to initiate CS synthesis in the presence of BFA, indicating that the β-xylosides are either unable to access the CS synthesizing machinery or that the machinery is no longer intact. Since BFA is thought to isolate the Golgi complex from the trans-Golgi network (TGN), the lack of CS synthesis by β-xylosides in the presence of BFA may indicate that CS initiation occurs in the Golgi complex while chain elongation occurs in the TGN. Finally, the intracellular pool of aggrecan core protein precursor accumulates in the presence of BFA. Release from BFA while blocking new protein synthesis (with cycloheximide) showed the core protein precursor to be chased into completed aggrecan. This indicates that reassembly of the CS synthesis apparatus is rapid and efficient after removal of the BFA block.

S5.4

Role of Sulfation in Formation and Stabilization of Matrix Dermatan Sulfate

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Formation of dermatan sulfate glycosaminoglycans proceeds from precursor chondroitin residues when the appropriate uronosyl epimerase is present to convert variable amounts of glucuronic acid to iduronic acid. Thus proteodermatan sulfate can be considered to be a form of proteochondroitin sulfate that contains some dermatan. When skin fibroblasts or other cells capable of producing proteodermatan sulfate were grown under low sulfate conditions, sulfation of *N*-acetylgalactosamine could be reduced to 50% or less. Treatment of the products with chondroitin AC lyase (which degrades chondroitin but not dermatan linkages) demonstrated that essentially all the non-sulfated residues were susceptible to the enzyme while essentially none of the sulfated residues were susceptible. This confirmed and extended the work of Malmstrom and his colleagues who used a human skin fibroblast microsomal system to establish a relationship of sulfation to epimerization during synthesis of dermatan sulfate. In agreement with this previous work, we found that epimerization and sulfation with a similar human skin fibroblast microsomal system was essentially complete when 3′ phosphoadenylyl 5′-phosphosulfate (PAPS) was present during the incubation. In contrast, as previously indicated, less than 10% of the glucuronic acid residues were epimerized in the absence of PAPS. Furthermore, we found that skin fibroblasts grown with concentrations of chlorate sufficient to abolish all sulfation produced little or no identifiable dermatan residues. This suggested to us that sulfation in an intact cell precedes epimerization, which disagrees with the previous proposal that epimerization occurs first. In either case, it is clear that the processes of epimerization and sulfation are connected. It is of note that the dermatan linkage is not susceptible to known animal endoglycosidases such as hyaluronidase, while the chondroitin linkage is readily cleaved. Thus the sulfation-linked epimerization converts some or all of the degradable portions of chondroitin to non-degradable dermatan sulfate, consequently protecting and stabilizing the glycosaminoglycan. We have found that skin fibroblasts require sulfate concentrations approaching 0.2 mM in order to obtain maximal sulfation/epimerization in converting chondroitin to dermatan sulfate. This concentration is at the lower end of sulfate concentrations found in the normal sera of various mammalian species, suggesting that sulfate deprivation *in vivo* might lead to an increase in the glucuronic acid residues susceptible to the degradative enzymes. This in turn would increase the susceptibility of proteodermatan sulfate glycosaminoglycans to degradation and subsequent turnover.

S5.5

HB-GAM (Pleiotrophin), an Extracellular Matrix-Associated Neurite Growth-Promoting Factor that Interacts with Heparin-type Carbohydrate Chains

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HB-GAM (heparin-binding growth-associated molecule; p 18) was isolated from rat brain as a neurite outgrowth-promoting protein, the expression of which in brain tissue corresponds to the perinatal stage of rapid axonal growth (1). Molecular cloning of HB-GAM revealed a novel, lysine-rich sequence that is homologous with the MK (midkine) sequence suggested to play a role in retinoic acid-induced cell differentiation (2). An identical sequence was found for the neurite-promoting and mitogenic protein pleiotrophin (3). Expression of the HB-GAM cDNA with a baculovirus vector produced a secreted protein, which had apparently identical properties as compared to brain-derived HB-GAM. *In situ* hybridization and immunohistochemistry using anti-synthetic peptide and anti-recombinant HB-GAM antibodies showed that HB-GAM accumulates to the pathways of axonal processes at the stage of rapid development *in vivo* but is absent or only occurs at low levels in the pathways after axonal growth has ceased. Tracts of HB-GAM were also created on culture wells *in vitro*, and were shown to induce a patterned outgrowth of neurites from embryonic rat brain neurons. The HB-GAM-induced neurite outgrowth requires the presence of heparin-type carbohydrate chains since excess amounts of low molecular weight heparin and treatment of brain neurons with heparitinase clearly reduce neurite outgrowth. We suggest that HB-GAM is a growth-promoting cue that is strongly expressed in the pathways of developing axons and requires the presence of heparin-type carbohydrates for biological activity.

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S5.6

Sponge Cell Aggregation — The Functional Implication of Carbohydrate Epitopes in Cellular Interactions

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Carbohydrates have been implicated in the species specific