single band of 73 kDa. Under reducing conditions, however, an additional component of 60 kDa was seen. Peptide mapping analysis indicated that both of these proteins were essentially identical, indicating that the 60 kDa component is probably a proteolytically cleaved from of the 73 kDa protein.

Studies on the activity of the enzyme toward a variety of pyridylaminated sugars showed that the enzyme is most active toward 2,2,4-branching triantennary and biantennary sugars. The *Km* value for biantennary sugar chain and UDP-GlcNAc were 133 μM and 3.5 mM, respectively.

Gu *et al.*, (1993) *J. Biochem.* in press.

S2.9

## Purification of Recombinant UDP-GlcNAc:α3-D-Mannoside β-1,2-*N*-Acetylglucosaminyltransferase I (GnT I) Expressed in Insect Cells

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UDP-GlcNAc:α3-D-mannoside β-1,2-*N*-acetyl-glucosaminyl-transferase I (GnT I; EC 2.4.1.101) catalyzes the conversion of ***1*** to ***2***, in the synthetic path from high mannose to complex and hybrid *N*-glycans. GnT I action is an essential pre-requisite for several enzymes in the pathway (GnT II-V; core α-1,6-fucosyltransferase). We have previously described the genes for rabbit, human and mouse GnT I [1-3]. GnT I, like other cloned glycosyltransferases [4,5], is a Golgi-localized type II transmembrane protein. A baculovirus shuttle vector was constructed in which the short *N*-terminal cytoplasmic tail and trans-membrane segment of the rabbit GnT I cDNA was replaced with an in-frame cleavable signal sequence. The modified GnT I gene was inserted into the genome of *Autographa californica* nuclear polyhedrosis virus (AcNPV) and Sf9 insect cells were infected with the recombinant baculovirus. High level expression of enzymatically active and soluble GnT I was achieved. About 90% of the GnT I was found in the cell culture medium at 1-5 mg/L. Kinetic analysis, Southern blots and immunoblots indicated that recombinant rabbit GnT I was being produced. Recombinant GnT I has been purified to near homogeneity by ion exchange and affinity chromatogaphy on UDP-hexanolamine agarose.

[Manα1-6(Manα1-3)Manα1-6][Rβ1-2Manα1-3]Manβ1-4GlcNAcβ1-4GlcNAc-Asn-X {***1***: R = H; ***2***: **R** = GlcNAc}.

(1) Sarkar, M., *et al., Proc. Natl. Acad. Sci. USA*, 1991, **88**, 234 – 238.
(2) Hull, E., *et al., Biochem. Biophys Res Commun*, 1991, **176**, 608 – 615.
(3) Pownall, S., *et al., Genomics*, 1992, **12**, 699 – 704.
(4) Paulson, J. C. and K. J. Colley, *J Biol Chem*, 1989, **264**, 17615 – 17618.
(5) Schachter, H., *Current Opinion in Structural Biology*, 1991, **1**, 755 – 765.

S2.10

## Genomic Mapping of The Rat UDP-*N*-Acetylglucosamine: α-6-D-Mannoside β-1,2-*N*-Acetylglucosaminyltransferase II Gene

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UDP-N-acetylglucosamine:α-6-D-mannoside β-1,2-*N*-acetyl-glucosaminyltransferase II (GnT II, EC 2.4.1.143) is a Golgi membrane-bound enzyme catalyzing an essential step in the biosynthetic pathway leading from high-mannose to complex *N*-linked oligosaccharides. A 840-bp fragment of the rat GnT II cDNA (D'Agostaro *et al.*, this volume) was used as a probe to screen a genomic library which was constructed from rat liver DNA partially digested with EcoRI and cloned into λCharon 4A phage vector. Approximately $4 \times 10^5$ phages were screened and six positive isolates were plaque purified. The restriction pattern upon digestion with EcoRI of the DNAs from the six phage clones was similar. The identity of these clones was verified by Southern blot analysis using three distinct restriction fragments of the rat cDNA for GnT II as hybridization probes. All three probes positively identified a 6.0 Kbp EcoRI fragment in all six clones. Similarly, a fragment of 6.0 Kbp was detected in Southern blots of rat liver DNA digested to completion with EcoRI and probed with the three restriction fragments of the rat GnT II cDNA. The 6.0 Kbp EcoRI fragment from three (RG1, RG4 and RG6) DNA clones was subcloned into pBSSKM plasmid vector. A fine restriction map was generated and the restriction fragments carrying the coding regions were identified by Southern blot analysis using cDNA fragments as hybridization probes. The nucleotide sequence of a genomic region spanning 3.3 Kbp was determined on both strands using the chain termination method. The sequence of the rat DNA for GnT II reveals a contiguous 1290-nucleotide open reading frame that codes for the entire GnT II polypeptide chain and is not interrupted by introns. The single-exon GnT II gene is flanked by a GC-rich 5′-untranslated region. The 3′-untranslated region contains multiple canonical polyadenylation signals and ATTTA motifs. This observation suggests that the expression of the GnT II gene may be regulated post-transcriptionally by a different utilization of polyadenylation signals.

S2.11

## Identification of Glycosyltransferase Genes in the Mollusc *Lymnaea Stagnalis*

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To study glycosylation in the mollusc *Lymnaea stagnalis* (LS) we set out a cloning strategy for the isolation of glycosyl-transferase genes from this snail. A LS genomic library was screened using the bovine β1→4galactosyltransferase (β4GalT) cDNA as a probe under low stringency hybridization conditions. Two positive clones were isolated and analyzed. In one of the clones 3 small regions were identified that appeared to correspond with exon 3,4 and 6 of the gene coding for murine β4-GalT. The deduced amino acid sequence of the LS and the murine β4GalT genomic sequences showed an identity of 42% in exon 3, 53% in exon 4 and 30% in exon 6.

In a Northern blot with mRNA derived from different tissues of the snail the LS genomic clone mainly hybridized