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What have we learned from glycosyltransferase knockouts in mice?

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Abstract

There are five major classes of glycan including N- and O-glycans, glycosaminoglycans, glycosphingolipids and glycosphosphatidylinositol anchors, all expressed at the molecular frontier of each mammalian cell. Numerous biological consequences of altering the expression of mammalian glycans are understood at a mechanistic level, but many more remain to be characterized. Mouse mutants with deleted, defective, or misexpressed genes that encode activities necessary for glycosylation have led the way to identifying key functions of glycans in biology. However, with the advent of exome sequencing, humans with mutations in genes involved in glycosylation are also revealing specific requirements for glycans in mammalian development. The aim of this review is to summarize glycosylation genes that are necessary for mouse embryonic development; pathway-specific glycosylation genes whose deletion leads to postnatal morbidity; and glycosylation genes for which effects are mild, but perturbation of the organism may reveal functional consequences. General strategies for generating and interpreting the phenotype of mice with glycosylation defects are discussed in relation to human congenital disorders of glycosylation (CDG).

Keywords

Glycosylation; glycosyltransferase; nucleotide sugar; glycosidase; mouse mutants; congenital disorders of glycosylation

INTRODUCTION

Glycosylation of proteins and lipids requires glycosyltransferases resident in the secretory pathway, and a variety of nucleotide sugar donor substrates that are synthesized in the cytoplasm and actively transported into the secretory pathway. Also required are glycosidases that process or remodel glycans. The evolutionary conservation of the large number of genes that encode these activities is testament to their functional roles in the development and homeostasis of mammals [1]. In fact, the major glycan pathways are conserved throughout the metazoa. These pathways may be quite simple, comprising the transfer of one or a few sugars in a linear or simply branched configuration, or very complex, consisting of long, linear or branched glycan chains. A biologically functional grouping of sugars (glycan epitope or determinant) may perform a specific function, and in a

given protein, this may require being located at a particular position. A well-characterized example of this is P-selectin glycoprotein ligand 1 (PSGL-1) in which the sialyl-Lewis X (sLeX) glycan determinant (NeuAc α 2,3Gal β 1,4[Fuc α 1,3]GlcNAc), attached to an O-GalNAc glycan on Thr19, is necessary for binding to P-selectin [2]. Many other glycoproteins on cells expressing PSGL-1 also carry glycans terminating in sLeX. However, sLeX on the O-glycan at Thr19, in addition to nearby sulfated tyrosines at the N-terminus of PSGL-1, together form the optimal ligand-binding domain for P-selectin [3]. Another example is the complex laminin-binding glycans of α -dystroglycan [4, 5], amongst which, those found at Thr317 and Thr319 in the mucin domain of α -dystroglycan are of key importance for interactions with ligands in the extracellular matrix [6]. Because this specificity occurs in a sea of related glycans on the same and other glycoproteins, defining structure/function relationships of glycan determinants requires a broad experimental approach that necessarily includes both biochemical and genetic strategies to define the basis of glycan functions in cells and organisms. Mouse mutants that synthesize altered glycans have provided many insights into essential glycan functions. This review will summarize mouse glycosylation mutants that reveal glycans required for embryonic development, pathway-specific glycosylation mutants that exhibit postnatal defects, and mutants in glycosylation activities affecting multiple classes of glycan that have mild, or no, apparent defects unless the mutant mouse is stressed in one way or another. Mouse glycosylation mutants provide valuable models of human disease and have been reviewed previously [7–9]. However, exome sequencing is rapidly revealing new glycosylation mutations and concomitant biological consequences in humans [10, 11]. Since glycosylation engineering of mammalian genomes has become relatively simple using zinc finger nucleases and CRISPR/Cas9 technologies [12, 13], the generation of precise mouse models of specific human diseases is now comparatively easy [14], and will greatly facilitate discoveries of the mechanistic bases of human diseases.

GLYCANS OF MAMMALIAN CELLS

The major glycans in mammalian cells are depicted in Fig. 1. The simplest modification is the transfer of a single sugar and this often occurs at multiple sites in a protein. For example, mammalian Notch receptors have up to 36 epidermal growth factor-like (EGF) repeats in their extracellular domain that contain distinct consensus sites with Ser or Thr that receive fucose (Fuc) from POFUT1, glucose (Glc) or xylose (Xyl) from POGLUT1, or N-acetylglucosamine (GlcNAc) from EOGT [15, 16] (Fig. 2). Fucose is also transferred to Ser or Thr in thrombospondin (TSP) repeats by protein O-fucosyltransferase 2 (POFUT2) and Fuc-O-TSP may be extended by the addition of Glc by B3GALTL [17]. TSP repeats may also carry C-linked mannose [18] (Fig. 2). Other types of relatively simple glycan include those that begin with O-GalNAc transferred to Ser or Thr, found in clusters in many mucins, and more sparsely or singly in other proteins [19]. There are about 20 polypeptide GalNAc-transferases (GALNTs) [20], but extension to core 1 and 2 O-GalNAc glycans is achieved by a single β (1,3)galactosyltransferase C1GALT1, and a dedicated chaperone called COSMC (C1GALT1C1) [21] (Fig. 2). Extension of O-GalNAc on Ser/Thr is also catalyzed by B3GNT6 to generate core 3 and core 4 O-GalNAc glycans (Fig. 2). GlcNAc is the only sugar transferred to Ser or Thr on many cytoplasmic and nuclear proteins (Fig. 1), and has

not been found in an extended form to date [22]. Glycolipids may have one or a few sugars attached to lipid in the outer leaflet of the plasma membrane, or have extended glycan chains that may be branched [23]. Glycophosphatidylinositol (GPI) anchors are specialized glycans that link a protein via its C-terminus and ethanolamine to phosphatidylinositol (Fig. 1). GPI-anchored proteins span the single outer leaflet of the plasma membrane [24].

The most complex glycans are Asn-linked glycans (N-glycans), glycosaminoglycans (GAGs), and the less abundant O-Man glycans, a subset of which mediate the binding of α -dystroglycan to ligands in the extracellular matrix (Fig. 1). The synthesis of N-glycans begins on the cytosolic face of the ER membrane with the transfer of GlcNAc-P to dolichol-phosphate and continues to Man₅GlcNAc₂-P-P-Dol, which is “flipped” across the ER membrane so the glycan faces the lumen of the ER [25]. This glycan is extended to Glc₃Man₉GlcNAc₂, which is transferred to protein by the action of oligosaccharyltransferase (OST), a complex of many subunits (Fig. 3). Mature glycoproteins with an Asn-X(not Pro)-Ser/Thr sequon may carry high mannose N-glycans formed in the ER or *cis*-Golgi, complex N-glycans that have a tri-mannose core (Man₃GlcNAc₂Asn) and are extended or modified in Golgi compartments (Fig. 4), and hybrid N-glycans that have features of high mannose and complex N-glycans [25]. Asn-X(not Pro)-Ser/Thr sequons do not necessarily carry an N-glycan, and there are also rare variations such as N-X-C or N-X-V that can carry an N-glycan [26]. Phosphorylated mannose residues in high mannose N-glycans occur on lysosomal hydrolases, and are recognized by the mannose-6-phosphate receptor that transports them to the lysosome [27]. Proteoglycans have a common core that begins with the transfer of Xyl to Ser or Thr [28]. The final core of four sugars (GlcA β 1,3Gal β 1,3Gal β 1,4Xyl β -O-Ser/Thr) is subsequently extended by disaccharide hexosamine repeats to form glycosaminoglycan (GAG) chains that include modifications such as epimerization and sulfation [28] (Fig. 1). A lengthy [Xyl α 1,3GlcA β 1,3]_n disaccharide polymer similar to a GAG and termed matriglycan is found attached to α -dystroglycan via GlcA β 1,4Xyl linked to two ribitol-5-phosphate units attached to the terminal GalNAc in a phosphorylated O-Man trisaccharide core [4, 5, 29] (Fig. 1). The matriglycan polymer, synthesized by the dual glycosyltransferase domain enzyme LARGE, has to date been found only on α -dystroglycan, but simple O-Man glycans (Figs. 1 and 2) are found on several other glycoproteins including the cadherins [30].

INTERPRETING GLYCOSYLATION DISRUPTION IN VIVO

Determining how individual sugars and glycan determinants influence the proteins or lipids to which they are attached in a mutant mouse is a challenge for several reasons. First, deleting an enzyme will affect all its substrates. To date there is no glycosyltransferase that is dedicated to a single protein, although lysosomal hydrolases have a unique conformation that allows their high mannose N-glycans to be acted on by the phospho-GlcNAc transferase GNPTAB/GNTPG [31], and a small group of proteins including luteinizing hormone (LH), contain a peptide recognition sequence for B4GALNT3 and B4GALNT4 [32]. Second, the substrate generated by deleting an enzyme may be acted on by another transferase to either “rescue” the mutant (if the same sugar is added in the same linkage), or to add a sugar that is not normally transferred to that glycan. Alternatively, the glycan generated may be acted on by a glycosidase, a modifying enzyme, and so on. Therefore, to conclude that the effect of

removing a glycosyltransferase from the mouse genome has consequences that reflect the loss of sugars or glycans from particular glycoconjugate(s) necessitates structural analysis of glycans from mutant compared to wild type. Determination of glycans accumulated in a mutant will allow compensatory glycosylation mechanisms (if any), or the nature of a new glycosylation landscape, to be defined. To conclude that a phenotype arising from a change in glycosylation is due to effects of truncated or altered sugars on a particular glycoprotein requires cell-based or biochemical assays in which the glycoprotein of interest is functionally characterized. Another strategy is to characterize the glycoprotein lacking one or more glycosylation sites. For example, a point mutation inactivating the O-fucose site in NOTCH1 EGF12 results in a hypomorphic *Notch1* allele [33, 34] due to the loss of O-fucose solely at that site [35]. However, it is important to determine that the change in amino acid to prevent glycosylation does not cause functional effects that are unrelated to the absence of the glycan. For example, loss of an O-fucose-modified Thr in Cripto was not responsible for its loss of activity, since regaining the O-fucose by introducing Ser did not restore activity [35]. As a first approach however, a simple analysis is sufficient to know if a glycosyltransferase and/or its glycan product(s) are necessary for embryonic or postnatal development, fertility, a normal life span, or the functions of individual cells and tissues, as can be seen from the examples described below. Important considerations are biological consequences that may occur only after a challenge, or only in a few cell types, or only at a certain stage of development. Wherever possible, an activity-dead glycosyltransferase should be compared to a null mutant.

There are many glycosylation-defective mice and not all could be included here due to space constraints. Publications on mice with targeted glycosylation mutations may generally be found, however, by searches using gene names from the Human Genome Nomenclature Committee (HGNC). Targeting constructs, ES cells and/or mice targeted in many glycosylation genes of glycan pathways are now available through consortia aiming to target every mouse gene [36], including KOMP (Knockout Mouse Project; <https://www.jax.org/research-and-faculty/tools/knockout-mouse-project>) the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk/science/groups/mouse-transgenic-technologies>) and the IMPC (International Mouse Phenotyping Consortium; <http://www.mousephenotype.org/>). Most of the glycosyltransferases and other glycosylation activities discussed in this review have numerous names in the literature. The names used here are based on the gene names recommended by the HGNC (<http://www.genenames.org/>)

GLYCANS ESSENTIAL FOR EMBRYONIC DEVELOPMENT

Mutations that severely truncate or prevent the synthesis of N-glycans, O-glycans, GPI-anchored glycoproteins, GAGs or glycolipids are lethal. This is not surprising because such mutations affect the properties of all, or a large number, of glycoproteins, proteoglycans or glycolipids (Table 1). The first mouse glycosylation mutant was developed ~20 years ago, and was not expected to be embryonic lethal [37, 38]. The deleted glycosyltransferase gene (*Mgat1*) encodes MGAT1 (GlcNAc-transferase I or GlcNAc-TI) that acts in the middle of the N-glycan synthetic pathway, and is not required for the viability of cultured cells [39] (Fig. 4). MGAT1 catalyzes the first step in complex N-glycan synthesis in the medial Golgi. Thus, glycoproteins with N-glycans acted on by MGAT1 are well beyond the endoplasmic

reticulum (ER), and have been correctly folded by ER chaperones that recognize immature N-glycans [40]. In mice lacking MGAT1, a simple Man₅GlcNAc₂ glycan is found at each Asn-X-Ser/Thr that would normally carry a hybrid or complex N-glycan (Fig. 4). In consequence, N-glycans lack Gal residues that interact with galectins and other glycan binding proteins, leading to profound effects on growth factor signaling [41]. There are several other consequences in *Mgat1* null embryos affecting signaling pathways and cell-cell interactions due to the concomitant loss of complex N-glycan sugars such as sialic acid, fucose and polylactosamine, as well as their modifications, such as sulfation. Mutations of genes that encode most activities acting in N-glycan synthesis prior to MGAT1 are also embryonic lethal (Table 1).

Once it was realized that N-glycans are essential for mammalian development, the generation of mouse mutants in the early reactions of N-glycan synthesis was confined to conditional knockout alleles. Conditional deletion or knockdown of complex and/or hybrid N-glycans has revealed insights into requirements for complex N-glycans in neurogenesis [42], oogenesis [43], spermatogenesis [44] immunity [45] and prostate cancer [46]. However, because complex and hybrid N-glycans modify the majority of glycoproteins, determining why differentiation processes are blocked in a particular cell type in their absence, is extremely difficult. Biochemical and genetic studies of mutations in humans with a CDG have identified phenotypic consequences of mutations inhibiting the early stages of N-glycan synthesis (Fig. 3) [11]. However, phenotypes are complicated, affect a subset of organs in each case, and do not suggest unique reactions that provide the mechanistic basis of a phenotype.

Most O-glycan pathways are also essential to mammalian development, and embryonic lethal phenotypes arising from O-glycan glycosyltransferase knockout have provided important insights into affected pathways. For example, when the *Pofut1* gene is inactivated, the phenotype of mutant embryos at E9.5 is very similar to embryos lacking a key member of the Notch signaling pathway such as NOTCH1, Presenilins 1 and 2, Mastermind, or RBP-Jκ [47, 48]. Again the phenotype is broad, affecting vasculogenesis, cardiogenesis, somitogenesis and neurogenesis. By contrast, lack of O-fucose on TSP type 1 repeats in *Pofut2* null embryos is associated with defects in the epiblast, gastrulation and epithelial to mesenchymal transition, leading to death much earlier at ~E6.5 with effects on Nodal, Wnt and FGF signaling pathways [49]. Embryos in which GlcNAc is not added to O-fucose on Notch EGF repeats due to ablation of the Lunatic Fringe gene (*Lfng*) are also embryonic lethal depending on genetic background [50, 51]. *Lfng* null mice have severe skeletal defects, an informative phenotype that revealed LFNG is essential for regulating Notch signaling during somitogenesis [52]. LFNG itself is regulated at transcriptional, translational and post-translational levels so that it is active for the precise window in which it is required during somitogenesis [53]. A mechanism by which LFNG controls Notch signaling during somitogenesis has been proposed based on analyses of mosaic mice [54]. In another O-glycan example, embryos that cannot transfer Gal to the O-GalNAc that initiates core 1 and core 2 O-glycan synthesis have a hemorrhagic phenotype that is probably responsible for the death of *C1galt1* (encodes T-synthase) or *C1galt1c1* (encodes the C1GALT1 chaperone COSMC) null embryos at around E13.5 [21, 55]. Interestingly, deletion of several polypeptide (pp) GalNAc-transferases that transfer the O-GalNAc substrate of C1GALT1

does *not* give rise to embryonic lethality [56]. This is presumably because there are about 20 ppGalNAc-transferases, and several are expressed in each tissue or cell type [20]. By contrast, the individual loss of 5 different ppGalNAc-transferases in *Drosophila* leads to embryonic lethality [56]. Interestingly, deletion of the O-GlcNAc-transferase OGT that adds GlcNAc to many cytosolic and nuclear proteins is lethal, not only for embryos, but also for cultured cells [57]. Many OGT substrates are regulatory proteins including transcription factors and thus multiple mechanisms may provide the basis for the lethal phenotype of cells lacking OGT.

Examples of glycosylation genes whose inactivation is embryonic lethal in mice are given in Table 1. Humans that are homozygous or compound heterozygotes for inactivating mutations in these genes are expected to die *in utero*. However, mutations in these genes that reduce, but do not eliminate activity, give rise to congenital diseases, including CDGs. For example, certain mutations in the *DPAGT1* gene that initiates N-glycan synthesis (Fig. 3), cause a limb-girdle myasthenic syndrome [58]; mutations that inactivate one allele of *POFUT1* or *POGLUT1* (Fig. 2) lead to the autosomal dominant disorder known as Dowling-Degos Disease [59, 60]. Phenotypes are consistent with disturbed Notch signaling but not yet defined at a mechanistic level. However, the muscular dystrophies termed dystroglycanopathies provide an excellent example of relating genotype, to effect on biochemical function of the encoded protein, to phenotype. Thus, mutations in *POMT1* or *POMT2* that form a complex required to form O-mannose glycans on α -dystroglycan (Figs. 1 and 2), span a range of phenotypes from the severe muscular dystrophy termed Walker Warburg Syndrome to a mild limb-girdle muscular dystrophy [61, 62]. Similarly, the β (1,4)GlcA-transferase B4GAT1, shown to transfer a GlcA that primes elongation of the [Xyl α 1,3GlcA β 1,3] polymer synthesized by LARGE [63, 64], is required for mouse development [65] (Table 1), and mutations in humans give rise to muscular dystrophy [66]. Each dystroglycanopathy mutation alters the O-mannose glycans attached to α -dystroglycan and thereby reduces critical interactions with extracellular matrix ligands including laminin, agrin and others, leading to defects in α -dystroglycan functions.

Important insights into the requirement for specific classes of mammalian glycans in different tissues and cell types have been obtained from conditional mouse mutants. In addition, and importantly, such studies have identified biological events that *do not* require particular glycans. This was the unexpected finding when *Pofut1* was deleted in spermatogonia. Spermatogenesis proceeds normally in the absence of POFUT1, indicating that Notch signaling is not an essential requirement in germ cells during spermatogenesis [44, 67]. This is consistent with the fact that mice in which *Notch1* was deleted in spermatogonia [44], or *Pofut1* was deleted in Sertoli cells [67], are also fertile.

PATHWAY-SPECIFIC GLYCANS THAT AFFECT POSTNATAL DEVELOPMENT

It is apparent from Table 1 that elimination of each class of glycan depicted in Fig. 1 is catastrophic for the organism. However, if the first one or two sugars of a glycan are transferred to protein, mice with glycoconjugates carrying truncated glycans are usually born, but may have a range of defects during postnatal development, and often die

prematurely. In this section, reactions that are specific to a particular pathway, and therefore affect essentially one class of glycan, will be discussed.

For N-glycans, *Mgat2* null mice survive at low frequency in certain genetic backgrounds, and exhibit phenotypic traits of patients with CDG IIa, but die prematurely [68]. MGAT2 transfers $\beta(1,2)$ GlcNAc to the $\alpha(1,6)$ Man of the $\text{Man}_3\text{GlcNAc}_2$ core linked to Asn, resulting in hybrid N-glycans lacking branches initiated from $\alpha(1,6)$ Man in the $\text{Man}_3\text{GlcNAc}_2$ core (Fig. 4). Conditional deletion of *Mgat2* in neurons gives no detectable phenotype, indicating that hybrid N-glycans are sufficient for viability and maintenance of neurons [42]. Deletion of other branch GlcNAc-transferases causes various degrees of morbidity. Thus, deletion of the *Mgat3* gene, precluding transfer of the bisecting GlcNAc to complex or hybrid N-glycans (Fig. 4, Bis), causes mild changes in B cell numbers in blood and altered vertical activity [69]. Deletion of the *Mgat4a* gene which initiates the $\beta(1,4)$ GlcNAc branch on N-glycans (Fig. 4) causes a metabolic phenotype and diabetes [70]. The basis of this phenotype is due to the requirement of MGAT4A to act on complex N-glycans of the glucose transporter GLUT2 in pancreatic beta cells. In the absence of MGAT4A, the N-glycans of GLUT2 lack a $\beta(1,4)$ GlcNAc branch, have a reduced residence time at the cell surface and accumulate intracellularly, reducing their ability to adequately transport glucose. MGAT4B performs the same reaction as MGAT4A, but is more restricted in its distribution, and does not compensate for the loss of MGAT4A in pancreas. By contrast, MGAT4A is ubiquitously expressed and can compensate for the loss of MGAT4B in a *Mgat4b* null mouse. Deletion of both *Mgat4a* and *Mgat4b* results in a phenotype similar to *Mgat4a* null mice [71]. However, N-glycans in the *Mgat4a/Mgat4b* double mutant lacking all $\beta(1,4)$ GlcNAc branches appear to have extra long polylactosamine extensions on other GlcNAc branches because terminal glycan levels are maintained, and relevant glycosyltransferase genes are upregulated. Therefore, compensation occurs at the level of N-glycans, apparently to maintain overall terminal sugar content on glycoconjugates. However, this compensation is not sufficient to rescue transporter function in *Mgat4a* null pancreas.

Deletion of the *Mgat5* gene has broad metabolic and phenotypic consequences [72]. MGAT5 transfers $\beta(1,6)$ GlcNAc to initiate a branch on the $\alpha(1,6)$ Man of the N-glycan core (Fig. 4). MGAT5B also transfers $\beta(1,6)$ GlcNAc to N-glycans but is distinct in that it efficiently transfers $\beta(1,6)$ GlcNAc to O-mannose glycans and is co-expressed with O-mannose glycans in brain [73]. The presence of the $\beta(1,6)$ GlcNAc branch on N-glycans is readily detected by enhanced binding of the plant lectin L-PHA to glycoproteins, and cannot occur on hybrid N-glycans. It has most recently been shown that by increasing GlcNAc levels in cells and mice, compensation of N-glycan levels may occur via enhanced metabolism and increased UDP-GlcNAc levels in *Mgat5* null mice [74]. In the absence of MGAT5, the glucagon receptor in liver and hepatocytes is functionally impaired, and hexosamine biosynthesis is reduced [75]. This phenotype correlates with the loss of galectin-9 binding to the N-glycans of the glucagon receptor, and appears similar to the mechanism of loss of GLUT2 function observed in *Mgat4a* null mice. *Mgat5* null mice exhibit increased T cell activation, enhanced susceptibility to autoimmunity, altered growth factor and cytokine signaling, a defect in behavior, accelerated aging and reduced fecundity [76, 77]. *Mgat5* null mice have been used to identify roles for N-glycans in retaining glycoproteins at the cell surface via interactions with a galectin lattice [78]. In particular, galectin-3 has been implicated in retaining EGF

and PDGF receptors as well as cytokine receptors at the cell surface [79]. Loss of MGAT5 causes a weakening of galectin lattice interactions, reduced cell surface residency of glycoprotein receptors, and a concomitant reduction in growth factor or cytokine signaling. Interestingly, loss of MGAT3, the GlcNAc-transferase that transfers the bisecting GlcNAc to N-glycans, has the opposite effect, serving to enhance growth factor signaling in mammary tumor cells [80], perhaps by increasing the accessibility of poly lactosamine on N-glycan branches. Consistent with this, overexpression of *Mgat3* causes suppression of chemically-induced hepatic tumors [81].

Another modification specific to N-glycans is catalyzed by FUT8 which transfers $\alpha(1,6)$ Fuc to the GlcNAc linked to Asn in the core of complex N-glycans (Fig. 4). Mice lacking FUT8 exhibit severe growth retardation and die shortly after birth, apparently due to defective lung development and emphysema [82]. The loss of the core Fuc on N-glycans of the TGF- β 1 receptor correlates with markedly reduced TGF- β signaling via SMAD2, and both cell and lung defects were at least partially rescued by TGF- β . Most recently it has been shown that mice lacking FUT8 exhibit reduced IgG production in response to antigenic stimulation due to a requirement for core Fuc on N-glycans of the IgG-B cell receptor [83].

O-glycan pathway-specific knockout mice affected in postnatal development have also been reported. The first was deletion of a $\beta(1,6)$ GlcNAc-transferase GCNT1 that generates core 2 O-GalNAc glycans (Fig. 2) [84]. Deletion of *Gcnt1* affects myeloid homeostasis and the inflammatory response, in large part due to severe reduction of P-selectin mediated leukocyte rolling [85]. *Gcnt2* null mice lack all core 2 O-glycans and branched core 1 O-glycans. They have a decreased mucosal barrier in the intestine and exhibit increased susceptibility to experimental colitis [86]. Deletion of *Gcnt3* has mild effects on behavior and gastrointestinal cell differentiation [86, 87]. Interestingly, elimination of all three O-glycan $\beta(1,6)$ GlcNAc-transferases does not have more serious *in vivo* consequences, though it causes a complete loss of core 2, core 4 and branched core 1 O-glycans, and revealed compensatory mechanisms for maintaining O-glycans such as elongating non-branched O-glycans [88].

Pathway specific enzymes related to the O-fucose glycan pathway are LFNG, MFNG and RFNG (Fig. 2). Mice that lack LFNG survive on certain backgrounds [89–91]. However, they have severe skeletal defects on all backgrounds, females are infertile, males are infertile and have a defective rete testis, and *Lfng* null mice die prematurely. Mice null for *Mfng* are viable but compound heterozygotes with *Jag1* exhibit bile duct defects [92], and MFNG is required for optimal production of marginal zone B cells [93]. Mice null for *Rfng* have mild defects in T and B cell production [94], and compound *Rfng/Jag1* heterozygotes have defective bile ducts [92]. In the Fuc-O-TSP pathway (Fig. 2), deletion of the Glc-transferase *B3galt1* in mouse has not been reported, but mutations in this gene cause Peters Plus Syndrome in humans [95]. The GlcNAc-transferase genes *Pomgnt1*, *Pomgnt2* (Fig. 2) and *Large* (Fig. 1), specific to the O-mannose pathway, have been inactivated in the mouse. Mice null for *Pomgnt1* which transfers $\beta(1,2)$ GlcNAc to O-mannose, are viable but have low fertility and usually die several months after birth with numerous defects in muscle, brain and eye development, a phenotype similar to humans with mutations in the *POMGNT1* gene [96]. Interestingly, inactivation of the *Pomgnt2* gene, which transfers $\beta(1,4)$ GlcNAc to O-

mannose, is lethal within one day after birth [97]. Embryonic *Pomgnt2* null brain exhibits major defects in neuronal migration. LARGE encodes two glycosyltransferase domains and generates a long polymer of [Xyl α 1,3GlcA β 1,3] repeats attached to O-mannose [63, 64, 98]. Inactivation of *Large* was discovered in the *myd* mouse to lead to muscular dystrophy [99], and is an excellent model for the human disease [100], one among the many dystroglycanopathies [101].

Mouse mutants with postnatal defects have also arisen from deletion of genes encoding enzymes predominantly specific to the generation of the GAGs HS, CS, dermatan sulfate (DS) or keratan sulfate (KS). Mice lacking HS3ST1, a heparan sulfate transferase that generates 3-O-GlcNAc in HS, have intrauterine growth retardation but no anticoagulant difficulties. However, on a C57BL/6 genetic background, they die in the early postnatal period [102]. Loss of HS glucuronyl C5-epimerase in *Hsepi* null mice also causes early postnatal death with respiratory failure, skeletal defects and kidney agenesis [103]. Kidney agenesis, skeletal defects, defects in the eye and perinatal death also occur in the absence of the HS 2-O-sulfotransferase in *Hs2st* null mice [104]. However, *Hs6st2* null mice lacking HS 6-O-sulfotransferase, do not die but have increased body weight, defective glucose and energy metabolism with insulin resistance, and altered axon guidance in the visual system [105]. Finally, mice with reduced activity of SGSH, the sulfamidase which removes sulfate from GlcNS in HS, have various defects including hepatosplenomegaly, lysosomal storage, motor deficits and early death around 10 months [106, 107]. Deletion of *Galns*, which encodes the related sulfamidase for GalNS in CS and KS, causes slowly developing, multi-organ, vacuolar and lysosomal storage disease [108]. Three different chondroitin/dermatan sulfotransferases have been deleted in the mouse, all with comparatively mild consequences. Mice lacking CHST5, a GlcNAc-6-O-sulfotransferase, exhibit disrupted organization of the cornea, reduced KS and increased amounts of CS and DS [109]; mice lacking CHST14, a predominantly DS GalNAc-4-O-sulfotransferase, exhibit reduced weight and fertility, a kinked tail and increased skin fragility [110], and reduced proliferation and neurogenesis of neural stem cells [111]; deletion of CHST11, a predominantly CS GalNAc-4-O-sulfotransferase, has no apparent effect on neural stem cell properties but *Chst11* null progeny die at birth [111]; deletion of CHST2 and CHST4 GlcNAc-6-O-sulfotransferases causes a marked reduction in homing of leukocytes to peripheral lymph nodes due to the loss of 6-O-sulfation on L-selectin ligands, and Lewis X on O-glycans [112, 113].

Mouse mutants with a postnatal defect specific to GSL synthesis have rather mild phenotypes. For example, deletion of the sialyltransferase gene *St3gal5* that encodes GM3 synthase causes an increased sensitivity to insulin that correlates with increased phosphorylation of the insulin receptor [114], and loss of hearing due to degeneration of the organ of Corti [115]. Deletion of the *B4galnt1* gene that encodes GM2/GD2 synthase and eliminates all complex GSLs, has no apparent effect on viability or fertility [116], similar to the phenotype of mice null for *St8sia1* that encodes GD3 synthase [117]. However, combining these two mutations to give a mouse that expresses only GM3 has long term deleterious effects on the nervous system, and results in sudden death in response to acoustic stimulation [117].

MUTATIONS IN GLYCOSYLTRANSFERASES THAT ACT IN MORE THAN ONE GLYCAN PATHWAY

The termini of N-glycans, O-glycans and GSLs may be generated by a group of glycosyltransferases that are not dedicated to one glycan pathway. The group includes GlcNAc-, GalNAc-, GlcA-, Gal-, NeuAc-, and Fuc-transferases. Together they generate polylactosamine chains of type I (Gal β 1,3GlcNAc) or type II (Gal β 1,4GlcNAc; LacNAc) that may be branched by β (1,6)GlcNAc-transferases and terminate with GalNAc, GlcA, sialic acid, fucose and sulfate in mammals.

Deletion of one of the six β (1,4)Gal-transferases that begin the extension of branches of complex and hybrid N-glycans (Fig. 1), as well as extend O-glycans (Fig. 2) and add LacNAc units (Figs. 1, 4), cause various defects in development. B4GALT1 has a long (L) and a short (S) form and deletion of both causes embryonic lethality (Table 1). However, deletion of only the long form allows retarded growth, causes defects in skin and intestine, and variable effects on fertility [118–120]. Numerous other effects on tissue homeostasis and interactions with pathogens are altered in *B4galt1* null mice [120–123]. Deletion of *B4galt2*, that is highly expressed in brain and transfers β (1,4)Gal to complex N-glycans, causes spatial learning and memory defects [124]. No mouse mutants lacking B4GALT3 or B4GALT4 have been reported, and *B4galt5* null embryos die at mid-gestation (Table 1). Mice with targeted deletion of the *B4galt6* gene are viable and fertile with a slight reduction in acidic GSLs in brain [125]. Deletions of the *B3galt1* through *B3galt4* genes, that initiate type I LacNAc chains, have not been reported to date.

The sugar transferred to β (1,4)Gal to generate LacNAc is β (1,3)GlcNAc. The gene initially shown to perform this reaction and designated *B3gnt1* has recently been found to encode a GlcA-transferase and renamed *B4gat1* [63, 64]. This is consistent with the absence of *B3gnt1* in sequence comparisons of genes that encode β (1,3)-linked glycosyltransferases [126]. However, *B3gnt2* null mice have markedly reduced polylactosamine on N- and potentially O-glycans, are viable and fertile, exhibit hyperactivation of B and T lymphocytes [126], and alterations in neuronal guidance [127]. Knockouts of *B3gnt3* (extends O-glycans), *B3gnt4* (generates polylactosamine), *B3gnt6* (core 3 O-glycan synthase), *B3gnt7* (KS synthase) or *B3gnt8* (generates polylactosamine) have not been reported. *B3gnt5* null mice have a variable phenotype. The first report found *B3gnt5* deletion to be embryonic lethal (Table 1). However, other mice lacking B3GNT5 (Lc3 synthase) were found to survive and be healthy [128], or to exhibit multi-organ and reproductive defects due to the loss of specific GSLs [129].

Depending on cell type, LacNAc or polylactosamine chains on N- and O-glycans, GSLs and KS may have β (1,6)GlcNAc branches, terminate in Fuc, NeuAc, GlcA or GalNAc, and may be sulfated on GlcNAc, GalNAc and GlcA. Genes have been inactivated for a number of the glycosyltransferases that catalyze the transfer of these sugars. In general, mouse mutants lacking these activities are viable and exhibit subtle defects in T or B cell development or functions, lymphocyte homing, neuronal migration, behavior, reproduction, or in responses to pathological conditions such as tumorigenesis or infection [7, 8]. One reason for this is that there are redundant activities that may compensate for the loss of a single transferase.

For example, examination of brain GSLs of mice null for four different $\alpha(2,3)$ sialyltransferase genes (*St3gal1*, *St3gal2*, *St3gal3*, *St3gal4*), individually or in combination, revealed that ST3GAL2 and ST3GAL3 are responsible for the synthesis of the major brain gangliosides GD1a and GT1b [130]. Deletion of *St3gal2* was the only single null in which a reduction of brain GSLs was observed, but deletion of both *St3gal2* and *St3gal3* prevented >95% synthesis of the major GSLs. In another example, both ST3GAL4 and ST3GAL6 were found to contribute to the synthesis of selectin ligands [131]. There are also examples in which loss of a single member of a family of glycosyltransferases can have important effects. One such case is *St3gal4*, deletion of which results in the clearance of unsialylated glycoproteins, including von Willerbrand factor and platelets, via the Ashwell-Morell receptor in liver, leading to thrombocytopenia and a bleeding disorder [132]. Amongst the $\alpha(1,3)$ fucosyltransferases which generate the stage-specific embryonic antigen (SSEA-1) or LeX (Gal β 1,4[Fuc α 1,3]GlcNAc) and/or sLeX (NeuAc α 2,3Gal β 1,4[Fuc α 1,3]GlcNAc), deletion of *Fut4*, *Fut7* and *Fut9*, and combinations have been reported. FUT7 is the major fucosyltransferase for the generation of sLeX on selectin ligands, but FUT4 also contributes [133]. *Fut9* null blastocysts do not express SSEA-1 but nevertheless progress through embryogenesis with no apparent problems [134]. This is surprising given previous reports that SSEA-1 is a critical adhesion molecule during mouse blastogenesis [135]. FUT1 and FUT2 transfer $\alpha(1,2)$ fucose to terminal Gal residues, and the loss of either or both in the mouse has no obvious consequences [136]. However, roles for these fucosyltransferases in gut homeostasis and disease are being uncovered. For example, following a systemic injection of lipopolysaccharide, intestinal epithelial cells of the small intestine are induced to add $\alpha(1,2)$ fucose to glycoconjugates expressed on the cell surface, and this fucosylation is greatly reduced in *Fut2* null mice [137]. Fucosylation induced by FUT2 is proposed to be part of a protective response to allow intestinal epithelial cells to maintain microbial interactions that preserve the gut during systemic stress.

An elegant example of roles for terminal sugars *in vivo* is the important requirement for correct terminal glycosylation and sulfation of the glycoprotein hormones [138]. The N-glycans of LH terminate specifically in GalNAc-4-SO₄ [32]. Deletion of GalNAc-4-sulfotransferase 1 (CHST8) increases the levels of circulating LH, leading to precocious sexual maturity in females with increased fecundity, and premature maturation of testis and seminal vesicles in males [139]. The generation of complex N-glycans terminating with GalNAc-4-SO₄ on LH depends on regulated expression of GalNAc- and sulfo-transferases and liver receptors that recognize these N-glycans, and clear them from the circulation [140]. Another sulfated glycan epitope with biological significance is HNK-1, expressed at the termini of N- and O-glycans and glycolipids, and generated by the action of GlcA-transferases and sulfotransferases. Deletion of GlcAT-P (B3GAT1) removes most HNK-1 from the mouse brain and leads to defects in higher brain functions like spatial memory [141]. A similar brain and learning phenotype was observed following deletion of B4GALT2, which led to a marked reduction in HNK-1, showing that B4GALT2 generates the major glycan substrate for B3GAT1 in brain [124].

CONCLUSIONS

This review has summarized examples of mouse mutants that have revealed insights into the functions of mammalian glycans, mainly at the level of cell, tissue and organ functions. Identifying how altered glycans on specific glycoconjugates give rise to a mouse phenotype is the next challenge. There are now hundreds of mice or ES cell lines with a targeted mutation in one of the many genes that affect glycosylation. Glycosyltransferase and glycosidase genes are described in the CaZY database <http://www.cazy.org/>; mice can be found at Mouse Genome Informatics (MGI) <http://www.informatics.jax.org/>, the KOMP Repository <https://www.komp.org/>, the Wellcome Trust Sanger Institute <https://www.sanger.ac.uk/resources/mouse/>, and the International Mouse Strain Resource (IMSR) <http://www.findmice.org/>.

In the future, biological effects of point mutations that inactivate a glycosyltransferase activity leaving gene products otherwise intact may be of more interest than gene deletions or disruptions. For example, in *Drosophila*, a point mutation inactivating the *Ofut1* gene has revealed effects due to loss of O-fucose on Notch, whereas gene deletion identified effects on Notch stability at the cell surface [142]. In addition, all genes involved in the generation of N-glycans, O-glycans, GAGs, GPI-anchors or GSLs are potential candidates for human CDGs that may manifest at very different stages of development. The diseases induced may be extremely debilitating [143], or cause a mild autism [144], or give rise to a phenotype in between those extremes. While identifying the glycosylation gene responsible can be complex [11], whole genome sequencing is greatly facilitating discoveries of new glycosylation gene disease associations [10]. Human diseases related to glycosylation genes are described in the databases Online Mendelian Inheritance in Man (OMIM) (<http://www.ncbi.nlm.nih.gov/omim>) and the Office of Rare Disease Research (ORDR) (<http://rarediseases.info.nih.gov/>). Identifying the genetic basis of glycosylation disorders and creating relevant mouse models to determine how each mutation disrupts development, differentiation or the functioning of mature cells, and ultimately deciphering mechanistic bases, is clearly of great importance to human health.

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Abbreviations

HS	heparan sulfate
CS	chondroitin sulfate
KS	keratan sulfate
DS	dermatan sulfate
LeX	Lewis X
sLeX	sialyl-Lewis X

GC	glycoconjugate
Fuc	fucose
Glc	glucose
Xyl	xylose
GlcNAc	N-acetylglucosamine
GalNAc	N-acetylgalactosamine
GlcA	glucuronic acid
TSP	thrombospondin
EGF	epidermal growth factor-like
GAG	glycosaminoglycan
GPI	glycophosphatidylinositol
pp	polypeptide
GSL	glycosphingolipid
SSEA-1	species specific embryonic antigen 1
LH	luteinizing hormone
α-DG	α -dystroglycan
OMIM	Online Inheritance in Man
HGNC	human genome nomenclature committee
KOMP	Knockout Mouse Project
IMPC	International Mouse Phenotyping Committee

References

1. Henrissat, B., Surolia, A., Stanley, P. A Genomic View of Glycobiology. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. Essentials of Glycobiology. 2. Cold Spring Harbor ; NY: 2009.
2. Leppanen A, Mehta P, Ouyang YB, Ju T, Helin J, Moore KL, et al. A novel glycosulfopeptide binds to P-selectin and inhibits leukocyte adhesion to P-selectin. J Biol Chem. 1999; 274:24838–48. [PubMed: 10455156]
3. Leppanen A, White SP, Helin J, McEver RP, Cummings RD. Binding of glycosulfopeptides to P-selectin requires stereospecific contributions of individual tyrosine sulfate and sugar residues. J Biol Chem. 2000; 275:39569–78. [PubMed: 10978329]
4. Praissman JL, Wells L. Mammalian O-mannosylation pathway: glycan structures, enzymes, and protein substrates. Biochemistry. 2014; 53:3066–78. [PubMed: 24786756]
5. Yoshida-Moriguchi T, Campbell KP. Matriglycan: a novel polysaccharide that links dystroglycan to the basement membrane. Glycobiology. 2015; 25:702–13. [PubMed: 25882296]

6. Hara Y, Kanagawa M, Kunz S, Yoshida-Moriguchi T, Satz JS, Kobayashi YM, et al. Like-acetylglucosaminyltransferase (LARGE)-dependent modification of dystroglycan at Thr-317/319 is required for laminin binding and arenavirus infection. *Proc Natl Acad Sci U S A*. 2011; 108:17426–31. [PubMed: 21987822]
7. Lowe JB, Marth JD. A genetic approach to mammalian glycan function. *Annu Rev Biochem*. 2003; 72:643–491. [PubMed: 12676797]
8. Haltiwanger RS, Lowe JB. Role of glycosylation in development. *Annu Rev Biochem*. 2004; 73:491–537. [PubMed: 15189151]
9. Stanley P, Okajima T. Roles of glycosylation in Notch signaling. *Curr Top Dev Biol*. 2010; 92:131–64. [PubMed: 20816394]
10. Matthijs G, Rymen D, Millon MB, Souche E, Race V. Approaches to homozygosity mapping and exome sequencing for the identification of novel types of CDG. *Glycoconj J*. 2013; 30:67–76. [PubMed: 22983704]
11. Freeze HH, Chong JX, Bamshad MJ, Ng BG. Solving glycosylation disorders: fundamental approaches reveal complicated pathways. *Am J Hum Genet*. 2014; 94:161–75. [PubMed: 24507773]
12. Steentoft C, Bennett EP, Schjoldager KT, Vakhrushev SY, Wandall HH, Clausen H. Precision genome editing: a small revolution for glycobiology. *Glycobiology*. 2014; 24:663–80. [PubMed: 24861053]
13. Wijshake T, Baker DJ, van de Sluis B. Endonucleases: new tools to edit the mouse genome. *Biochim Biophys Acta*. 2014; 1842:1942–50. [PubMed: 24794718]
14. Yang H, Wang H, Shivalila CS, Cheng AW, Shi L, Jaenisch R. One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. *Cell*. 2013; 154:1370–9. [PubMed: 23992847]
15. Rana NA, Haltiwanger RS. Fringe benefits: functional and structural impacts of O-glycosylation on the extracellular domain of Notch receptors. *Curr Opin Struct Biol*. 2011; 21:583–9. [PubMed: 21924891]
16. Ogawa M, Furukawa K, Okajima T. Extracellular O-linked beta-N-acetylglucosamine: Its biology and relationship to human disease. *World journal of biological chemistry*. 2014; 5:224–30. [PubMed: 24921011]
17. Kozma K, Keusch JJ, Hegemann B, Luther KB, Klein D, Hess D, et al. Identification and characterization of abeta1,3-glucosyltransferase that synthesizes the Glc-beta1,3-Fuc disaccharide on thrombospondin type 1 repeats. *J Biol Chem*. 2006; 281:36742–51. [PubMed: 17032646]
18. Doucey MA, Hess D, Cacan R, Hofsteenge J. Protein C-mannosylation is enzyme-catalysed and uses dolichyl-phosphate-mannose as a precursor. *Mol Biol Cell*. 1998; 9:291–300. [PubMed: 9450955]
19. Brockhausen I, Schachter H, Stanley P. O-GalNAc Glycans. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. *Essentials of Glycobiology*. 2. Cold Spring Harbor; NY: 2009.
20. Bennett EP, Mandel U, Clausen H, Gerken TA, Fritz TA, Tabak LA. Control of mucin-type O-glycosylation: a classification of the polypeptide GalNAc-transferase gene family. *Glycobiology*. 2012; 22:736–56. [PubMed: 22183981]
21. Wang Y, Ju T, Ding X, Xia B, Wang W, Xia L, et al. Cosmc is an essential chaperone for correct protein O-glycosylation. *Proc Natl Acad Sci U S A*. 2010; 107:9228–33. [PubMed: 20439703]
22. Ma J, Hart GW. O-GlcNAc profiling: from proteins to proteomes. *Clinical proteomics*. 2014; 11:8. [PubMed: 24593906]
23. Schnaar RL, Suzuki A, Stanley P. Glycosphingolipids. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. *Essentials of Glycobiology*. 2. Cold Spring Harbor; NY: 2009.
24. Ferguson, MAJ., Kinoshita, T., Hart, GW. Glycosylphosphatidylinositol Anchors. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. *Essentials of Glycobiology*. 2. Cold Spring Harbor; NY: 2009.

25. Stanley, P., Schachter, H., Taniguchi, N. N-Glycans. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. Essentials of Glycobiology. 2. Cold Spring Harbor; NY: 2009.
26. Zielinska DF, Gnäd F, Wisniewski JR, Mann M. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. *Cell*. 2010; 141:897–907. [PubMed: 20510933]
27. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. *Nat Rev Mol Cell Biol*. 2003; 4:202–12. [PubMed: 12612639]
28. Esko, JD., Kimata, K., Lindahl, U. Proteoglycans and Sulfated Glycosaminoglycans. In: Varki, A.Cummings, RD.Esko, JD.Freeze, HH.Stanley, P.Bertozzi, CR., et al., editors. Essentials of Glycobiology. 2. NY: Cold Spring Harbor; 2009.
29. Kanagawa M, Kobayashi K, Tajiri M, Many H, Kuga A, Yamaguchi Y, et al. Identification of a Post-translational Modification with Ribitol-Phosphate and Its Defect in Muscular Dystrophy. *Cell reports*. 2016; 14:2209–23. [PubMed: 26923585]
30. Vester-Christensen MB, Halim A, Joshi HJ, Steentoft C, Bennett EP, Lavery SB, et al. Mining the O-mannose glycoproteome reveals cadherins as major O-mannosylated glycoproteins. *Proc Natl Acad Sci U S A*. 2013; 110:21018–23. [PubMed: 24101494]
31. Kornfeld S. Lysosomal enzyme targeting. *Biochem Soc Trans*. 1990; 18:367–74. [PubMed: 2164980]
32. Fiete D, Beranek M, Baenziger JU. Molecular basis for protein-specific transfer of N-acetylgalactosamine to N-linked glycans by the glycosyltransferases beta1,4-N-acetylgalactosaminyl transferase 3 (beta4GalNAc-T3) and beta4GalNAc-T4. *J Biol Chem*. 2012; 287:29194–203. [PubMed: 22722937]
33. Ge C, Stanley P. The O-fucose glycan in the ligand-binding domain of Notch1 regulates embryogenesis and T cell development. *Proc Natl Acad Sci U S A*. 2008; 105:1539–44. [PubMed: 18227520]
34. Ge C, Stanley P. Effects of varying Notch1 signal strength on embryogenesis and vasculogenesis in compound mutant heterozygotes. *BMC Dev Biol*. 2010; 10:36. [PubMed: 20346184]
35. Shi S, Ge C, Luo Y, Hou X, Haltiwanger RS, Stanley P. The threonine that carries fucose, but not fucose, is required for Cripto to facilitate Nodal signaling. *J Biol Chem*. 2007; 282:20133–41. [PubMed: 17504756]
36. Sung YH, Baek IJ, Seong JK, Kim JS, Lee HW. Mouse genetics: catalogue and scissors. *BMB reports*. 2012; 45:686–92. [PubMed: 23261053]
37. Ioffe E, Stanley P. Mice lacking N-acetylglucosaminyltransferase I activity die at mid-gestation, revealing an essential role for complex or hybrid N-linked carbohydrates. *Proc Natl Acad Sci USA*. 1994; 91:728–32. [PubMed: 8290590]
38. Metzler M, Gertz A, Sarkar M, Schachter H, Schrader JW, Marth JD. Complex asparagine-linked oligosaccharides are required for morphogenic events during post-implantation development. *EMBO Journal*. 1994; 13:2056–65. [PubMed: 8187759]
39. Chen W, Stanley P. Five Lec1 CHO cell mutants have distinct Mgat1 gene mutations that encode truncated N-acetylglucosaminyltransferase I. *Glycobiology*. 2003; 13:43–50. [PubMed: 12634323]
40. Caramelo JJ, Parodi AJ. Getting in and out from calnexin/calreticulin cycles. *J Biol Chem*. 2008; 283:10221–5. [PubMed: 18303019]
41. Boscher C, Dennis JW, Nabi IR. Glycosylation, galectins and cellular signaling. *Curr Opin Cell Biol*. 2011; 23:383–92. [PubMed: 21616652]
42. Ye Z, Marth JD. N-glycan branching requirement in neuronal and post-natal viability. *Glycobiology*. 2004; 14:547–58. [PubMed: 15044398]
43. Shi S, Williams SA, Seppo A, Kurniawan H, Chen W, Ye Z, et al. Inactivation of the Mgat1 gene in oocytes impairs oogenesis, but embryos lacking complex and hybrid N-glycans develop and implant. *Mol Cell Biol*. 2004; 24:9920–9. [PubMed: 15509794]
44. Batista F, Lu L, Williams SA, Stanley P. Complex N-Glycans Are Essential, but Core 1 and 2 Mucin O-Glycans, O-Fucose Glycans, and NOTCH1 Are Dispensable, for Mammalian Spermatogenesis. *Biol Reprod*. 2012; 86(179):1–12.

45. Zhou RW, Mkhikian H, Grigorian A, Hong A, Chen D, Arakelyan A, et al. N-glycosylation bidirectionally extends the boundaries of thymocyte positive selection by decoupling Lck from Ca(2)(+) signaling. *Nat Immunol.* 2014; 15:1038–45. [PubMed: 25263124]
46. Beheshti Zavareh R, Sukhai MA, Hurren R, Gronda M, Wang X, Simpson CD, et al. Suppression of Cancer Progression by MGAT1 shRNA Knockdown. *PloS one.* 2012; 7:e43721. [PubMed: 22957033]
47. Shi S, Stanley P. Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways. *Proc Natl Acad Sci U S A.* 2003; 100:5234–9. [PubMed: 12697902]
48. Okamura Y, Saga Y. Pofut1 is required for the proper localization of the Notch receptor during mouse development. *Mech Dev.* 2008; 125:663–73. [PubMed: 18547789]
49. Du J, Takeuchi H, Leonhard-Melief C, Shroyer KR, Dlugosz M, Haltiwanger RS, et al. O-fucosylation of thrombospondin type 1 repeats restricts epithelial to mesenchymal transition (EMT) and maintains epiblast pluripotency during mouse gastrulation. *Dev Biol.* 2010; 346:25–38. [PubMed: 20637190]
50. Evrard YA, Lun Y, Aulehla A, Gan L, Johnson RL. lunatic fringe is an essential mediator of somite segmentation and patterning. *Nature.* 1998; 394:377–81. [PubMed: 9690473]
51. Zhang N, Gridley T. Defects in somite formation in lunatic fringe-deficient mice. *Nature.* 1998; 394:374–7. [PubMed: 9690472]
52. Shifley ET, Vanhorn KM, Perez-Balaguer A, Franklin JD, Weinstein M, Cole SE. Oscillatory lunatic fringe activity is crucial for segmentation of the anterior but not posterior skeleton. *Development.* 2008; 135:899–908. [PubMed: 18234727]
53. Williams DR, Shifley ET, Lather JD, Cole SE. Posterior skeletal development and the segmentation clock period are sensitive to Lfng dosage during somitogenesis. *Dev Biol.* 2014; 388:159–69. [PubMed: 24560643]
54. Okubo Y, Sugawara T, Abe-Koduka N, Kanno J, Kimura A, Saga Y. Lfng regulates the synchronized oscillation of the mouse segmentation clock via trans-repression of Notch signalling. *Nat Commun.* 2012; 3:1141. [PubMed: 23072809]
55. Xia L, Ju T, Westmuckett A, An G, Ivanciu L, McDaniel JM, et al. Defective angiogenesis and fatal embryonic hemorrhage in mice lacking core 1-derived O-glycans. *J Cell Biol.* 2004; 164:451–9. [PubMed: 14745002]
56. Tran DT, Ten Hagen KG. Mucin-type O-glycosylation during development. *J Biol Chem.* 2013; 288:6921–9. [PubMed: 23329828]
57. Shafi R, Iyer SP, Ellies LG, O'Donnell N, Marek KW, Chui D, et al. The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc Natl Acad Sci U S A.* 2000; 97:5735–9. [PubMed: 10801981]
58. Belaya K, Finlayson S, Slater CR, Cossins J, Liu WW, Maxwell S, et al. Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. *Am J Hum Genet.* 2012; 91:193–201. [PubMed: 22742743]
59. Li M, Cheng R, Liang J, Yan H, Zhang H, Yang L, et al. Mutations in POFUT1, encoding protein O-fucosyltransferase 1, cause generalized Dowling-Degos disease. *Am J Hum Genet.* 2013; 92:895–903. [PubMed: 23684010]
60. Basmanav FB, Oprisoreanu AM, Pasternack SM, Thiele H, Fritz G, Wenzel J, et al. Mutations in POGLUT1, encoding protein O-glucosyltransferase 1, cause autosomal-dominant Dowling-Degos disease. *Am J Hum Genet.* 2014; 94:135–43. [PubMed: 24387993]
61. Wallace SE, Conta JH, Winder TL, Willer T, Eskuri JM, Haas R, et al. A novel missense mutation in POMT1 modulates the severe congenital muscular dystrophy phenotype associated with POMT1 nonsense mutations. *Neuromuscul Disord.* 2014; 24:312–20. [PubMed: 24491487]
62. Nigro V, Savarese M. Genetic basis of limb-girdle muscular dystrophies: the 2014 update. *Acta myologica: myopathies cardiomyopathies: official journal of the Mediterranean Society of Myology / edited by the Gaetano Conte Academy for the study of striated muscle diseases.* 2014; 33:1–12.
63. Willer T, Inamori KI, Venzke D, Harvey C, Morgensen G, Hara Y, et al. The glucuronyltransferase B4GAT1 is required for initiation of LARGE-mediated alpha-dystroglycan functional glycosylation. *eLife.* 2014;3.

64. Praissman JL, Live DH, Wang S, Ramiah A, Chinoy ZS, Boons GJ, et al. B4GAT1 is the priming enzyme for the LARGE-dependent functional glycosylation of alpha-dystroglycan. *eLife*. 2014;3.
65. Wright KM, Lyon KA, Leung H, Leahy DJ, Ma L, Ginty DD. Dystroglycan organizes axon guidance cue localization and axonal pathfinding. *Neuron*. 2012; 76:931–44. [PubMed: 23217742]
66. Buysse K, Riemersma M, Powell G, van Reeuwijk J, Chitayat D, Roscioli T, et al. Missense mutations in beta-1,3-N-acetylglucosaminyltransferase 1 (B3GNT1) cause Walker-Warburg syndrome. *Hum Mol Genet*. 2013; 22:1746–54. [PubMed: 23359570]
67. Hasegawa K, Okamura Y, Saga Y. Notch signaling in Sertoli cells regulates cyclical gene expression of Hes1 but is dispensable for mouse spermatogenesis. *Mol Cell Biol*. 2012; 32:206–15. [PubMed: 22037762]
68. Wang Y, Tan J, Sutton-Smith M, Ditto D, Panico M, Campbell RM, et al. Modeling human congenital disorder of glycosylation type IIa in the mouse: conservation of asparagine-linked glycan-dependent functions in mammalian physiology and insights into disease pathogenesis. *Glycobiology*. 2001; 11:1051–70. [PubMed: 11805078]
69. Orr SL, Le D, Long JM, Sobieszczuk P, Ma B, Tian H, et al. A phenotype survey of 36 mutant mouse strains with gene-targeted defects in glycosyltransferases or glycan-binding proteins. *Glycobiology*. 2013; 23:363–80. [PubMed: 23118208]
70. Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Takeuchi M, Marth JD. Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. *Cell*. 2005; 123:1307–21. [PubMed: 16377570]
71. Takamatsu S, Antonopoulos A, Ohtsubo K, Ditto D, Chiba Y, Le DT, et al. Physiological and glycomic characterization of N-acetylglucosaminyltransferase-IVa and -IVb double deficient mice. *Glycobiology*. 2010; 20:485–97. [PubMed: 20015870]
72. Cheung P, Pawling J, Partridge EA, Sukhu B, Grynepas M, Dennis JW. Metabolic homeostasis and tissue renewal are dependent on beta1,6GlcNAc-branched N-glycans. *Glycobiology*. 2007; 17:828–37. [PubMed: 17483135]
73. Alvarez-Manilla G, Troupe K, Fleming M, Martinez-Urbe E, Pierce M. Comparison of the substrate specificities and catalytic properties of the sister N-acetylglucosaminyltransferases, GnT-V and GnT-Vb (IX). *Glycobiology*. 2010; 20:166–74. [PubMed: 19846580]
74. Ryczko MC, Pawling J, Chen R, Abdel Rahman AM, Yau K, Copeland JK, et al. Metabolic Reprogramming by Hexosamine Biosynthetic and Golgi N-Glycan Branching Pathways. *Scientific reports*. 2016; 6:23043. [PubMed: 26972830]
75. Johswich A, Longuet C, Pawling J, Abdel Rahman A, Ryczko M, Drucker DJ, et al. N-glycan remodeling on glucagon receptor is an effector of nutrient sensing by the hexosamine biosynthesis pathway. *J Biol Chem*. 2014; 289:15927–41. [PubMed: 24742675]
76. Dennis JW, Pawling J, Cheung P, Partridge E, Demetriou M. UDP-N-acetylglucosamine:alpha-6-D-mannoside beta1,6 N-acetylglucosaminyltransferase V (Mgat5) deficient mice. *Biochim Biophys Acta*. 2002; 1573:414–22. [PubMed: 12417426]
77. Grigorian A, Mkhikian H, Demetriou M. Interleukin-2, Interleukin-7, T cell-mediated autoimmunity, and N-glycosylation. *Ann N Y Acad Sci*. 2012; 1253:49–57. [PubMed: 22288682]
78. Lau KS, Dennis JW. N-Glycans in cancer progression. *Glycobiology*. 2008; 18:750–60. [PubMed: 18701722]
79. Lau KS, Partridge EA, Grigorian A, Silvescu CI, Reinhold VN, Demetriou M, et al. Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. *Cell*. 2007; 129:123–34. [PubMed: 17418791]
80. Song Y, Aglipay JA, Bernstein JD, Goswami S, Stanley P. The bisecting GlcNAc on N-glycans inhibits growth factor signaling and retards mammary tumor progression. *Cancer Res*. 2010; 70:3361–71. [PubMed: 20395209]
81. Ekuni A, Miyoshi E, Ko JH, Noda K, Kitada T, Ihara S, et al. A glycomic approach to hepatic tumors in N-acetylglucosaminyltransferase III (GnT-III) transgenic mice induced by diethylnitrosamine (DEN): identification of haptoglobin as a target molecule of GnT-III. *Free Radic Res*. 2002; 36:827–33. [PubMed: 12420740]

82. Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, et al. Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. *Proc Natl Acad Sci U S A*. 2005; 102:15791–6. [PubMed: 16236725]
83. Li W, Yu R, Ma B, Yang Y, Jiao X, Liu Y, et al. Core fucosylation of IgG B cell receptor is required for antigen recognition and antibody production. *J Immunol*. 2015; 194:2596–606. [PubMed: 25694612]
84. Ellies LG, Tsuboi S, Petryniak B, Lowe JB, Fukuda M, Marth JD. Core 2 oligosaccharide biosynthesis distinguishes between selectin ligands essential for leukocyte homing and inflammation. *Immunity*. 1998; 9:881–90. [PubMed: 9881978]
85. Sperandio M, Thatte A, Foy D, Ellies LG, Marth JD, Ley K. Severe impairment of leukocyte rolling in venules of core 2 glucosaminyltransferase-deficient mice. *Blood*. 2001; 97:3812–9. [PubMed: 11389021]
86. Stone EL, Ismail MN, Lee SH, Luu Y, Ramirez K, Haslam SM, et al. Glycosyltransferase function in core 2-type protein O glycosylation. *Mol Cell Biol*. 2009; 29:3770–82. [PubMed: 19349303]
87. Lee SH, Fukuda M. Study of the biological functions of mucin type core 3 O-glycans. *Methods Mol Biol*. 2013; 1022:41–50. [PubMed: 23765652]
88. Ismail MN, Stone EL, Panico M, Lee SH, Luu Y, Ramirez K, et al. High-sensitivity O-glycomic analysis of mice deficient in core 2 {beta}1,6-N-acetylglucosaminyltransferases. *Glycobiology*. 2011; 21:82–98. [PubMed: 20855471]
89. Moran JL, Shifley ET, Levorse JM, Mani S, Ostmann K, Perez-Balaguer A, et al. Manic fringe is not required for embryonic development, and fringe family members do not exhibit redundant functions in the axial skeleton, limb, or hindbrain. *Dev Dyn*. 2009; 238:1803–12. [PubMed: 19479951]
90. Hahn KL, Beres B, Rowton MJ, Skinner MK, Chang Y, Rawls A, et al. A deficiency of lunatic fringe is associated with cystic dilation of the rete testis. *Reproduction*. 2009; 137:79–93. [PubMed: 18801836]
91. Hahn KL, Johnson J, Beres BJ, Howard S, Wilson-Rawls J. Lunatic fringe null female mice are infertile due to defects in meiotic maturation. *Development*. 2005; 132:817–28. [PubMed: 15659488]
92. Ryan MJ, Bales C, Nelson A, Gonzalez DM, Underkoffler L, Segalov M, et al. Bile duct proliferation in Jag1/fringe heterozygous mice identifies candidate modifiers of the Alagille syndrome hepatic phenotype. *Hepatology*. 2008; 48:1989–97. [PubMed: 19026002]
93. Tan JB, Xu K, Cretigny K, Visan I, Yuan JS, Egan SE, et al. Lunatic and manic fringe cooperatively enhance marginal zone B cell precursor competition for delta-like 1 in splenic endothelial niches. *Immunity*. 2009; 30:254–63. [PubMed: 19217325]
94. Song Y, Kumar V, Wei HX, Qiu J, Stanley P. Lunatic, Manic, and Radical Fringe Each Promote T and B Cell Development. *J Immunol*. 2016; 196:232–43. [PubMed: 26608918]
95. Reis LM, Tyler RC, Abdul-Rahman O, Trapane P, Wallerstein R, Broome D, et al. Mutation analysis of B3GALTL in Peters Plus syndrome. *American journal of medical genetics Part A*. 2008; 146A:2603–10. [PubMed: 18798333]
96. Liu J, Ball SL, Yang Y, Mei P, Zhang L, Shi H, et al. A genetic model for muscle-eye-brain disease in mice lacking protein O-mannose 1,2-N-acetylglucosaminyltransferase (POMGnT1). *Mech Dev*. 2006; 123:228–40. [PubMed: 16458488]
97. Yagi H, Nakagawa N, Saito T, Kiyonari H, Abe T, Toda T, et al. AGO61-dependent GlcNAc modification primes the formation of functional glycans on alpha-dystroglycan. *Scientific reports*. 2013; 3:3288. [PubMed: 24256719]
98. Yoshida-Moriguchi T, Yu L, Stalnaker SH, Davis S, Kunz S, Madson M, et al. O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. *Science*. 2010; 327:88–92. [PubMed: 20044576]
99. Grewal PK, Hewitt JE. Mutation of Large, which encodes a putative glycosyltransferase, in an animal model of muscular dystrophy. *Biochim Biophys Acta*. 2002; 1573:216–24. [PubMed: 12417403]
100. Holzfeind PJ, Grewal PK, Reitsamer HA, Kechvar J, Lassmann H, Hoeger H, et al. Skeletal, cardiac and tongue muscle pathology, defective retinal transmission, and neuronal migration

- defects in the Large(myd) mouse defines a natural model for glycosylation-deficient muscle - eye - brain disorders. *Hum Mol Genet.* 2002; 11:2673–87. [PubMed: 12354792]
101. Endo T. Glycobiology of alpha-dystroglycan and muscular dystrophy. *J Biochem.* 2015; 157:1–12. [PubMed: 25381372]
 102. HajMohammadi S, Enjyoji K, Princivalle M, Christi P, Lech M, Beeler D, et al. Normal levels of anticoagulant heparan sulfate are not essential for normal hemostasis. *J Clin Invest.* 2003; 111:989–99. [PubMed: 12671048]
 103. Li JP, Gong F, Hagner-McWhirter A, Forsberg E, Abrink M, Kisilevsky R, et al. Targeted disruption of a murine glucuronyl C5-epimerase gene results in heparan sulfate lacking L-iduronic acid and in neonatal lethality. *J Biol Chem.* 2003; 278:28363–6. [PubMed: 12788935]
 104. Bullock SL, Fletcher JM, Beddington RS, Wilson VA. Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfotransferase. *Genes Dev.* 1998; 12:1894–906. [PubMed: 9637690]
 105. Nagai N, Habuchi H, Sugaya N, Nakamura M, Imamura T, Watanabe H, et al. Involvement of heparan sulfate 6-O-sulfation in the regulation of energy metabolism and the alteration of thyroid hormone levels in male mice. *Glycobiology.* 2013; 23:980–92. [PubMed: 23690091]
 106. Bhattacharyya R, Gliddon B, Beccari T, Hopwood JJ, Stanley P. A novel missense mutation in lysosomal sulfamidase is the basis of MPS III A in a spontaneous mouse mutant. *Glycobiology.* 2001; 11:99–103. [PubMed: 11181566]
 107. Hemsley KM, Hopwood JJ. Development of motor deficits in a murine model of mucopolysaccharidosis type IIIA (MPS-III A). *Behavioural brain research.* 2005; 158:191–9. [PubMed: 15698885]
 108. Tomatsu S, Orii KO, Vogler C, Nakayama J, Levy B, Grubb JH, et al. Mouse model of N-acetylgalactosamine-6-sulfate sulfatase deficiency (Galns^{-/-}) produced by targeted disruption of the gene defective in Morquio A disease. *Hum Mol Genet.* 2003; 12:3349–58. [PubMed: 14583446]
 109. Hayashida Y, Akama TO, Beecher N, Lewis P, Young RD, Meek KM, et al. Matrix morphogenesis in cornea is mediated by the modification of keratan sulfate by GlcNAc 6-O-sulfotransferase. *Proc Natl Acad Sci U S A.* 2006; 103:13333–8. [PubMed: 16938851]
 110. Akyuz N, Rost S, Mehanna A, Bian S, Loers G, Oezen I, et al. Dermatan 4-O-sulfotransferase1 ablation accelerates peripheral nerve regeneration. *Exp Neurol.* 2013; 247:517–30. [PubMed: 23360803]
 111. Bian S, Akyuz N, Bernreuther C, Loers G, Laczynska E, Jakovcevski I, et al. Dermatan sulfotransferase Chst14/D4st1, but not chondroitin sulfotransferase Chst11/C4st1, regulates proliferation and neurogenesis of neural progenitor cells. *J Cell Sci.* 2011; 124:4051–63. [PubMed: 22159417]
 112. Kawashima H, Petryniak B, Hiraoka N, Mitoma J, Huckaby V, Nakayama J, et al. N-acetylglucosamine-6-O-sulfotransferases 1 and 2 cooperatively control lymphocyte homing through L-selectin ligand biosynthesis in high endothelial venules. *Nat Immunol.* 2005; 6:1096–104. [PubMed: 16227985]
 113. Uchimura K, Gauguier JM, Singer MS, Tsay D, Kannagi R, Muramatsu T, et al. A major class of L-selectin ligands is eliminated in mice deficient in two sulfotransferases expressed in high endothelial venules. *Nat Immunol.* 2005; 6:1105–13. [PubMed: 16227986]
 114. Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. *Proc Natl Acad Sci U S A.* 2003; 100:3445–9. [PubMed: 12629211]
 115. Yoshikawa M, Go S, Takasaki K, Kakazu Y, Ohashi M, Nagafuku M, et al. Mice lacking ganglioside GM3 synthase exhibit complete hearing loss due to selective degeneration of the organ of Corti. *Proc Natl Acad Sci U S A.* 2009; 106:9483–8. [PubMed: 19470479]
 116. Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. *Proc Natl Acad Sci U S A.* 1996; 93:10662–7. [PubMed: 8855236]

117. Kawai H, Allende ML, Wada R, Kono M, Sango K, Deng C, et al. Mice expressing only monosialoganglioside GM3 exhibit lethal audiogenic seizures. *J Biol Chem*. 2001; 276:6885–8. [PubMed: 11133999]
118. Asano M, Furukawa K, Kido M, Matsumoto S, Umesaki Y, Kochibe N, et al. Growth retardation and early death of beta-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. *EMBO Journal*. 1997; 16:1850–7. [PubMed: 9155011]
119. Lu Q, Hasty P, Shur BD. Targeted mutation in beta1,4-galactosyltransferase leads to pituitary insufficiency and neonatal lethality. *Dev Biol*. 1997; 181:257–67. [PubMed: 9013935]
120. Rodeheffer C, Shur BD. Targeted mutations in beta1,4-galactosyltransferase I reveal its multiple cellular functions. *Biochim Biophys Acta*. 2002; 1573:258–70. [PubMed: 12417408]
121. Nishie T, Miyaishi O, Azuma H, Kameyama A, Naruse C, Hashimoto N, et al. Development of IgA nephropathy-like disease with high serum IgA levels and increased proportion of polymeric IgA in Beta-1,4-galactosyltransferase-deficient mice. *Contrib Nephrol*. 2007; 157:125–8. [PubMed: 17495449]
122. Chen J, Lu L, Shi S, Stanley P. Expression of Notch signaling pathway genes in mouse embryos lacking b4galactosyltransferase-1. *Gene Expr Patterns*. 2006; 6:376–82. [PubMed: 16412699]
123. Shinzaki S, Iijima H, Fujii H, Kuroki E, Tatsunaka N, Inoue T, et al. Altered oligosaccharide structures reduce colitis induction in mice defective in beta-1,4-galactosyltransferase. *Gastroenterology*. 2012; 142:1172–82. [PubMed: 22333949]
124. Yoshihara T, Sugihara K, Kizuka Y, Oka S, Asano M. Learning/memory impairment and reduced expression of the HNK-1 carbohydrate in beta4-galactosyltransferase-II-deficient mice. *J Biol Chem*. 2009; 284:12550–61. [PubMed: 19265195]
125. Tokuda N, Numata S, Li X, Nomura T, Takizawa M, Kondo Y, et al. beta4GalT6 is involved in the synthesis of lactosylceramide with less intensity than beta4GalT5. *Glycobiology*. 2013; 23:1175–83. [PubMed: 23882130]
126. Togayachi A, Kozono Y, Kuno A, Ohkura T, Sato T, Hirabayashi J, et al. Beta3GnT2 (B3GNT2), a major polylactosamine synthase: analysis of B3GNT2-deficient mice. *Methods Enzymol*. 2010; 479:185–204. [PubMed: 20816167]
127. Henion TR, Madany PA, Faden AA, Schwarting GA. beta3GnT2 null mice exhibit defective accessory olfactory bulb innervation. *Mol Cell Neurosci*. 2013; 52:73–86. [PubMed: 23006775]
128. Fan X, Kondo Y, Tokuda N, Ohmi Y, Ando R, Umezue T, et al. Strong antibody reaction against glycosphingolipids injected in liposome-embedded forms in beta3GN-T5 knockout mice. *Nagoya journal of medical science*. 2011; 73:137–46. [PubMed: 21928695]
129. Kuan CT, Chang J, Mansson JE, Li J, Pegram C, Fredman P, et al. Multiple phenotypic changes in mice after knockout of the B3gnt5 gene, encoding Lc3 synthase--a key enzyme in lacto-neolacto ganglioside synthesis. *BMC Dev Biol*. 2010; 10:114. [PubMed: 21087515]
130. Sturgill ER, Aoki K, Lopez PH, Colacurcio D, Vajn K, Lorenzini I, et al. Biosynthesis of the major brain gangliosides GD1a and GT1b. *Glycobiology*. 2012; 22:1289–301. [PubMed: 22735313]
131. Yang WH, Nussbaum C, Grewal PK, Marth JD, Sperandio M. Coordinated roles of ST3Gal-VI and ST3Gal-IV sialyltransferases in the synthesis of selectin ligands. *Blood*. 2012; 120:1015–26. [PubMed: 22700726]
132. Ellies LG, Ditto D, Levy GG, Wahrenbrock M, Ginsburg D, Varki A, et al. Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands. *Proc Natl Acad Sci U S A*. 2002; 99:10042–7. [PubMed: 12097641]
133. Homeister JW, Thall AD, Petryniak B, Maly P, Rogers CE, Smith PL, et al. The alpha(1,3)fucosyltransferases FucT-IV and FucT-VII exert collaborative control over selectin-dependent leukocyte recruitment and lymphocyte homing. *Immunity*. 2001; 15:115–26. [PubMed: 11485743]
134. Kudo T, Kaneko M, Iwasaki H, Togayachi A, Nishihara S, Abe K, et al. Normal embryonic and germ cell development in mice lacking alpha 1,3-fucosyltransferase IX (Fut9) which show disappearance of stage-specific embryonic antigen 1. *Mol Cell Biol*. 2004; 24:4221–8. [PubMed: 15121843]

135. Fenderson BA, Zehavi U, Hakomori S. A multivalent lacto-N-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective. *J Exp Med*. 1984; 160:1591–6. [PubMed: 6491606]
136. Domino SE, Zhang L, Gillespie PJ, Saunders TL, Lowe JB. Deficiency of reproductive tract alpha(1,2)fucosylated glycans and normal fertility in mice with targeted deletions of the FUT1 or FUT2 alpha(1,2)fucosyltransferase locus. *Mol Cell Biol*. 2001; 21:8336–45. [PubMed: 11713270]
137. Pickard JM, Maurice CF, Kinnebrew MA, Abt MC, Schenten D, Golovkina TV, et al. Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. *Nature*. 2014; 514:638–41. [PubMed: 25274297]
138. Baenziger JU. Glycoprotein hormone GalNAc-4-sulphotransferase. *Biochem Soc Trans*. 2003; 31:326–30. [PubMed: 12653629]
139. Mi Y, Fiete D, Baenziger JU. Ablation of GalNAc-4-sulfotransferase-1 enhances reproduction by altering the carbohydrate structures of luteinizing hormone in mice. *J Clin Invest*. 2008; 118:1815–24. [PubMed: 18431515]
140. Mi Y, Lin A, Fiete D, Steirer L, Baenziger JU. Modulation of mannose and asialoglycoprotein receptor expression determines glycoprotein hormone half-life at critical points in the reproductive cycle. *J Biol Chem*. 2014; 289:12157–67. [PubMed: 24619407]
141. Yamamoto S, Oka S, Inoue M, Shimuta M, Manabe T, Takahashi H, et al. Mice deficient in nervous system-specific carbohydrate epitope HNK-1 exhibit impaired synaptic plasticity and spatial learning. *J Biol Chem*. 2002; 277:27227–31. [PubMed: 12032138]
142. Ishio A, Sasamura T, Ayukawa T, Kuroda J, Ishikawa HO, Aoyama N, et al. O-fucose monosaccharide of Drosophila Notch has a temperature-sensitive function and cooperates with O-glucose glycan in Notch transport and Notch signaling activation. *J Biol Chem*. 2015; 290:505–19. [PubMed: 25378397]
143. Sparrow DB, Chapman G, Wouters MA, Whittock NV, Ellard S, Fatkin D, et al. Mutation of the LUNATIC FRINGE Gene in Humans Causes Spondylocostal Dysostosis with a Severe Vertebral Phenotype. *Am J Hum Genet*. 2006; 78:28–37. [PubMed: 16385447]
144. Kamien B, Harraway J, Lundie B, Smallhorne L, Gibbs V, Heath A, et al. Characterization of a 520 kb deletion on chromosome 15q26.1 including ST8SIA2 in a patient with behavioral disturbance, autism spectrum disorder, and epilepsy. *American journal of medical genetics Part A*. 2014; 164A:782–8. [PubMed: 24357335]
145. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Marth JD, et al. Symbol nomenclature for glycan representation. *Proteomics*. 2009; 9:5398–9. [PubMed: 19902428]
146. Varki A, Cummings RD, Aebi M, Packer NH, Seeberger PH, Esko JD, et al. Symbol Nomenclature for Graphical Representations of Glycans. *Glycobiology*. 2015; 25:1323–4. [PubMed: 26543186]

Figure 1.

Glycans of mammalian cells. This diagram presents the different classes of mammalian glycans using the symbols shown for each sugar [145, 146]. The filled boxes identify the initiating sugar or core of each glycan. The major classes of glycan are labeled and attached to generic proteins. On the left, GPI-anchored glycoproteins have sugars attached at the C-terminus and are often modified with N- and/or O-glycans in the luminal domain, and anchored in the outer leaflet of the plasma membrane. The protein labeled EGF/TSP contains EGF repeats (pink) and thrombospondin repeats (gray). HS and CS GAG chains are shown attached by the proteoglycan core. Hyaluronan is a GAG found in the extracellular matrix, unattached to protein. In the cytosol is a protein modified with O-GlcNAc, an intracellular glycosylation modification that does not occur in the secretory pathway. The protein with the oval extracellular domain is α -dystroglycan (α -DG) associated non-covalently to its transmembrane β -dystroglycan subunit. The complex O-mannose glycan on α -DG has a polymer of $[Xyl\alpha 1,3GlcA\beta 1,3]_n$ termed matriglycan attached via a $GlcA\beta 1,4Xyl$ linked to two ribitol-5-phosphate residues attached to GalNAc in the phospho-O-Man trisaccharide core. The simple O-mannose glycan adjacent is also essential for α -

dystroglycan function. Examples of glycolipids are represented to the right of dystroglycan. The protein at right (red) carries an oligomannose N-glycan (left side), a complex N-glycan (right side), an O-GalNAc glycan (upper) and an O-mannose glycan (lower). The glycans shown are relatively simple examples of each class and are found on a large number of proteins. Complex N-glycans may be large with up to five branches and numerous lactosamine units $[\text{Gal}\beta 1,4\text{GlcNAc}]_n$ extending each branch, and various terminal sugars. This figure is modified from Fig. 1.6, and reproduced with permission of the Consortium of Glycobiology editors from chapter 1, “Essentials of Glycobiology” 2nd edition, Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW and Etzler ME, editors. Cold Spring Harbor (NY). Cold Spring Harbor Laboratory Press, 2009.

Figure 2. O-Glycans of mammalian cells. The protein motifs that receive O-glycans and the sugars attached are summarized using symbols shown in the figure. The glycosyltransferases that catalyze the transfer of each sugar to generate each class of O-glycan are also shown.

Figure 3.

N-glycan synthesis in the cytoplasm and ER. The reactions catalyzed by enzymes in the cytosol and ER membrane are depicted. Oligosaccharyltransferase (OST) is a complex of many subunits that catalyzes the transfer of the mature N-glycan precursor to protein. Most activities shown have been found mutated in humans, giving rise to CDGs [11]. This figure is modified from Fig. 8.3 and reproduced with permission of the Consortium of Glycobiology editors from chapter 8, “Essentials of Glycobiology” 2nd edition, Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW and Etzler ME, editors. Cold Spring Harbor (NY). Cold Spring Harbor Laboratory Press, 2009.

Figure 4.

Complex N-glycan synthesis in mammalian cells. The initiation of complex and hybrid N-glycan synthesis is catalyzed by MGAT1. The GlcNAc transferred may be extended by Gal and sialic acid to form a hybrid N-glycan. Branching GlcNAc-transferases and GlcNAc residues as shown. Bis identifies the bisecting GlcNAc transferred by MGAT3. Transferases that may extend each branch with LacNAc or polylactosamine, or terminate complex N-glycans with different sugars, act in the trans Golgi and trans Golgi network (TGN). The ovals encompass the LeX and sLeX glycan determinants respectively.

Table 1
Glycosylation genes essential for mouse embryonic development

Gene ^a	Glycan Substrate Accumulated	Glycan(s) Not Generated	Stage of Death	Refs.
N-Glycans				
<i>Mpi</i>	Fructose-6-P	Man-6-P	~E11.5	[144]
<i>Pmm2</i>	Man-6-P	Man-1-P	~E3.5	[145]
<i>Dpagt1</i>	Dol-P	GlcNAc-P-P-Dol	E4–5	[146]
<i>Mgat1</i>	Man ₅ GlcNAc ₂ Asn	GlcNAc-Man ₅ GlcNAc ₂ Asn	~E9.5	[36, 37]
<i>Man2a1+Man2a2</i>	GlcNAcMan ₅ GlcNAc ₂ Asn	GlcNAc-Man ₃ GlcNAc ₂ Asn	E15.5–18.5-P0	[147]
GPI-anchors				
<i>Piga</i>	Naked GPI-anchor	GlcNAc-GPI-anchor	E7.5–9.5	[148]
O-GalNAc Glycans				
<i>C1galt1</i>	GalNAc-O-Ser/Thr	Gal-GalNAc-Ser/Thr	~E13.5	[54]
<i>C1galt1c1</i>	GalNAc-O-Ser/Thr	Gal-GalNAc-Ser/Thr	~E13.5	[21]
O-Fucose Glycans				
<i>Pofut1</i>	C ₂ XXXXXS/TC ₃ EGF repeats	Fuc-O-EGF	~E9.5	[46]
<i>Lfng</i>	Fuc-O-EGF	GlcNAc-Fuc-O-EGF	P0	[49, 50]
<i>Pofut2</i>	TSR type 1 repeats	Fuc-O-TSR type 1	~E6.5	[48]
O-Mannose Glycans				
<i>Pomt1</i>	Unmodified Ser/Thr	Man-O-Ser/Thr	E7.5–9.5	[149]
<i>Pomt2</i>	Unmodified Ser/Thr	Man-O-Ser/Thr	~E9.5	[150]
<i>B4gat1</i>	GlcNAc(GlcNAc)Man(P)-O-Ser/Thr	Priming GlcA for Large XlyGlcA polymer	~E9.5	[62, 64]
O-Glucose Glycans				
<i>Poglut1</i>	C ₁ XSXPTC ₂ EGF repeats	Glc-O-EGF repeat	~E10.5	[151]
Glycosamino-glycans (GAGs)				
<i>Ext1</i>	Core GAG lacks HS	Heparan sulfate	E6.5–8.5	[152]
<i>Ext2</i>	Core GAG lacks HS	Heparan sulfate	E6.5–8.5	[153]
<i>Ndst1+Ndst2</i>	No N sulfation of HS	HS with GlcNS	~E12.5	[154]
<i>Hs6st1</i>	Reduced 6-O-sulfation GlcN in HS	HS with GlcN6S	E15.5	[155]
<i>Hs6st1+Hs2st</i>	Very reduced 2-O- and 6-O-sulfation GlcN in HS	HS with GlcN-O-2S and GlcN-O-6S	<E15.5	[156]
<i>Chst11</i>	Reduced 4-O-sulfation of GalN in CS	CS with GalN-O-4S	~P0	[110]
Glycolipids				
<i>Ugcg</i>	Ceramide lacks Glc	Glc-Cer	E9.5	[157]
<i>B3gnt5</i>	GalGlc-Cer	GlcNAcGalGlc-Cer	~E3.5	[158]
<i>B4galt5</i>	GlcNAcGalGlc-Cer	GalGlcNAcGalGlc-Cer	~E10.5	[159, 160]
Nucleotide Sugars				
<i>Gne</i>	Mannosamine	GCs without sialic acid	~E9.5	[161]
<i>Ugdh</i>	UDP-Glc	UDP-GlcA	~E4.5	[162]

Gene ^a	Glycan Substrate Accumulated	Glycan(s) Not Generated	Stage of Death	Refs.
<i>Slc35c1</i>	GDP-Fuc	GCs with little Fuc	~P0	[163]
<i>Slc35d1</i>	High UDP-GlcA and UDP-GalNAc in cytoplasm	CS GAGs	~P0	[164]

^aRefer to figures to identify reaction catalyzed

GC, glycoconjugate; HS, heparan sulfate; CS, chondroitin sulfate; KS, keratan sulfate.