FUNCTIONAL RELEVANCE of MEMBRANE LIPID ASYMMETRY AND FLUIDITY IN *C. Albicans*, A PATHOGENIC YEAST

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Candida albicans is an opportunistic diploid fungus that causes infection in immunocompromised and debilitated patients. Wide spread and prolonged usage of azoles in recent years has led to the rapid development of the phenomenon of azole resistance which poses a major threat to antifungal therapy. Various mechanisms which contribute towards the development of azole resistance have been implicated in *Candida* such as overexpression of, or mutations in the target enzyme of azoles, lanosterol 14a - demethylase as well as overexpression of drug efflux pumps encoding genes belonging to ATP-Binding Cassette (ABC) namely, *CDR1, CDR2* and to Major Facilitator Superfamilies of transporters (MFS) i.e. *MDR1*.

It has been shown by us and as well as by others that the action of antifungals is modulated by subtle modification of the membrane lipid composition. Of note clinical as well as adapted azole resistant isolates of *C. albicans* exhibit altered membrane phospholipid and sterol compositions. Biological membranes are organized assemblies of lipids and proteins with small amounts of carbohydrate. They regulate the composition of the intracellular medium by controlling the flow of nutrients, waste products, ions etc., into and out of the cell by the membrane embedded pumps that transport specific substances against an electrochemical gradient. The major lipid components of yeast membrane are **a)** saturated and unsaturated long chain fatty acids, **b)** triacylglycerols (fatty acid triesters of glycerol), **c)** glycerophospholipids (or phosphoglycerides) which consists of phospholipids like phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol (cardiolipins), **d)** sphingolipids (C_{18} derivatives of sphingosine and dihydrosphingosine) and **e)** ergosterol (C27 membrane sterol and has C3-OH group and C7 to C8 double bond.

Alteration in the degree of unsaturation, carbon chain length, positional distribution of acyl lipids and change in the membrane lipid composition contribute to the physical state of the membrane and thereby alter the membrane fluidity by altering the membrane order. The membrane order can be altered by manipulation in the levels of ergosterol, phospholipid, sphingolipid or fatty acids.

Phospholipids are asymmetrically distributed in the membrane and the loss of this distribution functions as a cue for several physiological processes. In *C. albicans*, the ABC transporters namely Cdr1p and Cdr2p are mainly responsible for the maintenance of the asymmetric distribution of the phospholipids. Altering the membrane environment by altering the membrane lipids alter the functioning of these ABC

transporters which are transmembrane proteins.

In this study we have examined the functional relevance of membrane fluidity and membrane lipid asymmetry in *Candida albicans*.

Our results suggested that *CDR1* under normal physiological conditions functions as floppase for PtdEtn thereby maintaining membrane lipid asymmetry whereas the same translocates PtdSer to the outer leaflet of the membrane in aged stationary phase *Candida* cells. Like other yeast cells, *Candida albicans* cells also probably have an altruistic response that eliminates aged and defective cells that would otherwise compete with healthy cells for nutrients and this membrane lipid asymmetrical changes are correlated to *CDR1*.

The morphogenetic mutants were analyzed for change in their membrane lipid composition. The defect in signaling pathways of morphogenesis resulted in change of the physical state of the membrane and membrane lipid composition which subsequently resulted in altered phospholipid translocation by the membrane transporter, Cdr1p.

In the subsequent sections of this study, the membrane lipid composition was manipulated and the relevance of this altered lipid composition in drug resistance and morphogenesis was examined. No correlation between membrane fluidity and hyphal induction was detected. We favor a model, in which stress responses and induction of morphogenesis do not share the same dependence on membrane fluidity. We rather postulate that levels of oleic acid have a direct effect on specific components of the hyphal induction machinery.

Ole1p may serve as a suitable target for future antifungals. Because *OLE1* is essential it is likely that potential Ole1 inhibitors will prevent cell growth and lead to a rapid loss of viability. This would be an advantage compared to azole inhibitors of ergosterol biosynthesis, which do not kill fungal pathogens. Even at low doses of inhibitors, which reduce but do not eliminate Ole1 function, hyphal morphogenesis and consequently virulence of *C. albicans* would be blocked. A major structural difference between the mammalian and fungal Ole1 proteins is the presence of an integral cytochrome b_5 domain in fungal desaturases, which allow the development of selective inhibitors.

Altering membrane fluidity by fluidizers did not alter drug susceptibility or resulted in morphogenetic defect. Rather the ergosterol or sphingolipid contents and their close interaction was functionally relevant for drug resistance and morphogenesis in *Candida albicans*.

For our study, we exploited *erg* mutants defective in ergosterol biosynthesis and Fumonisin B1 as biochemical inhibitor of sphingolipid biosynthesis. Sphingolipid composition also was subsequently altered by *IPT1* disruption. We observed that reduction in either ergosterol inor sphingolipid, two major membrane lipid constituents had deleterious effects on drug resistance wherein *Candida* cells turned hypersensitive to most of the drugs tested. Our results further suggest that sphingolipid-ergosterol interactions are important determinants for the surface localization of the major drug extrusion pump protein Cdr1p, which in turn affects drug susceptibilities of *C. albicans* cells.

Morphogenic defect also was observed if the ergosterol or sphingolipid composition was altered. Recent reports suggest the involvement of sterol- and sphingolipid- enriched microdomains in hyphal morphogenesis in *C. albicans* wherein membrane lipid polarization appear to contribute to the ability of this pathogen to grow in highly

polarized manner to form hyphae. Our results are in agreement with such reports since we also observe that disruption of ergosterol/$M(IP)_2C$-rich domains in the plasma membrane of the *IPT1* mutants results in defective hyphal morphogenesis. It appears that not only there is a close interaction between membrane raft constituents and drug susceptibilities of yeast cells, there is also a well coordinated control of their regulation.
Our observations acquire significance if one considers recent reports, which show the existence of discrete membrane microdomains, known as lipid rafts within lipid bilayers, predominantly composed of sphingolipid and sterol. We could demonstrate the existence of such close interactions in *Candida albicans* and disruption of this interaction resulted in increased drug susceptibility and defective morphogenesis.
IPT1 disruption resulted in depletion of $M(IP)_2C$ and accumulation of MIPC in the *ipt1* mutant cells which again caused disruption of their interactions with ergosterol which also demonstrated that ($M(IP)_2C$) interacts with ergosterol with greater propensity among the three major species of sphingolipids namely, inositol phosphoceramide (IPC), mannosyl inositol phosphoceramide (MIPC) and mannosyl diinositol diphosphoceramide ($M(IP)_2C$).

The ease which enables us to dial lipid composition of *Candida albicans*, prompted us to exploit the role of membrane lipids in cellular physiology, particularly in drug susceptibilities of this pathogen. Our previous study has provided hints for a close association between azole resistance and membrane lipid fluidity and asymmetry. The first section of this study shows altered membrane lipid asymmetry in the aged *Candida albicans* cells and in the second section, we find altered membrane fluidity and membrane lipid asymmetry in the morphological mutants of *Candida albicans* which are defective in hyphal formation. In the subsequent sections we have manipulated the lipid composition to check the relevance of this altered membrane properties in drug resistance and morphogenesis of *Candida albicans*. By specifically altering the membrane lipid composition in this study, either genetically (homozygous disruption of lipid biosynthetic genes) or by employing inhibitors which block lipid synthesis, we have further examined these aspects in greater depths. The thesis embodies three sections dealing with these aspects. Taken together, the results presented in the thesis clearly establishes that lipid composition cannot be ignored when one considers drug susceptibilities and morphogenesis of this pathogen. This would pave way for further investigation, if lipid- dependent processes can become potential novel targets for a new class of antifungal agents.