boardering the most highly conserved region of the sialylmotif resulted in the isolation of three new members of the sialyltransferase gene family. Each of them contains the conserved sialylmotif and exhibits a type 2 transmembrane topology characteristic of glycosyltransferases. Two of them, STX and STY, which were isolated from a rat newborn or an adult brain cDNA library, respectively, had no homology outside of the sialylmotif. In contrast, STZ isolated from a human placenta cDNA library had more than 30% homology with Galβ 1-3(4)GlcNAc α2-3sialyltransferases (ST3N). In addition, we have cloned homolog of rat ST3N from human placenta because it was reported that ST3N enzyme in human placenta prefered type 2 chain acceptors while the one in rat liver prefered type 1. Northern analysis of these newly cloned genes has revealed remarkable tissue specific and developmentally regulated expression. In order to facilitate functional analysis of these genes, soluble forms of the proteins have been generated. Transient expression experiments are now in progress to evaluate the enzymatic activity of these proteins.

(1) Gillespie, W., *et al.* (1992) *J. Biol. Chem.*, **267**, 21004 – 21010.
(2) Wen, D. X., *et al.* (1992) *J. Biol. Chem.*, **267**, 21011 – 21019.
(3) Drickamer, K. (1993) *Glycobiology*, **3**, 2 – 3.

S2.6

Evolutionary Studies on Bacterial Sialidases — Common Origin and Irregular Distribution

P. Roggentin and R. Schauer
Biochemisches Institut der Univ., Olshausenstr. 40, 2300 Kiel, Germany.

The preferred substrates for microbial sialidases are produced by animals which appeared late in evolution (*Echinodermata* to *Mammalia*). Sialidases are common in these animals, but are irregularly distributed among microbial species or even strains. Bacteria possessing sialidase generally have close contact with animals. These observations make sialidases interesting subjects for evolutionary studies. Bacterial sialidases are very variable in terms of their molecular and enzymic properties. However, a common origin of sialidases is indicated by comparison of the primary structures deduced from cloned and sequenced bacterial sialidase genes. Homology is mainly based on conserved and repeated sequences (1). From sequence alignments, 23 amino acids were found to be conserved in 11 sialidase proteins. Further identical amino acids were present in each pair of sequences compared, which allowed the calculation of similarity values among sialidases. Some sialidases exhibit sequence similarities in agreement with the phylogenetic distances of their producers, while others do not. The latter group contains mainly enzymes, which are apparently not adapted for microbial purposes. A spreading of the sialidase gene information by mobile elements among *Bacteria* during evolution is thus indicated.

(1) P. Roggentin, B. Rothe, J. B. Kaper, J. Galen, L. Lawrisuk, E. R. Vimr and R. Schauer (1989) *Glycoconj. J.* **6**:349 – 353.

S2.7

Molecular Cloning and Expression of cDNA Encoding Rat UDP-*N*-Acetylglucosamine:α-6-D-Mannoside β-1,2-*N*-Acetylglucosaminyltransferase II

G. A. F. D'Agostaro[1], A. Zingoni[1], R. J. Simpson[2], R. L. Moritz[2], H. Schachter[3] and B. K. Bendiak[4]
[1]*Lab. Biophysics, ENEA, Roma, Italy;* [2]*Joint Protein Structure Laboratory, Ludwig Inst. Cancer Research and The Walter and Eliza Hall Inst. for Medical Research, Melbourne, Australia;* [3]*Research Institute, Hospital for Sick Children, Toronto, Ont. M5G 1X8, Canada;* [4]*The Biomembrane Institute, Univ. Washington, Seattle, WA, USA.*

UDP-*N*-acetylglucosamine:α-6-D-mannoside β-1,2-*N*-acetylglucosaminyltransferase II (GnT II, EC 2.4.1.143) is a Golgi membrane-bound enzyme catalyzing the conversion of Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4R to **GlcNAcβ1-2**Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4R where R is GlcNAcβ1-4(±Fucα1-6)GlcNAc-Asn-X. This reaction is an essential step in the biosynthetic pathway leading from high-mannose to complex *N*-linked oligosaccharides. GnT II was purified from rat liver (Bendiak and Schachter, *J Biol Chem.* 262: 5775-5783, 1987); SDS-PAGE revealed a 42 KDa polypeptide. Sequence analysis of the *N*-terminus and a tryptic peptide identified 14 and 18 amino acid residues respectively. Synthetic oligonucleotides were used to isolate from rat liver cDNA libraries in λgt11 overlapping cDNA clones containing a 1290 bp open reading frame encoding a 430 amino acid polypeptide. The colinearity between the cDNA sequence and the amino acid sequences from GnT II was consistent with the translation frame and confirmed the authenticity of the cDNA clones. A GenBank database search showed no significant sequence similarity to other previously cloned glycosyltransferases including β-1,2-*N*-acetylglucosaminyltransferase I. The GnT II sequence predicts a domain structure and Type II integral membrane protein topology typical of all previously cloned glycosyltransferases, i.e., a short *N*-terminal cytoplasmic segment, a single 20 amino acid hydrophobic domain (presumptive non-cleavable signal/anchor domain), and a large C-terminal lumenal region. The C-terminal 389 amino acids of rat GnT II were linked in frame to the cleavable signal sequence of the Il-2 receptor and expressed under control of the Rous sarcoma virus promoter in COS-7 cells. A similar construct carrying GnT II cDNA out of frame was used as a negative control. Using a standard enzyme assay for GnT II activity in the culture medium, a 77-fold enhancement of GnT II activity over the negative control was detected at 72 hr after transfection in transient expression experiments. These data unambiguously verify the identity of the cloned cDNA sequences for rat GnT II and demonstrate that the C-terminal region of the GnT II polypeptide chain includes the catalytic site.

S2.8

Purification and Characterization of *N*-Acetylglucosaminyltransferase

A. Nishikawa, J. Gu, M. Ohno and N. Taniguchi
Department of Biochemistry, Osaka University Medical School, Japan.

A β1-6*N*-acetylglucosaminyltransferase V (GnT-V) has been purified up to 20,000-fold from the cultured supernatant of the QG small lung cancer cell line with a 37% yield. The isolation procedure included chromatography on phenyl Sepharose, hydroxylapatite, UDP-hexamolamine Sepharose and substrate-conjugated Sepharose. Sodium dodecyl sulfate gel electrophoresis under non-reducing conditions showed a