The O-Mannosylation Pathway: Glycosyltransferases and Proteins Implicated in Congenital Muscular Dystrophy*

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Several forms of congenital muscular dystrophy, referred to as dystroglycanopathies, result from defects in the protein O-mannosylation biosynthetic pathway. In this minireview, I discuss 12 proteins involved in the pathway and how they play a role in the building of glycan structures (most notably on the protein α-dystroglycan) that allow for binding to multiple proteins of the extracellular matrix.

Although O-mannosylation of mammalian proteins was observed almost 35 years ago (1), it was not until the turn of the millennium that the importance of this protein post-translational modification pathway began to be appreciated. In the early 2000s, multiple groups established that deficiencies of enzymes in this pathway result in multiple forms of congenital muscular dystrophy (CMD) that have now been termed dysenzymes in this pathway result in multiple forms of congenital muscular dystrophy, which is the most common form of muscular dystrophy, is an X-linked recessive disorder resulting from loss of expression of functional dystrophin, a cytoplasmic actin-binding protein (16). Dystrophin is connected to a set of proteins at the plasma membrane, most notably dystroglycan (18). Dystroglycan is a single gene product (DAG1) that is processed into two subunits: β-dystroglycan, that is a transmembrane protein that interacts with dystrophin in the cytoplasm, and α-dystroglycan, which is a soluble secreted glycoprotein that interacts with both β-dystroglycan and multiple components of the extracellular matrix, such as laminin, perlecain, pikachurin, neurexin, and agrin (18–23). These extracellular matrix proteins recognize and bind the unusual glycan structures on α-dystroglycan. Thus, proper glycosylation of α-dystroglycan is essential for binding to extracellular matrix components (24).

Although α-dystroglycan is both N- and O-linked glycosylated, it is the O-linked glycans that are essential for proper function (25). In terms of O-linked glycosylation, α-dystroglycan contains both classical mucin-like O-GalNAc-initiated glycans and the more unusual O-Man-initiated glycans (11). Multiple studies have clearly demonstrated that it is the O-mannosylated glycan structures that serve as binding sites for laminin and presumably other extracellular matrix proteins (18–23). Interestingly, it appears to be these same essential structures that are recognized by the antibody IIH6 and that are used as cellular binding sites by some arenaviruses (9, 26).

The initial O-mannose residue is added to serines and threonines of α-dystroglycan and other proteins that have not been clearly defined but certainly must exist in the endoplasmic reticulum (ER) (27, 28). Multiple sites of O-mannosylation (and O-GalNAcylation) on α-dystroglycan have been established (8, 11, 12). This O-mannose can then be extended to create a variety of glycan structures (Fig. 1) (reviewed recently in Refs. 13 and 29). In terms of how O-mannose-extended glycan structures are important for binding to the extracellular matrix, two recent studies have made substantial contributions (7, 10). It has been demonstrated on α-dystroglycan that a GalNAc-β3-GlcNAc-β4-Man structure that is phosphorylated at the 6-position of mannose and further extended by an unknown moiety on the distal side of the phosphate, forming a phosphodiester structure, is essential for binding to extracellular matrix proteins (10). Most recently, it has been proposed that a key component of this unknown extension from the phosphate contains the repeating disaccharide α3-GlcUA-β3-Xyl- (7).

Other recent reviews have focused on the structures, substrates, and functional implications of the O-mannosylation pathway and the phenotypes observed in the various muscular dystrophies (13, 14, 17, 24, 29, 30). Here, I review the enzymes/proteins of the pathway that have been implicated in CMD.

Enzymes/Proteins of the Pathway

Over the last decade, a variety of enzymes and proteins have been implicated in the O-mannosylation pathway. Here, I focus primarily on the human proteins involved in the pathway that, when defective, have been shown to cause CMD, specifically dystroglycanopathies (Table 1). It should be noted that at least one-third of dystroglycanopathies are of unknown genetic etiology and do not have defects in the known gene products.
analyzed by the DPM synthase complex (34). The catalytic activity on the cytosolic side of the ER (34). This reaction is catalyzed from GDP-Man and dolichol phosphate via an inverting mechanism from yeast to man, and several model systems have provided invaluable insights into the pathway (31–33). DPM is synthesized provided linear or branched structures. A key structure for binding to extra-cellular matrix proteins is not fully resolved but contains a phosphodiester linkage, and a component of the X moiety is likely to be the LARGE-catalyzed repeating disaccharide. Green circles, Man; blue squares, GlcNAc; yellow square, GalNAc; yellow circle, Gal; pink diamonds, Neu5Ac; red triangles, Fuc; orange star, Xyl; blue/white diamond, GlcUA. GlcNAc residues on the O-Man added in the 2-position are drawn up to the left, in the 4-position straight up, and in the 6-position up to the right. Asymmetric branched structures are drawn in only one possible configuration, although isomeric structures are likely to exist.

involved to date in the O-mannosylation pathway. Furthermore, several of the enzymes needed to build the array of structures observed (Fig. 1) are common to multiple pathways, such as the sialyltransferases, fucosyltransferases, and galactosyltransferases, and are not discussed here, as there is no evidence to date for them being defective in CMD. Finally, O-mannosylation is an evolutionarily conserved post-translational modification from yeast to man, and several model systems have provided invaluable insights into the pathway (31–33).

Dolichyl-phosphate Mannose Synthase—Dolichyl-phosphate mannose (DPM) is the donor for luminal ER mannosylation, including N-, O-, and C-glycosylation as well as glycosphatidylinositol anchor biosynthesis (34). DPM is synthesized from GDP-Man and dolichyl phosphate via an inverting mechanism on the cytosolic side of the ER (34). This reaction is catalyzed by the DPM synthase complex (34). The catalytic activity is performed by DPM1, a dolichyl-phosphate β-1,4-mannosyltransferase belonging to the glycosyltransferase 2 (GT2) family of the CAZy (Carbohydrate-Active enZymes) Database (34). DPM2 and DPM3 are ER-localized transmembrane proteins that interact with the catalytic DPM1 protein to form a fully active DPM synthase complex (35). Causal mutations for a dystroglycanopathy phenotype along with type I congenital disorders of glycosylation (CDG) have been observed in DPM2 and DPM3 (36, 37). Although patient mutations in DPM1 cause a severe form of CDG (38), surprisingly, no dystroglycanopathies or muscular dystrophy has been noted. Given the vital role of DPM in multiple forms of glycosylation, it is perhaps not surprising that mutations in DPM2 and DPM3 cause severe pleiotropic phenotypes. Whether DPM2/3 mutations are truly causal for CMD remains controversial in the field. The proteins responsible for flipping the DPM to the lumen of the ER have not been determined, and impairment of function of these proteins would also likely lead to a plethora of complications resembling both CMD and CDG, as observed for DPM2 and DPM3.

Dolichyl-phosphate-mannose:Protein Mannosyltransferase (POMT1/2)—Initial O-mannosylation of proteins in the ER is catalyzed by POMT1/2 using DPM as the donor (39, 40). POMT1 and POMT2 belong to the inverting GT39 family in the CAZy Database. The proper expression of both proteins together is required for the catalysis of this first step in the O-mannosylation pathway (40, 41). Multiple mutations in both genes are causal for CMD, and complete loss-of-function mutations cause Walker-Warburg syndrome, the most severe of the dystroglycanopathies (42–49). Localization of these enzymes in the ER infers that O-mannosylation precedes classical mucin-like O-GalNAcylations of proteins in the secretory pathway. A recent study has demonstrated that O-mannosylation appears to modulate O-GalNAc addition and site selection (50). Furthermore, loss of O-mannosylation would potentially provide novel sites for the polypeptide GalNAc transferases in the cis-Golgi. Thus, loss of O-mannosylation may alter O-GalNAc addition on proteins, and this “gain of modification” could be responsible for some of the observed phenotypes in CMD.

2-C-Methyl-D-Erythritol 4-Phosphate Cytidylyltransferase-like Protein (Isoprenoid Synthase Domain-containing (ISPD))—ISPD is not predicted to be a glycosyltransferase, yet mutations in this protein cause Walker-Warburg syndrome with clear loss of α-dystroglycan functional glycosylation (51, 52). This enzyme has high similarity to an enzyme in the non-mevalonate pathway for isoprenoid synthesis (53). However, mammals are thought to use only the mevalonate pathway, and several other enzymes in the bacterial non-mevalonate pathway are not obviously conserved in higher animals (53). Thus, the role for this putative enzyme remains unclear, although it clearly impacts the ability of POMT1/2 to transfer O-mannose (52). Does the defect in ISPD affect other types of glycosylation that depend on dolichol-linked sugars? Does ISPD play a role in modification of dolichol-linked mannose? These questions have yet to be fully explored.

UDP-GlcNAc:O-Linked Mannose β1,2-N-Acetylglucosaminyltransferase (POMGnT1)—POMGnT1 catalyzes the extension of the O-mannose-initiated structure with a GlcNAc in a β2-linkage and is a member of the CAZy GT13 family of inverters (6). Mutations in this gene are observed in patients with multiple forms of dystroglycanopathy (6, 54–59). Mice with a knock-out of this enzyme present with phenotypes consistent with human muscle-eye-brain disease, a severe form of CMD (55). Genotype-phenotype correlations have begun to be established for this enzyme (54). This enzyme is localized in the cis-Golgi, and its action appears to be essential for not only 2-extension but also 6-branching of the O-mannose moiety with GlcNAc (28). 6-Branching of the O-mannose is catalyzed by UDP-GlcNAc:mannose β1,6-N-acetylglucosaminyltransferase, GnT-Vb (GnT-IX) (60). Although GnT-Vb and O-mannose branching is localized primarily to the brain, making the gene an attractive potential affected target for undiagnosed CMD with neurological complications, mice lacking GnT-Vb alone or in combination with a knock-out of GnT-Va (which can partially compensate for O-Man branching in the absence of GnT-Vb) do not display any gross brain abnormalities or muscular dystrophy (61). Given the recent finding that the 6-phosphomannose structure that was presumably extended...
with the functional glycan that is LARGE-dependent had an extension with β4-GlcNAc instead of β2-GlcNAc raises several questions (see Fig. 1) (10). Was the β4-GlcNAc structure observed a cell culture artifact from overexpression of a recombinant α-dystroglycan fragment in HEK293 cells? If not, which GlcNAc transferase is responsible for this activity (multiple GlcNAc transferases can add a β4-GlcNAc structure onto mannoside residues in N-linked structures, including GnT-III, GnT-IV, and GnT-VI), and does its loss induce dystroglycanopathy? Also, if the functionally glycosylated structure is extended via a phosphodiester (68). Recent work by Beedle et al. (69) has demonstrated that α-dystroglycan isolated from fukutin knock-out animals has exposed phosphates as opposed to phosphodiester (69). Although it has been suggested that they are putative Golgi-localized glycosyltransferases, they do not fit into any established CAZy glycosyltransferase family, and mutation of the DXD domain in FKRP does not appear to alter functional glycosylation of α-dystroglycan (67). Both proteins are part of the nucleotidytransferase superfamily and do contain LicD domains, which have been implicated in phosphorylcholine transfer to sugars in a 6-linkage, forming a phosphodiester (68). Recent work by Beedle et al. (69) has demonstrated that α-dystroglycan isolated from fukutin knock-out animals has exposed phosphates as opposed to phosphodiester(CDN), which are critical for functional glycosylation. Thus, although these putative enzymes are clearly involved in the formation of the functional O-mannose-initiated glycans important for binding to extracellular proteins, their exact functions remain a mystery.

UDP-Xyl:GlcUA α1,3-Xylosyltransferase and UDP-GlcUA: Xyl β1,3-Glucoacetylglycosyltransferase (LARGE1 and LARGE2)—The most recent protein associated with CMD is GTDC2 (75). This glycosyltransferase belongs to the CAZy inverting family. Defects in this gene are seen in patients presenting with Walker-Warburg syndrome, and knockdown of this gene in zebrafish recapitulated the phenotype of the knockdown of POMT1 (75). It was speculated that this enzyme might be a xylosyltransferase (GT61 does contain β1,2-xylosyltransferases) (75). Sequence comparison showed that this enzyme also has high homology to the recently described secretary pathway-localized protein O-β-N-acetylgalcosaminyltransferase (EGF domain-specific O-GlcNAc transferase) (76, 77). The actual activity of this enzyme and what role it plays specifically in producing functional glycan-dependent protein associations related to CMD remain to be elucidated.

**Conclusions**

Although substantial progress has been made in the last decade in uncovering enzymes and proteins that modulate the O-mannosylation pathway and that cause CMD, many questions remain to be answered. As noted above, several of the
putative enzymes are poorly characterized in terms of their preferred substrates and actual enzymatic activity. For the more defined glycosyltransferases, little work has been done to establish genotype-phenotype correlations with the existing mutations. Clearly, based on the fact that at least one-third of patients with dystroglycanopathy do not have defects in the described gene products, there are other enzymes/proteins to be discovered and characterized. Although substantial progress in determining the extracellular matrix-binding glycan structures has been made in the last few years, a full description of these structures remains to be presented. The protein substrates for the O-mannosylation pathway have yet to be elucidated, with only a few putative proteins besides α-dystroglycan being identified to date. This may be particularly important given that the phenotypes observed in the dystroglycanopathies clearly overlap but also exceed those observed in Duchenne muscular dystrophy (65, 78). Thus, like all good science, the cohort of scientists/clinicians in this field have made substantial advances while creating more questions that need to be pursued if we are to better understand the disease-relevant pathway of protein O-mannosylation.

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