Dystrophin-glycoprotein complex: Post-translational processing and dystroglycan function

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The dystrophin-glycoprotein complex (DGC) is a multimeric transmembrane protein complex first isolated from skeletal muscle membranes (1). The central protein of the DGC is dystroglycan (Figure 1A). In addition to skeletal muscle, dystroglycan is strongly expressed in heart, smooth muscle, as well as many non-muscle tissues including brain and peripheral nerve. In vertebrates, dystroglycan is generated from a single gene (DAG1), which is cleaved into a peripheral α-dystroglycan protein and a transmembrane β-dystroglycan protein (2). At the sarcolemma in muscle, β-dystroglycan binds intracellularly to dystrophin (which binds the actin cytoskeleton) and extracellularly to α-dystroglycan. α-dystroglycan completes the link from the cytoskeleton to the basal lamina by calcium-dependent binding with high affinity to extracellular matrix proteins (Figure 1B) containing LamG domains, such as laminin (3), neurexin (4), agrin (5-8), and perlecan (9). In addition to dystroglycan and dystrophin, the DGC in muscle cells contains a sarcoglycan complex composed of four sarcoglycan proteins (α, β, γ, δ) and sarcospan (1,10). Intracellularly, the sarcolemma DGC, through dystrophin, interacts with a pair of syntrophins (α1 and β1) (11), and α-dystrobrevin (12). α-syntrophin and α-dystrobrevin can interact with nNOS and localize it to the sarcolemma (13,14). Syntrophin also can interact with aquaporin 4 through a PDZ domain and stabilizes it in the sarcolemma (15). The C-terminal tail of β-dystroglycan also contains a PPXY motif that can interact with dystrophin or caveolin 3(16). The exact function of the entire DGC is not completely determined but it is thought to contribute the structural stability of the muscle cell membrane during cycles of contraction and relaxation (17). In humans, mutations in dystrophin cause Duchenne and Becker’s muscular dystrophy, mutations in sarcoglycans in skeletal muscle cause limb girdle muscular dystrophy, and mutations in α2 laminin cause congenital muscular dystrophy (18). Despite the central role of dystroglycan in the DGC, no primary mutations in dystroglycan have been identified in any
human disease. However, mutations in dystrophin do cause a secondary reduction in sarcolemma expression of dystroglycan (2). Disruption of the DAG1 gene in mice results in embryonic lethality and dystroglycan appears essential for the formation of the basement membrane (Reichert’s membrane) that separates the embryo from the maternal circulation (19,20).

Emerging genetic data have shown that mutations in proteins with homology to glycosyltransferases are linked to murine and human muscular dystrophies. Biochemical analysis of muscle biopsies has revealed a convergent role for these proteins in the glycosylation of $\alpha$-dystroglycan, a process that is required for its functional activity. The loss of dystroglycan function by incomplete glycosylation can lead to a variety of clinical symptoms including muscular dystrophy and abnormal central nervous system development and function. Here we review what is known about the biosynthetic pathway of dystroglycan required for its normal structure and function, and the new insights into dystroglycan function revealed from the study of mouse models and human patients with incomplete glycosylation induced "dystroglycanopathies." Because the only detected DGC defect in these "dystroglycanopathies" is the disruption of the dystroglycan ligand binding domain, the recent work supports the proposal that the functions of components of the DGC in the sarcolemma of differentiated skeletal muscle are largely to support the integrity and sarcolemma localization of the central extracellular matrix receptor, dystroglycan.

**Post-translational processing and structure of dystroglycan**

A N-terminal signal peptide directs insertion of dystroglycan into the endoplasmic reticulum membrane with the N-terminus in the lumen (Figure 2A). Dystroglycan is then cleaved by an unidentified protease at amino acid 653 into the $\alpha$- and $\beta$-dystroglycan subunits
The significance of this cleavage is unknown, particularly because the amino acid sequence around the cleavage site in vertebrate dystroglycan is not conserved in *C. elegans* and *Drosophila melanogaster*, and Western blotting with a C-terminal antibody suggests the dystroglycan peptide is not cleaved into two subunits in *Drosophila* (22). N-linked and full O-linked glycosylation, and plasma membrane trafficking of dystroglycan is not required for cleavage of dystroglycan suggesting it may occur in the endoplasmic reticulum (23,24). The C-terminal region of α-dystroglycan (residues 550-585) binds to the N-terminus of β-dystroglycan (residues 654-750) independently of glycosylation (25). The sarcoglycan complex also appears to be required for a strong interaction of α-dystroglycan with the DGC in skeletal muscle. Sarcoglycan null mutations, resulting in the loss of the sarcoglycan complex, result in dissociation of α-dystroglycan from muscle membranes and the DGC (10).

Dystroglycan undergoes N-linked and extensive O-linked glycosylation, which causes α-dystroglycan to migrate on SDS-PAGE at a broad band with an approximate molecular weight of 120-180 kDa depending on tissue type (156kDa in muscle, predicted molecular weight is approximately 75 kDa) (2). α-dystroglycan contains a large mucin-like domain with a number of Ser or Thr residues, which are potential sites for O-glycosylation (2). Dystroglycan also contains four potential N-linked glycosylation sites, three in α-dystroglycan, one in β-dystroglycan (2). Exhaustive treatment of dystroglycan with N-glycanases does not alter its activity as an extracellular matrix receptor suggesting N-linked sugars are not required for ligand binding (3). However, full chemical deglycosylation of dystroglycan results in the complete loss of ligand binding activity (3). Interestingly, the same chemical deglycosylation also results in the loss of reactivity of two monoclonal antibodies raised against dystroglycan, clones IIH6 and VIA41(3). IIH6 antibody inhibits laminin binding, suggesting that O-linked sugars are required
for both ligand binding and the reaction of these monoclonal antibodies with dystroglycan (3).
The O-linked glycoconjugates contain a fairly unique sugar linkage where mannose is directly
coupled to serine or threonine in the dystroglycan peptide (26,27). This O-mannosyl linkage has
only been found in a few other mammalian proteins (28). In peripheral nerve dystroglycan, this
Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man-Ser/Thr glycoconjugate has been proposed to be
required for laminin binding (26). However, millimolar concentrations of this glycoconjugate
are required to competitively inhibit laminin binding to dystroglycan (26). Sialic acid has also
been reported to competitively inhibit laminin binding to peripheral nerve dystroglycan at lower
concentrations than the above glycoconjugate (29). However, enzymatic removal of sialic acid
from skeletal muscle dystroglycan has no effect on laminin binding (30). Furthermore,
dystroglycan from brain and cardiac muscle has decreased terminal sialoglycosylation compared
to skeletal muscle dystroglycan but dystroglycan from all three tissues binds tightly to laminin
(30). Therefore, although it is well accepted that glycoconjugates are indeed important for ligand
binding, the hypothesis that specific glycoconjugates on dystroglycan are involved directly in
ligand binding is still controversial.

The extensive glycosylation of dystroglycan has made the elucidation of the structure of
the peptide and glycoconjugates, and generation of peptide antibodies technically challenging.
Rotary shadowing and electron microscopy of purified chick α-dystroglycan showed dumbbell
shaped particles with two globular domains linked by a flexible segment (31). Presumably, the
flexible linker domain corresponds to the mucin domain extended by the large number of
attached glycoconjugates. The crystal structure of the C-terminus of dystrophin with a β-
dystroglycan peptide has revealed that an EF hand domain in dystrophin stabilizes an interaction
of a WW motif (PpxY) in the C-terminal tail of β-dystroglycan with a WW domain in dystrophin.
The crystal structure of α2 laminin G-domains reveals that a coordinated calcium ion is surrounded by a basic surface that is thought to provide the site for interactions with acid glycoconjugates (such as sialic acid) on dystroglycan (33). How this structural surface on the G-domain confers a specific interaction with dystroglycan and not with other sialic acid containing glycoproteins is not clear. The complete enzymatic pathway for dystroglycan processing, the exact binding site for ligands on dystroglycan, and the direct or structural role of dystroglycan glycoconjugates in ligand binding activity is still not determined.

**Post-translational disruption of dystroglycan function in muscular dystrophy.**

Despite the fact that mutations in dystroglycan in humans have not been found, recent studies have indicated, rather surprisingly, that the dystroglycan post-translational processing pathway is a convergent target for many human muscular dystrophies (Table I). Fukuyama congenital muscular dystrophy (FCMD), muscle-eye brain-disease (MEB), and Walker Warburg Syndrome (WWS) are severe congenital muscular dystrophies with mental retardation, neuronal migration defects including cobblestone lissencephaly, and variable ocular anomalies (MEB and WWS) (34). The FCMD gene mutation was first identified as a retrotransposon insertion into the *fukutin* gene (35). The function of fukutin is unknown, but the protein has homology to the *fringe*-like family of enzymes that modify glycolipids and glycoproteins (36). More recently, the gene responsible for MEB was identified as an O-mannosyl-β1,2-N-acetylglucosaminyltransferase (POMGNT-1) (37). A homologue for fukutin was also identified in humans, termed fukutin related protein (FKRP), and is mutated in a form of congenital muscular dystrophy and a milder dystrophy, limb girdle muscular dystrophy 2I (38,39).

The identification of dystroglycan as the post-translational target responsible for the phenotype in these enzyme disorders originated largely from the study of human patient muscle
biopsies. A few preliminary reports had suggested that α-dystroglycan was missing in the muscle fiber membrane in MEB and FCMD (40,41). However, recent data convincingly show in MEB and FCMD muscle that core α-dystroglycan protein and the entire DGC are present at the sarcolemma, but α-dystroglycan is shifted in molecular weight by approximately 60 kDa (24). The apparent altered post-translational modification of α-dystroglycan removes epitopes for the monoclonal antibodies, IIH6 and V1A41, similar to the chemical deglycosylation experiments performed by Ervasti and Campbell (3). Based also on findings of mutations in POMGNT-1 in the MEB patients (24), this shift can be attributed to abnormal O-mannosyl glycosylation of α-dystroglycan. This hypoglycosylation causes α-dystroglycan to be non-functional as a receptor for its known extracellular matrix proteins, including laminin, neurexin and agrin (24). Interestingly, sarcolemma glycoproteins prepared by lectin chromatography (that also enriched both normal and mutant dystroglycan) revealed an almost complete loss of total high affinity laminin binding activity, suggesting dystroglycan is one of the major glycoprotein laminin receptors in human muscle sarcolemma (24). Because mutations in laminin α2 (42) and dystrophin (43) also cause muscular dystrophy, the muscle phenotype is likely due to a similar loss of the functional link across the sarcolemma by dystroglycan from dystrophin to laminin.

However, a major question remained whether dystroglycan was responsible for the neuronal migration phenotype in human patients. A mutation in the spontaneous mutant myodystrophy (myd) mouse was identified by positional cloning in the gene LARGE, which also encodes a putative glycosyltransferase (44). Despite over 25 years since the identification of the myd mutant mouse, no brain phenotype had been described (45). Careful examination of the brains of the myd mice revealed abnormal glycosylation of α-dystroglycan leading to the functional loss of ligand binding activity similar to myd skeletal muscle, abnormal cerebral...
cortical layering resembling human lissencephaly and defects in cerebellar granule cell migration (24). Because a number of proteins could be targeted by this enzymatic pathway, it was still unclear if the functional defect in $\alpha$-dystroglycan was sufficient to account for the brain phenotype. Using cre-LoxP gene targeting, the dystroglycan gene was deleted in the mouse brain and the abnormal neuronal migration exactly mimicked the $myd$ mouse and resembled lissencephaly seen in FCMD, MEB and WWS patients (46). The very recent identification of the human WWS gene as a putative O-mannosyl transferase (POMT-1) and the demonstrated loss of glycosylated $\alpha$-dystroglycan epitopes in WWS muscle biopsies (47), suggests MEB, WWS, FCMD and $myd$ muscle and brain phenotypes can be explained by a loss of function of $\alpha$-dystroglycan due to abnormal glycosylation.

Likely, not all the genes participating in this $\alpha$-dystroglycan processing pathway have been identified and mutations in these genes may be responsible for unexplained forms of muscular dystrophy and diseases of abnormal neuronal migration (Figure 2b). In patients with FKRP mutations, no developmental brain phenotype is apparent suggesting another enzyme(s) might compensate for FKRP activity in brain. This also raises the intriguing possibility that enzymes involved in glycosylating dystroglycan specifically in brain may be responsible for unexplained forms of human lissencephaly without muscular dystrophy. $Myd$ mice also escape the embryonic lethality seen in dystroglycan knock-out mice suggesting a potential developmentally regulated compensatory enzyme for LARGE. The POMT-1 gene is a homologue of rotated abdomen in Drosophila (48). Rotated abdomen mutants show defects in myogenesis (49), although it has not been demonstrated that this phenotype is caused by a defect in Drosophila dystroglycan. Finally, the cellular biology and enzymatic activities of fukutin, FKRP, POMT1 and LARGE have not been determined. Surprisingly, mutations in LARGE and
fukutin cause a similar molecular weight shift (~60kDa) of α-dystroglycan as MEB patients (24). This suggests that LARGE and fukutin may interact with POMGNT-1 or POMT1 by supplying precursor sugars or directly modulating their enzymatic activity (Figure 2b).

**Insights into dystroglycan function from human patients and mouse models**

The studies of α-dystroglycan post-translational processing in human patients and mouse models have revealed important new insights into the function of dystroglycan and the DGC. Although the genetic role of the DGC in muscular dystrophy is well established, the functional role of dystroglycan in skeletal muscle is still debated. Because dystroglycan is important for assembly of Reichert’s membrane in developing embryos (19,20), it was hypothesized that skeletal muscle basement membranes would be severely disrupted when dystroglycan was functionally or genetically disrupted. However, despite large disruptions of basement membrane and matrix protein isoform expression in myd mouse brains, the skeletal muscle basement membranes in myd mice are grossly normal in terms of matrix protein isoform expression and localization (24). Muscle basement membrane formation is also normal when the dystroglycan gene is specifically targeted in muscle (50). This suggests dystroglycan may not be essential as a basement membrane organizer in muscle and additional matrix receptors may partially compensate for the functional loss of dystroglycan. Integrins containing β1 isoforms are also required in concert with dystroglycan for complete embryonic basement membrane assembly (51-53), and may in part compensate for the loss dystroglycan as a basement membrane organizer in muscle (54,55).

The function of dystroglycan in muscle has also been examined by specific genetic targeting of the DAG1 gene in mice. Chimeric mice generated from targeted embryonic stem cells showed that the genetic reduction of dystroglycan expression in muscle could caused
muscular dystrophy (56). Similar to myd mice, muscle basement membrane formation was grossly normal in dystroglycan null chimeric mice (56). In addition, although dystroglycan may still play an important role as an agrin receptor in the development of the neuromuscular junction, dystroglycan expression is not required for initial formation of this sarcolemma specialization in response to agrin (57,58). More recently, the DAG1 gene was targeted by cre-LoxP technology in differentiated skeletal muscle and resulted in normal muscle basement membrane formation, but a surprisingly mild dystrophic phenotype (50). The creatine kinase promoter used to express cre-recombinase in these studies failed to target dystroglycan expressed in satellite cells, and the mice displayed a remarkable ability to continuously regenerate muscle compared to other DGC associated dystrophic mouse models (50). This suggests that dystroglycan may play an important role in satellite cell survival or function. Interestingly, muscle biopsies from human patients with a dystroglycan post-translational processing defect and mild limb girdle muscular dystrophy were also examined (most likely LGMD2I, although not determined in this study). These patients showed a similar expression pattern of residual normally processed dystroglycan from satellite cells after regeneration (50). This could be explained by an additional enzyme in satellite cells that compensates for the mutant enzyme in these LGMD patients but whose expression is turned off after muscle regeneration. This phenomenon of residual normal dystroglycan expression from regenerating satellite cells is not demonstrated in the severe muscular dystrophies of myd mice, and FCMD or MEB patients (24). Therefore, precise understanding of the dystroglycan processing pathway and its developmental regulation, may shed important light into how dystroglycan modulates satellite cell function, and how dystroglycan processing might be targeted therapeutically to increase functional dystroglycan expression, promote muscle regeneration, and improve the dystrophic phenotype.
Despite the loss of laminin binding activity of dystroglycan in myd, FCMD and MEB muscle, the remaining DGC proteins can still localize to the sarcolemma (24,50,59). Also, dystrophin and dystrophin associate proteins can still localize to the sarcolemma after the genetic deletion of the entire DG protein in muscle (50,59). In contrast, the abnormal glycosylation of α-dystroglycan in myd mouse brain causes the failure of targeting of many normally expressed DGC related proteins, including dystrophin, to neural synapses and glial end feet (24). For muscle, this suggests the DGC may be targeted to the sarcolemma either by a stronger association with the actin cytoskeleton through the 400 kDa skeletal muscle dystrophin isoform, or perhaps an additional sarcolemma protein that stabilizes dystrophin or the DGC at the sarcolemma. In brain, dystroglycan, specifically by its ability to bind ligands, has unique scaffold function in brain to recruit proteins associated with the DGC to the localized structures with glia and neurons. It is still unknown which molecules are targeted to the synapse by dystroglycan, which ligands are important, and if those molecules are directly responsible for the functional synaptic defect in brain specific dystroglycan knockout mice (46). It is tempting to speculate that the failure of recruiting functional molecules to the synapse by dystroglycan and dystrophin, may in part underlie the cognitive impairment in FCMD, MEB, WWS and a subset of dystrophin associated muscular dystrophies (34,60).

The finding that the DGC proteins can still localize to the sarcolemma in dystroglycan glycosylation deficient muscular dystrophies (24,50,59), also sheds new light on how the entire DGC may function. Several studies in mouse models of DGC associated muscular dystrophies have attempted to assign a signaling molecule scaffold function to the DGC or direct roles of functional molecules associated with the DGC (such as aquaporin 4 and nNOS) in the pathogenesis of muscular dystrophy [reviewed in (61,62)]. However, in the abnormal
glycosylation dystroglycanopathies, dystroglycan, the DGC and associated proteins are correctly targeted to the sarcolemma. Only the extracellular ligand binding domain of α-dystroglycan is disrupted and muscular dystrophy still develops. Therefore, the functional roles of the DGC proteins, such as the sarcoglycans and to some extent dystrophin, in relationship to dystroglycan, may be more similar to the relationship of structural accessory proteins to the pore forming subunits of ion channels. Sarcoglycans may function primarily to stabilize the dystroglycan alpha and beta subunit interactions (10), and dystrophin provides cytoskeleton interactions to stabilize and target dystroglycan to the sarcolemma. Dystroglycan, as the central component, contributes the important function as a ligand receptor and adhesive protein that helps stabilize the sarcolemma relative to the extracellular matrix. The lack of a critical signaling role of the DGC associated proteins would be consistent with a lack of muscular dystrophy phenotype in aquaporin 4 null, nNOS null mice, and syntrophin null mutations that dissociate nNOS from the sarcolemma (13,63,64). The function of α-dystrobrevin as a cytoplasmic protein of the DGC in this hypothesis is less certain, since dystrobrevin null mice have normal DGC and dystroglycan localization to the sarcolemma but still have a very mild myopathy (14). However, because dystrobrevin isoforms can interact with dystrophin (12) and sarcoglycans (65), it remains to be tested whether or not dystrobrevin may indirectly destabilize the α-dystroglycan association with the DGC resulting in a partial phenotype (i.e. by altering sarcoglycan function) without modulating the sarcolemma DGC localization.

Finally, the function of dystroglycan outside of muscle is also becoming more clear. In myd and dystroglycan knockout mice brains, a complete loss of high affinity laminin binding receptor activity in glycoprotein preparations and focal disruptions of the brain basement membrane, termed the glia limitans, are observed (24,46). The glia limitans normally provides
an important anchoring site for endfeet of glial cells that provide tracts for migrating neurons during development (66). A similar defect in cortical layering and disruption of the glia limitans can be seen by genetic ablation in brain of B1 integrin or the nidogen binding domain on laminin γ1 (67,68), suggesting a general disruption of glial cell-extracellular matrix interactions may cause glia limitans disruptions, disruption of radial glia tracts and abnormal neuronal migration. The reports so far do not preclude a role for neuronal expressed dystroglycan in the neuronal migration phenotype because dystroglycan is functionally disrupted in both neurons and glia in myd and dystroglycan knockout mice (24,46). In fact, neuronal migration defects in reelin knockout mice (69), inhibition of integrin dependent neuronal adhesion by reelin (70), and evidence that reelin serves as a detachment signal for neuronal migration (71), suggest that alterations in neuronal cell adhesion by dystroglycan could also play an important role in cortical neuronal migration. Finally, prenatal lymphocytic choriomeningitis virus exposure can produce CNS and retinal abnormalities similar to MEB and WWS. The findings that pathogenic organisms such as LCMV use dystroglycan as a receptor and compete for ligand binding (72,73), suggests dystroglycan may also play a role in acquired forms of neurological disease.

**Perspective**

In summary, the work over the last ten years on the biochemistry of dystroglycan and its interaction with ligands, and the development of specific antibody reagents, allowed for the identification of the mechanism causing several inherited human muscular dystrophies. In turn, the genetic data on human patients and mutant mice is identifying the important players in the basic biology of the O-mannosylation pathway that is required for dystroglycan function. With the combination of appropriate genetic modeling in mice, the full circle is being completed to fully understand the enzymatic processing and function of dystroglycan in muscle and non-
muscle tissues. Hopefully, through this work, appropriate therapeutic targets might be revealed to restore normal dystroglycan processing and/or function to prevent the development of dystroglycan-associated muscular dystrophy.

Acknowledgements

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References


TABLE 1. Mutations in post-translational processing proteins in muscular dystrophy. The glycosylated epitope and core dystroglycan data is from immunohistochemistry on muscle biopsies. The MW shift of dystroglycan indicated is from Western blots of muscle biopsy glycoproteins. * In myd mouse, glycosylated dystroglycan epitopes are also missing in brain, heart, and retina. #The mutant core protein in myd brain migrates at a MW similar to the mutant core protein in myd muscle (~30 kDa shift). References are indicated parenthetically.

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FIGURE 1. The muscle dystrophin-glycoprotein complex. A) The components of the core dystrophin-glycoprotein complex that have been demonstrated to copurify in a large MW complex from digitonin solubilized skeletal muscle membranes (1). B) The ligands and associated proteins that can interact with the dystrophin-glycoprotein complex. Laminin 2 is the predominant laminin isoform in skeletal muscle basement membranes.

FIGURE 2. The post-translational processing of dystroglycan (DG). A) The domain structure of dystroglycan. SP indicates the signal peptide directing insertion into the endoplasmic reticulum. The propetide is cleaved into two subunits by an unknown protease (star) with β-dystroglycan containing the transmembrane domain (TM). The mucin-like domain contains a large number of Ser/Thr residues for O-linked glycosylation. Consensus N-linked glycosylation sites are indicated by arrows. The deglycosylated molecular weight of dystroglycan is from experiments using enzymatic and/or complete chemical deglycosylation (1,3). B) The O-mannosylation of α-dystroglycan is a target for muscular dystrophies in mice and man. Only POMGNT-1 has been experimentally demonstrated to have glycosyltransferase activity (37,74), although all mutated proteins indicated have demonstrated homology to glycosyltransferases. The activity of POMT1 is inferred by its homology to known mannosyltransferases. The function of fukutin and LARGE are unknown but similar molecular weight shifts of α-dystroglycan in myd mice and FCMD and MEB patients suggests both may interact with early steps in the O-mannosylation pathway (dotted arrows), perhaps by supplying donor sugars to POMGNT1 or POMT1.
Michele and Campbell, Figure 1
Michele and Campbell, Figure 2
TABLE 1. Mutations in post-translational processing proteins in muscular dystrophy. The glycosylated epitope and core dystroglycan data is from immunohistochemistry on muscle biopsies. The MW shift of dystroglycan indicated is from Western blots of muscle biopsy glycoproteins. * In myd mouse, glycosylated dystroglycan epitopes are also missing in brain, heart, and retina. #The mutant core protein in myd brain migrates at a MW similar to the mutant core protein in myd muscle (~30 kDa shift). References are indicated parenthetically.

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