bovine CD36 prepared from milk fat globule membranes (MFGM) derived from mammary gland epithelial cell membranes contains hybrid-type and bi-, tri- and tetra-antennary complex-type sugar chains with the ±Neu5Acα2→6GalNAcβ1→4GlcNAc groups which amounted to 28% of the total sugar chains (1). Lectin blot analysis of bovine MFGM glycoproteins with use of *Wistaria floribunda* agglutinin (WFA) indicated that many glycoproteins including CD36 have the sugar chains with the groups. Structural analysis of the sugar chains released by hydrazinolysis from these glycoproteins by sequential exoglycosidase digestion and by methylation analysis revealed that the sugar chains which bound to a WFA-agarose column are mainly of biantennary complex-type and hybrid-type with the ±Neu5Acα2→6 GalNAcβ1→4GlcNAc groups. In contrast, lectin blotting of glycoproteins obtained from primary cultured cells derived from bovine mammary gland epithelial cells revealed that there were few glycoprotein bands stained with WFA. However, the bands appeared in the glycoprotein samples obtained from the cultured cells treated with prolactin, insulin and hydrocortison, suggesting that *N*-acetylgalactosaminylation of glycoproteins in bovine mammary gland epithelial cells is expressed by differentiation of cells under the influence of the hormones.

(1) Nakata, N., Furukawa, K., Greenwalt, D. E., Sato, T. and Kobata, A. (1993) Biochemistry, in press.

S1.14

Glucuronyltransferases in Nervous System Associated with the Biosynthesis of HNK-1 Carbohydrate Epitope

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The HNK-1 carbohydrate epitope is expressed on many neural adhesion molecules such as N-CAM, Ll, J1, MAG, and Po and also on unique glycolipids, 3-sulfoglucuronyl neolactotetraosyl ceramide and its homologues. The terminal 3-sulfoglucuronyl moiety is essential for the recognition by the monoclonal antibody HNK- 1. This epitope is temporally and specially regulated during the development of the nervous system and suggested to play important roles in cell – cell adhesion and recognition. Recently, we found the presence of two types of glucuronyltransferase activity in the brain: one transfers glucuronic acid from UDP-glucuronic acid to glycolipid acceptors, e.g., neolactotetraosylceramide, (GlcAT-L) and the other transfers glucuronic acid to glycoprotein acceptors, e.g., asialoorosomucoid (GlcAT-P) (Oka, S., Terayama, K., Kawashima, C., and Kawasaki, T. (1992) J. Biol. Chem., *267*, 22711 – 22714). These two types of glucuronyltransferase were different from the liver glucuronyltransferases associated with detoxication and also from those involved in the glycosaminoglycan synthesis. GlcAT-L and GlcAT-P shared some properties in common such as requirement of a terminal lactosamine structure in an acceptor molecule, predominant localization in the brain, activity changes with development and requirement of manganese. However, they showed differences in their phospholipid dependency and pH profiles, besides their respective acceptor preference for glycolipids and glycoproteins. The acceptor specificity and the tissue distribution suggest that these glucuronyltransferases are involved in the biosynthesis of the HNK-1 carbohydrate epitope on glycolipids and glycoproteins associated with neural cell adhesion and recognition.

S1.15

TGF-β Uncouples the Inhibitory Effect of Increasing Cell Density on the Amount of Chondroitin Sulfate Proteoglycan Synthesized Per Cell

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Anchorage-dependent cultures of a population of cells derived from the outer part of the rat calvarium demonstrated decreased net accumulation of radiolabelled chondroitin sulfate (CS) and hyaluronic acid (HA) per cell as the cell density of the cultures increased. The addition of TGF-β resulted in large stimulations of the net CS, but not the net HA, accumulating in the medium at all cell densities and an abolition of the density-dependent effect. The accumulation of CS and HA in the medium was shown to be due largely to increases in the newly synthesized glycoconjugates. The results suggest a relatively specific upregulation of CS proteoglycan (PG) synthesis and an uncoupling of the inhibitory effect of high cell density on CS PG synthesis.

Two hypotheses are being examined to account for these effects. One hypothesis is based on the relation between cell density and the number and affinity of TGF-β receptors. The other is based on the presence of progressively steeper diffusion gradients that are set up in stationary cultures as cell density increases.

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S1.16

N-Glycan Patterns and GlcNAc – Transferase I Activities of Three Insect Cell Lines

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(I) Membrane glycoproteins from *Bombyx mori* (Bm-N), *Mamestra brassicae* (Mb 0503) and *Spodoptera frugiperda* (Sf-21) cells were compared with respect to their *N*-glycosidically linked oligosacharides.

After release with PNGase A, oligosaccharides were reductively aminated with 2-aminopyridine, separated and identified by 2D-HPLC-mapping in combination with exoglycosidase digestions.

Oligomannose type structures ranging from $(Man)_2(GlcNAc)_2$ to $(Man)_9(GlcNAc)_2$ could be detected in all three cell lines. The small (Man_2, Man_3) oligosaccharides were found to be partially fucosylated at the asparagine-linked GlcNAc residue. Additionally, a small fraction of less then 5% of the total oligosaccharides was found to contain terminal GlcNAc residues. In Mb 0503-cells, the following two structures could be found: