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Review Article

Membrane homoeostasis and multidrug resistance in yeast

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Synopsis

The development of MDR (multidrug resistance) in yeast is due to a number of mechanisms. The most documented mechanism is enhanced extrusion of drugs mediated by efflux pump proteins belonging to either the ABC (ATP-binding cassette) superfamily or MFS (major facilitator superfamily). These drug-efflux pump proteins are localized on the plasma membrane, and the milieu therein affects their proper functioning. Several recent studies demonstrate that fluctuations in membrane lipid composition affect the localization and proper functioning of the MDR efflux pump proteins. Interestingly, the efflux pumps of the ABC superfamily are particularly susceptible to imbalances in membrane-raft lipid constituents. This review focuses on the importance of the membrane environment in functioning of the drug-efflux pumps and explores a correlation between MDR and membrane lipid homoeostasis.

Key words: *Candida*, ergosterol, lipid raft, multidrug resistance (MDR), sphingolipid, transporter

INTRODUCTION

In both pathogenic (*Candida* sp., *Aspergillus* and *Cryptococcus*) and non-pathogenic (*Saccharomyces cerevisiae*) yeast, several mechanisms can contribute to the development of MDR (multidrug resistance). Some of the most common strategies employed by drug-resistant yeast include mutation or overexpression of the drug target, decrease in the import of drugs and enhanced efflux of drugs etc. [1–3]. Extrusion of noxious compounds from the cell by efflux pumps is one of the most frequently used strategies for the development of drug resistance in yeast, and it holds true for several others [4–6].

Efflux pump proteins of the ABC (ATP-binding cassette) superfamily and MFS (major facilitator superfamily) of transporters are common exporters of structurally unrelated drugs. The ABC transporters use the energy derived from ATP hydrolysis to power the efflux, whereas the MFS transporters make use of proton gradient across the plasma membrane for the extrusion of drugs. Genomic analyses of *S. cerevisiae* and of the pathogenic yeast *Candida albicans* reveal the existence of 30 and 28 putative ABC transporters respectively, of which only a few have been found to function as drug transporters [7,8].

In cancer cell lines, the overexpression of human Pgp (P-glycoprotein)*/MDR1*, a homologue of yeast ABC proteins, is invariably associated with the development of drug resistance, which has been the major cause of failure of cancer chemotherapy [4]. Interestingly, human Pgp/*MDR1* is predominantly localized in plasma-membrane domains enriched in cholesterol (mammalian sterol), and the depletion of cholesterol impairs drug transport in a substrate- and cell-type-specific manner [9]. It has also been observed that human Pgp/*MDR1* contributes to stabilizing the cholesterol-rich microdomains by mediating cholesterol redistribution within the cell membrane [10]. It has been suggested that the activities of the yeast efflux pumps, particularly those that belong to the ABC superfamily, are also influenced by subtle modifications in membrane lipid composition [11–13]. For example, fluctuations in sterol level, particularly, result in destabilization of the membrane and a decrease in drug resistance of yeast cells [11–13]. These observations acquire significance with the reported existence, within the lipid bilayer, of discrete microdomains in yeast membranes (lipid rafts), predominantly composed of sphingolipids and sterols [14,15]. Of note, the acquisition of the MDR phenotype in certain mammalian cell lines also is accompanied by an up-regulation of genes encoding proteins and the metabolism of lipids that constitute membrane

Abbreviations used: ABC, ATP-binding cassette; Ca, *Candida albicans*; FLR/FLU, fluconazole resistance; *HSP12*, heat-shock protein 12; IPC, inositol phosphoceramide; IPT, inositol phosphotransferase; LCB, long-chain base; LCBP, LCB phosphate; $M(IP)_2C$, mannosyl bi-inositol diphosphoceramide; MDR, multidrug resistance; MFS, major facilitator superfamily; MIPC, mannosyl IPC; NBD, nucleotide-binding domain; NDT, non-dityrosine; PDR, pleiotropic drug resistance; PDRE, pleiotropic drug-response element; Pgp, P-glycoprotein; RSB, resistance to sphingoid base; RTA, resistance to 7-aminocholesterol; SNQ, sensitivity to 4-nitroquinoline-*N*-oxide; Tac, transcription activator of Cdr genes; TF, transcription factor; TMD, transmembrane domain; TMS, transmembrane-spanning segment; Upc, uptake control; YAP1, yeast activator protein 1; YOR, yeast oligomycin resistance; YRR, yeast reveromycin resistance.

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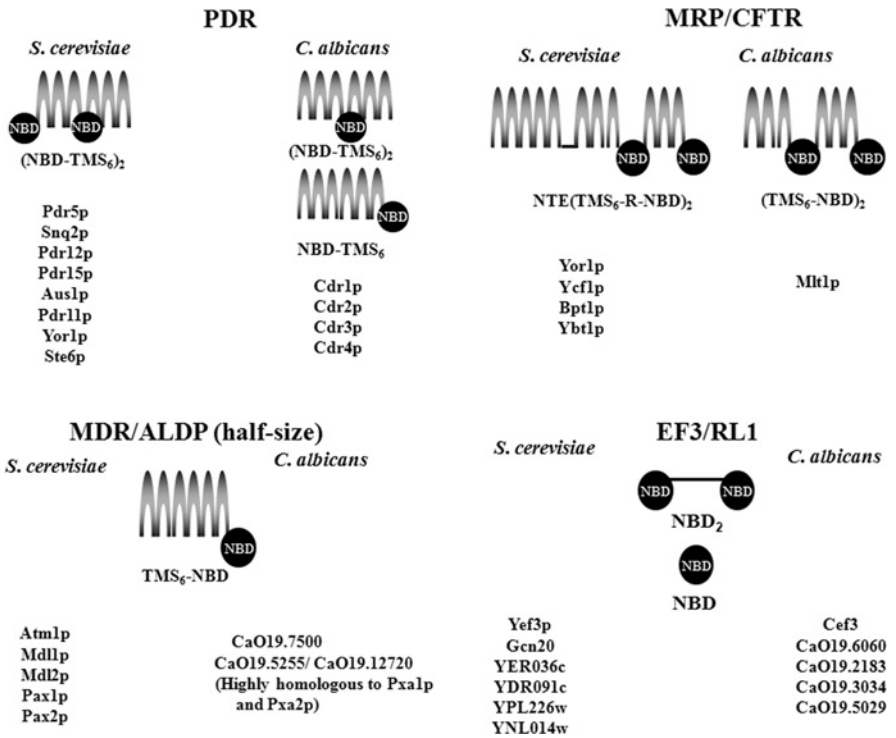

Figure 1 Predicted topology of the yeast ABC proteins
The various ABC proteins are divided into subfamilies. Full transporters have 12 TMSs and two NBDs. Half transporters have six TMSs and a single NBD. The NBDs are the ATP-binding domains comprising Walker A, signature sequence and Walker B motifs, in the order mentioned. Key proteins from both *S. cerevisiae* and *C. albicans* are listed in respective panels (for more details, see references [7,8]). ALDP, adrenoleukodystrophy protein; CFTR, cystic fibrosis transmembrane conductance regulator; EF3/RL1, elongation factor 3/RNase L inhibitor.

rafts and caveolar membranes [16]. Coupled together, it appears that membrane sphingolipids and sterols, both individually and through their mutual interactions, can critically affect the functioning of drug-efflux pump proteins. This review focuses on the roles of these important membrane microdomain lipids in influencing the function of drug-efflux pumps in yeast. The discussion on the role of raft lipids is preceded by a short account of ABC and MFS drug-transporter proteins in yeast.

YEAST MULTIDRUG TRANSPORTERS

ABC multidrug-efflux proteins

Yeast ABC proteins are generally made up of two TMDs (transmembrane domains) and two cytoplasmically located NBDs (nucleotide-binding domains), although putative 'half-proteins', which probably dimerize to become fully functional, are also known to exist in yeast [7,8]. These half-protein transporters in some prokaryotes are known to function as MDR pumps (Figure 1) [17,18]. Pdr5p, Snq2p (sensitivity to 4-nitroquinoline-*N*-oxide 2) and Yor1p (yeast oligomycin resistance 1) are the major full drug transporters in *S. cerevisiae*, whereas Cdr1p and Cdr2p (Pdr5p homologues) contribute to drug resistance in *C. albicans* [19–20d]. Typically, each of the two TMDs of full ABC proteins comprise six transmembrane-spanning segments (TMSs) that are preceded or followed by the NBDs (NBD–TMS2) or TMS–NBD2 (Figure 1). Although it appears that several TMSs come together to form the substrate-binding site(s), this alone does not appear to be sufficient for drug transport across the membrane bilayer. Given their varied roles and the different structural characteristics of substrates that members of ABC superfamily appear to efflux, it is hardly surprising that, despite an overall conservation of the domain architecture of TMDs, their primary sequences are significantly different. On the other hand, the NBDs of ABC transporters, which power drug transport, are highly conserved, both in terms of primary structure and architecture (Figure 2) [21]. TMDs and NBDs, together, form the minimal functional unit necessary for substrate transport. Unlike most other ABC transporters, the NBDs of fungal transporters have unique positioning of typical, but critical, amino-acid residues within the conserved N-terminal NBD domain, such as in the Walker A and Walker B motifs (Figure 2). On the other hand, the C-terminal NBD of fungal ABC transporters have fully conserved motifs, which are essentially identical to motifs in non-fungal transporters [22]. The structural and functional analyses of human Pgp/MDR1 and its other homologues have demonstrated the importance of NBDs and TMDs in drug extrusion [23]. In

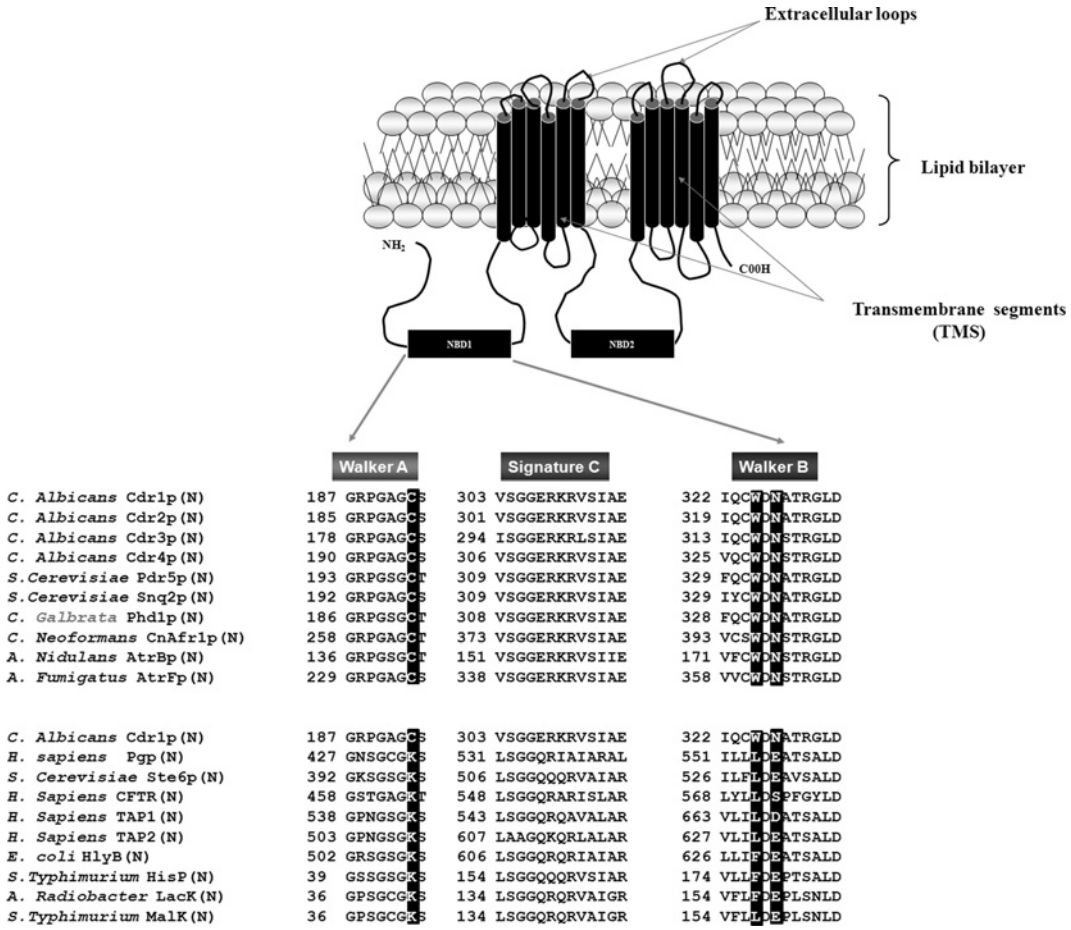

Figure 2 **Structural organization of the CaCdr1p of ABC superfamily in *C. albicans***
The cartoon (top) shows the presence of 12 TMSs and two NBDs for Cdr1p. The conserved Walker A, Walker B and signature sequences are enlarged and aligned with conserved sequences of other fungal and non-fungal ABC transporters to show the degree of similarity and unique placement of certain amino acids. CFTR, cystic fibrosis transmembrane conductance regulator; N, N-terminal. *A. Fumigatus, Aspergillus fumigatus*; *A. Nidulans*, *Aspergillus nidulans*; *A. Radiobacter*, *Agrobacterium radiobacter*; *C. Galbrata*, *Candida glabrata*; *C. Neoformans*, *Cryptococcus neoformans* ; *E. coli*, *Escherichia coli*; *H. Sapiens*, *Homo sapiens*; *S. Typhimurium*, *Salmonella typhimurium*.

comparison, studies pertaining to the identification of the molecular determinants of yeast ABC drug transporters have only been initiated recently [22,24].

MFS multidrug-efflux proteins

The MFS was originally defined as a superfamily of permeases that are characterized by two structural units of six TMS–α-helical segments, which are linked by a cytoplasmic loop. It consists of evolutionary conserved membrane transport proteins involved in the symport, antiport or uniport of various substrates [25–29]. One major cluster of this family consists of the PMF (proton motive force)-dependent drug-efflux proteins and some substrate-specific drug-efflux proteins, such as the well-studied tetracycline exporter, TetB [30–33]. The phylogenic analyses of MFS drug exporters show that its members possess either 12 or 14 TMSs [34].

MFS proteins also function as major drug transporters, which are involved in drug efflux, thus contributing to MDR in yeast. Similar to the ABC protein superfamily, very few members of the MFS family are drug exporters. For example, in a total of 62 proteins, only *FLR1* in *S. cerevisiae* (fluconazole resistance) has been shown to confer resistance to fluconazole, 4-NQO (4-nitroquinoline-*N*-oxide), cycloheximide, benomyl, methotrexate, cerulenin and diazaborine etc. [35]. Also, in the pathogenic *C. albicans*, from a total of 71 MFS proteins, only CaMDR1 (*C. albicans* MDR1) is known to extrude drugs, where its overexpression has been linked to azole resistance in clinical isolates [36–39]. *FLU1* (fluconazole resistance), another member of the MFS of *C. albicans*, was cloned by functional complementation of a fluconazole-sensitive strain of *S. cerevisiae*. However, *FLU1* is not involved in the development of fluconazole resistance in clinical isolates of *C. albicans*. Interestingly, studies revealed that

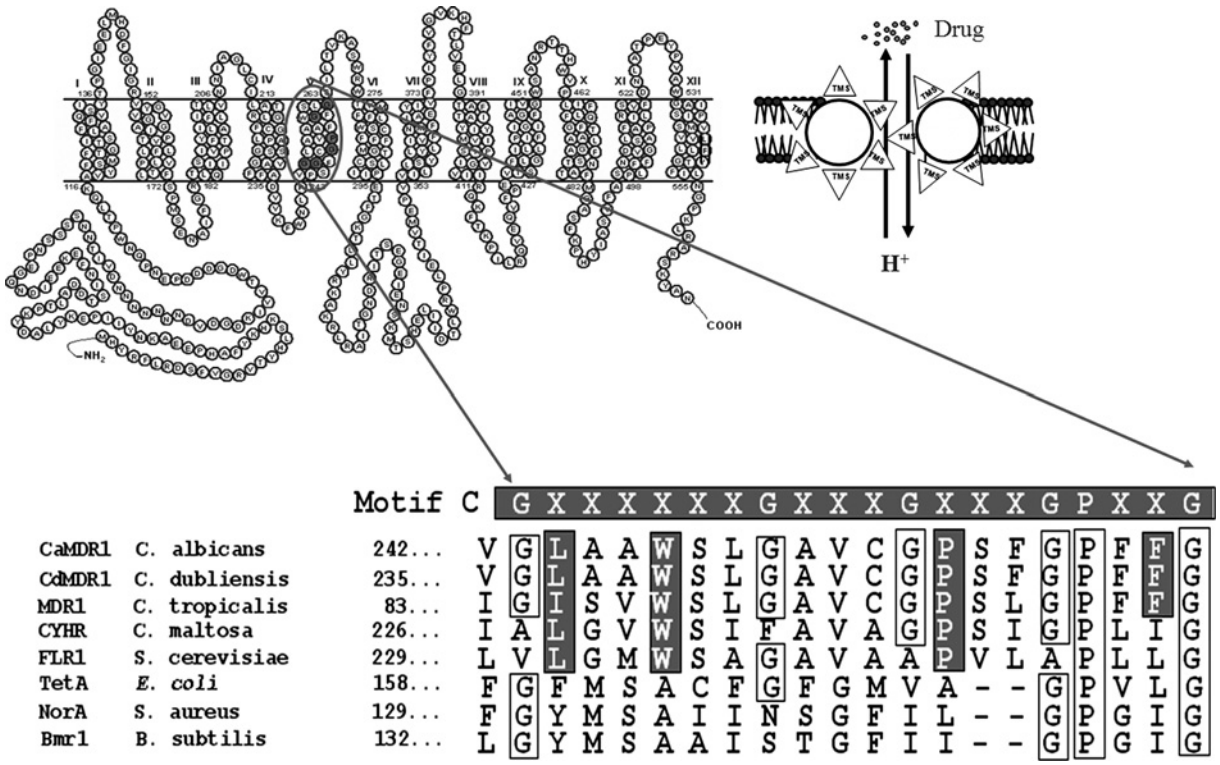

Figure 3 **Structural organization of the CaMdr1p of MFS superfamily in *C. albicans***
The 'antiporter motif' or 'motif C' in the transmembrane helix V is enlarged and aligned with other drug transporters to show the degrees of similarity. Right-hand panel: antiporter activity of CaMdr1p. B. subtilis, *Bacillus subtilis*; C. dubliensis, *Candida dubliensis*; C. maltosa, *Candida maltosa*; C. tropicalis, *Candida tropicalis*; E. coil, *Escherichia coli*; S. aureus, *Staphylococcus aureus*.

the preferred substrate of Flu1p is mycophenolic acid, rather than fluconazole [40].

The antiporter motif in the predicted TMS5 of MFS transporters is well conserved in all of the functionally related subgroups of bacteria and plants [29,34,41]. MFS drug exporters in yeast also possess this motif. Alanine scanning of all the 21 amino acids of TMS5 of CaMdr1p has recently highlighted the residues that probably contribute to the efflux activity of CaMdr1p [38] (Figure 3). Additionally, there are many conserved motifs present within TMDs of MFS proteins which may contribute to drug specificities [34]. Multiple-sequence analyses of the MFS proteins suggest that proteins within this family share greater similarity between their N-terminal domains than in their C-terminal domains, which is consistent with the hypothesis that the latter domain is responsible for substrate recognition [34].

MDR AND LIPIDS

On the basis of several studies, a close interaction between membrane lipids and drug-extrusion pump proteins has been recognized [11–13,42]. On the one hand, it has been observed that the ABC drug-efflux proteins in human (Pgp/*MDR1*) and yeast (Pdr5p and Yor1p in *S. cerevisiae*, and CaCdr1p and CaCdr2p in *C. albicans*) can translocate phospholipids between the two monolayers of the plasma membrane, whereas, on the other hand, human Pgp/*MDR1* is also known to participate in sterol homoeostasis [43,44]. Additionally, these drug-extrusion pumps are found to be particularly sensitive to the nature and the physical state of the surrounding lipids [11–13,42]. For example, both CaCdr1p and Pdr5p are sensitive to fluctuations in the lipid environment where selective functions mediated by these drug-extrusion pumps are affected [11,13,34].

Ergosterol levels affect drug susceptibilities

Various studies employing *erg* (enzymes involved in ergosterol biosynthesis) mutants of *S. cerevisiae* and *C. albicans* have revealed a close relationship between MDR and sterol levels of the plasma membrane [13,42]. In azole-resistant yeast, a decreased level of ergosterol and increased membrane fluidity is commonly observed [45]. Further investigations confirmed a correlation between drug susceptibility and an altered membrane composition [11,12,42]. The increased drug susceptibility of the *erg* mutants was attributed to an increased membrane fluidity, which leads to an enhanced passive diffusion of drug molecules across the plasma membrane [11,42,47]. The lack of ergosterol in the *erg* mutants could also destabilize or disrupt the ergosterol-rich microdomains, thus affecting membrane organization and

function of drug-export proteins that are preferentially localized within these domains [11,12].

Sphingolipid levels affect drug susceptibilities

The early steps in mammalian and fungal sphingolipid synthesis are conserved, but they diverge later in the pathway to produce structurally and chemically different types of sphingoid bases, ceramides and complex sphingolipids [49,50]. Therefore, over the years, the sphingolipid biosynthetic pathway has been exploited as an antifungal drug target in pathogenic yeast [51,52]. Unlike mammals, fungi do not have phosphatidylcholine as part of their polar head group in sphingolipids; instead they have phosphoinositol which is transferred on to ceramide to make IPC (inositol phosphoceramide) [53,54]. IPC is further modified by the addition of mannose to produce MIPC (mannosyl IPC), and the addition of a second inositol phosphate to make $M(IP)_2C$ (mannosyl bi-inositol diphosphoceramide) [53,55]. Although the synthesis of these sphingolipids is critical to maintain normal plasma-membrane function, synthesis of $M(IP)_2C$ is not critical for viability [55]. In *S. cerevisiae*, the *IPT1* (inositol phosphotransferase 1) deletion mutant grows normally, but displays sensitivity to calcium and increased resistance to the polyene antibiotic nystatin [55]. Hallstrom et al. [56] observed that loss of *IPT1* has complex effects on drug resistance in *S. cerevisiae*, mediated through Pdr1p and Pdr3p TFs (transcription factors), which regulate MDR genes in *S. cerevisiae* (see below). In order to explore the role of sphingolipids, a specific inhibitor fumonisin B1, which blocks the synthesis of phytoceramide, a precursor for the three major sphingolipid species was employed [11]. The study revealed a close interaction between plasma membrane ergosterol and sphingolipids in *C. albicans* cells [11]. The depletion of either of the two impaired the function of a major drug-efflux pump Cdr1p, which resulted in the *Candida* cells becoming hypersensitive to several drugs. In another study, when sphingolipid synthesis was specifically blocked by homozygous disruption of the *CaIPT1* gene, it led to decrease in drug resistance due to altered sphingolipid composition [11]. Besides this, *ipt1* mutants of *C. albicans* were unable to form hyphae. The effect of MIPC accumulation and absence of $M(IP)_2C$ in *ipt1* mutants on efflux of drug substrates was very selective in terms of the effects on different efflux pump proteins. For example, in comparison with the efflux of fluconazole, a substrate of CaCdr1p, the efflux of methotrexate, a specific substrate of MFS CaMdr1p, remained unaffected in *ipt1* mutant cells. Taken together, it appears that similar to ergosterol levels, altered sphingolipid composition, both of which are among the major constituents of membrane rafts, affect drug susceptibilities and morphogenesis in *C. albicans.*

ERGOSTEROL AND SPHINGOLIPIDS ARE MEMBRANE-RAFT CONSTITUENTS

Yeast sphingolipids and ergosterol are important components of distinct membrane lipid domains known as rafts [14,57,58]. In several organisms, various proteins with diverse functions in cellular processes, such as signal transduction, membrane trafficking, lipid and protein sorting, and even receptors for certain pathogens, 'home' to the rafts [60,61]. The presence of rafts offers a platform for the cell to allow interactions between different partners of metabolic cascades, ensuring their efficiency. The existence of rafts acquired significance in the area of MDR due to the finding that 24–40% of human Pgp*/MDR1* is present in these detergent-resistant plasma-membrane domains [62]. The presence of lipid rafts has been associated with hyphal growth in *C. albicans* [14]. In yeast, the mating tips projection 'shmoos' are enriched in these microdomains [63,64]. The presence of these domains in the shmoos is probably required for specific interactions between various mating partners or with any other process where cell–cell fusion is necessary [64]. The following part of this review highlights evidence that supports a direct relationship between membrane-raft constituents and MDR, preceded by a short overview of regulation of MDR genes in yeast.

REGULATION OF MDR

The transcriptional activation of MDR genes, leading to overexpression of drug-efflux pumps in the development of azole resistance, is well known in pathogenic yeast [65,66]. However, the mechanisms by which the expression of the MDR genes is altered in clinical azole-resistant *Candida* isolates are not fully understood. On the other hand, the mechanisms underlying the up-regulation of MDR genes in the development of MDR are relatively well described in *S. cerevisiae*, wherein three networks of *trans*-acting factors, such as PDR (pleiotropic drug resistance), YAP1 (yeast activator protein 1)-like factor and YRR (yeast reveromycin resistance), are mainly involved in controlling the expression of MDR genes [2,67,68].

The TFs *PDR1* and *PDR3* of the zinc-cluster protein family regulate the transcription of genes encoding ABC drug transportes, such as *PDR5* and *SNQ2* [2,67,68]. YAP-like factor of the bZip family of TFs confers resistance to a variety of toxicants. YAP-like factor 1 targets include an MFS-type drug extrusion pump *FLR1* [69] and a glutathione reductase gene, *GLR1*, involved in conferring oxidative tolerance to yeast cells [70]. Additionally, YAP1 also activates *PDR5* expression under stress conditions, such as heat shock [71]. A link between the YAP and PDR networks has also been established in *S. cerevisiae* [72]. Another zinc-finger-containing TF, YRR1 is involved in complex PDR network regulation by directly activating *SNQ2* and *YOR1* [73], both of which are also common targets of Pdr1p, Pdr3p and Yap1p [74]. In addition to *PDR1*, *PDR3*, *YAP1* and *YRR1*, a transcriptional repressor of *PDR*, called *RDR1* (repressor of drug resistance) [75,76], has been shown to bind to the PDRE (pleiotropic drug-response element), a *cis*-acting regulatory element on the *PDR5* promoter [75]. PDREs are also required for the regulatory actions of Pdr1p and Pdr3p. Furthermore, Pdr1p

and Pdr3p have been shown to cross-talk with another TF, Stb5p. Yrr1p, on the other hand, mainly exists as a homodimer. This indicates a complex regulatory circuit that is required for the expression of *PDR* genes in *S. cerevisiae* [77].

As mentioned above, the up-regulation of genes encoding drug-extrusion pumps of either the ABC (*CaCDR1* and *CaCDR2*) or MFS (*CaMDR1*) superfamilies represents one of the most prevalent mechanisms of drug resistance in *Candida* [78,79]. The various possibilities that may affect the expression of *CaCDR1* in azole-resistant clinical isolates of *C. albicans* include mutations in the promoter region (*cis* element) of the gene, altered regulation by *trans*-regulatory factors controlling expression of these genes or molecular changes taking place during mRNA processing [80–83]. In one study, the molecular changes responsible for *CaMDR1* activation in matched fluconazole-sensitive and -resistant isolates were examined by Wirsching et al. [81]. Since sequence analyses of the *CaMDR1* promoter region did not reveal any mutation in the matched pair of fluconazole-sensitive/resistant isolates, it was proposed that the *CaMDR1* promoter was probably activated by a *trans*-regulatory factor(s) that might be mutated in fluconazole-resistant isolates [81]. A mutation in the *trans*-regulatory factor has been reported as the cause for the activation of the *PDR16* gene (phosphatidylinositol inositol transfer protein), which is co-ordinately regulated with *CaCDR1* and *CaCDR2* in clinical isolates of *C. albicans* [80]. A search for homologues of Pdr1p/Pdr3p in *C. albicans* identified regulators that turned out to be negative regulators of genes encoding *CaCDR1* and *CaCDR2*. One such TF, *FCR1* (fluconazole resistance 1), was isolated using the strategy of functional complementation in a *pdr1*/*pdr3* mutant strain of *S. cerevisiae* [84]. Although Fcr1p was able to up-regulate the expression of Pdr5p in *S. cerevisiae* in a manner similar to Pdr1p/Pdr3p, it acted as a negative regulator of CaCdr1p in *C. albicans*. Consistent with this result, its deletion made *C. albicans* cells resistant to fluconazole [84]. This showed that, although the regulators in these two yeast might be orthologues, they have evolved to perform different functions. Another gene responsible for regulating the expression of CaCdr1p is called *CaNDT80* (*Ca* non-dityrosine) [85]. CaNdt80p in *S. cerevisiae* functions as a regulator of meiosis genes. Interestingly, in *C. albicans*, it was shown to up-regulate CaCdr1p expression. Furthermore, a *Candt80*-null mutant was susceptible to antifungals, which was consistent with the abolishment of CaCdr1p expression in this strain. Recently, another novel zinc-finger-containing regulator, Tac1p (transcription activator of Cdr genes 1), was identified as the main positive regulator of CaCdr1p and CaCdr2p [86]. In terms of the drug-response elements that it binds to in the promoters of CaCdr1p and CaCdr2p, it appears to be the closest functional homologue of Pdr1p/ Pdr3p [86]. Hyperactive alleles of Tac1p have been isolated from azole-resistant clinical isolates, indicating that, indeed, a mutation in this TF in clinical isolates enables overexpression of genes of Tac1p targets therein [86]. This probably is one of the mechanisms by which *C. albicans* cells develop azole resistance during prolonged exposure to these antifungals.

In *S. cerevisae* Ecm22 (extracellular mutant 22) and Upc2p (uptake control 2) are known to be involved in the regulation of genes involved in the ergosterol biosynthesis pathway. CaUpc2p, a homologue of *S. cerevisiae* Upc2p, in *C. albicans* has also been shown to regulate ergosterol biosynthetic pathway genes. Interestingly, *C. albicans* cells with *CaUPC2* deleted are susceptible to antifungal therapy [87,88]. This study provides additional evidence of a link between membrane sterols and their effects on function of efflux pumps, leading to sensitivity to antifungals.

MEMBRANE RAFT CONSTITUENTS AND GENES ENCODING DRUG-EFFLUX PUMPS ARE CO-ORDINATELY REGULATED

Microarray analysis has provided an insight into the genes that are co-regulated with the drug-resistance genes [89,90]. This would indicate that these genes, which are co-regulated with *MDR* genes, either share a common function or common regulatory sequences. The most interesting genes co-ordinately regulated with the *MDR* genes are those involved in sphingolipid biosynthesis and in lipid metabolism, which contribute to the formation of the membrane rafts [89,90]. As drugs are not a part of the normal cell mileu, this raises the question as to the normal physiological substrates for these efflux pumps. The fact that drug-efflux pump proteins, such as Pdr5p and Yor1p of *S. cerevisiae* and CaCdr1p and CaCdr2p of *C. albicans*, are also phospholipid translocators suggests that these pumps might co-ordinate the synthesis and transport of the important lipid constituents of the plasma membrane [92]. Superimposed with these facts, the requirement of lipid rafts for the proper membrane localization of the drug-efflux pump proteins further strengthens the possibility of a connection between the raft constituents and *MDR* genes, particularly those encoding drug-extrusion pumps. We have demonstrated recently [93] that CaCdr1p is exclusively localized within membrane rafts, whereas CaMdr1p does not show such selectivity (Figure 4). Additionally, any imbalance in lipid metabolism, particularly with sphingolipid or ergosterol synthesis, leads to selective mislocalization of the ABC transporter CaCdr1p [93].

The first evidence for the existence of such a co-regulation came from the studies performed with cancer cells. In cancer cells, the development of drug resistance involves the up-regulation of human Pgp*/MDR1*, with a simultaneous overexpression of lipid and protein constituents that are required for the formation of lipid rafts and caveolar membranes [16]. Typical caveolae are defined as 50–100 nm non-clathrin-coated invaginations of the plasma membrane, which are characterized by the presence of an integral membrane protein called caveolin. Drug-resistant cell lines have high levels of caveolin, as compared with drug-sensitive cell lines [16]. Pgp*/MDR1* is shown to be associated with caveolin-rich membrane domains, which may be required to ensure its proper functioning.

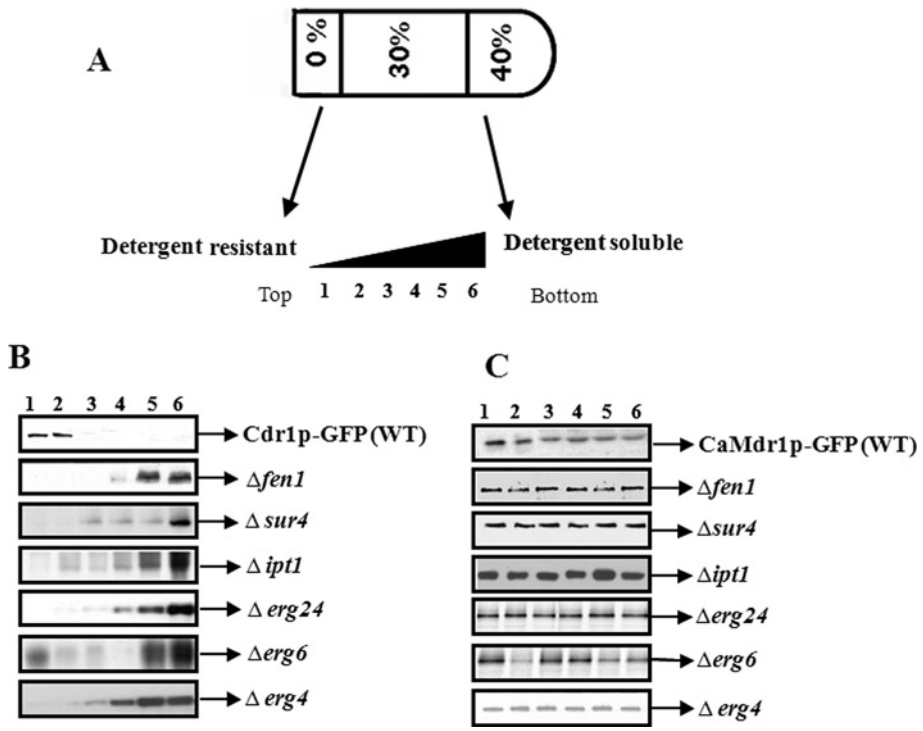

Figure 4 Localization of Cdr1p and CaMdr1p within the plasma membrane

An OptiPrep® gradient showing distribution of the detergent-resistant and detergent-soluble membrane fractions. Membrane lipid rafts are localized in the top two fractions. (B) CaCDR1–GFP (green fluorescent protein) and CaMDR1–GFP were overexpressed in a wild-type *S. cerevisiae* strain. Immunoblot analysis with anti-GFP antibody of the lipid-raft fractions clearly show the presence of CaCdr1p in the top two floating raft fractions (upper panel). The rest of the panels show the mislocalization of CaCdr1p–GFP in lipid metabolism mutants. (C) The distribution of CaMdr1p in OptiPrep® gradient shows that CaMdr1p is a non-raft protein, the localization of which is not affected in various lipid mutants. *fen1*, *sur4* and *ipt1* are enzymes of the sphingolipid biosynthesis pathway, whereas *erg24*, *erg6* and *erg4* are enzymes of the ergosterol biosynthesis pathway. WT, wild-type.

Although the link between membrane-raft lipids and their impact on drug transporters has been partly evaluated in mammalian systems, such a correlation in yeast is only beginning to be realized. Interestingly, microarray analysis performed with the hyperactive alleles of TFs such as Pdr1p (Pdr1-3) and Pdr3p (Pdr3-7) of *S. cerevisiae* confirms this assumption [89]. One study showed that a number of genes involved in sphingolipid metabolism and in the homoeostasis of its precursors are up-regulated along with the ABC transporter genes *PDR5*, *SNQ2* and *YOR1* [89]. Additionally, *PDR16* and *IPT1* which have a known role in sphingolipid metabolism and genes such as *RTA1* (resistance to 7-aminocholesterol 1) and *RSB1* (resistance to sphingoid base 1), which are integral membrane proteins, were also up-regulated along with the ABC transporter genes [94–97].

The first experimental evidence demonstrating a link between the PDR pathway and sphingolipid biosynthesis came from studies involving *IPT1* in *S. cerevisiae* [56]. The gene *IPT1* catalyses the last step in the sphingolipid biosynthetic pathway in yeast. The transcriptional induction of *IPT1* in the presence of hyperactive alleles of Pdr1p and Pdr3p occurs via a single PDRE in its promoter region. An *IPT1* null mutant displayed increased resistance to cycloheximide and decreased resistance to oligomycin [56]. This study showed that alterations in the sphingolipid levels in the plasma membrane selectively affect the drug resistance and membrane drug transporters. Interestingly, all the membrane drug-transporter proteins are not necessarily susceptible to membrane lipid fluctuations. For example, it has been observed that the MFS transporter of *C. albicans*, CaMdr1p, does not respond to lipid changes and its functions remain unaffected, whereas the functions of the ABC transporter CaCdr1p are abrogated under similar conditions [98].

Analysis of other genes of the sphingolipid pathway revealed that LAC (longevity-assurance gene cognate 1) is also responsive to the changes in the activity of the PDR pathway [99]. In the sphingolipid biosynthetic pathway, enzymes such as Dpl1p, ceramidases and transporters, which are responsible for the efflux of LCBs (long-chain bases) out of the cell, comprise of a regulatory circuit that maintains normal concentrations of the LCBs. LCBs are the precursors of LCBPs (LCB phosphates) and ceramide, which promote cell proliferation and trigger apoptosis in the cell. AS LCBPs and ceramide control antagonistic processes, an appropriate balance of LCBP/ceramide is required by the cell [50,101].

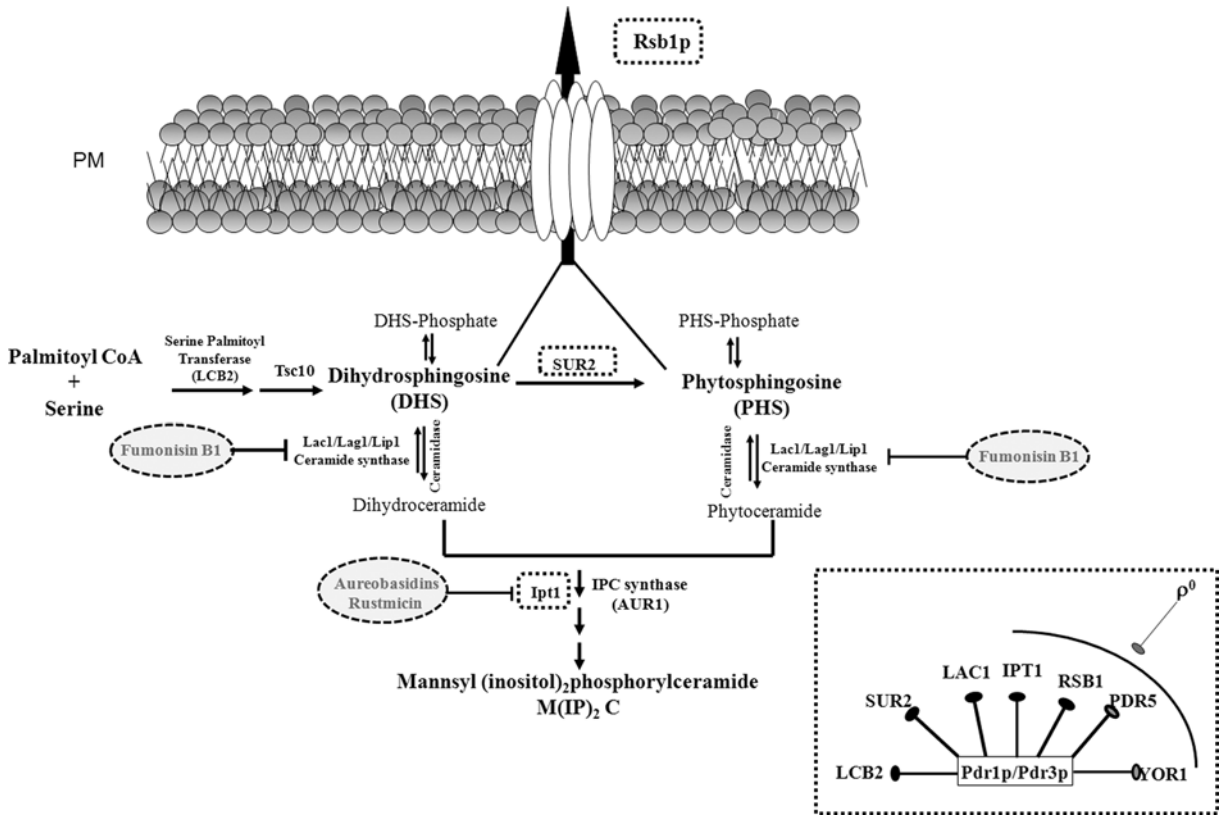

Figure 5 **Co-ordinate regulation of the sphingolipid biosynthetic pathway and drug-efflux pumps in *S. cerevisiae***
Genes regulated by Pdr1p/Pdr3p are shown in the sphingolipid biosynthetic pathway. Inhibitors used to block this pathway are shown in grey circles. Rsb1p, a membrane protein that effluxes the LCBs (dihydrosphingosine/DHS and PHS), is also a part of the PDR regulon. The inset shows targets for Pdr1p/Pdr3p, some of which are up-regulated in ρ^0 cells. Pdr5p and Yor1p function as phospholipid translocators in *S. cerevisiae*. PM, plasma membrane.

RSB1, a recently identified membrane transporter, has been shown to be responsible for the efflux of LCBs in *S. cerevisiae* (Figure 5). *RSB1* was identified on the basis of its ability, when expressed via a high-copy number plasmid, to suppress sensitivity to PHS (phytosphingosine; one of the LCBs) of *dpl1*Δ cells [97]. The expression of Rsb1p was up-regulated in *pdr5* null cells in a Pdr1p-dependent manner [102]. These strains therefore were highly resistant to exogenously added PHS, with a concomitant increase in LCB efflux. Two independent microarray analyses demonstrated that this gene is overexpressed with Pdr5p and other ABC drug transporters in the presence of a hyperactive allele of Pdr1p and in ρ^0 cells [89,90]. The role of Pdr5p as a phospholipid translocator and its connection with Rsb1p shows that the PDR pathway probably regulates the transport of phospholipids and LCBs to ensure proper membrane-raft mileu [103]. Kihara and Igarashi [102] showed that there was a reciprocal relationship between Pdr5p and Rsb1p, where Rsb1p is overexpressed in *pdr5* null cells. Contradictory to this, another study showed that, although Δ*pdr5* cells are highly resistant to PHS, it is not necessarily by activation of Rsb1p [104]. This would suggest an independent mechanism is activated in the absence of *pdr5* and renders the cells resistant to PHS. The latter study [104] instead points to the fact that both Pdr5p and Rsb1p are up-regulated in ρ^0 cells, which places them both in the retrograde regulon in *S. cerevisiae*. Together, these studies support the idea that the PDR pathway has a major role to play in controlling the lipid mileu in the plasma membrane.

In *C. albicans*, comparison of gene expression profiles in the azole-resistant clinical isolates and drug (fluphenazine)-induced laboratory strains show that, in addition to the multidrug transporters CaCdr1p and CaCdr2p, genes such as *RTA3* (homologue of Rsb1p), *IFU5* and *HSP12* (heat-shock protein 12) and *IPF4065* (putatively involved in stress response) are the most highly expressed genes. A total of 42 genes were commonly regulated when the fluphenazine-exposed cells were compared with the azole-resistant isolates. All the above genes, except for *HSP12* and *IPF4065*, have a drug-response element in their promoter regions and hence are targets for Tac1p [86,105].

The studies conducted so far establish that MDR in yeast is a result of the simultaneous activation of many factors in the cells in response to a drug. While it involves the overexpression of genes encoding drug-efflux pumps, it is also accompanied by

additional biochemical changes, which include up-regulation of the lipid-raft constituents. The results discussed above imply that yeast possess a co-ordinated programme for up-regulation of both lipid-raft constituents and drug-efflux pumps.

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