice guidelines for the management of cryptococcal disease. Infectious diseases society of America. Clin. Infect. Dis. 30 (4), 710–718.

Saini, P., Prasad, T., Gaur, N.A., Shukla, S., Jha, S., et al., 2005. Alanine scanning of transmembrane helix 11 of Cdr1p ABC antifungal efflux pump of *Candida albicans*: identification of amino acid residues critical for drug efflux. J. Antimicrob. Chemother. 56 (1), 77–86.

Sanglard, D., Bille, J., 2002. Current understanding of the modes of action of and resistance mechanisms to conventional and emerging antifungal agents for treatment of *Candida* infections. In: Calderone, R.A. (Ed.), *Candida* and Candidiasis. ASM Press, Washington, DC, pp. 349–383.

Sanglard, D., Ischer, F., Bille, J., 2001. Role of ATP-binding-cassette transporter genes in high-frequency acquisition of resistance to azole antifungals in *Candida glabrata*. Antimicrob. Agents Chemother. 45 (4), 1174–1183.

Sanglard, D., Ischer, F., Calabrese, D., Majcherczyk, P.A., Bille, J., 1999. The ATP binding cassette transporter gene *CgCDR1* from *Candida glabrata* is involved in the resistance of clinical isolates to azole antifungal agents. Antimicrob. Agents Chemother. 43 (11), 2753–2765.

Sanglard, D., Kuchler, K., Ischer, F., Pagani, J.L., Monod, M., et al., 1995. Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. Antimicrob. Agents Chemother. 39 (11), 2378–2386.

Sanguinetti, M., Posteraro, B., La Sorda, M., Torelli, R., Fiori, B., et al., 2006. Role of *AFR1*, an ABC transporter-encoding gene, in the in vivo response to fluconazole and virulence of *Cryptococcus neoformans*. Infect. Immun. 74 (2), 1352–1359.

Sarvikivi, E., Lyytikainen, O., Soll, D.R., Pujol, C., Pfaller, M.A., et al., 2005. Emergence of fluconazole resistance in a *Candida parapsilosis* strain that caused infections in a neonatal intensive care unit. J. Clin. Microbiol. 43 (6), 2729–2735.

Sauna, Z.E., Bohn, S.S., Rutledge, R., Dougherty, M.P., Cronin, S., et al., 2008. Mutations define cross-talk between the N-terminal nucleotide-binding domain and transmembrane helix-2 of the yeast multidrug transporter Pdr5: possible conservation of a signaling interface for coupling ATP hydrolysis to drug transport. J. Biol. Chem. 283 (50), 35010–35022.

Saurin, W., Hofnung, M., Dassa, E., 1999. Getting in or out: early segregation between importers and exporters in the evolution of ATP-binding cassette (ABC) transporters. J. Mol. Evol. 48 (1), 22–41.

Schneider, E., Hunke, S., 1998. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. FEMS Microbiol. Rev. 22 (1), 1–20.

Schumacher, M.A., Brennan, R.G., 2003. Deciphering the molecular basis of multidrug recognition: crystal structures of the *Staphylococcus aureus* multidrug binding transcription regulator QacR. Res. Microbiol. 154 (2), 69–77.

Shahi, P., Moye-Rowley, W.S., 2009. Coordinate control of lipid composition and drug transport activities is required for normal multidrug resistance in fungi. Biochim. Biophys. Acta 1794 (5), 852–859.

Shao, P.L., Huang, L.M., Hsueh, P.R., 2007. Recent advances and challenges in the treatment of invasive fungal infections. Int. J. Antimicrob. Agents 30 (6), 487–495.

Shukla, S., Saini, P., Smriti, Jha, S., Ambudkar, S.V., et al., 2003. Functional characterization of *Candida albicans* ABC transporter Cdr1p. Eukaryot. Cell 2 (6), 1361–1375.

Silveira, F.P., Husain, S., 2007. Fungal infections in solid organ transplantation. Med. Mycol. 45 (4), 305–320.

Singh, N., Paterson, D.L., 2005. *Aspergillus* infections in transplant recipients. Clin. Microbiol. Rev. 18 (1), 44–69.

Sobel, J.D., 2007. Vulvovaginal candidosis. Lancet 369 (9577), 1961–1971.

Strausbaugh, L.J., Sewell, D.L., Ward, T.T., Pfaller, M.A., Heitzman, T., et al., 1994. High frequency of yeast carriage on hands of hospital personnel. J. Clin. Microbiol. 32 (9), 2299–2300.

Taglicht, D., Michaelis, S., 1998. *Saccharomyces cerevisiae* ABC proteins and their relevance to human health and disease. Methods Enzymol. 292, 130–162.

Tanaka, A.R., Ikeda, Y., Abe-Dohmae, S., Arakawa, R., Sadanami, K., et al., 2001. Human ABCA1 contains a large amino-terminal extracellular domain homologous to an epitope of Sjogren's syndrome. Biochem. Biophys. Res. Commun. 283 (5), 1019–1025.

Thornewell, S.J., Peery, R.B., Skatrud, P.L., 1997. Cloning and characterization of *CneMDR1*: a *Cryptococcus neoformans* gene encoding a protein related to multidrug resistance proteins. Gene 201 (1–2), 21–29.

Tsao, S., Rahkhoodaee, F., Raymond, M., 2009. Relative contributions of the *Candida albicans* ABC transporters Cdr1p and Cdr2p to clinical azole resistance. Antimicrob. Agents Chemother. 53 (4), 1344–1352.

Tusnady, G.E., Sarkadi, B., Simon, I., Varadi, A., 2006. Membrane topology of human ABC proteins. FEBS Lett. 580 (4), 1017–1022.

Tutulan-Cunita, A.C., Mikoshi, M., Mizunuma, M., Hirata, D., Miyakawa, T., 2005. Mutational analysis of the yeast multidrug resistance ABC transporter Pdr5p with altered drug specificity. Genes Cells 10 (5), 409–420.

van den Brule, S., Smart, C.C., 2002. The plant PDR family of ABC transporters. Planta 216 (1), 95–106.

Verrier, P.J., Bird, D., Burla, B., Dassa, E., Forestier, C., et al., 2008. Plant ABC proteins – a unified nomenclature and updated inventory. Trends Plant Sci. 13 (4), 151–159.

von Heijne, G., 1995. Membrane protein assembly: rules of the game. Bioessays 17 (1), 25–30.

Wada, S., Tanabe, K., Yamazaki, A., Niimi, M., Uehara, Y., et al., 2005. Phosphorylation of *Candida glabrata* ATP-binding cassette transporter Cdr1p regulates drug efflux activity and ATPase stability. J. Biol. Chem. 280 (1), 94–103.

Wang, N., Silver, D.L., Costet, P., Tall, A.R., 2000. Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. J. Biol. Chem. 275 (42), 33053–33058.

White, T.C., 1997. Increased mRNA levels of *ERG16*, *CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob. Agents Chemother. 41 (7), 1482–1487.

White, T.C., Marr, K.A., Bowden, R.A., 1998. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin. Microbiol. Rev. 11 (2), 382–402.

Wilson, J., 1998. The aetiology, diagnosis and management of denture stomatitis. Br. Dent. J. 185 (8), : 380–384.

Wingard, J.R., 1995. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin. Infect. Dis. 20 (1), : 115–125.

Wisplinghoff, H., Bischoff, T., Tallent, S.M., Seifert, H., Wenzel, R.P., et al., 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39 (3), 309–317.