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JB Special Review—Fundamental Roles of Glycans in Eukaryotes

Glycobiology of α-dystroglycan and muscular dystrophy

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Most proteins are modified by glycans, which can modulate the biological properties and functions of glycoproteins. The major glycans can be classified into *N*-glycans and *O*-glycans according to their glycan-peptide linkage. This review will provide an overview of the *O*-mannosyl glycans, one subtype of *O*-glycans. Originally, *O*-mannosyl glycan was only known to be present on a limited number of glycoproteins, especially α-dystroglycan (α-DG). However, once a clear relationship was established between *O*-mannosyl glycan and the pathological mechanisms of some congenital muscular dystrophies in humans, research on the biochemistry and pathology of *O*-mannosyl glycans has been expanding. Because α-DG glycosylation is defective in congenital muscular dystrophies, which also feature abnormal neuronal migration, these disorders are collectively called α-dystroglycanopathies. In this article, I will describe the structure, biosynthesis and pathology of *O*-mannosyl glycans.

Keywords: dystroglycan/glycan biosynthesis/glycosyltransferase/muscular dystrophy/*O*-mannosyl glycan.

Abbreviations: α-DG, α-dystroglycan; Dol-P-Man, dolichol phosphate mannose; ER, endoplasmic reticulum; Fuc, fucose; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; Man, mannose; MEB, muscle-eye-brain disease; POMGNT1, protein *O*-mannose β1, 2-*N*-acetylglucosaminyltransferase; POMT1, protein *O*-mannosyltransferase 1; POMT2, protein *O*-mannosyltransferase 2; Sia, sialic acid; WWS, Walker–Warburg syndrome; Xyl, xylose.

Proteins produced by mammalian cells are frequently post-translationally modified by glycans. The glycan moieties affect the stability and conformation of those glycoproteins and play roles in molecular recognition processes that occur during viral infection and in cell adhesion during inflammation and metastasis, differentiation, development and many other events involving intercellular communication. The mechanisms underlying the many glycan-mediated recognition processes are not fully understood.

The major glycoprotein glycans can be classified into two groups according to their glycan-peptide linkages. Glycans linked to Asn residues are *N*-glycans, and glycans linked to Ser or Thr residues are *O*-glycans. In *N*-glycans, the reducing terminal *N*-acetylglucosamine (GlcNAc) is linked to the amide group of Asn via an aspartylglycosylamine linkage. In *O*-glycans, the reducing terminal *N*-acetylgalactosamine (GalNAc) is attached to the hydroxyl groups of Ser and Thr. In addition to abundant *O*-GalNAc forms, several unique types of *O*-glycosylation have been reported, including *O*-mannosylation. Recently, the importance of α-dystroglycan (α-DG) *O*-mannosylation in muscle and brain development was demonstrated. In this review, I will describe the structures and biosynthetic pathways of *O*-mannosyl glycans and the relationship between muscular dystrophy and α-DG glycosylation.

Dystroglycan

α-DG is an extracellular peripheral membrane glycoprotein anchored to the cell membrane by binding to a transmembrane glycoprotein, β-DG (Fig. 1A). These two subunits were originally identified as a component of the sarcolemmal dystrophin–glycoprotein complex (DGC) (*1*). DG is encoded by a single gene (*DAG1*) (*2*) and is cleaved into two proteins (α-DG and β-DG) by post-translational processing (Fig. 1B). DGC is thought to act as a linker between the extracellular matrix and the intracellular cytoskeleton because α-DG binds with high affinity to the extracellular matrix component laminin, and the intracellular domain of β-DG binds to the cytoskeletal protein dystrophin. β-DG has a single transmembrane domain, and its C-terminus faces the cytoplasm. The last 15 amino acids of β-DG bind directly to the Cys-rich region of dystrophin. This region of β-DG is also Pro rich and contains a Tyr phosphorylation site. The N-terminal domain of α-DG is cleaved by furin-like protease after glycosylation is completed (Fig. 1B). The significance of the cleavage is not fully understood (*3*).

α-DG is heavily glycosylated. Whereas the deduced amino acid sequence predicts an ~74 kDa core peptide, α-DG appears as a broad smear in SDS–PAGE with apparent molecular masses of ~156 kDa in skeletal muscle and 120 kDa in brain and peripheral nerves. Differences in the molecular masses of α-DGs obtained from different tissues are thought to be due to tissue-specific differential glycosylation. Chemical modification by periodic acid or trifluoromethanesulfonic acid treatment resulted in the loss of laminin-binding, suggesting that the glycan moiety is essential for this activity. However, the enzymatic removal of *N*-glycans did not affect the laminin-binding activity. In addition, nitrous acid treatment to degrade heparan sulfate or

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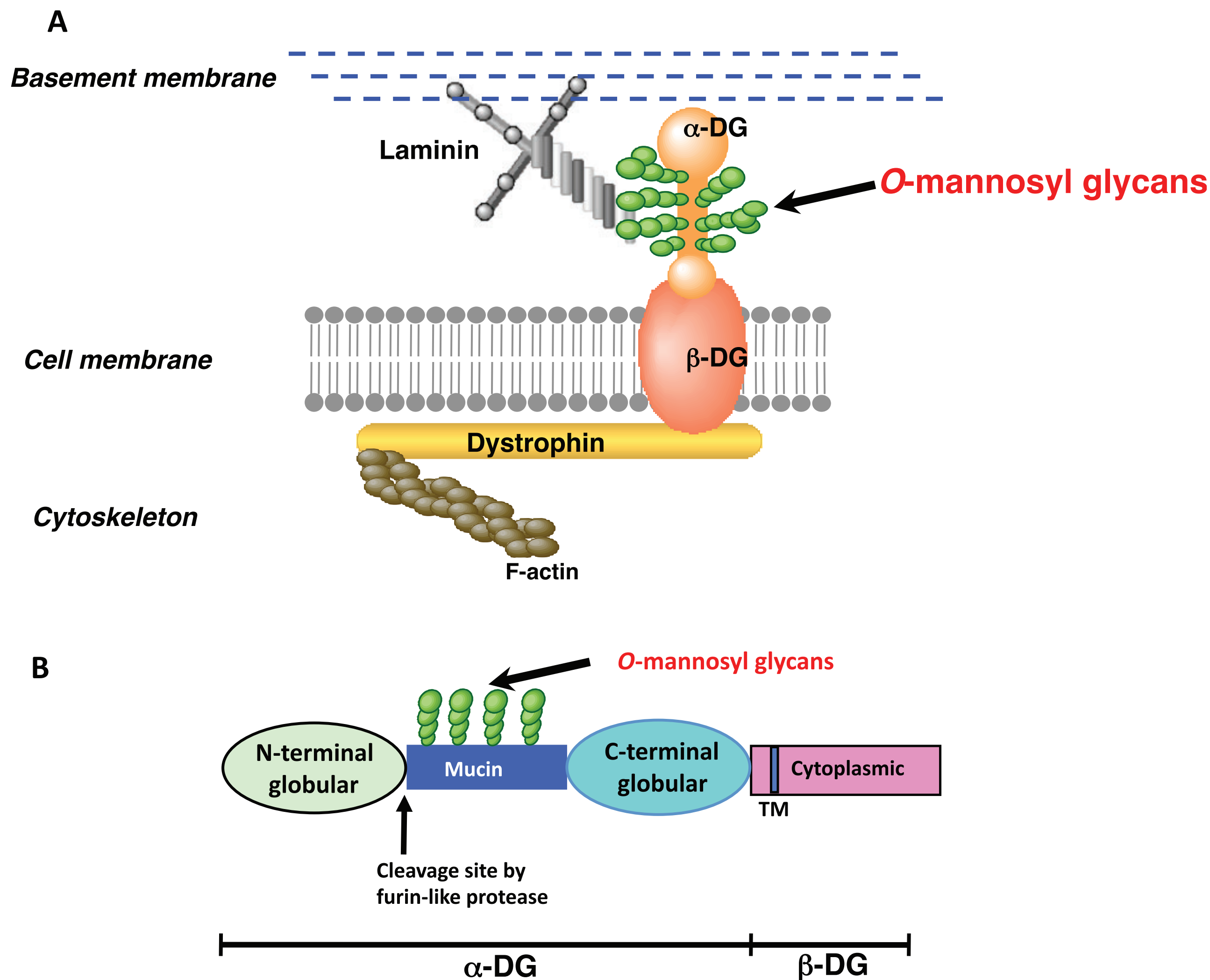

Fig. 1 Molecular organization of the DGC (A) and dystroglycan (B). α-DG links extracellular components containing LG domains, such as laminin, with β-DG, which is a transmembrane glycoprotein, and it also binds to dystrophin, which in turn binds to the actin cytoskeleton. α-DG is heavily glycosylated, and its glycans play a role in binding to laminin. (B) Domain structure of dystroglycan. TM, transmembrane.

glycosaminoglycan lyase digestion did not affect laminin binding. These results suggest that the *O*-glycans of α-DG are important for laminin-binding. α-DG has a mucin-type *O*-glycosylation site in the central region of the molecule (Fig. 1B). The mucin-type domain contains more than 40 Ser/Thr residues that form an *O*-glycan cluster. Some of these *O*-glycan (*O*-mannose and *O*-GalNAc) attachment sites have been elucidated (*4*).

The function of DG has been examined by targeting the *DAG1* gene in mice. However, the disruption of this gene in mice is embryonic lethal. Therefore, the function of DG was examined using the Cre/LoxP system for tissue-specific disruptions. The *DAG1* knockout in skeletal muscle did not affect muscle basement membrane formation but resulted in a mild dystrophic phenotype (*5*). The brain-specific targeting caused abnormal cerebral cortical layering resembling human cobblestone lissencephaly and abnormal cerebellar granule cell migration (*6*). Peripheral nerve-specific disruption caused defects in both myelination and nodal architecture (*7*). These results indicate that DG is essential for normal development and number of cellular functions. Originally, it was thought that the core DG protein could be responsible for these functions, but recent data have suggested that *O*-mannosyl glycans are indeed required for the functions.

DG is also involved in pathogen adhesion to host cells. Several members of the arenavirus family, including the Lassa fever virus and *Mycobacterium leprae*, the bacterium responsible for leprosy, bind to target cells through interactions with a common receptor, α-DG. Although the contribution of the glycan moieties of α-DG to viral entry has been suggested, details of the underlying molecular events remain to be determined (*8*).

O-mannosyl glycan structures

O-mannosylation is a modification that is well known in yeast; *O*-mannosylated proteins are abundant in the yeast cell wall (*9*). In yeast, all *O*-mannosyl glycan

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structures elucidated thus far are neutral linear glycans consisting of 1–7 mannose residues. Protein *O*-mannosylation is vital in yeast because its absence affects cell wall structure and rigidity.

Mammalian *O*-mannosylation was first identified in brain chondroitin sulfate proteoglycans, but the details were unclear. Because *O*-glycans were reported to be important in laminin-binding, we started structural studies on the *O*-glycans of α-DG. We determined that the major *O*-glycan of bovine peripheral nerve α-DG is a novel *O*-mannosyl glycan, Siaα2-3Galβ1-4GlcNAcβ1-2Man (*10*). Interestingly, rabbit skeletal muscle α-DG has the same *O*-mannosyl glycan (*11*). In addition to the *O*-mannosyl glycan, we found almost equal amounts of the core 1 structure, Galβ1-3GalNAc, in rabbit skeletal muscle α-DG. Subsequently, an HNK-1 epitope carrying an *O*-mannosyl glycan (HSO_3-3GlcAβ1-3Galβ1-4GlcNAcβ1-2Man) was detected in total rabbit brain glycopeptides (*12*). Furthermore, α-DG from sheep brain has a Galβ1-4(Fucα1-3)GlcNAcβ1-2Man (the Le^x epitope) structure (*13*). These oligosaccharides also contain 2,6-substituted mannose [GlcNAc β1-6(GlcNAcβ1-2)Man] (*14*). Recently, it was proposed that a GalNAcβ1-3GlcNAcβ1-4 branch and a phosphodiester-linked modification [GalNAcβ1-3GlcNAcβ1-4(phosphate-6)Man] occur on the *O*-mannosyl glycan of α-DG (*15*). A novel glycosaminoglycan-like disaccharide repetitive structure $[\text{-3GlcA}\beta\text{1-3Xyl}\alpha\text{1-}]_n$ attached to α-DG has also been reported. The detailed understanding of how the $[\text{-3GlcA}\beta\text{1-3Xyl}\alpha\text{1-}]_n$ glycan is attached to α-DG has not yet been elucidated, but it is most likely linked via phosphorylated *O*-mannosyl structures (Fig. 2). Therefore, mammals have a series of *O*-mannosyl glycans with heterogeneity of mannose branching and peripheral structures. Based on this diversity and complexity of *O*-mannosyl glycans, Campbell's group proposed three categories of *O*-mannosyl glycans in terms of GlcNAc extension of the *O*-mannose residue (Fig. 2). Core M1 consists of only β1,2-linked GlcNAc. Core M2 is branched β1,6-linked GlcNAc, and core M3 is initiated by β1,4-linked GlcNAc and branched at the 6-position by phosphate (*16*).

α-DG glycan moieties have been proposed to be essential for its biological functions. However, the reason for heterogeneous α-DG glycans in different tissues remains unclear. Tissue-specific α-DG ligand binding mechanisms may provide an explanation, as will be discussed.

O-mannosyl glycan biosynthesis

Core M1 and Core M2

The core M1 biosynthetic pathway is initiated by protein *O*-mannosylation (Fig. 3). Protein *O*-mannosyltransferases catalyse the transfer of a mannosyl residue from dolichol phosphate mannose (Dol-P-Man) to the Ser/Thr residues of certain proteins. This step occurs in the endoplasmic reticulum (ER). In total, seven genes (*pmt1-7*) that are responsible for the formation of mannosyl-peptide linkages have been identified in the *Saccharomyces cerevisiae* genome (*9*).

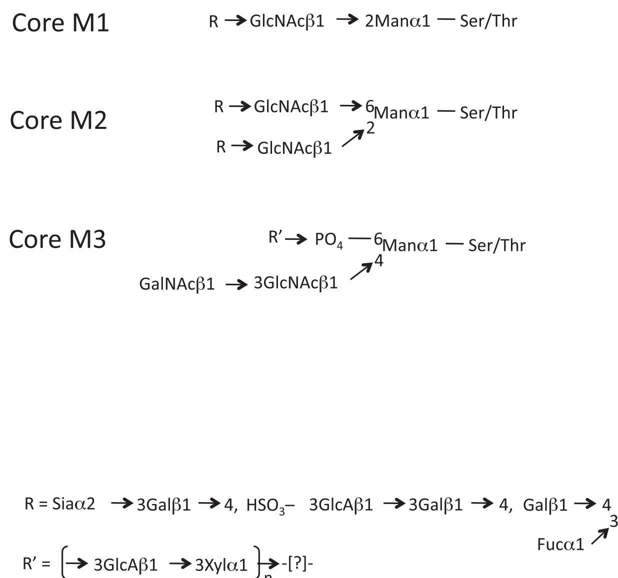

Fig. 2 Proposed *O*-mannosyl glycans (core structures and peripheral structures). Fuc, fucose; Gal, galactose; Man, mannose; Sia, sialic acid. Because a linker portion is not determined, it is marked as '?' in the figure.

Two *pmt* homologues, *POMT1* and *POMT2*, were discovered in humans (Fig. 3). Protein *O*-mannosyltransferase 1 (POMT1) and protein *O*-mannosyltransferase 2 (POMT2) share almost identical hydropathy profiles, which predict both to be integral membrane proteins with multiple transmembrane domains. We demonstrated that human POMT1 and POMT2 have protein *O*-mannosyltransferase activity but only when they are co-expressed (*17*). This finding suggests that POMT1 and POMT2 form a hetero-complex to show enzymatic activity. In addition, POMT1 and POMT2 *N*-glycosylation are essential for their enzymatic activities (*18*). *POMT1* and *POMT2* are expressed in all human tissues, but *POMT1* is highly expressed in foetal brain, testis and skeletal muscle, and *POMT2* is predominantly expressed in testis (*19*, *20*). It should be prudent to determine the regulatory mechanisms for protein *O*-mannosylation in each tissue. It is noteworthy that *O*-mannosylation contributes to ER protein quality control (*21*).

The GlcNAcβ1-2Man linkage is formed by the UDP-GlcNAc protein *O*-mannose β1,2-*N*-acetylglucosaminyltransferase (POMGNT1) (*22*). POMGNT1 catalyses the transfer of GlcNAc from UDP-GlcNAc to *O*-mannosyl glycoproteins within the Golgi apparatus. Human POMGNT1 is a 660 amino acid type II membrane protein with four domains: an N-terminal cytoplasmic tail, a transmembrane domain, a stem domain and a catalytic domain (*23*). The deletion of 298 amino acids from the N-terminus of POMGNT1 including the stem domain does not affect its activity. Therefore, the stem domain function remains unknown. We previously examined the substrate requirement by POMGNT1 in terms of the sequence and length of amino acids using a series of chemically synthesized mannosyl peptides (*24*). POMGNT1

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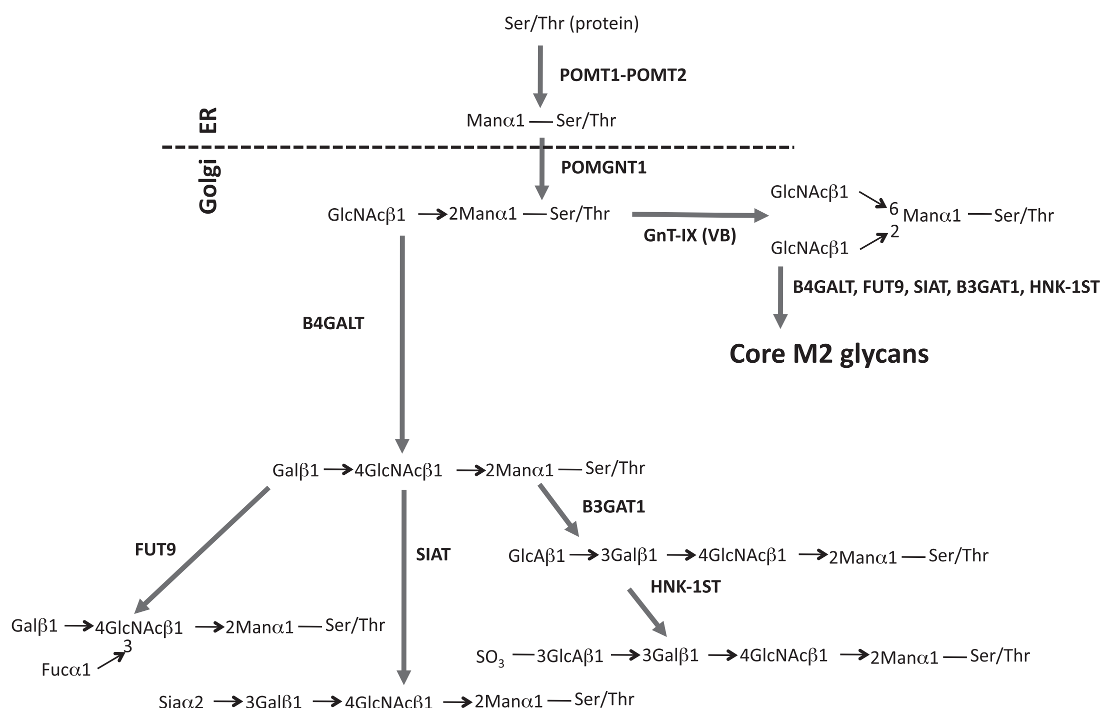

Fig. 3 Possible biosynthetic pathway of *O*-mannosylglycans (core M1 and core M2). GnT-IX(VB), β1,6-*N*-acetylglucosaminyltransferase IX(VB); B3Gat1, β1,3-glucuronyltransferase.

requires three or more amino acid residues to transfer GlcNAc on mannosyl peptides and the amino acid sequence also affects its activity (*25*), suggesting that peptide sequences may determine the efficiency of GlcNAc transfer. Recently, the presence of unextended *O*-mannose in rabbit skeletal muscle T-cadherin was reported (*26*), suggesting that POMGNT1 may not transfer GlcNAc to this mannose residue.

As described earlier, core M2 has the 2,6-substituted *O*-mannose structure GlcNAcβ1-6(GlcNAcβ1-2)Man (Fig. 2). A gene for this 6-branching enzyme (GnT-IX/GnT-VB) has been cloned and is exclusively expressed in the brain (*27*, *28*). After the GlcNAcβ1-2Man linkage is formed by POMGNT1, GnT-IX acts on the product and forms GlcNAcβ1-6(GlcNAcβ1-2)Man (Fig. 3) (*29*). GnT-IX is involved in forming the HNK-1 structure of the 2,6-branched *O*-mannosyl glycan on RPTPβ, which affects oligodendrocyte survival and recovery from demyelinating disease (*30*), and the presence of 2,6-branched *O*-mannosyl glycan regulates its phosphatase activity (*31*). Recently, we demonstrated that *O*-mannose-linked HNK-1 in the brain is primarily carried by phosphacan, a secreted splicing variant of RPTPβ (*32*).

Seven human β1,4-galactosyltransferases (B4GALTs) have been identified and characterized. However, it remains unclear which enzymes can act on a disaccharide (GlcNAcβ1-2Man) in *O*-mannosyl glycans. A recent study suggested that among human B4GALTs, both B4GALT-I and B4GALT-II could efficiently act on the *O*-mannose substrate, but the relative activity of human B4GALT-II was higher than B4GALT-I (*33*). *B4GALT-I* is expressed in many tissues but not in the brain, whereas *B4GALT-II* is expressed at high levels in the brain. These results suggest that B4GALT-II is a major regulator of *O*-mannosylglycan synthesis in the brain.

Peripheral structures are synthesized by GALTs, sialyltransferases (SIATs), glucuronyltransferases (GLCATs), sulfotransferase (HNK-1ST) and α1,3-fucosyltransferase-9 (FUT9), which comprise a series of Golgi-resident glycosyltransferases, but the details of how they control *O*-mannosylglycan synthesis have not been fully elucidated (Fig. 3).

Core M3

The biosynthetic pathway of core M3, phosphorylated *O*-mannosyl trisaccharide [GalNAcβ1-3GlcNAcβ1-4(phosphate-6)Man], has recently been proposed (*16*). First, glycosyltransferase-like domain-containing 2 (GTDC2/POMGNT2) catalyses the transfer of GlcNAc to a mannose residue, demonstrating that it contains protein *O*-mannose β1,4-*N*-acetylglucosaminyltransferase activity. Next, this disaccharide (GlcNAcβ1-4Man) is modified by β1,3-*N*-acetylgalactosaminyltransferase 2 (B3GALNT2) and then by the protein kinase-like protein SGK196/POMK, which is a specific *O*-mannose kinase. However, because SGK196/POMK lacks certain amino acid residues necessary for kinase catalysis, the mechanism of the phosphotransfer reaction remains unclear. Processing up to GalNAcβ1-3GlcNAcβ1-4(phosphate-6)Man occurs in the ER (*16*).

LARGE is a glycosyltransferase-like protein that contains two catalytic domains and is involved in α-DG glycosylation. LARGE generates polymers of alternating xylose (Xyl) and glucuronic acid (GlcA) residues (*34*). One of its catalytic domains exhibits

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xylosyltransferase activity, and the other catalytic domain exhibits GLCAT activity. A detailed understanding of how the xylosyl–glucuronyl polysaccharide is attached to α-DG has not been obtained, but it is most likely linked via phosphorylated *O*-mannosyl structures (as demonstrated in Figs 2 and 4). Interestingly, HNK-1ST suppresses LARGE-dependent α-DG glycosylation and reduces the ligand binding activity of α-DG (*35*), suggesting that sulfate transfer plays an important role in α-DG glycosylation. LARGE2, a paralogue of LARGE, catalyses the same glycosylation reactions as LARGE and exhibits xylosyltransferase and GLCAT activities as well as polymerizing activity (*34*, *36*). LARGE is widely expressed, and the highest levels of this gene are found in the brain, heart and skeletal muscle. In contrast, LARGE2 is highly expressed in the kidney and placenta but not in the brain. It is of interest to determine the contribution of both paralogues to the functional modification of α-DG *in vivo*.

It is clear that POMGNT1 mutations cause laminin-binding insufficiency (*22*, *37*, *38*), likely because of defective post-phosphoryl modification in core M3, which suggests that the 2-substituted *O*-mannose structure is required for post-phosphoryl modification; however, the role of the GlcNAcβ1-2Man linkage in the post-phosphoryl modification remains unclear. Further studies are necessary to clarify the regulatory mechanisms for the formation of the *O*-mannosyl branching structure.

Ligands of α-DG and *O*-mannosylated proteins

Laminin is the most well-known ligand of α-DG, and *O*-mannosyl glycans of α-DG play an important role in laminin binding. Laminin binds to α-DG through its laminin globular (LG) domain in a Ca^{++}-dependent manner. The GlcA-Xyl repeating structure on α-DG is reportedly required for laminin interaction, as demonstrated in Figs 1 and 2 (*39*, *40*). Further studies revealed that *O*-mannosyl glycans of α-DG also play a role in binding to extracellular matrix components and synaptic molecules, such as agrin (*41*), perlecan (*42*), neurexin (*43*), pikachurin (*44*) and slit (*45*). The failure of α-DG to bind to these ligands because of a defect in α-DG glycosylation affects normal muscular function and interrupts neuronal migration in the developing brain. It is important to elucidate the role of α-DG glycans in the binding and recognition of these ligands by α-DG. Many common structural features and tissue-specific differences in glycosylation have also been elucidated through the characterization of α-DG that was purified from different tissues (*46*, *47*). Different α-DG glycoforms may have unique or variable functions in different tissues.

For a long time, α-DG was the most well known *O*-mannosylated protein. However, it has recently become evident that many *O*-mannosylated proteins exist, including recombinant IgG2 (*48*), phosphacan/RPTPβ (*30–32*), CD24 (*49*), neurofascin 186 (*50*), lecticans (*51*), plexins and cadherins (*52*). Cadherins are type I transmembrane proteins that play important roles in calcium-dependent cell adhesion. Classic cadherins are involved in the formation and maintenance of cellular binding within tissues through adherens junction formation. Interestingly, protein *O*-mannosylation is crucial for E-cadherin-mediated cell adhesion (*53*). Because the above *O*-mannosylated proteins are highly important for nervous system development due to their roles in adherence to the cell surface or the extracellular matrix, elucidating the role of *O*-mannosyl glycans in these adhesion and recognition molecules will be an important future goal.

α-Dystroglycanopathy and uncharacterized causative genes

Muscular dystrophies are genetic diseases that cause progressive muscle weakness and wasting. The best-known muscular dystrophy was described by Duchenne and results from mutations in the gene encoding dystrophin (Fig. 1A). In congenital muscular dystrophies, muscle weakness is apparent at birth or shortly thereafter. Recent data indicate that the aberrant *O*-mannosylation of α-DG is the cause of some forms of congenital muscular dystrophy, and these muscular dystrophies are termed α-dystroglycanopathies.

α-DG hypoglycosylation greatly reduces its binding affinity for extracellular matrix components such as laminin, thereby disrupting the dystroglycan-extracellular matrix linkage and leading to membrane fragility. The severity of the clinical manifestations of α-dystroglycanopathy is partly correlated with the genotype of the responsible gene. However, even when the causative gene is the same, the clinical manifestations exhibit a broad spectrum from the severe type that results in fatality with brain malformation in early childhood to the milder form that presents as adult-onset limb-girdle muscular dystrophy without brain malformation (*54*). It is unclear whether defects in additional *O*-mannosylated proteins beyond α-DG contribute to the observed disease complexity. Details regarding the functions of the causative gene products for the α-dystroglycanopathies are listed in Table I.

Muscle–eye–brain disease (MEB) is an autosomal recessive disorder that is characterized by congenital muscular dystrophy, ocular abnormalities and brain malformation (type II lissencephaly). The *POMGNT1* gene is responsible for MEB (*22*). All of the known mutant POMGNT1 proteins discovered in patients with MEB did not exhibit enzymatic activity and could not form the GlcNAcβ1-2Man linkage. Together, these findings indicate that MEB is inherited as a loss-of-function of the *POMGNT1* gene. In addition, a selective deficiency of glycosylated α-DG was found in MEB patients, suggesting that α-DG is a potential target of POMGNT1 and that α-DG hypoglycosylation is the pathological mechanism for MEB.

POMT1 and POMT2 are responsible for catalysing the first step in *O*-mannosyl glycan synthesis (*17*). *POMT1* and *POMT2* mutations result in Walker–Warburg syndrome (WWS), an autosomal

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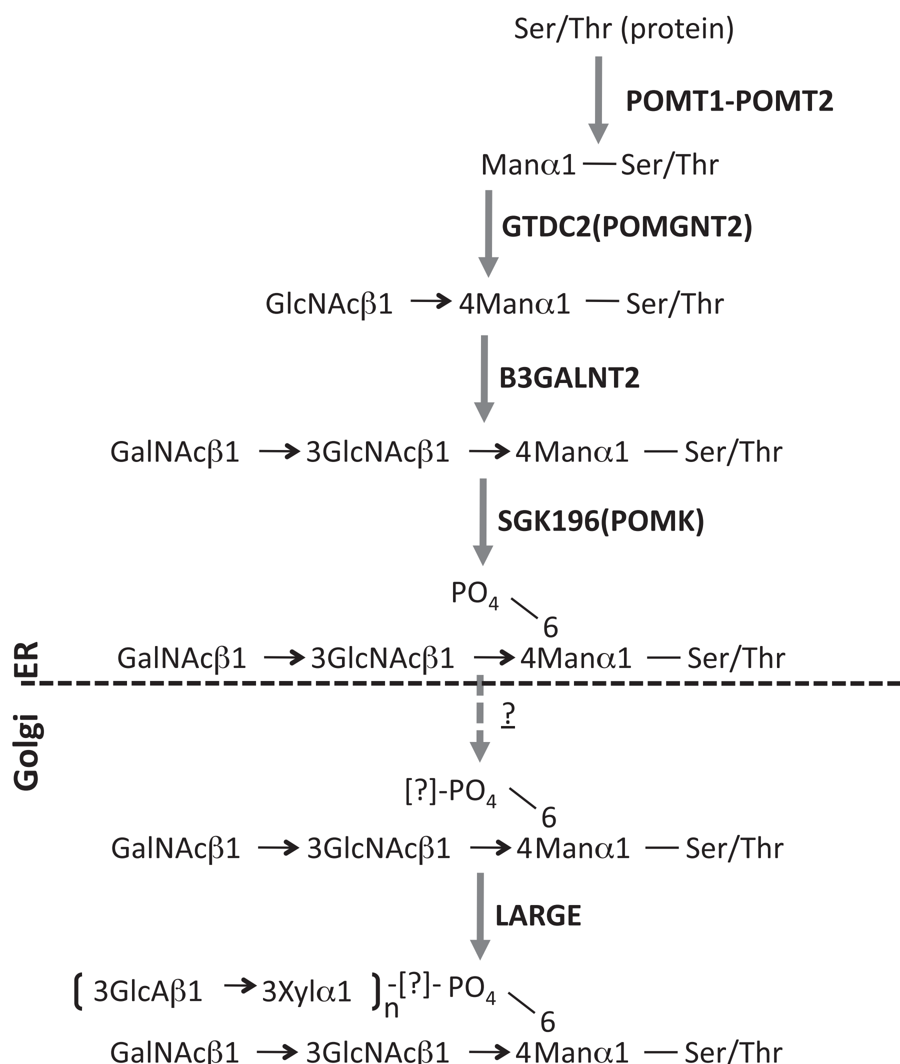

Fig. 4 Possible biosynthetic pathway of *O*-mannosylglycans (core M3). LARGE, acetylglucosaminyltransferase-like; GTDC2(POMGNT2), glycosyltransferase-like domain-containing protein 2; SGK196(POMK), protein *O*-mannose kinase. POMT1 and POMT2 are the same as Fig. 3.

recessive developmental disorder that is associated with congenital muscular dystrophy, neuronal migration defects and ocular abnormalities. Mutations in the POMT1 and POMT2 proteins in WWS patients do not prevent their complex formation, but the mutations do abolish the *O*-mannosyltransferase activity of the complex (*79*, *80*).

Patients with congenital muscular dystrophy, profound mental retardation, white matter changes, subtle structural abnormalities in the brain and a reduction in immunologically detectable α-DG have been reported (congenital muscular dystrophy type 1D, MDC1D), and these patients had mutations in the *LARGE* gene. As described, LARGE is a polymerizing enzyme of alternating Xyl and GlcA residues (*34*).

GTDC2(*POMGNT2*), *B3GALNT2* and *SGK196* *(POMK)* are responsible for the formation of core M3 as described (*16*) and their dysfunction can also cause WWS (*66*), most likely due to a defect in the formation of scaffolding core structures for the xylosyl–glucuronyl polysaccharide. *B3GNT1* may encode a glycosyltransferase that plays a critical role in the synthesis of the laminin-binding glycan on α-DG by collaborating with LARGE (*81*). Reduced expression leads to increased tumour formation, but the glycan structures synthesized by the B3GNT1/LARGE complex were not identified accurately (*81*). Recently, *B3GNT1* mutations were identified in WWS patients (*82*).

FKTN, *FKRP*, *TMEM5* and *ISPD* are uncharacterized, but their mutations lead to defects in α-DG glycosylation. FKTN and FKRP are type II membrane proteins that have some similarities to glycosyltransferases and may be involved in post-phosphoryl modifications. However, their exact functions have not been determined. Fukuyama-type congenital muscular dystrophy, which results from mutations in *FKTN*, is an autosomal recessive disorder that is characterized by congenital muscular dystrophy, lissencephaly and eye anomalies (*57*). MDC1C is caused by a defect in fukutin-related protein (*FKRP*), a homologue of

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Table I. α-Dystroglycanopathy genes encoding established and hypothesized functions.

Genes	Protein function	OMIM
POMT1 (*55*)	Protein *O*-mannosyltransferase 1 (*17*)	607423
POMT2 (*56*)	Protein *O*-mannosyltransferase 2 (*17*)	607439
POMGNT1 (*22*)	Protein *O*-mannose β1, 2-*N*-acetylglucosaminyltransferase (*22*)	606822
FKTN (*57*)	Unknown	607440
FKRP (*58*, *59*)	Unknown	606596
LARGE (*60*)	β1,3-GlcA and α1,3-Xyl transferases (*40*)	603590
GTDC2/POMGTN2 (*61*)	Protein *O*-mannose β1, 4-*N*-acetylglucosaminyltransferase (*16*)	614828
B3GALNT2 (*62*)	β1, 3-*N*-acetylgalactosaminyltransferase 2 (*63*)	610194
B3GNT1 (*64*)	β1, 3-*N*-acetylglucosaminyltransferase 1 (*65*)	605517
SGK196/POMK (*66*)	Protein *O*-mannose kinase (*16*)	615247
TMEM5 (*67*)	Unknown	605862
GMPPB (*68*)	GDP-mannose pyrophosphorylase B (*69*)	615320
DPM1 (*70*)	Dolichol-phosphate mannosyltransferase 1 (*71*)	603503
DPM2 (*72*)	Dolichol-phosphate mannosyltransferase 2 (*71*)	603564
DPM3 (*73*)	Dolichol-phosphate mannosyltransferase 3 (*71*)	605951
DOLK (*74*)	Dolichol kinase (*75*)	610746
ISPD (*76*, *77*)	Unknown	614631
DAG1 (*78*)	Dystroglycan	613818

OMIM, Online Mendelian Inheritance in Man.

FKTN, and is characterized by severe weakness, muscle degeneration and cardiomyopathy (*58*). Mental retardation and cerebellar cysts have been observed in some cases. Allelic mutations in the *FKRP* gene also cause a milder and more common form of muscular dystrophy termed LGMD2I, which is frequently associated with cardiomyopathy and exhibits variable onset, ranging from adolescence to adulthood (*59*). Patients with *FKRP* mutations invariably exhibit reduced α-DG glycosylation. Although the function of FKRP is unknown, it has been suggested that it is a Golgi-resident protein involved in α-DG glycosylation as a glycosyltransferase or a modulator (*46*). It is worth noting that patients with *FKRP* mutations do not exhibit reduced POMT or POMGNT1 activity (*79*), suggesting that FKRP is not associated with POMT1, POMT2 or POMGNT1. Conversely, fukutin is reportedly associated with POMGNT1 in the Golgi compartment. Interestingly, a transgenic knock-in mouse model carrying a retrotransposon insertion in the *FKTN* gene exhibits a 30% reduction in POMGNT1 activity (*83*), suggesting that fukutin modulates POMGNT1 activity.

Mutations in *TMEM5* cause severe cobblestone lissencephaly, consistent with α-dystroglycanopathy (*66*, *67*). TMEM5 is a type II membrane protein that is similar to exostosin (EXT1), which is a glycosyltransferase for heparan sulfate proteoglycan synthesis (*67*), but the function of TMEM5 is not clear.

Isoprenoid synthase domain-containing protein (ISPD) belongs to the family of 4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME) synthases in the 2-C-methyl-D-erythritol 4-phosphate pathway (the MEP pathway, the biosynthetic pathway producing isoprenoid precursors from pyruvic acid and glyceraldehyde 3 phosphoric acid). Mutations in *ISPD* reportedly cause WWS (*76*, *77*) and decrease POMT activity (*76*). However, the precise function of *ISPD* and the mechanism of mutation-induced POMT activity reduction are still unknown. Although isoprenoids serve as precursors to cholesterol and dolichol, the MEP pathway is not essential in mammals. Conversely, the mevalonate pathway, which incorporates isoprenoids from acetyl CoA, is essential in mammals. Based on homology, ISPD may be involved in the synthesis of sugar donor substrates, such as CDP-sugar or dolichol-sugar. Further studies will be necessary to elucidate the function of ISPD.

DPM1, DPM2, *DPM3*, *DOLK* and *GMPPB* encode proteins that are involved in the synthesis of Dol-P-Man, an essential mannose donor required for *O*-mannosylation, *N*-glycosylation, C-mannosylation and the formation of glycosyl-phosphatidylinositol anchors. DOLK encodes the dolichol kinase responsible for dolichol-phosphate formation. A combined *N*-glycosylation and *O*-mannosylation deficiency due to *DOLK* mutations has been observed in patients who present predominant dilated cardiomyopathy (*74*). GMPPB catalyses GDP-Man synthesis from GTP and mannose-1-phosphate (*68*). GDP-Man is required for Dol-P-Man synthesis. No evidence of abnormal *N*-glycosylation has been found for mutant *GMPPB*. Dol-P-Man synthesis is catalysed from dolichol-phosphate and GDP-Man by the DPM synthase complex, which comprises three subunits: DPM1, DPM2 and DPM3. A defect in DPM3 causes abnormal *O*-mannosylation but only mildly abnormal *N*-glycosylation (*73*). Recently, DPM2 defects were identified in patients with abnormal *N*-glycosylation and *O*-mannosylation (*72*). Although mutations in *DPM1* have long been studied with relation to congenital disorders of glycosylation due to *N*-glycosylation defects, DPM1 defects have also been described in an α-dystroglycanopathy (*70*). These data indicate that available Dol-P-Man also affects protein *O*-mannosylation.

As described herein, LARGE is involved in the processing of the N-terminal domain of α-DG by furin (Fig. 1B), which is necessary for α-DG maturation (*3*). A physical interaction between LARGE and α-DG is required for α-DG glycosylation by LARGE,

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and the processing of α-DG occurs after glycosylation. Notably, a *DAG1* Thr192Met substitution (T192M) was identified in a patient with limb-girdle muscular dystrophy (*78*). This mutation reduces the interaction of α-DG with LARGE and leads to defective α-DG glycosylation. Therefore, it is included in the list of genes that cause α-dystroglycanopathies.

Recently, a method to identify the genes that play a role in pathologies related to *O*-mannosylation has been established (*66*). It is the gene disruption screening method to detect molecules associating with the formation of functional α-DG glycans. Because Lassa fever virus entering cells is an α-DG glycan-dependent manner (*8*), genes involved in α-DG glycosylation could be identified by analysing virus-resistant cells. By this method, many known causative genes and many candidate genes for α-dystroglycanopathies have been found. Mutations in these candidate genes may be present in uncharacterized patients. In the future, a molecular diagnosis of each patient will be necessary to gain an improved understanding of the pathology of α-dystroglycanopathy. This information will improve our understanding of the biosynthetic pathways and the regulatory mechanisms of protein *O*-mannosylation.

Perspectives

O-mannosyl glycan glycobiology has greatly progressed because of the combined effects of improvements in glycan analysis technology and gene sequencing technology. Glycan analysis has revealed various new *O*-mannosyl glycan structures, whereas gene sequencing has identified many new causative genes for uncharacterized α-dystroglycanopathies. Biochemical research has significantly contributed to an improved understanding of α-dystroglycanopathies. Finally, because the hypoglycosylation of α-DG is a common feature in α-dystroglycanopathies, enhanced α-DG glycosylation may be a novel strategy for curing these diseases in the future.

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Conflict of Interest

None declared.

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