

# Congenital disorders of glycosylation: a concise chart of glycocalyx dysfunction

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# Congenital disorders of glycosylation – a consice chart of glycocalyx dysfunction

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**Abstract**

Glycosylation is a ubiquitous modification of lipids and proteins. Despite the essential contribution of glycoconjugates to the viability of all living organisms, diseases of glycosylation in humans have only been identified over the past few decades. The recent development of next-generation DNA sequencing techniques has accelerated the pace of discovery of novel glycosylation defects. The description of multiple mutations across glycosylation pathways has revealed a tremendous diversity of functional impairments bus also pointed to phenotypic similarities emphasizing the interconnected flow of substrates underlying glycan assembly. The current list of 100 known glycosylation disorders provides an overview on the significance of glycosylation in human development and physiology.

**Highlights**

- Congenital disorders underline the role of glycosylation in human development.
- Next-gen sequencing techniques expanded the discovery of glycosylation gene defects.
- The clinical variability of glycosylation disorders implies they are underdiagnosed.

**Glycosylation disorders**

Glycosylation is by far the most complex form of protein [1, 2] and lipid modification [3, 4] in all domains of life. The tremendous diversity of glycoconjugate structures resulting from intricate biosynthetic pathways is a major factor hampering the assignment of functions to glycans chains. Much has been learnt from the study of disrupted glycosylation genes in model organisms, thereby establishing numerous essential contributions of glycans in regulating cell and organ functions [5]. The study of human diseases of glycosylation brings additional insights by providing a more differentiated view on glycan functions. Indeed, most human mutations are hypomorphic, thus causing partial loss of glycosylation reactions that lead to variable clinical manifestations.

Diseases of glycosylation are also referred to as congenital disorders of glycosylation (CDG). Given the heterogeneity of glycans, the clinical scope of CDG is considerable, ranging from nearly normal phenotypes to severe multi-organ dysfunctions causing infantile lethality. CDG are rare diseases. The prevalence among CDG types is very different from one type to another, but is largely unknown. The difficulty in identifying patients is another reason behind the rarity of CDG. Unspecific symptoms and the lack of simple laboratory tests make the recognition of CDG cases extremely challenging. The identification of CDG has long relied on the detection of under-glycosylated serum transferrin by

isoelectric focusing [6]. While easy to perform and requiring only a few microliters of blood, this test exclusively reveals alterations of N-glycosylation. Similar blood tests have unfortunately not been established to reliably diagnose defects in other classes of glycosylation. The simplicity of the serum transferrin test also explains why disorders of N-glycosylation account for the majority of known CDG.

Recent developments in genome-wide DNA sequencing technology enable the identification of mutations without a priori knowledge of candidate genes. As in other fields of biology, next-generation sequencing approaches have increased the pace of discovery for new types of CDG [7]. The barrier of 100 genes defects impairing glycosylation has just been passed (see www.physiol.uzh.ch/Glycosylation for a graphical overview). These defects encompass nearly all glycosylation pathways and affect different molecular processes from substrate biosynthesis up to protein trafficking [8, 9]. The recent application of unbiased strategies such as exome and whole-genome sequencing have further revealed CDG-causing mutations in genes previously not associated with glycosylation, thereby expanding our view on these complex pathways.

CDG have originally been classified in two groups. So-called CDG type-I included defects of lipid-linked oligosaccharide assembly from the formation of dolichol-PP-GlcNAc up to their transfer to asparagine residues on nascent proteins. CDG type-II, by contrast, included defects of N-glycan trimming and elongation as well as defects in any other class of glycosylation [10]. Because several defects affect multiple glycosylation pathways, the artificial distinction between CDG type-I and -II has been replaced by a flat nomenclature simply associating implied genes with the suffix CDG [11]. Functionally, defects can also be grouped based on their contribution to glycosylation reactions (**Figure 1**). Accordingly, the present review discusses glycosylation disorders through five functional categories, featuring 1) genes encoding glycosyltransferase enzymes, 2) genes involved in donor substrate biosynthesis, 3) genes mediating the translocation of donor substrates, 4) genes regulating glycosyltransferase localization, and 5) genes affecting the homeostasis of secretory organelles.

**Glycosyltransferases**

The human genome includes close to 200 glycosyltransferase genes [12]. Glycosyltransferases are the enzymes shaping glycans through the formation of glycosidic linkages. The majority of these glycosyltransferases are transmembrane proteins anchored in the endoplasmic reticulum (ER) and Golgi membranes [13]. Defects of ER glycosyltransferases involved in the assembly of the lipid-linked oligosaccharide $GlcNAc_2Man_9Glc_3$ limit the availability of this substrate for transfer to N-glycosylation sites on acceptor proteins during translation (**Figure 2**). Such defects of N-glycosylation lead to glycoproteins lacking whole N-glycan chains. Depending on the glycoproteins affected, non-occupancy of N-glycosylation sites can impair protein folding, secretion and stability. At the level of the organism, such defects lead to multiple organ dysfunctions. Neurological symptoms are frequent, including psychomotor retardation, ataxia, and hypotonia. Liver and cardiac dysfunctions are also frequently observed as well as endocrine disorders, which mainly affect the sexual maturation of female patients [14].

The functional impairments associated with some glycosyltransferase deficiencies reflect the functional relevance of the involved glycoproteins. For example, O-mannosylation [15] is an essential modification of $\alpha$-dystroglycan that ensures proper interactions between the dystroglycan complex and proteins of the extracellular matrix [16]. Such interactions are essential for the integrity of

muscular fibers, for the migration of neurons in the cortex, and for the retinal architecture [17]. Because α-dystroglycan is the main carrier of O-mannose chains, the manifestations of O-mannosylation disorders relate to α-dystroglycan functions, and therefore encompass muscular degeneration, brain abnormality, and blindness. Clinically, these disorders belong to the congenital muscular dystrophies and are known as Walker-Warburg syndrome, Muscle-Eye-Brain disease, Fukuyama-type congenital muscular dystrophy, and Limb-girdle muscular dystrophy. The most severe cases are usually associated with mutations in the core mannosyltransferase genes *POMT1* [18] and *POMT2* [19] and in the β1-2 GlcNAc-transferase gene *POMGNT1* [20] (**Figure 2**), but other gene defects also account for severe cases of Walker-Warburg syndrome and Muscle-Eye-Brain disease. To date, defects in 12 genes are known to cause congenital muscular dystrophies, although the functions of some of these genes are still unclear. For example, the *FKTN* and *FKRP* genes encode putative glycosyltransferases involved in O-mannosylation, but their exact substrate specificity and activity remain unknown [21].

Another form of O-linked glycosylation is characterized by the addition of fucose (Fuc) to serine and threonine in the context of the epidermal growth factor (EGF)-like domains and thrombospondin-1 (TSP1) domains. Typical acceptor proteins are members of the Notch family including the ligands Jagged and Delta-like, which are signaling proteins involved in morphogenetic processes [22]. Complete deficiency of core O-fucosyltransferases POFUT1 and POFUT2 has not been described yet, but heterozygous mutations in the *POFUT1* gene have been identified in cases of Dowling-Degos disease, an autosomal dominant pigmentation disorder [23]. Furthermore, mutations in the downstream acting glycosyltransferases, that is, the β1-3 GlcNAc-transferase LFNG and the β1-3 Glc-transferase B3GALTL (**Figure 2**), have been associated with disorders of vertebral segmentation [24] and to multiple developmental defects known as Peters-Plus syndrome [25], respectively.

In general, defects of core glycosyltransferases are more severe than defects of terminal glycosylation. Nevertheless, the severity of the disease and the scope of organ involvements are also influenced by the functional redundancy inherent to specific glycosyltransferase reactions in the biosynthesis of classes of glycosylation. For example, mucin-type O-glycosylation is initiated by a large family of polypeptide GalNAc-transferases [26]. The partial redundancy in this enzyme family prevents a major loss of this type of glycans in humans, which explains why there is only one known disease of mucin-type O-glycosylation, called familial tumoral calcinosis, which is associated with mutations in the polypeptide GalNAc-transferase *GALNT3* gene [27]. The hormone FGF23 requires for its secretion O-GalNAc glycans specifically added by GALNT3 in the Golgi apparatus [28]. Loss of FGF23 secretion leads to hyperphosphatemia and tissue calcification, which are the cardinal symptoms of tumoral calcinosis.

**Donor substrates**

Despite the hundreds of glycosyltransferases expressed in human cells, only eleven building blocks are used as donor substrates for the assembly of all human glycans. These substrates include nine nucleotide-activated sugars and two dolichol-phosphate (P) linked sugars (**Figure 3A)**. Donor substrates are biosynthesized in the cytosol, or in the nucleus in the case of CMP-sialic acid (Sia), through multiple steps including interconversion between monosaccharide isomers. Donor substrates are used across classes of glycosylation, meaning that defects in the formation of individual nucleotide-activated sugars have a broad impact on glycan structures and lead to severe multiorgan disorders. However, the clinical severity of a given gene defect widely varies based on the

level of residual activity enabled by individual mutations. For example, about 100 mutations have been described for the phosphomannomutase *PMM2* gene [29], which represents by far the most frequent form of CDG. Mutations completely abrogating PMM2 activity lead to embryonic lethality [30] whereas point mutations that have minimal impact on the enzymatic activity will only cause mild intellectual disabilities. PMM2 activity mediates the conversion of mannose (Man)-6-P to Man-1-P, which is an early step in the biosynthesis of GDP-Man (**Figure 3B**). GDP-Man is further converted to dolichol-P-Man by an enzymatic complex encoded by the *DPM1*, *DPM2*, and *DPM3* genes. Dolichol-P-Man is a substrate used in N-glycosylation, O-mannosylation, and for the biosynthesis of the glycosylphosphatidylinositol (GPI) anchors. Accordingly, decreased dolichol-P-Man availability causes of range of diseases sharing features of classical N-glycosylation disorders, but also of the congenital muscular dystrophies caused by O-mannosylation defects.

Sometimes, exome sequencing of untyped CDG cases reveals mutations in genes that were previously associated with diseases unrelated to glycosylation. For example, phosphoglucomutase deficiency resulting from mutations in the *PGM1* gene causes glycogen storage disease XIV, characterized by accumulation of glycogen in muscles because of reduced formation of Glc-6-P from Glc-1-P occurring during breakdown of glycogen [31]. The reverse reaction catalyzed by PGM1 (i.e., the formation of Glc-1-P from Glc-6-P) is also important for the subsequent formation of UDP-Gal, which is utilized for glycan formation (**Figure 3B**). Indeed, mutations in *PGM1* have been identified as causing CDG with multiple clinical involvements such as growth retardation, cleft palate, muscular and cardiac disorders, and liver dysfunction among other manifestations [32].

The study of glycosylation diseases occasionally points to unexpected findings relative to the biological importance of donor substrate biosynthesis. The *GNE* gene encodes the bifunctional enzyme UDP-GlcNAc 2-epimerase/ManNAc kinase, which catalyzes a rate-limiting step in the biosynthesis of Sia [33]. The disruption of the *Gne* gene in mice is embryonic lethal [34], but decreased GNE activity in human beings is mainly associated with adult-onset, progressive limb-girdle muscle weakness with a remarkable sparing of quadriceps muscles [35]. This rather mild disease suggests that Sia can be efficiently salvaged in humans to bypass any defect of biosynthesis.

Because the biosynthetic pathways of most donor substrates are interconnected, it is tempting to try to therapeutically circumvent specific defects by increasing the supply of alternative carbohydrates that can be converted to the missing substrate. Unfortunately, such an approach has only been successful to treat the deficiency of Man-P isomerase (MPI), which catalyzes the interconversion of fructose-6-P and Man-6-P (**Figure 3B**). MPI deficiency is mainly a hepatic-intestinal disease and thus lacks the neurological involvement often found in CDG [36]. The decreased formation of Man-6-P accompanying MPI deficiency can be efficiently compensated by dietary Man supplementation, thereby alleviating disease symptoms [37]. Similarly, dietary supplementation with Gal has recently been shown to normalize serum transferrin glycosylation in patients affected of PGM1 deficiency [32], suggesting that Gal supplementation may alleviate some of the defects associated with the disease.

**Localization of donor substrates**

Nucleotide-activated sugars are synthesized in the cytosol and nucleus, but need to be transported to the lumen of the ER and Golgi apparatus for glycosylation reactions. Dedicated antiporters mediate the coupled translocation of nucleotide-activated sugars into the organelles and the return

of corresponding nucleotide-monophosphates into the cytosol (**Figure 1**). Most antiporters are specific for a given nucleotide-activated sugar, although multi-specific transporters have also been described. For example, SLC35D1 transports UDP-GlcA and UDP-GalNAc into the ER in exchange for returning UMP to the cytosol. These two nucleotide-activated sugars are involved in the biosynthesis of chondroitin sulfate, a main component of proteoglycans secreted by chondrocytes. Mutations in the *SLC35D1* gene cause a skeletal disease called Schneckenbecken dysplasia, characterized by severe bone abnormalites leading to neonatal lethality [38]. It is likely that other classes of glycosylation are affected by the decreased transport of UDP-GlcA and UDP-GalNAc, but the extent of such alterations has not been addressed yet.

Additional defects of nucleotide-activated sugar transport have been associated with diseases, the symptoms of which reflect the importance of the implied carbohydrate for specific cellular functions. Mutations in the *SLC35C1* gene encoding a Golgi GDP-Fuc transporter impairs terminal fucosylation, which yields epitopes such as ABO and Lewis blood group antigens [39]. Some of these fucosylated epitopes function as ligands for selectins [40] and thereby participate to leukocyte adhesion and extravasation reactions [41]. Accordingly, the shortage of GDP-Fuc in the Golgi caused by defective transport impairs leukocyte trafficking and leads to increased bacterial infections. In addition, affected patients present with short stature, intellectual disability, and mild facial dysmorphism. Hematologic defects and susceptibility to infections were reverted by oral supplementation with Fuc [42]. By comparison, mutations in the CMP-Sia transporter gene *SLC35A1* were found in a patient with intellectual impairment, seizures, ataxia, thrombocytopenia, renal and cardiac disorders [43]. A general conclusion about the role of sialylation cannot be drawn from these two cases, but the symptoms confirm the importance of Sia for leukocyte and platelet functions.

Dolichol-linked substrates do not use dedicated transporters to reach the ER lumen but proteins have been described that facilitate the translocation of these substrates across membranes. The first of these proteins is called MPDU1 and is required for making dolichol-P-Man and dolichol-P-Glc available to ER mannosyltransferases and glucosyltransferases [44]. These enzymes mediate the elongation of the dolichol-PP-oligosaccharide substrate for N-glycosylation and participate in O-mannosylation and GPI anchor biosynthesis. The mechanism of MPDU1 action is still unknown, but mutations in the *MPDU1* gene lead to a form of CDG featuring symptoms typical of N-glycosylation disorders, including psychomotor disability, hypotonia, and seizures [45, 46]. Symptoms typical of CDG were also observed in patients harboring mutations in the *RFT1* gene [47], which encodes a protein involved in the translocation of the precursor dolichol-PP-linked $GlcNAc_2Man_5$ from the cytosolic to the luminal side of the ER membrane [48] (**Figure 2**). Defective RFT1 activity results in the accumulation of dolichol-PP-$GlcNAc_2Man_5$, which remains unavailable for further extension by luminally-oriented ER mannosyl- and glucosyltransferases.

**Localization of glycosyltransferases**

A precise localization of glycosyltransferases is also required for proper glycan maturation in the Golgi apparatus. Some glycosyltransferases concentrate in the cisternae of the cis-Golgi, whereas others accumulate in the trans-Golgi. The mechanisms underlying the distribution of glycosyltransferases are not completely understood, but proteins regulating vesicle transport are involved in the process. The Conserved Oligomeric Golgi (COG) complex orchestrates the recycling of medial- and cis-Golgi resident proteins by acting as a tether to connect COPI vesicles with cis-Golgi membranes [49]. COG defects lead to abnormal glycosylation [50] because of missorting of

glycosylation enzymes and sugar transporters [51]. Whereas multiple classes of glycosylation are impaired, COG-related disorders are usually identified by detection of underglycosylated serum transferrin just like defects of N-glycosylation.

To date, mutations in seven out of eight COG subunit genes have been described. The most severe diseases are observed for *COG6*, *COG7* and *COG8* mutations, associated with severe neurological impairment, liver dysfunction, and infantile lethality [52-55]. The identification of milder cases of COG6 and COG7 deficiency harboring different mutations [56, 57] however shows that the severity of the disease does not simply relate to the subunit affected but rather to the capability of forming a fully functional COG complex. Besides the severe diseases observed for *COG6* and *COG7* defects, moderate clinical manifestations have been associated with mutations in *COG1* [58, 59], *COG2* [60], *COG4* [61, 62], and *COG5* [63-65].

COG subunits build a complex of two lobes, including COG1 to COG4 in lobe A and COG5 to COG8 in lobe B (**Figure 4**). In general, defects in lobe A lead to milder disease than defects in lobe B. Lobe A appears to be important for overall Golgi architecture, playing a role in Golgi organization and cis-Golgi sorting [66]. Alterations in lobe A lead to accumulation of late glycosylation enzymes in COG complex vesicles, thereby preventing interaction with their substrate. Lobe B rather mediates vesicular sorting of trans-Golgi enzymes through functional interactions with the tethering and fusion machinery of trans-Golgi cisternae [67, 68]. Accordingly, glycosyltransferases from early Golgi cisternae, such as the β1-2 GlcNAc-transferase *MGAT1*, are more affected by a defect in lobe A [66]. By contrast, Gal-transferases and Sia-transferases residing in trans-Golgi cisternae are more influenced by lobe B alterations [67]. Furthermore, lobe B deficiency mainly results in altered steady state levels of these enzymes due to their translocation to the ER and subsequent proteasomal degradation [65]. The broad involvement of lobe B in regulating glycosyltransferase and other trans-Golgi proteins probably account for the increased severity of lobe B mutations.

Whereas COG defects demonstrate the importance of glycosyltransferase localization for glycosylation, the characterization of another disease called Tn syndrome has pointed to the importance of chaperones in supporting folding and trafficking of specific glycosyltransferases. The Tn syndrome is a clonal defect of core 1 β1-3 Gal-transferase activity limited to a subset of hematopoietic cells. The presentation of bare O-linked GalNAc (the Tn antigen) on erythrocytes leads to the binding of naturally-occurring anti-Tn antibodies and hence to agglutination and hemolysis [69]. Tn antigen presentation on leukocytes and platelets may cause mild leukopenia and thrombocytopenia. Although core 1 β1-3 Gal-transferase activity is decreased in Tn syndrome, no mutations have been found yet in the corresponding *C1GALT1* gene. Rather, mutations in the *COSMC* gene encoding an ER-localized chaperone required for C1GALT1 folding have been identified as causing Tn syndrome [70]. C1GALT1 is the only glycosyltransferase known to undergo chaperone-assisted folding, but the example shows that proper glycosylation also relies on specific proteins such as COSMC that regulate the trafficking of glycosyltransferases from the ER to the Golgi.

**Organelle milieu**

Glycosyltransferases require co-factors, such as the metal ion $Mn^{2+}$, and a range of environmental conditions to catalyze glycosylation reactions. The recent application of unbiased genetic approaches, such as homozygosity mapping and exome sequencing, has pointed to novel genes that affect glycosylation by regulating the acidification and ionic constituents of the secretory pathway.

The *ATP6V0A2* gene encodes a subunit of an $H^+$-ATPase proton pump localized in the Golgi apparatus [71], which likely regulates pH in Golgi cisternae. Defective ATP6V0A2 action yields structural alterations of Golgi architecture but also causes accumulation of abnormal intracellular vesicles [72]. These changes affect multiple classes of glycosylation as shown by the abnormal N-glycosylation and mucin type O-glycosylation of blood serum proteins. Clinically, mutations in *ATP6V0A2* lead to multiple abnormalities including growth delay and psychomotor disability, but also to skin wrinkling and connective tissue alterations referred to as cutis laxa [73]. Skin and skeletal phenotypes are likely related to alterations of extracellular matrix secretion as indicated by changes of TGF-β signaling observed in affected fibroblasts [71].

Homozygosity mapping and exome sequencing also revealed mutations in the *TMEM165* gene as causing a glycosylation disorder with broad clinical involvement. The five patients identified to date present with growth and developmental delay, hypotonia, skeletal abnormalities, and hepatomegaly [74]. TMEM165 is a transmembrane protein localized in the Golgi membrane but also found in the plasma membrane, late endosomes and lysosomes. The function of TMEM165 is unclear but appears to be related to the transport of calcium [75], which is normally found in high concentrations in the Golgi apparatus [76]. It is possible, but not yet tested, that TMEM165 dysfunctions might also affect $Mn^{2+}$ import mediated by the SPCA1 $Ca^{2+}$ pump [77], which would explain the broad glycosylation defect observed in TMEM165 patients.

Exome sequencing will continue to unravel genes of previously unknown or unclear function as causes of CDG. This growing catalog of gene defects will broaden our knowledge of the factors regulating glycosylation, but it will also bring forward new questions regarding the underlying mechanisms of such regulatory pathways. The recent characterization of TMEM165 is a good example outlining the difficult path following the description of mutations in a new gene. This work shows that biochemical and cell biological investigations are always required to understand the biological impact of genetic alterations.

**Concluding remarks**

This brief review of CDG illustrates the diversity of glycan functions by outlining the widespread consequences of alterations at specific points along biosynthetic pathways. While fascinating, the complexity of CDG and the broad range of disease severity within a CDG type, renders CDG diagnosis challenging. This suggests that CDG is probably underdiagnosed. Accordingly, the application of unbiased sequencing approaches will certainly reveal further gene defects as cause of CDG, but also unravel glycosylation defects in mild disorders such as non-syndromic intellectual disability [78]. Looking back at the evolving CDG landscape of the past 20 years, it has become clear that the description of these diseases has greatly increased the awareness of the biomedical community for the significance of glycosylation in human development and physiology.

**TEXT BOX: Glycosaminoglycans**

Disorders of glycosylation are not limited to altered glycosyltransferase activity and localization, donor substrate biosynthesis and translocation, and homeostasis of secretory organelles. After assembly of glycan chains, several carbohydrates are further modified by methylation, acylation, phosphorylation, and sulfation, thereby granting additional properties to glycoconjugates.

Sulfation is for example a prominent modification found on glycosaminoglycans [79], which decorate proteoglycans and other extracellular matrix proteins. Glycosaminoglycans are linear chains featuring repeats of disaccharide motifs, which defines subclasses named heparan sulfate, chondroitin sulfate, dermatan sulfate, and keratan sulfate. The extensive sulfation of glycosaminoglycans increases water retention, thereby contributing to tissue hydration and swelling. In addition to affecting physical properties, the arrangement of sulfated groups on glycosaminoglycan chains generate specific binding sites for soluble proteins, such as growth factors, cytokines, and coagulation enzymes. Through this binding of growth and differentiation factors, glycosaminoglycans are essential for the formation of morphogenetic gradients [80], which direct organ formation during embryogenesis and postnatal tissue growth. Accordingly, general alterations of glycosaminoglycan biosynthesis are embryonic lethal. Despite their essential functions during embryogenesis, eight genetic defects of glycosaminoglycan assembly have been identified to date. These defects consists of the genes *XYLT1*, *B3GALT7*, *B3GALT6*, *B3GAT3* encoding glycosyltransferases building the tetrasaccharide core Xyl(β1-4)Gal(β1-3)Gal(β1-3)GlcA, the polymerizing glycosyltransferase *EXT1*, *EXT2*, and *CHSY1* genes, and the dermatan sulfate epimerase *DSE* gene [81]. These defects of glycosaminoglycan biosynthesis are associated with skeletal and connective tissue disorders such as multiple hereditary exostoses, Desbuquois dysplasia, and different forms of the Ehlers-Danlos syndrome.

Alterations of glycosaminoglycan sulfation also cause different connective tissue disorders. To date, mutations in the three *CHST3* [82], *CHST6* [83], and *CHST14* [84] sulfotransferase genes have been recognized in cases of spondyloepiphyseal dysplasia, macular corneal dystrophy, and Ehlers-Danlos syndrome musculocontractural type 1, respectively. The CHST3 sulfotransferase catalyzes the 6-O-sulfation of GalNAc in chondroitin and dermatan sulfate, whereas CHST6 catalyzes the 6-O-sulfation of GlcNAc in keratin sulfate, and CHST14 the 4-O-sulfation of GalNAc in dermatan sulfate. Limited availability of the sulfotransferase donor substrate 3'-phosphoadenosine-5'-phosphosulfate caused by mutations in the *PAPSS2* synthase gene also lead to a skeletal disease [85], which shares phenotypic similarities with several defects of glycosaminoglycan biosynthesis and sulfate.

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**Figure legends**

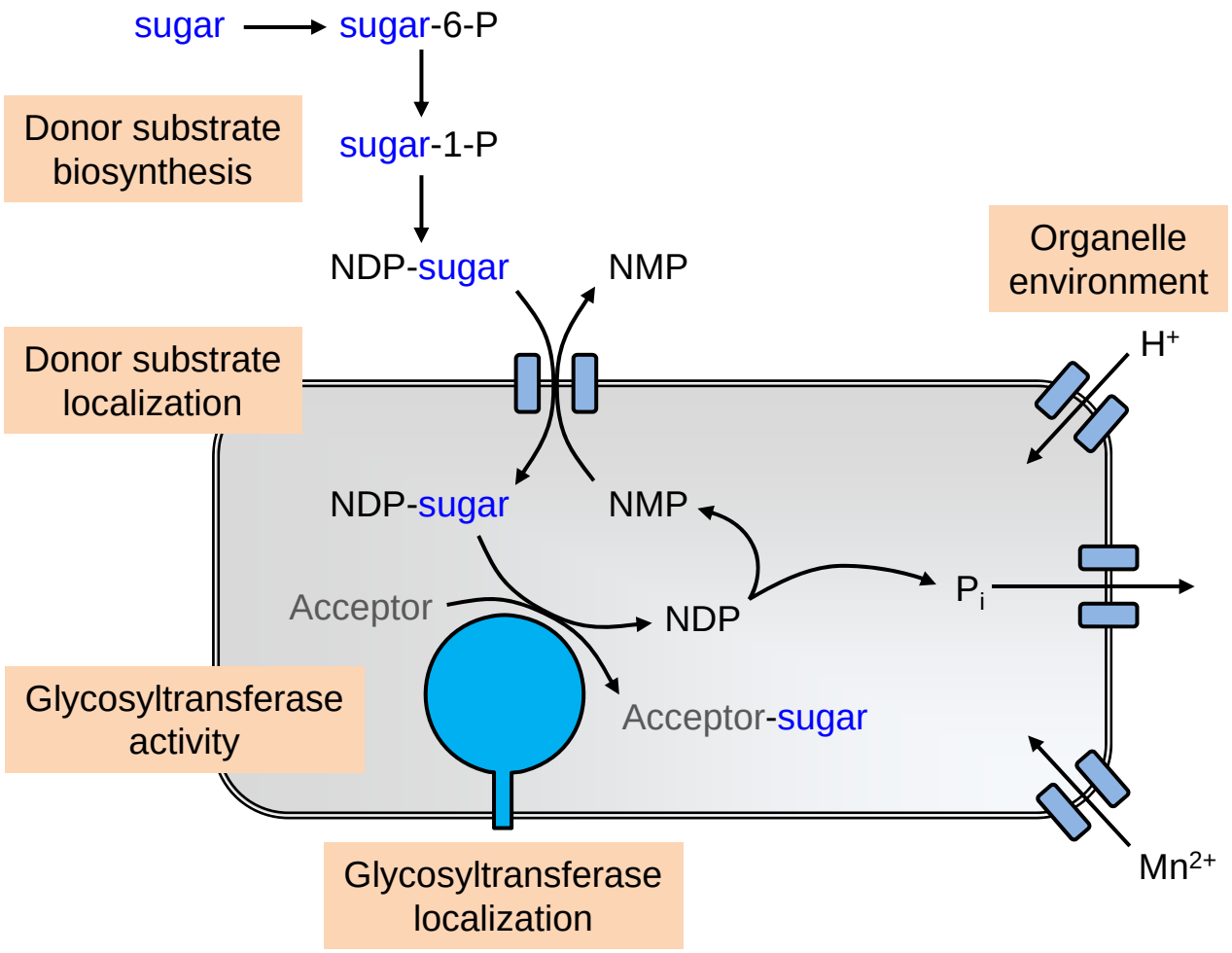
**Figure 1.** Glycosylation reaction. Schematic representation of the key players required for glycosylation reactions occurring in the Golgi apparatus. The biosynthesis of nucleotide-activated sugars (NDP-sugar) takes place in the cytosol whereas glycosyltransferase enzymes are localized on the luminal side of the endomembranes of the secretory pathway. Transporter systems are required for the import of nucleotide-activated sugars into the Golgi apparatus and for maintaining optimal ionic conditions in the organelle, thereby regulating pH, $Mn^{2+}$ import and P export.

**Figure 2.** Biosynthesis of core structures for N-glycosylation, O-mannosylation, and O-fucosylation. N-glycosylation begins at the ER membrane by the stepwise assembly of dolichol-PP-$GlcNAc_2Man_9Glc_3$, which is transferred to the selected Asn residues of nascent glycoproteins by the oligosaccharyltransferase complex (OST). O-Mannosylated and O-fucosylated glycans are shaped by the sequential addition of different monosaccharides based on the acceptor specificity of glycosyltransferases.

**Figure 3.** Donor substrates of glycosyltransferases. **A**, List of donor substrates utilized in human cells for glycosylation reactions. The substrates are grouped by nucleotide types and dolichol-P (DolP). **B**, Biosynthesis pathways of UDP-Gal, UDP-Glc, DolP-Glc, GDP-Man, and DolP-Man. The positions of known gene defects are marked with the corresponding gene symbols. *GALK1*, galactokinase; *GALT*, Gal-1-P uridylyltransferase; *GALE*, UDP-Gal 4-epimerase; *PGM1*, phosphoglucomutase 1; *MPI*, Man-P isomerase; *PMM2*, phosphomannomutase 2; *GMPPA*/*GMPPB*, GDP-Man pyrophosphorylase A/B; *DPM1/2/3*, DolP-Man synthase 1/2/3.

**Figure 4.** Schematic organization of the Conserved Oligomeric Golgi (COG) complex with display of lobe contributions to organelle architecture and protein trafficking. The eight COG subunits form two lobes, designated A (blue) and B (red). Interactions between subunits of a lobe are indicated with triple and quadruple lines. Lobe A and lobe B interact through COG1 and COG8 subunits (black line). Both lobes interact with COPI tether proteins (Golgin84, p115) and several SNARE proteins (STX5, STX6, Sly1, GS27, SNAP29), which are outlined in orange boxes. Lobe A and B mediate vesicular retrograde transport of cis (MGAT1, MAN2A1, ST3GAL5) and medial (B4GALT1, ST3GAL1, ST6GAL1) Golgi enzymes, respectively. Golgi glycosyltransferases are outlined in green boxes.

Figure 1


sugar
sugar-6-P
sugar-1-P
NDP-sugar
NMP
Donor substrate biosynthesis
Donor substrate localization
Organelle environment
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NMP
Acceptor
NDP
Pi
Glycosyltransferase activity
Acceptor-sugar
Mn2+
Glycosyltransferase localization

Figure 2

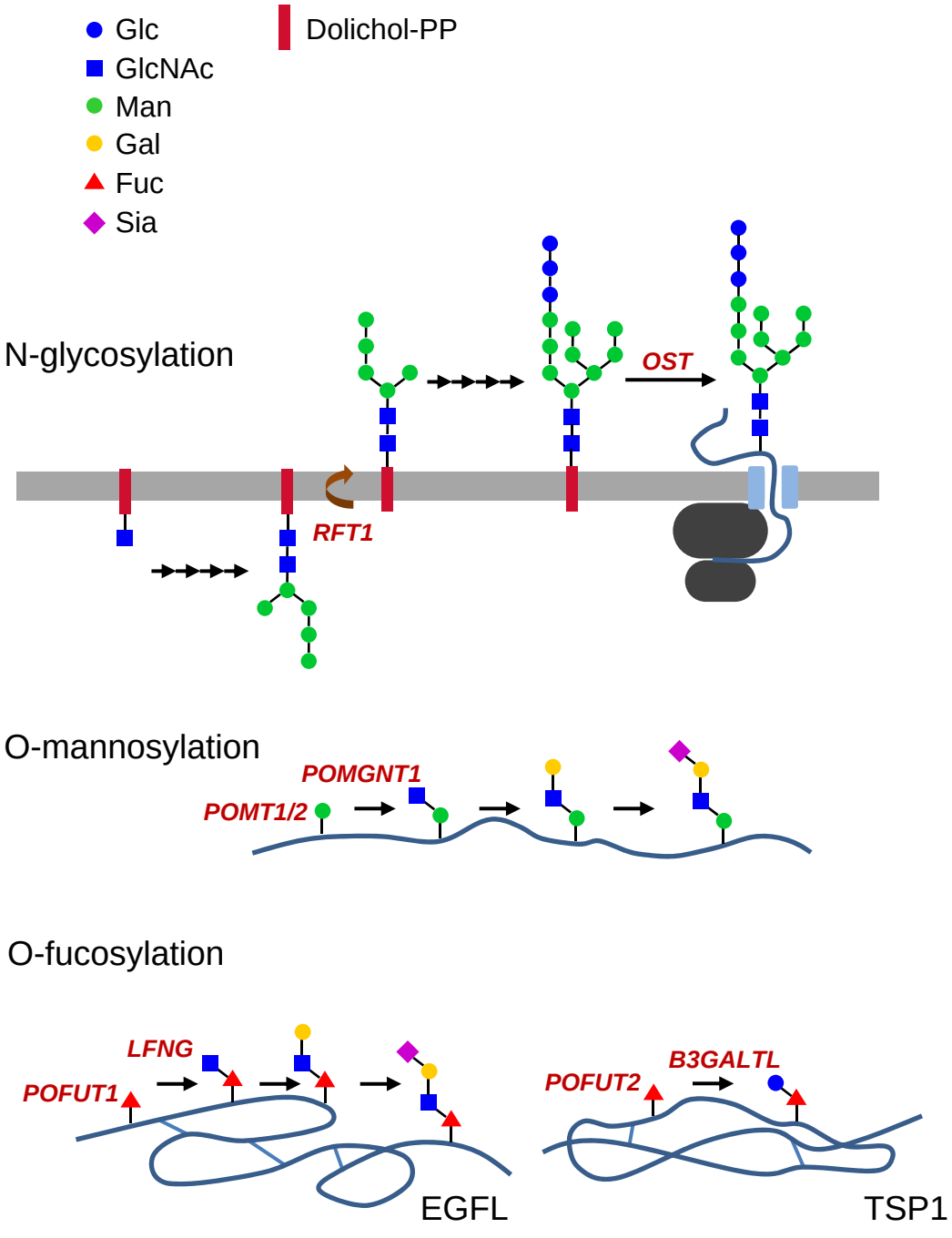

Glc
GlcNAc
Man
Gal
Fuc
Sia
Dolichol-PP
N-glycosylation
OST
RFT1
O-mannosylation
POMGNT1
POMT1/2
O-fucosylation
LFNG
POFUT1
POFUT2
B3GALTL
EGFL
TSP1

Figure 3

**A**

UDP-Glc
UDP-GlcNAc
UDP-Gal
UDP-GalNAc
UDP-GlcA
UDP-Xyl

GDP-Man
GDP-Fuc

DolP-Man
DolP-Glc

CMP-Sia

**B**

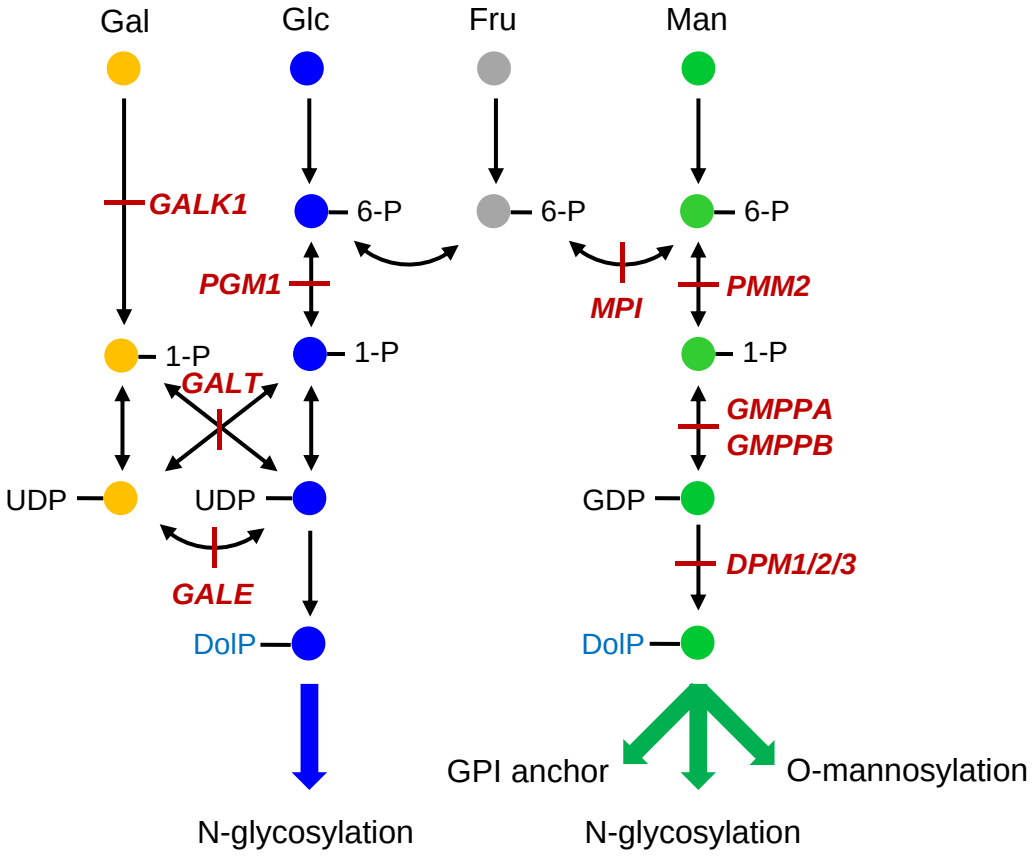
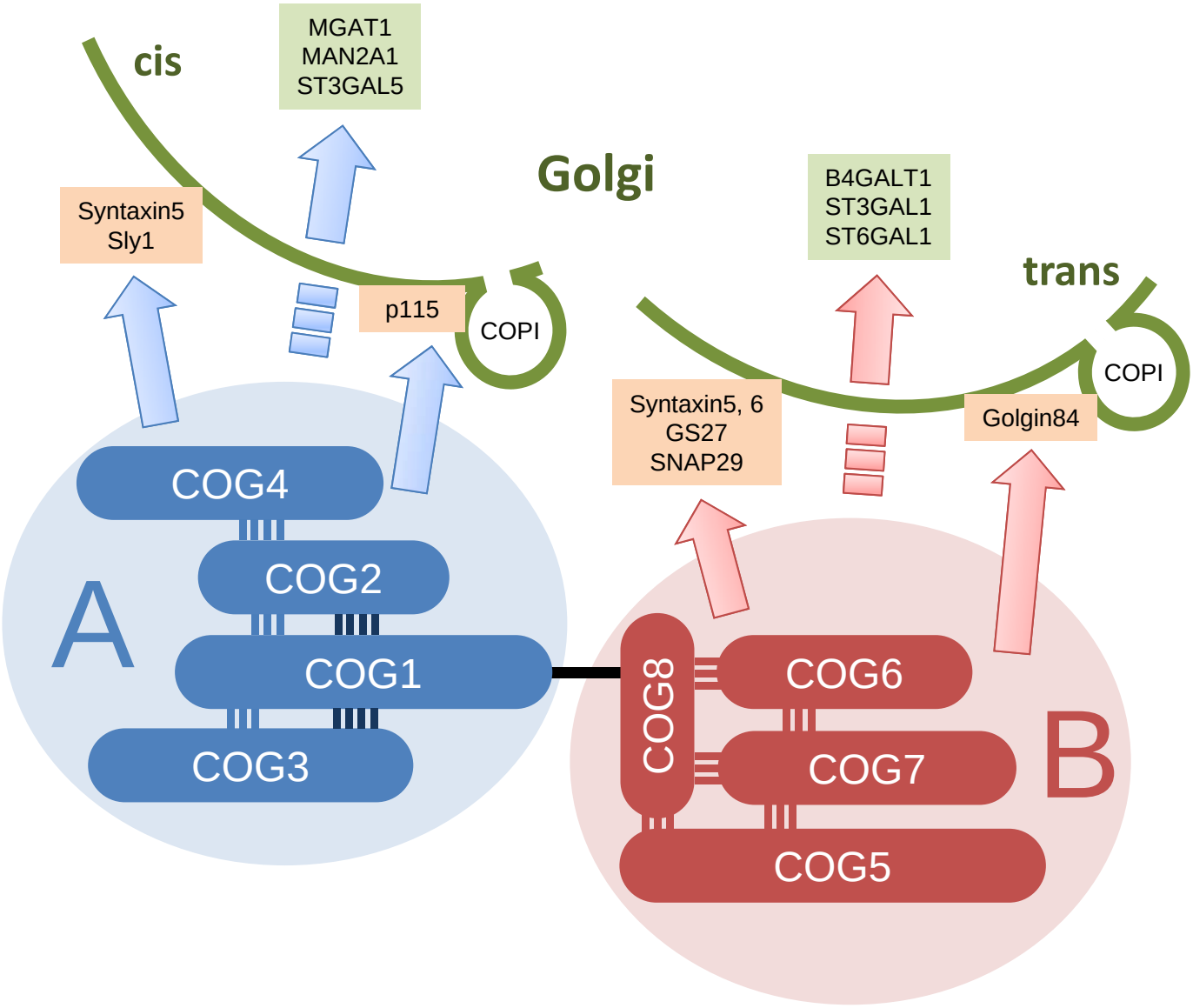


Figure 4


cis
MGAT1
MAN2A1
ST3GAL5
Golgi
Syntaxin5
Sly1
p115
COPI
B4GALT1
ST3GAL1
ST6GAL1
trans
COPI
Syntaxin5, 6
GS27
SNAP29
Golgin84
COG4
COG2
COG1
COG3
A
COG8
COG6
COG7
COG5
B