muscle in order to investigate *in vivo* the metabolic pathway from glycogenin to glycogen and vice versa. We find several intermediates between glycogenin and glycogen containing, we believe, different amounts of carbohydrate but all of them appear to contain one molecular proportion of glycogenin. One of them, of Mr 400 kDa, which we term proglycogen, is a stable intermediate in glycogen synthesis and degradation. It is broken down to glycogenin when cells are treated with ammonium ions or sulfonylureas. When these factors are removed from the growth media, a rapid resynthesis of glycogen takes place.

Recently the cDNA for glycogenin from rabbit skeletal muscle has been cloned by Roach and independently by us. We have also cloned the cDNA for glycogenin from human skeletal muscle. Comparing the deduced amino acid sequences, we find that glycogenin structure is closely conserved. Of the 332 amino acids present in glycogenin, there are only 36 differences between the two species. Some regions, such as the first 30 *N*-terminal amino acids, and the sequence surrounding the glycosylation site, are identical in both human- and rabbit-muscle glycogenins.

These findings open new aspects of the biogenesis of storage polysaccharides in general and, in particular, may help to explain aberrations in glycogen synthesis that are presently unresolved.

S1.3

Alterations of the Glycosylation of Human Transferrin Secreted by HepG2 Cells

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Serotransferrins and lactotransferrins are glycoproteins of the transferrin family involved in the iron transfer to target cells. A high homology in the amino acid sequences of different transferrins has been observed and the X-Ray diffraction analysis of human and bovine lactotransferrins and rabbit serotransferrin has revealed that the folding of polypeptide chains into two lobes is very similar.

The most important structural differences between the transferrins concern the number, the structure and the location of the glycans on the polypeptide chain, our previous studies have shown that *in vivo* glycosylation of transferrin glycans depends on the species, the tissues involved in the biosynthesis and the physiological and pathological states.

In our recent studies we have analyzed the alterations of the glycosylation of human serotransferrin secreted by HepG2 cells by using the sensitive method of antibody-affinity blotting to detect the three transferrin glycovariants separated by concanavalin A affinity electrophoresis in agarose gels.

The results obtained demonstrated that an important increase of the proportion of biantennary glycans was obtained in the presence of IL-6 in association with dexamethasone and during the storage at low temperature of the cells. In contrast, addition of glucose in the culture medium was shown to induce an increase of the proportion of triantennary glycans.

These results suggest that the activity of the *N*-acetylglucosaminyl transferases IV and V is submitted to an important regulation.

S1.4

Up-Regulation of Two Glycosyltransferases, Lc3 Synthase and GM3 Synthase, is a Key in Biosynthesis of Bioactive Gangliosides for Determining Differentiation-Directions of Human Leukemia HL-60 Cells

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Ganglioside GM3 was remarkably increased during monocytoid differentiation of human myelogenous leukemia HL-60 cells, and neolacto-series gangliosides (NeuAc-nLc) were enriched during granulocytoid differentiation. In addition, HL-60 was differentiated into monocytic lineage by exogenous GM3 and into granulocytoid by NeuAc-nLc[1]. In the present report, the enzymatic bases of glycosphingolipid biosynthesis in HL-60 during differentiation were investigated. We have elucidated the followings; (i) during monocytic differentiation, CMP-NeuAc:lactosylceramide $\alpha2\rightarrow3$sialyltransferase (GM3 synthase) was up-regulated, resulting in dramatic GM3 increase,and the upstream UDP-GlcNAc: lactosylceramide $\beta1\rightarrow3$N-acetylglucosaminyltransferase (Lc3 synthase) was down-regulated, resulting in decrease of NeuAc-nLc, although the downstream glycosyltransferases (for synthesis of NeuAc-nLc), UDP-Gal:GlcNAc$\beta1\rightarrow3$Gal$\beta1\rightarrow$R $\beta1\rightarrow4$galactosyltransferase, UDP-GlcNAc:Gal$\beta1\rightarrow4$GlcNAc$\beta1\rightarrow$R $\beta1\rightarrow3$*N*-acetylglucosaminyltransferase, CMP-NeuAc: Gal$\beta1\rightarrow4$GlcNAc$\beta1\rightarrow$R $\alpha2\rightarrow3$sialyltransferase, and CMP-NeuAc: Gal$\beta1\rightarrow4$GlcNAc$\beta1\rightarrow$R $\alpha2\rightarrow6$sialyltransferase, were ready to catalyze the enzyme reactions. (ii) On the other hand, Lc3 synthase was up-regulated together with activation of the downstream glycosyltransferases, resulting in notable increase of NeuAc-nLc, while GM3 synthase is unchanged, resulting in relative decrease of GM3 during granulocytic differentiation[2]. These results suggest that two key upstream glycosyltransferases, GM3 synthase and Lc3 synthase, play critical roles in regulating the total metabolic flow of glycosphingolipid biosynthesis in HL-60 cells during differentiation. This switch ON/OFF mechanism of these two glycosyltransferases, together with our previous findings, might be one of the most important parts of determining system of differentiation direction.

(1) Proc. Natl. Acad. Sci. USA, **83**, 782, 1986.
(2) J. Biol. Chem., **267**, 23507, 1992.

S1.5

The Use of Inhibitors of Glycosylation and Processing to Study Synthesis and Function of Complex Carbohydrates

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Complex carbohydrates are widespread in nature and include various types of glycoproteins as well as glycolipids and glycosylphosphatidylinositol (GPI) anchors. Except for a few cases (i.e., mannose-6-P receptor, asialoglycoprotein receptor, selectins, etc.), the role of the carbohydrate is not well understood. One approach to help understand the function of the carbohydrate is to find and utilize inhibitors that alter the