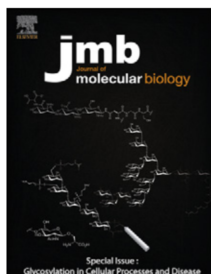
Review



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 glycophosphatidylinositol 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).
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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 the 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 (NeuAcα2,3Galβ1,4[Fucα1,3]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 (α-DG) [4,5], among which, those found at Thr317 and Thr319 in the mucin domain of α-DG 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 glycopconjugates, defining the 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 (pp) 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-DG 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 $Man_5GlcNAc_2$-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 $Glc_3Man_9GlcNAc_2$, which is transferred to protein by the action of oligosaccharyltransferase, 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 ($Man_3GlcNAc_2$Asn) 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 (GlcAβ1,3-Galβ1,3Galβ1,4Xylβ-O-Ser/Thr) is subsequently extended by disaccharide hexosamine repeats to form GAG chains that include modifications such as epimerization and sulfation [28] (Fig. 1). A lengthy $[Xyl\alpha1,3GlcA\beta1,3]_n$ disaccharide polymer similar to a GAG and termed matriglycan is found attached to α-DG via GlcAβ1,4Xyl 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) contains a peptide recognition sequence for B4GALNT3 and B4GALNT4 [32].

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