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S1. CONTROL OF GLYCOCONJUGATE METABOLISM INCLUDING NOVEL PATHWAYS ENZYMES AND ENZYME INHIBITORS

S1.1
On the Origin of Oligosaccharide Species

D. H. Van den Eijnden
Dept. Medical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands.

Oligosaccharides either free or protein- or lipid-linked are secondary gene products, that are formed by the sequential action of a group of specific enzymes: the glycosyltransferases (being the primary gene products). Together the expression levels of these enzymes make up for the glycosylation potential of a cell. In addition the acceptor specificity of glycosyltransferases as well as their localization in the cell are of major importance in the synthesis of specific oligosaccharide structures.

In the pathway leading to *N*-acetyllactosamine (LacNAc) type *N*-linked glycans the activity of the elongating and terminating enzymes is strongly dependent on the degree and type of branching of the acceptor substrates as brought about by the action of the branching *N*-acetylglucosaminyltransferases (GlcNAc-T) I-V. Thus prior action of GlcNAc-T IV will result in a shift from α6-sialylation to α3-sialylation; action of GlcNAc-T V will strongly reduce α6-sialylation and will promote poly-*N*-acetyllactosaminoglycan (poly-LAG) formation; action of GlcNAc-T III will result in a reduced further glycan processing yielding incomplete structures. In addition prior action of each of the branching GlcNAc-T's may have an effect on the branch specificity of the elongating and terminating enzymes. For instance α3-sialyltransferase (α3-NeuAc-T) shows no branch specificity with diantennary substrates, whereas it prefers the β1→2/αl→3 and 1→4/α1→3 branches after GlcNAc-T IV action. Similarly the β3-GlcNAc-T ("i-enzyme"; initiation of poly-LAG extensions on *N*-linked chains) shows no branch specificity with diantennary acceptors, but strongly prefers the β1→2/α1→6 and the β1→6/α1→6 branches of tri- and tetra-antennary glycans that result from GlcNAc-T V action. These changes in activities and branch specifities form the basis for the preferred terminal glycosylation patterns found on *N*-glycoproteins.

Recently a novel pathway leading to the formation of GalNAcβ1→4GlcNAc- (*N,N'*-diacetyllactosediamine; LacdiNAc) based structures has been identified. This LacdiNAc pathway is controlled by an UDP-GalNAc:GlcNAc-R β4-*N*-acetylgalactosaminyltransferase (β4-GalNAc-T) that has been found in cercariae of the avian schistosome *Trichobilharzia ocellata* as well as in the albumen gland of the mollusc *Lymnaea stagnalis*. In higher animals this enzyme is likely to act in competion with the β4-galactosyltransferase (β4-Gal-T) with which it shares a very similar acceptor specificity. Further processing of LacdiNAc chains has appeared so far to be very analogous to that of the LacNAc based chains. cDNA clones that may encode the β4-GalNAc-T have been isolated from different tissues of *L. stagnalis*.

Studies on the *N*- and *O*-glycosylation in 3T3 fibroblasts transfected with the N-*ras* proto-oncogene have shown that overexpression of this gene results in the altered expression of several key glycosyltransferases involved in these processes. Analysis of the *N*- and *O*-linked chains revealed the formation of increased amounts of branched poly-LAG on both types of chains as well as an overall increase in sialylation. These structural results could be explained from the altered glycosyltransferase activities by assuming that enzymes that can act on the same oligosaccharide intermediate structure may in fact not compete in the cell because of a differential localization. These studies thus have provided indirect evidence for a physical separation of GlcNAc-T III and V from β4-Gal-T and of the β6-GlcNAc-T (*O*-linked core 2 synthase) and β3-GlcNAc-T (initiation of poly-LAG extensions on *O*-linked chains) from α3-NeuAc-T involved in *O*-glycan synthesis.

S1.2
The Biogenesis of Glycogen and the Role of Glycogenin

J. Lomako, W. M. Lomako, M. D. Alonso and W. J. Whelan
Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101, USA.

It is now accepted that glycogen contains a covalently attached protein in 1:1 molecular proportion to polysaccharide. This protein (glycogenin) when purified to homogeneity in a native state, is able to attach to itself a maltosaccharide chain up to eight glucose units in an autocatalytic manner and when so glucosylated, serves as a primer for glycogen synthase. This is how glycogen synthesis is initiated. It has been found in this laboratory that the first glucose unit is attached to glycogenin through the novel alpha-glycosidic bond to the hydroxyl group of a tyrosine residue. This has been confirmed by Cohen's group where, after sequencing glycogenin, they located the glucosylated tyrosine at position 194. In our most recent work we have used cultured rat-brain astrocytes and quail-embryo