The cell surface invertase was purified from *S. cerevisiae och1:LEU2* disruptant cells *(Δoch1)* , which have a defect in elongation of outer chain attached to the *N*-linked core oligosaccharides (K. Nakayama *et al.* (1992) *EMBO J.* 11, 2511-2519). Structure analysis of the pyridylaminated (PA)-oligosaccharides confirmed that the *och1* mutation causes a complete loss of α-1,6-poly-mannose outer chain, although the PA-oligosaccharides, in which one or two α-1,3-linked mannose(s) attached to the endoplasmic reticulumn (ER)-form core oligosaccharide ($Man_8GlcNAc_2$), were also detected. Analysis of the *Δoch1 mnn1* strain oligosaccharides released from total cell mannoprotein manifested that the *Δoch1 mnn1* mutant eliminates the α-1,3-mannose attached to the core and accumulates predominantly a single ER-form oligosaccharide species, suggesting the potential advantage of this strain as a host cell to produce mammalian high mannose type glycoproteins. The *Δoch1 mnn1 alg3* mutants accumulated $Man_5GlcNAc_2$-PA and $Man_8GlcNAc_2$-PA from total cell mannoprotein, confirming the lack of outer chain addition to the incomplete core-like oligosaccharide and the leaky phenotype of *alg3* mutation. All the results suggest that the *OCH1* gene encodes α-1,6-mannosyltransferase which is functional in the initiation of α-1,6-poly-mannose outer chain addition to the *N*-linked core oligosaccharide in yeast.

S2.19

The Hydrophobic Region of Dolichyl Phosphoryl Mannose Synthase is not Essential for Enzyme Activity or for Growth in *S. Cerevisiae*

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Dolichyl phosphoryl mannose (Dol-P-Man) synthase from *Saccharomyces cerevisiae* is encoded by the essential gene *DPM1*. Evidence indicates that the enzyme is oriented on the cytoplasmic face of the endoplasmic reticulum, where it forms Dol-P-Man from GDP-Man and Dol-P. It has also been shown that it functions in *O*-linked glycosylation, *N*-linked glycosylation, and glycosyl phosphatidylinositol anchor formation through its product, Dol-P-Man, which donates its mannose residue in all three pathways. The sole predicted membrane-spanning domain of the enzyme is found at the C-terminus, which includes a consensus sequence for the putative "dolichol recognition sequence". This sequence has been found in practically all enzymes which utilize some form of the lipid as a substrate. We have constructed mutant forms of Dol-P-Man synthase which have allowed us to address two questions: i) is the consensus sequence required for enzyme activity or to support growth in yeast, and ii) is the predicted C-terminal membrane-spanning domain the only anchor of the protein to the endoplasmic reticulum?

Through several deletion and substitution mutants we have found that the consensus sequence of Dol-P-Man synthase can be removed or altered, and the resulting mutants still retain some, albeit reduced, enzymatic activity. Several of these mutants still support growth in yeast, and two mutant proteins apparently function well in *N*-linked glycosylation, as judged by analysis of the precursor lipid-linked oligosaccharides. These results indicate that the hydrophobic region of Dol-P-Man synthase containing the consensus sequence is not required for enzyme activity or for growth in *S. cerevisiae.*

Mutant proteins that support growth in yeast were still found to be membrane-associated, but those containing deletions of the membrane-spanning domain were found to be peripheral, as opposed to the wild-type enzyme which is integral. The possibility of interactions of Dol-P-Man synthase with other membrane proteins is being examined.

S2.20

PMT1, Gene for the Key Enzyme of Protein *O*-Glycosylation in *Saccharomyces cerevisiae*

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O-glycosylation proceeds differently as far as known in mammalian, plant and fungal cells. In the yeast *Saccharomyces cerevisiae* as well as in a number of other fungi the initial steps of protein *O*-glycosylation take place at the endoplasmic reticulum.

I $\text{GDP-Man} + \text{Dol-P} \overset{Mg2+}{\rightleftharpoons} \text{Dol-P-Man} + \text{GDP}$

II $\text{Dol-P-Man} + \text{Protein (Ser/Thr)} \overset{Mg2+}{\rightleftharpoons} \text{Protein (Ser/Thr)Man} + \text{Dol-P}$

III $\text{Protein(Ser/Thr)Man} + n\text{GDP-Man} \overset{Mn2+}{\rightleftharpoons} \text{Protein(Ser/Thr)Man}_{1+n} + n\text{GDP}$

Enzyme II was purified to homogeneity. A protein band with an apparent molecular weight of 92 kD correlated well with enzyme activity. A polyclonal antibody raised against the 92 kD protein precipitates the mannosyltransferase activity [1]. Tryptic peptides of the protein were generated. Oligodeoxynucleotides corresponding to the peptide sequences were used to screen yeast genomic DNA. An open reading frame (ORF) of 2451 bp was identified encoding a polypeptide of 817 amino acids. Expression in *E. coli* proved that the 92 kDa protein is the mannosyltransferase. Haploid gene disruptants show identical growth rate as wild type cells, but have a defect in cell separation and agglutination. They show neither the 92 kD protein nor *in vitro* mannosyltransferase activity. *In vivo* *O*-glycosylation is reduced but not completely destroyed. *A*-agglutinin and chitinase, exclusively *O*-glycosylated proteins, show reduced glycosylation in mutant cells; their sensitivity towards the killer toxin K1, which depends on *O*-linked saccharide chains, is also decreased to about 50%.

Using a genetic as well as a biochemical approach and the PMT1 disruptant we characterized a new mannosyltransferase activity responsible for the remaining *O*-glycosylation.

(1) Strahl-Bolsinger, S. and Tanner, W. (1991) *Eur. J. Biochem.* **196**, 185 – 190.

S2.21

The Primary Structure of the "Large" Sialidase Isoenzyme of *Clostridium Perfringens* A99 and its Comparison with Further Sialidases

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C. perfringens strains produce two sialidases, which have quite different molecular weights (42/71 kDa), substrate specificities (limited/broad), and location (intracellular/