

to a novel metabolic relationship between the two, or to the presence of new ribosyl polymers in mycobacteria.

#### S1.41

### Human UDP-Galnac: Polypeptide N-Acetylgalactosaminyl Transferase: Identification of Two Distinct Threonine Transferase Activities

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Purification of UDP-GalNAc: polypeptide N-acetylgalactosaminyl transferase from human placenta and ovine and porcine submaxillary glands by affinity chromatography on a defined synthetic peptide containing multiple threonine acceptor substrate sites resulted in separation of at least two distinct threonine transferase activities. A panel of synthetic peptides was utilized as acceptor substrates on transferase preparations before and after affinity purification on the synthetic peptide in order to evaluate the substrate specificities. Only a fraction of the transferase activity available was bound to the affinity column evaluated by a number of acceptor substrate peptides including the peptide used for the affinity chromatography column. Interestingly, one peptide containing a single threonine glycosylation site was not glycosylated by the affinity purified transferase and the transferase activity to this peptide passed quantitatively through the affinity column. Furthermore, kinetic analysis of crude and purified transferase preparations revealed

significant differences between the two transferase preparations. The results suggest that more than one threonine transferase activity exist, and it is likely to be due to different structural proteins.

#### S1.42

### Purification to Apparent Homogeneity of Human UDP-Galnac: Polypeptide N-Acetylgalactosaminyl Transferase

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A soluble UDP-GalNAc: polypeptide N-acetylgalactosaminyl transferase was purified 1500-fold to apparent homogeneity from human placenta tissue. The transferase was solubilized with 1% Triton X-100 and affinity purified by chromatography using a synthetic peptide containing multiple threonine acceptor substrate sites. The specific activity of the purified soluble transferase in the presence of detergent and 2-Mercaptoethanol was 0.75 U/mg measured with the synthetic peptides used in the affinity purification. The purified transferase had an apparent molecular weight of 50 kd by S12 gel filtration run without detergent and 55 kd as measured by SDS-PAGE under reducing conditions. When the passthrough of the affinity chromatography column run in Triton-100 was reapplied to the same column after detergent exchange to octylglucoside an apparent membrane bound transferase was obtained.

## S.2 MOLECULAR BIOLOGY OF GLYCOSYLTRANSFERASES AND CARBOHYDRATE TRIMMING ENZYMES

### S2.1

#### Structure and Function of Mammalian Glycosyltransferase Genes

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Mammalian cell surfaces display a variety of distinct glycoconjugate molecules, whose structures change during development and differentiation. Fucosylated glycans are one interesting class of such molecules. Some of these glycoconjugates, including the sialyl Lewis x and sialyl Lewis a determinants, function as ligands for the selectin family of cell adhesion molecules. These molecules are constructed by a family of fucosyltransferases. We have used gene transfer and cross-hybridization approaches to isolate several mammalian fucosyltransferase genes. Structural and functional analyses indicate that these can be classified into distinct families, based on similarities and differences between their structures and their enzymatic activities. In the human species, the  $\alpha(1,3)$  fucosyltransferase gene family contains at least four members. Three of these share more than 90% primary amino acid sequence identity, and are physically and genetically linked on chromosome 19. Structure/function analyses indicate that the portions of these enzymes that determine their ability to

distinguish amongst a variety of acceptor substrates correspond to peptide sequences within and adjacent to the "stem" region. Representative human  $\alpha(1,3)$ fucosyltransferases have been expressed in *E. coli*, and used to prepare antisera against these enzymes. Biosynthetic studies with these reagents indicate that these polypeptides are glycosylated, exist as membrane-associated molecules within the cell, and are also released from cells as soluble molecules. Studies are in progress to define the positions of proteolytic cleavage within these molecules, that yield these soluble, secreted, catalytically-active fragments, and to further refine our understanding of the primary sequence determinants within these enzymes that are most important in dictating their acceptor substrate specificity.

### S2.2

#### Molecular Cloning and Localization to Chromosome 14 of The Human UDP-N-Acetylglucosamine: $\alpha$ -6-D-Mannoside $\beta$ -1,2-N-Acetylglucosaminyltransferase II Gene (MGAT2)

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