From glycosylation disorders back to glycosylation: what have we learned?

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Abstract

Diseases of glycosylation have long remained confined to the rare hematological disorders, the Tn-syndrome [1] and paroxysmal nocturnal hemoglobinuria [2]. This rarity was often interpreted as a sign that defects of glycosylation are either lethal, or remain asymptomatic because of the large redundancy found in glycosylation pathways. The description of multiple glycosylation disorders over the last years has definitively settled the issue and demonstrated the broad range of biological processes relying on proper glycosylation. However, beyond establishing the developmental and physiological roles of glycosylation how did glycosylation disorders contribute to new insights in the field of glycobiology?
Diseases presenting similar clinical features often share common causes. Such phenotypic similarities have helped expanding the inventory of genes involved in common glycosylation pathways. This was the case for various forms of congenital muscular dystrophies, which allowed associating putative glycosyltransferase genes with α-dystroglycan glycosylation. In other instances, similar clinical features pointed to relationships between glycosyltransferases and their protein substrates. Typically, the identification of the polypeptide GalNAc-transferase-3 mutations as a cause of tumor calcinosis shed new light on the significance of O-GalNAc modifications in the context of FGF23 functions. Furthermore, the identification of glycosylation disorders linked to defects of vesicular transport has advanced the understanding the mechanisms involved in glycosyltransferase localization. Sometimes, the description of novel glycosylation disorders yielded unexpected findings, which raised new questions on the functions of previously described genes. For example, mutations in the UDP-GlcNAc-2-epimerase/N-acetylmannosamine kinase gene caused a relatively mild myopathy, which was in strongly contrast to the embryonic lethality observed in the corresponding knockout mouse model. In conclusion, glycosylation disorders can reveal interesting features of glycoconjugates or open new perspectives on specific glycobiology pathways.

**Dystroglycan glycosylation**

The description of several glycosylation disorders among the family of congenital muscular dystrophies certainly expanded the visibility and biological significance of the O-mannosylation pathway. Protein O-mannosylation was long considered to be specific to fungal cell wall glycosylation. Whereas O-linked mannosylated glycans had been documented in multicellular organisms including mammals [3], the structure of the O-mannosylated chains and the nature of the dedicated glycosyltransferases have long remained unclear. The description of the POMGNT1 glycosyltransferase and its association to Muscle-Eye-Brain disease [4] rapidly brought O-mannosylation to the central stage of congenital muscular dystrophies. The successive description of FKRT [5], POMT1 [6], POMT2 [7], LARGE [8] and the reinterpretation of the fukutin defect [9] definitively established the importance of α-dystroglycan glycosylation in the etiology of congenital muscular dystrophies. Whereas the involvement of the aforementioned proteins in α-dystroglycan glycosylation is undisputed, to date only POMT and POMGNT1 have been assigned a clear glycosyltransferase activity. LARGE, fukutin and FKRP show structural features typical of glycosyltransferases but their enzymatic activities remain unknown. Studies performed in mutant CHO cells have shown that LARGE is capable of elongating both O- and N-glycan chains [10]. The presence of two distinct glucosyltransferase- and N-acetylgalactosaminytransferase-like domains in LARGE suggests that this protein may have a carbohydrate polymerizing activity as observed in the
biosynthesis of glycosaminoglycan chains [11, 12]. The large carbohydrate moiety of the α-dystroglycan protein certainly supports this notion. So why have the activity of LARGE, fukutin and FKRP remained intractable in spite of numerous efforts? Like for other orphan glycosyltransferases, several options could account for the missing activity. It could be that these enzymes require a co-factor that has not been identified yet. Alternatively, it is possible that these enzymes build heteromeric complexes with partners that have not been considered yet. It could also be that these enzymes transfer carbohydrates to acceptor substrates modified by sulfation or phosphorylation as it has been described for the formation of the glycosaminoglycan tetrasaccharide core [13]. Although the puzzle of α-dystroglycan biology is not complete, the description of glycosylation defects in CMDs has allowed grouping some pieces, i.e. putative glycosyltransferases, around the central figure of α-dystroglycan.

Mucin O-glycosylation

Mucin-type O-glycosylation is initiated by the transfer of α-linked GalNAc to the hydroxyl group of serine and threonine. This step is catalyzed by a family of polypeptide GalNAc-transferases comprising at least 20 members [14]. This multitude of isoforms makes it challenging to predict the outcome of single enzyme defects. Therefore, the association of the polypeptide GalNAc-transferase-3 GALNT3 gene with a form of tumoral calcinosis [15] came as a surprise. Tumoral calcinosis, a disorder of calcium and phosphate metabolism, is also caused by mutations in the FGF23 [16] and KLOTHO [17] genes. The discovery of the GALNT3 defect as a cause of tumoral calcinosis demonstrated the role of O-glycosylation in the secretion and functions of the FGF23 glycoprotein. In fact, FGF23 is O-glycosylated and it has been shown that a specific O-glycan chain protects the FGF23 protein from cleavage by subtilisin-like proteases while transiting through the secretory pathway [18]. This case is not unique since O-glycosylation has also been shown to protect the intracellular proteolytic cleavage of the endopeptidase meprin [19] and to be required for the expression of the LDL receptor [20]. Additional examples are likely to follow, thereby drawing functional relationships between O-glycosyltransferases and specific glycoproteins. So far, the GALNT3 defect contributes to establish the importance of O-glycosylation for the intracellular processing of glycoproteins.

Oligosaccharyltransferase

Several disorders of N-glycosylation have been characterized in the last decade, largely thank to the easy detection of abnormally glycosylated serum transferrin by isoelectric focusing. Abnormal transferrin glycosylation was observed for defects of lipid-linked oligosaccharide (LLO) assembly and for defects of N-glycan processing. Even defects of glycosyltransferase localization, such as encountered in COG deficiency, affect the N-glycosylation of transferrin. Whereas most genes of the
N-glycosylation pathway encoding ER-localized proteins have been related to glycosylation disorders, the oligosaccharyltransferase complex has remained a lonely exception. This multiprotein complex is expressed ubiquitously and it was expected that defective oligosaccharyltransferase activity would result in abnormal transferrin glycosylation. However, the recently described mutations in the TUSC3/N33 gene [21, 22], which encodes the oligosaccharyltransferase subunit OST3, did not affect the N-glycosylation of transferrin. The OST3 protein is homologous to the OST6 subunit and analyses conducted in yeast have shown that OST3 and OST6 form distinct complexes with the other oligosaccharyltransferase subunits [23]. OST3 and OST6 are functionally related since OST3 deficiency can be partially rescued by OST6 and vice versa [24]. This functional overlap explains why the deletion of the OST3 gene in yeast yielded only minor N-glycosylation defects. Along this line, the normal glycosylation of serum transferrin detected in human OST3 deficiency may be related to such a redundancy between OST3 and OST6. Despite the partial interchangeability of OST3 and OST6, the association of OST3 mutations with nonsyndromic mental retardation [21, 22] demonstrated the unique contribution of OST3 towards specific glycoproteins. At the biomedical level, two lessons can be learned from this result. Firstly, the normal N-glycosylation of serum transferrin seen in OST3 deficiency suggests that additional glycosylation disorders have certainly remained undiagnosed to date and that other tests should be developed to detect potential restricted glycosylation defects. Secondly, the mental retardation phenotype indicated that OST3 is required for the proper glycosylation and functions of proteins in the central nervous system. Nonsyndromic mental retardation has also been related to mutations in the CRBN, CC2D1A, PRSS12 and GRIK2 genes. The latter gene encodes a subunit of the glutamate receptor, which carries eight N-glycosylation sites. The phenotypic similarity between the OST3 and GRIK2 defects may indicate that OST3 is required for GRIK2 expression or activity. Although representing a difficult task, the description of the OST3 deficiency prompted for the search of specifically underglycosylated proteins. The characterization of OST3 substrate specificity would contribute to a better understanding of the mechanisms underlying the recognition of glycosylation substrates during protein translation.

**LLO glucosyltransferases**

The comparison of the clinical features linked to different defects of LLO assembly pointed to a peculiar observation. The last steps of LLO assembly are catalyzed by the ALG6, ALG8 and ALG10 glucosyltransferases (Fig. 1). Whereas ALG6 and ALG8 defects lead to the accumulation of very similar LLO structures, the clinical outcome of ALG8 deficiency is more severe than that of ALG6 deficiency. The main features associated to ALG6 deficiency, which causes CDG-Ic [25], are central hypotonia and mild coagulopathy [26]. By contrast, ALG8 deficiency, which causes CDG-Ih [27, 28], leads to cardio-respiratory problems, dysmorphism, oedema in addition to a central hypotonia and...
coagulopathy as found in CDG-Ic. Most ALG8 patients died in their infancy, with one exception presenting a mild clinical outcome [28]. What are the reasons for the clinical discrepancy between the ALG6 and ALG8 deficiencies? It is possible that the mutations found in the ALG8 gene lead to a stronger underglycosylation than found in ALG6 deficiency. However, the comparison of the level of transferrin glycosylation between ALG6 and ALG8 deficiency does not support this hypothesis. Alternatively, the different clinical outcome may be related to different functions of the ALG6 and ALG8 glucosyltransferases. The characterization of the ALG8 deficient fibroblasts revealed an unexpected glucosidase activity targeting LLOs. Indeed, ALG8 deficiency was accompanied by the accumulation of the incomplete LLOs dolichyl-pyrophosphate-GlcNAc2Man9 and -GlcNAc2Man9Glc1 [27, 28], whereas only the latter structure was expected to accumulate. This presence of the LLO dolichyl-pyrophosphate-GlcNAc2Man9 in ALG8-deficient cells indicated that glucosylated LLOs are substrates of the glucosidase-II enzyme, which removes Glc residues on N-glycans after they have been transferred to proteins [29]. The accumulation of incomplete glucosylated LLOs in the ALG8-deficient cells may divert or impair glucosidase-II activity and thereby worsen the impact of the ALG8 defect itself on protein glycosylation, folding and secretion. Although the involvement of glucosidase-II in the pathogenesis of CDG-Ih is hypothetical at this stage, the question of the distinct severity between ALG6 and ALG8 deficiency shows how the study of glycosylation disorders lead to new questions and thereby to new concepts on the mechanisms underlying protein glycosylation.

Glycosyltransferase localization

The localization of glycosyltransferases is a dynamic process tightly regulated with the flow of secreted proteins. Recently, defects of glycosylation have been related to the abnormal assembly of the Conserved Oligomeric Golgi (COG) complex, which is a tethering complex involved in vesicular transport. The COG complex is found in eukaryotes from yeast up to human cells. Although the cellular functions of COG are still being established, the discovery of COG defects among glycosylation disorders demonstrated the importance of COG for proper glycosyltransferase localization. Especially interesting was the strong differences in clinical severity between the COG defects described to date. In fact, whereas the deficiency of the COG7 subunit lead to a multiorgan disorder and to infant mortality in the index patients [30], the deficiency of the COG1 subunit lead to a milder disease [31]. As discussed by Foulquier et al. [31], this difference is likely related the specific involvement of COG subunits in regulating the localization of Golgi-resident proteins. In any case, the availability of fibroblast cells from patients with COG deficiency allows a systematic investigation of the role of the COG complex on the regulation of glycosylation pathways.

CMP-sialic acid biosynthesis
The UDP-GlcNAc 2-epimerase/N-acetylmannosamine kinase (GNE) enzyme catalyzes a rate-limiting step in the synthesis of sialic acid. Accordingly, GNE activity also correlates with the level of sialylation on many cell types [32]. Therefore, the association of GNE mutations to a mild form of neuromuscular disorder called hereditary inclusion body myopathy (HIBM) [33] was quite surprising. HIBM is characterized by a late onset myopathy mostly limited to leg muscles. Histological examination of HIBM tissue revealed typical intramuscular vacuoles and filamentous inclusions of unknown origin. By contrast, the disruption of the GNE gene in mice lead to embryonic lethality [34] and even the introduction of the frequent GNE mutation M712T by knockin into the mouse genome has been shown to result in perinatal lethality [35]. The striking discrepancy between the outcome of GNE deficiency in humans and mice illustrates the different requirement for sialic acid in both species. This statement does not mean that sialic acid is dispensable in humans. In fact, the severe hematological disorder associated with a defect of the CMP-sialic acid transporter in a child [36] supports the essential role of sialylated glycans for human physiology. The rather weak phenotype of GNE deficiency could be related to the activity of possible salvage pathways that could compensate for the loss of endogenous sialic acid biosynthesis. At any rate, additional work is required to understand the relationship between the pathogenesis of HIBM, the level of residual GNE activity and the contribution of alternate sources of sialic acid in human biology.

As evident from the examples discussed here, glycosylation disorders provide new insights on the role of glycosylation in humans and highlight differences to animal models. Several glycosylation disorders have been described in the last decade and the list is certainly going to expand steadily in the years to come. The recent investigation of N-glycosylation in the context of autoimmune diseases like multiple sclerosis [37, 38] is promising to broaden the biomedical significance of glycosylation even more. The study of glycosylation disorders has definitively become an integral part of the current research landscape on glycosylation. With this concluding statement, I would also like to remind that this achievement has only been made possible by the combined effort of clinicians and glycobiologists.

Acknowledgements
This review is dedicated to Eric Berger at the occasion of his retirement and in recognition to his pioneer role in the study of glycosylation disorders. This work was supported by a grant from the Swiss National Science Foundation (3100A0-116039) and by the European Union project Euroglycanet (LSHM-2005-512131).

References


Figure legend
Figure 1. Glucosylation and deglucosylation of lipid-linked oligosaccharides in the endoplasmic reticulum. The ALG6, ALG8 and ALG10 glucosyltransferases add each a Glc residue to the dolicholpyrophosphate-GlcNAc₂Man₉ oligosaccharide. The characterization of CDG-Ih has demonstrated that the glucosidase-II (GLS2) enzyme is capable of deglucosylating LLOs. A similar activity of the glucosidase-I (GLS1) enzyme on LLOs has not been shown yet.
Figure 1 - Hennet