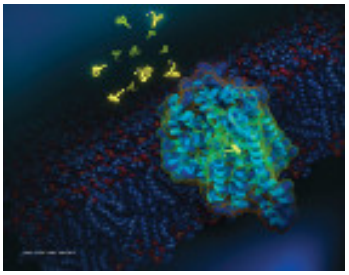

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Probing the mechanism of sterol extraction by Amphotericin B in yeast membranes using neutron reflection

Amphotericin B (AmB) is a naturally occurring antibiotic with a broad spectrum against systemic fungal and parasitic infections. AmB interacts preferentially with ergosterol in cell membranes, giving rise to the fungal specificity, but it also interacts with a lower affinity with cholesterol, giving rise to toxic side effects that are often dose-limiting. The classical AmB mechanism is based on aqueous pores formed by AmB-sterol complexes, but it has recently been shown that ergosterol-binding alone [1], or extraction of ergosterol [2] form the basis of antifungal activity. We have used neutron reflection (NR) and deuterium labeling to study ergosterol and cholesterol extraction by AmB in yeast and model membranes, coupled to analysis of the lipid composition of both pathogenic and non-pathogenic yeast cells to elucidate the effect of membrane composition on AmB activity.

The structure of yeast membranes and their response to AmB [3,4] differ considerably from typical model lipid bilayers and depends on the degree of lipid polyunsaturation. AmB inserts in yeast membranes both in the absence and presence of ergosterol, but forms no aqueous pores. We have also observed in-situ a highly hydrated extramembraneous AmB layer, which does not form on simple POPC-sterol membranes. While AmB inserts to a much higher degree in cholesterol containing membranes, the amount of cholesterol extracted is very limited.

In membranes from the pathogenic yeast Candida glabrata, genetically manipulated to show either increased or decreased AmB resistance, resistance is related to changes in both AmB insertion and ergosterol extraction.

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