Establishing the Mammalian Cholesterol Biosynthetic Pathway in the Yeast *Saccharomyces cerevisiae* Fosters Functional Expression of Mammalian Membrane Proteins

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Saccharomyces cerevisiae is an easy-to-grow, easy-to-handle model organism for heterologous protein expression. Recent efforts to optimize yeast for this aim have mainly addressed the humanization of the glycosylation pathways. However, when considering the functional expression of membrane proteins, the use of this expression system is often limited by the yeast-specific lipid composition. Yeast plasma membrane has a sterol composition different from mammalian cells. Cholesterol is the main plasma membrane sterol in mammalian cells in contrast to ergosterol in yeast cells.

Most integral membrane proteins are protein-lipid complexes and the presence of specific phospholipids and sterols in their membrane environment has been shown to be crucial for their activity (Opekarova M., Tanner W. (2003) *Biochem Biophys Acta*, **1610**: 11-22).

We here report on engineered yeast strains that contain the mammalian sterol biosynthetic pathway. These strains are able to synthesize the mammalian post zymosterol sterols cholesta-7,24-dienol, cholesta-5,7,24-trienol and 7-dehydrocholesterol. Cholesterol, as the end product of the mammalian sterol biosynthetic pathway is also produced. We analysed the whole cell and membrane sterol pattern and evaluated the expression and functionality of a cholesterol dependent mammalian transporter in some of these “humanized” yeast strains.