The major goal of this thesis has been to gain some insights into the molecular basis of pleiotropic drug resistance and pleiotropic drug sensitivity in the yeast *Saccharomyces cerevisiae.* We have attempted to investigate specific questions regarding the role of the membranes, the multidrug resistance proteins and the glutathione S-transferases in multidrug resistance.

We constructed an isogenic panel of strains, bearing disruptions in the late steps of ergosterol biosynthetic pathway in the protease deficient background. These strains displayed hypersensitivity to various drugs. We have also compared the resistance profiles of *erg* strains towards different polyene antibiotics. The *erg2Δ* and *erg6Δ* strains were resistant to all the three antibiotics *i.e.* nystatin, amphotericin B and hamycin. The *erg3Δ* strain was found to be nystatin resistant but amphotericin B and hamycin sensitive while the *erg4Δ* strain exhibited sensitivity to all the three antibiotics. Our findings suggest that there is a difference in the binding affinity of each polyene antibiotic with the sterol molecule predominantly accumulated in each of the mutant strain.

The drug sensitivity profile of some of the *erg* strains (*erg6Δ and erg2Δ*) was found to be similar to that of *pdr5Δ* strain which bears a disruption in the yeast multidrug resistance protein, Pdr5p. This observation prompted us to examine whether the drug sensitivity of *erg* strains is solely due to an increased permeability and uptake of various compounds as has been considered or whether it is also due to the poor functioning of various pumps that are involved in the efflux of these compounds. To study this, we measured the rate of rhodamine 6G efflux, which is known to be effluxed by Pdr5p, in various strains. We observed a reduced efflux of rhodamine 6G in *erg* strains as compared to the isogenic wild type strain. As these could be a result of either the reduced efficiency or an altered substrate specificity of the Pdr5 pump, we extended this study to other drugs to examine if there was an altered substrare specificity.

Pdr5p was overexpressed from a multicopy plasmid in *erg* strains and the resistance profiles of Pdr5p towards structurally diverse compounds like cycloheximide, emetine, crystal violet and estradiol were compared in these strains. Interestingly, Pdr5p-mediated resistance to the above drugs was found to be significantly diminished in the erg strains. Furthermore, this decreased level of resistance to different drugs was not solely due to the increased permeability of the strains and it was found to be both strain and drug specific suggesting that not only the efficiency but also the substrate specificity of Pdr5p was affected. For example, though *erg6Δ* and *pdr5Δ* strains show similar sensitivity to cycloheximide, crystal violet *etc.*, the level of resistance conferred in the *pdr5Δ* strain was much higher than that in the *erg6Δ* strain. Moreover, Pdr5p overexpression was found to restore the wild type growth in the estradiol sensitive *erg6Δ* strain but virtually failed to confer any resistance to emetine in an otherwise emetine sensitive *erg6Δ* strain.

As we could demonstrate that an alteration in the sterol composition leads to a change in the efficiency and substrate specificity of Pdr5p, we wished to examine if a change in the phospholipid composition of the membrane also affects the functioning of Pdr5p. To investigate this, we initally constructed a *cho1Δ* strain in a similar protease deficient background. This strain was defective in the synthesis of phosphatidylserine synthase (encoded by *CHO1*). However, we observed that Pdr5p overexpression was deleterious to these cells resulting in the loss of Pdr5p overexpressing plasmid in this strain background, but when we constructed a *cho1Δ* strain in a protease plus background and expressed Pdr5p, the Pdr5p overexpressing plasmid was found to be stable and it was able to confer resistance to both cycloheximide and crystal violet, though, not to the same extent. This drug selectivity suggests that the phospholipid composition of the membrane also plays a role in the modulation of Pdr5p function.

We also attempted to correlate the altered efficiency and substrate specificity of PDr5p in various strains with their membrane fluidities. Although all the *erg* strains

showed an increased fluidity, that extent of the fluidity could not be correlated with their drug sensitivities.

To address the role of another family of proteins, the glutathione S-transferases (GSTs), which are implicated in determining the substrate specificity of another class of multidrug resistance proteins *i.e.,* the multidrug resistance-associated proteins, (MRPs), we decided to investigate the occurence of GSTs in *S. cerevisiae* and the role that they might play in the drug resistance of this yeast. We identified and created disruptions in two open reading frames (ORFs) *YLL060C* and *YIR038C* that appeared to be putative GSTs. Deletions of these genes were not lethal, and our attempts to detect, any drug resistance or sensitivity phenotype associated with the single or double disruption of these two ORFs or with the overexpression of these proteins did not yield any clues on the function of these proteins. We constructed a reporter gene fusion with the *GST1* (*YLL060C*) promoter and were able to show through a β-galactosidase reporter gene assay that the promoter region (460 bp) of the ORF, *YLL060C*, can be induced, though weakly, by the compounds which are known to be GST substrates like ethacrynic acid, *p*-nitrobenzoyl chloride, *o*-DNB etc. and also by the compounds that cause oxidative stress like H_2O_2, *t*-BOOH and diamide. This finding suggests that the YLL060Cp may indeed be a real GST, though, we have yet to show the GST activity of this protein. Apart from this, the exact role of the proteins encoded by *YLL060C* and *YIR038C* in drug detoxification process and the other enzymatic properties associated with these proteins have to be determined.

In this thesis, we have also attempted to extend, the observations obtained with *S. cerevisiae*, to the fission yeast, *Schizosaccharomyces pombe.* It was in this direction that we checked the feasibility of simple PCR-mediated gene disruption technique in *S. pombe* which has been routinely used for the functional analysis of genes in *S. cerevisiae.* We have demonstrated that such a method of gene disruption is feasible in *S. pombe* though

with a very low efficiency of about 2%, and that this efficiency of targeted gene disruption increases with an increase in the homology length for the target gene. However, detailed investigations regarding the various factors involved in regulating the targeted gene disruption by simple PCR-based approach are needed.