small amount of 42k protein and was found to have β-galactoside α2,6-sialyltransferase activity, indicating a part of the enzyme was renatured. These results suggest that the bacterial expression system, with the optimization of the renaturing condition, will be a powerful tool for elucidating molecular mechanisms of sialyltransferases.

S2.15
Minimal Size of Active Primate α1,3 Galactosyltransferase

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The cDNA encoding the marmoset New World Monkey α1,3-galactosyltransferase (α1,3 GT) enzyme was cloned from a cDNA library of the B95.8 cell line and sequenced. To determine the minimal size necessary to retain catalytic activity a series of deletions were made in the coding region using specific sets of PCR primers. The truncated products were expressed as fusion proteins with staphylococcal protein A using the vector pPROTA. Up to 68 amino acids downstream of the transmembrane domain could be removed without decreasing the ability of the enzyme to transfer [^{3}H] galactose to *N*-acetyllactosamine acceptor. The enzyme was remarkably sensitive, however, to truncations at the C-terminus, as removal of only two amino acids was sufficient to decrease activity of the enzyme by 95%. A comparison of the wild-type enzyme activity with that of α1,3 GT truncated by 64 amino acids downstream of the transmembrane domain revealed a similar rate of enzyme activity and a similar specificity for various carbohydrate acceptors, including a low but detectable utilization of galactose and *N*-acetylglucosamine. These results suggest an important role for amino acids at the C-terminus, either in catalysis or in maintenance of proper protein conformation, and define the length of the stem region of the enzyme, which extends the catalytic domains into the lumen of the Golgi apparatus, as 68 amino acids beyond the transmembrane domain.

S2.16
Molecular Cloning and Expression of β-Galactoside α2,3-Sialyltransferase from Mouse Brain

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Sialyltransferases are a family of glycosyltransferases which catalyze the transfer of sialic acid to terminal positions on the carbohydrate groups of glycoproteins and glycolipids. They appear to be expressed in a tissue-restricted manner and produce diverse carbohydrate structure which recognized as key determinants in biological recognition. To investigate the tissue-specific structure and function among the family of them, DNA clones encoding β-galactoside α2,3-sialyltransferase were isolated from mouse brain cDNA libraries using sequence information obtained from the conserved amino acid sequence of the previously cloned enzymes, rat liver α2,6- (1) and porcine submaxillary gland α2,3-sialyltransferases (2). The cDNA sequence revealed an open reading frame coding for 337 amino acids and amino acid sequence showed 80% identity with that of the above α2,3-sialyltransferase. The primary structure of this enzyme suggested that a putative domain structure, like other glycosyltransferases, is consisted of a short NH_2-terminal cytoplasmic domain (7 residues), a signal-membrane anchor sequence (29 residues) and a large COOH-terminal catalytic domain (301 residues). The identity of this enzyme was confirmed by construction of a recombinant sialyltransferase in which the cytoplasmic domain and signal-anchor sequence was replaced with the immunoglobulin signal sequence, and expression in COS-7 cells, which resulted in secretion into the medium in soluble and active form. This result would provide new insight into the molecular biology of glycosylation as well as the structural relationships among the family of sialyltransferases.

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S2.17
Characterization of Substrate Specificity of Yeast Mannosyltransferase

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We have already reported that the OCH1 protein, which is isolated as a complemental gene of *och1* mutant that is deficient in the oligosaccharide elongation of yeast, transfers a mannose to the oligosaccharide of mannoprotein accumulated in the *och1* mutant and is responsible for the mannose outer chain elongation (K. Nakayama *et al.*, (1992), *EMBO J.*, **11**, 2511-2519). Here, we report the substrate specificity of this OCH1 protein by measuring the mannose transfer activity to various acceptors. The combination of pyridylamino (PA) labeled *N*-linked oligosaccharides and unlabeled GDP-mannose, or unlabeled *N*-linked oligosaccharides and [^{14}C] labeled GDP-mannose were used as acceptors and donors, respectively. The OCH1 protein dependent mannosyltransferase activity was compared between the microsomal membranes obtained from Δ*och1* (*OCH1* gene disruptant), KK4 (single copy of *OCH1* chromosomal gene), and KK4/YEp51-OCH1 (multi copy of *OCH1* gene) cells. The OCH1 protein utilized $Man_8GlcNAc_2$ and $Man_8GlcNAc$ as an acceptor. Mannose transfer occurred in $Man_{8\text{-}10}GlcNAc_2$-PA isolated from Δ*och1* mannoprotein and $Man_{6\text{-}9}GlcNAc_2$-PA isolated from mammalian glycoprotein. All the results suggest that the OCH1 protein is an initiation specific α-1,6-mannosyltransferase.

S2.18
Structure of the *N*-Linked Oligosaccharides from *OCH1, OCH1 MNN1* and *OCH1 MNN1 ALG3* Mutants of *Saccharomyces Cerevisiae*

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