Dystroglycan: Extracellular Matrix Receptor that Links to Cytoskeleton

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Abstract

Dystroglycan is a highly glycosylated extracellular matrix receptor that is linked to the cytoskeleton and expressed in many tissues. It plays a crucial role in a variety of biological processes, including maintenance of the skeletal muscle membrane integrity and development of the central nervous system. Dystroglycanopathies are muscular dystrophies in which aberrant posttranslational modification results in a reduction in the number of [xylose-glucuronic acid] repeats on the α-dystroglycan subunit. This repeat is essential for binding between α-dystroglycan and its laminin-G domain-containing ligands within the extracellular matrix (i.e., laminin, perlecan, and agrin). Recent genetic and biochemical data have shown that mutations in at least 17 genes that encode enzymes

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implicated in posttranslational processing (including known and putative glycosyltransferases and a novel mannose kinase) lead to such reductions and cause congenital or limb-girdle muscular dystrophies that are sometimes accompanied by brain and eye abnormalities. In this chapter, we review the history of dystroglycan research, as well as our current understanding of the molecular underpinnings of dystroglycan function, in particular its posttranslational modifications.

Keywords
Dystroglycan • Extracellular matrix • LARGE • Muscular dystrophy • Posttranslational modification • Protein *O*-mannose kinase (SGK196)

The History of Dystroglycan Research

Dystroglycan was originally identified as a constituent of the dystrophin-glycoprotein complex (DGC). Several co-migrating proteins were identified by analysis of rabbit dystrophin purified from skeletal muscle using wheat germ agglutinin (WGA)-Sepharose and diethylaminoethanol (DEAE)-Sepharose followed by sucrose-gradient centrifugation (Ervasti et al. 1990; Ervasti and Campbell, 1991). Subsequent studies revealed that, among the co-fractioned proteins, a 156 kDa and a 43 kDa protein were derived from the same gene, *DAG1* (Ibraghimov-Beskrovnaya et al. 1992). These proteins are now known to be an extracellular subunit (α-dystroglycan) and a transmembrane-spanning intracellular subunit (β-dystroglycan), respectively.

α-Dystroglycan binds to several components of the extracellular matrix, including laminin. In contrast, β-dystroglycan binds to the intracellular protein dystrophin, thereby providing a link between the extracellular matrix and the cytoskeleton (Fig. 1). Biochemical analysis using a deglycosylation reagent (trifluoromethanesulfonic acid) impaired dystroglycan's laminin-binding activity (Ervasti and Campbell 1993), suggesting that the glycosylation of dystroglycan was critical for its function as a laminin receptor. Given that deletions in the dystrophin gene cause Duchenne and Becker muscular dystrophies (Hoffman et al. 1987), the dysfunction of other DGC components was proposed to also cause muscular dystrophy. Indeed, dystroglycan hypoglycosylation occurs in various muscular dystrophies, including Walker-Warburg syndrome (WWS), muscle-eye-brain disease (MEB), Fukuyama congenital muscular dystrophy (FCMD), and some limb-girdle muscular dystrophies. Together, these diseases are designated as "dystroglycanopathies." The genes currently known to cause these disorders are listed in Table 1.

The biological importance of dystroglycan function was revealed by gene knockout studies in mice. Since systemic deletion of the *Dag1* gene results in embryonic lethality, due to the disruption of Reichert's membrane, conditional knockout mice were generated using Cre-recombinase. Dystroglycan deficiency in mature skeletal myofibers (with Cre-recombinase driven by the muscle creatine-kinase promoter) was found to cause a remarkably mild form of muscular dystrophy

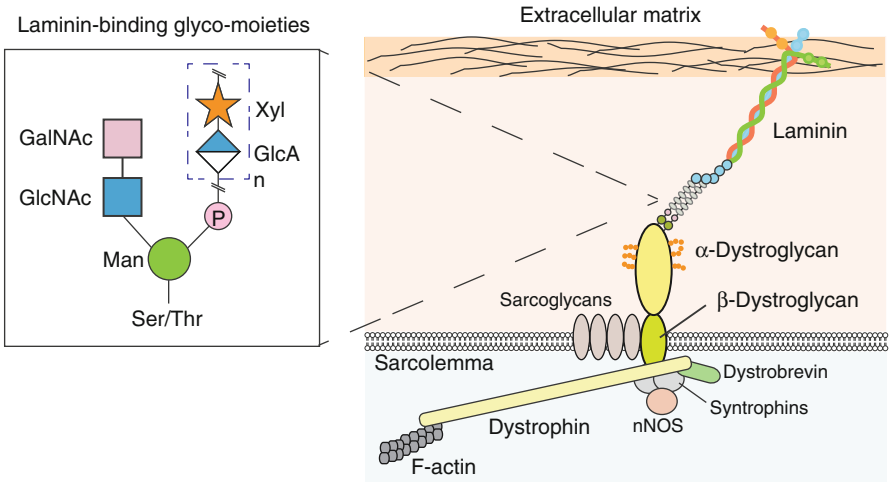

Fig. 1 Schematic illustration of the dystrophin-glycoprotein complex and recently identified glycostructures that are essential for α-dystroglycan's ability to bind laminin α2. Dystroglycan maintains the stability of the plasma membrane (sarcolemma) by linking the cytoskeleton to the extracellular matrix. Its function as a laminin receptor depends on posttranslational modifications that are carried out by a series of enzymes (as shown in inset): (i) attachment of oligosaccharides (designated as M3 glycans; Yoshida-Moriguchi et al. 2013) to *O*-mannosyl glycans, by GTDC2 (POMGNT2) and B3GALNT2; (ii) phosphorylation of *O*-mannosyl glycans bearing the M3 glycans, by SGK196 (POMK); and (iii) post-phosphoryl modification with polymers composed of xylose (Xyl) and glucuronic acid (GlcA), by LARGE

(Cohn et al. 2002). Presumably, this was because dystroglycan expression was maintained in satellite cells, which have the capacity to differentiate into myofibers in response to muscle-fiber damage. These data suggest that dystroglycan functions normally in satellite cells even in the absence of function in the surrounding mature muscle. This preserved expression is sufficient to prevent muscular dystrophy from becoming severe.

Tissue-specific *Dag1* knockout strategies were found to phenocopy brain abnormalities characteristic of patients with WWS, MEB, and FCMD. In this case, *Dag1* knockout was carried out in glial fibrillary acid protein (GFAP)-cre mice to elucidate the dystroglycan function in the brain, and the abnormalities observed were a variety of disorganized neuronal migration patterns, likely due to interruption of glia limitans integrity. Moreover, electrophysiological analyses of GFAP-cre *Dag1* null mice demonstrated the blunting of long-term potentiation, probably due to a defect in the postsynaptic region (Moore et al. 2002). These data further emphasize the role of dystroglycan in maintaining elements of brain structure that are crucial to normal function.

Notwithstanding that the hypoglycosylation of α-dystroglycan was established as a hallmark of the dystroglycanopathies some time ago, it long remained unclear as to whether this defect is sufficient to cause muscular dystrophy. Theoretically, similar defects in the posttranslational modification of other substrates could

Table 1 Genes involved in pathogenesis of dystroglycanopathies

Gene name	OMIM	Functions of gene products
DAG1	128239	Dystroglycan gene
FKTN	607440	Unknown
POMGNT1	606822	*N*-Acetylglucosaminyl transfer
FKRP	606596	Unknown
POMT1	607423	Protein *O*-mannosyl transfer, together with POMT2
LARGE	603590	Xylosyl and glucuronyl transfer
POMT2	607439	Protein *O*-mannosyl transfer, together with POMT1
DPM3	605951	Dolichol phosphate mannose biosynthesis
DOLK	610746	Dolichol phosphate biosynthesis
ISPD	614631	Unknown
DPM2	603564	Dolichol phosphate mannose biosynthesis
GTDC2 (POMGNT2)	614828	*N*-Acetylglucosaminyl transfer, positively regulates the SGK196-mediated kinase reaction
TMEM5	605862	Unknown
B3GALNT2	610194	*N*-Acetylgalactosaminyl transfer, positively regulates the SGK196-mediated kinase reaction
B3GNT1	605517	*N*-Acetylglucosaminyl transfer
SGK196 (POMK)	615247	*O*-Mannosyl glycan phosphorylation
GMPPB	615320	GDP-Mannose biosynthesis
DPM1	603503	Dolichol phosphate mannose biosynthesis

contribute to disease pathogenesis, a hypothesis that seemed to be supported by the fact that no patient had been identified with mutations in the *DAG1* gene. In 2011, however, a dystroglycan missense mutation (T192M) was discovered in a patient with limb-girdle muscular dystrophy and cognitive impairment (Hara et al. 2011). Further analysis of the effects of this mutation using a knock-in mouse model showed that α-dystroglycan was indeed hypoglycosylated in this context and that a consequent loss of laminin binding led to muscular dystrophy. These data provide direct genetic evidence that dystroglycan is the main – and most likely only – molecule that bears *O*-linked glycans whose loss causes muscular dystrophy accompanied by brain abnormalities.

Molecular Basis of α-Dystroglycan Posttranslational Modification

A previous study identified a rare tetrasaccharide structure (Neu5Ac-α2,3-Gal-β 1,4-GlcNAc-β1,2-Man) on α-dystroglycan, and it was proposed to be essential for dystroglycan's ability to bind ligand (see other chapters). This hypothesis was supported by the fact that the products of both *POMT1* and *POMT2*, mutations in which cause WWS, act as *O*-mannosyltransferases (Manya et al. 2004). However, it is unlikely that the entire tetrasaccharide is directly involved in ligand binding because treatment with various glycan-specific deglycosylation enzymes did not

reduce α-dystroglycan's ligand-binding activity (Combs and Ervasti 2005). Among the causative genes for dystroglycanopathies listed in Table 1, like-acetylglucosaminyltransferase (LARGE) was thought to play a fundamental role in attaching laminin-binding glycans to α-dystroglycan. In *Large*^{myd} mice, which harbor a deletion in the mouse *Large* gene, functional modification of α-dystroglycan is impaired and both severe muscular dystrophy and brain abnormalities are present (Grewal et al. 2001; Michele et al. 2002). Moreover, in muscle cells obtained from a patient with WWS, LARGE overexpression considerably increased the extent of α-dystroglycan functional glycosylation (Barresi et al. 2004). These observations indicate that LARGE plays a central role in attaching laminin-binding moieties to α-dystroglycan.

Recently, great progress has been made in identifying the glyco-moieties that govern dystroglycan function. Notably, treatment with cold aqueous hydrofluoric acid, which cleaves a phospho-ester linkage, was shown to abrogate the laminin-binding activity of α-dystroglycan, suggesting that phosphorylation is involved in the posttranslational modification of α-dystroglycan (Yoshida-Moriguchi et al. 2010). Indeed, mass spectrometry and NMR analyses revealed a phosphate residue at the C6 position of *O*-mannosyl glycans on α-dystroglycan. Subsequent biochemical analysis using immobilized metal affinity chromatography (IMAC) beads (which capture monoester-linked but not diester-linked phosphate residues) revealed that α-dystroglycan expressed in *Large*^{myd} mice and in a subject with FCMD was recognized by IMAC beads, whereas wild-type samples were not. These results indicate that "post-phosphoryl" modification is impaired in severe forms of α-dystroglycanopathies.

Then, what is the enzymatic activity of LARGE? Important insights to answer this question came from gas chromatography-mass spectrometry analysis of α-dystroglycan, which detected substantial amounts of xylose (Xyl) and glucuronic acid (GlcA), as well as known glycosyl moieties. The importance of Xyl residues with respect to α-dystroglycan modification was confirmed using a CHO cell line that is defective for xyloside synthesis. In light of the finding that α-dystroglycan contains substantial amounts of two glycans, the presence of two distinct glycosyltransferase-like motifs in LARGE was intriguing and led to the hypothesis that LARGE might be involved in the attachment of both sugars. This possibility was tested by enzymatic analyses, using a recombinant form of LARGE lacking its transmembrane domain. Indeed, LARGE was found to transfer repeating disaccharide composed of Xyl and GlcA to α-dystroglycan. Moreover, a mutation in either catalytic site abrogated the ability of LARGE to transfer Xyl and GlcA, respectively (Inamori et al. 2012). Given that the positive charges of laminin's globular domain are likely important for its interaction with α-dystroglycan, polymerization of the negatively charged Xyl-GlcA unit may contribute to dystroglycan ligand binding.

The enzyme responsible for the phosphorylation of *O*-mannosyl glycans has also been identified (Yoshida-Moriguchi et al. 2013). This discovery was facilitated by technological advances that made it possible to identify new causative genes for dystroglycanopathies, including *GTDC2*, *B3GALNT2*, and *SGK196* (Manzini et al. 2012; Stevens et al. 2013; Jae et al. 2013). Among these, GTDC2 and

B3GALNT2 were found to act as a β1,4-*N*-acetylglucosaminyltransferase and a β1,3-*N*-acetylgalactosaminyltransferase, respectively. Further analysis revealed that these enzymes attach the disaccharide (GalNAc-β3-GlcNAc) to *O*-mannosyl glycans on α-dystroglycan (Fig. 1). The implication of *SGK196* in the etiology of dystroglycanopathies was interesting because although the encoded protein has a motif that shares a weak homology with known kinases, it had been classified as an inactive protein kinase. The discovery that SGK196 is capable of phosphorylating *O*-mannosyl groups was thus surprising. However, this analysis also revealed that this enzyme acts as a protein *O*-mannose kinase (POMK) only under special circumstances: the *O*-mannosyl substrate must first be modified by both GTDC2 and B3GALNT2. Thus, the phosphorylation of α-dystroglycan is strictly controlled by surrounding glycans that do not directly contribute to recognition of the extracellular matrix-localized ligand.

Future Perspectives

Since the discovery of dystroglycan, many researchers have worked toward elucidating the molecular mechanisms that underlie the posttranslational modification of this vital protein. These efforts, facilitated by the emergence of new methodologies, have revealed that the enzymatic activities of LARGE are essential for dystroglycan's ligand-binding activity and revealed some of the key glyco-moieties involved in dytroglycan's laminin-binding activity. Nevertheless, important details of the molecular mechanisms underlying the dystroglycanopathies remain to be elucidated. For example, the roles of several dystroglycanopathy-associated enzymes (Fukutin, FKRP, ISPD, and TMEM5) in the functional glycosylation of α-dystroglycan remain undetermined. Further studies are expected not only to shed light on these molecular mechanisms, but also to facilitate the development of therapeutic strategies for the treatment of patients with dystroglycanopathies.

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References

Barresi R, Michele DE, Kanagawa M, Harper HA, Dovico SA, Satz JS, Moore SA, Dumanski JP, Schachter H, Cohn RD, Nishino I, Campbell KP (2004) LARGE can functionally bypass α-dystroglycan glycosylation defects in distinct congenital muscular dystrophies. Nat Med 10:696–703

Cohn RD, Henry MD, Michele DE, Barresi R, Saito F, Moore SA, Flanagan JD, Skwarchuk MW, Robbins ME, Mendell JR, Williamson R, Campbell KP (2002) Disruption of *Dag1* in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. Cell 110:639–648

Combs AC, Ervasti JM (2005) Enhanced laminin binding by α-dystroglycan after enzymatic deglycosylation. Biochem J 390:303–309

Ervasti JM, Campbell KP (1991) Membrane organization of the dystrophin-glycoprotein complex. Cell 66:1121–1131

Ervasti JM, Campbell KP (1993) A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol 122:809–823

Ervasti JM, Ohlendieck K, Kahl SD, Gaver MG, Campbell KP (1990) Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. Nature 345:315–319

Grewal PK, Holzfeind PJ, Bittner RE, Hewitt JE (2001) Mutant glycosyltransferase and altered glycosylation of α-dystroglycan in the myodystrophy mouse. Nat Genet 28:151–154

Hara Y, Balci B, Kanagawa M, Beltrán-Valero de Bernabé D, Gundesli H, Yoshida-Moriguchi T, Willer T, Satz JS, Burden SJ, Oldstone MBA, Accardi A, Talim B, Muntoni F, Topaloglum H, Dincer P, Campbell KP (2011) A dystroglycan mutation associated with limb-girdle muscular dystrophy. N Eng J Med 364:939–946

Hoffman EP, Brown RH Jr, Kunkel LM (1987) Dystrophin: the protein product of the Duchenne muscular dystrophy locus. Cell 51:919–928

Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP (1992) Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 355:696–702

Inamori K, Yoshida-Moriguchi T, Hara Y, Anderson ME, Yu L, Campbell KP (2012) Dystroglycan function requires xylosyl- and glucuronyltransferase activities of LARGE. Science 335:93–96

Jae LT, Raaben M, Riemersma M, van Beusekom E, Blomen VA, Velds A, Kerkhoven RM, Carette JE, Topaloglu H, Meinecke P, Wessels MW, Lefeber DJ, Whelan SP, van Bokhoven H, Brummelkamp TR (2013) Deciphering the glycosylome of dystroglycanopathies using haploid screens for lassa virus entry. Science 340:479–483

Manya H, Chiba A, Yoshida A, Wang X, Chiba Y, Jigami Y, Margolis RU, Endo T (2004) Demonstration of mammalian protein *O*-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. Proc Natl Acad Sci U S A 101:500–505

Manzini MC, Tambunan DE, Hill RS, Yu TW, Maynard TM, Heinzen EL, Shianna KV, Stevens CR, Partlow JN, Barry BJ, Rodriguez J, Gupta VA, Al-Qudah AK, Eyaid WM, Friedman JM, Salih MA, Clark R, Moroni I, Mora M, Beggs AH, Gabriel SB, Walsh CA (2012) Exome sequencing and functional validation in zebrafish identify *GTDC2* mutations as a cause of Walker-Warburg syndrome. Am J Hum Genet 91:541–547

Michele DE, Barresi R, Kanagawa M, Saito F, Cohn RD, Satz JS, Dollar H, Nishino I, Kelley RI, Somer H, Straub V, Mathews KD, Moore SA, Campbell KP (2002) Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. Nature 418:417–422

Moore SA, Saito F, Chen J, Michele DE, Henry M, Messing A, Cohn RD, Barta SR, Westra S, Williamson R, Hoshi T, Campbell KP (2002) Deletion of brain dystroglycan recapitulates aspects of congenital muscular dystrophy. Nature 418:422–425

Stevens E, Carss KJ, Cirak S, Foley AR, Torelli S, Willer T, Tambunan DE, Yau S, Brodd L, Sewry CA, Feng L, Haliloglu G, Orhan D, Dobyns WB, Enns GM, Manning M, Krause A, Salih MA, Walsh CA, Hurles M, Campbell KP, Manzini MC (2013) UK10K consortium, stemple D, Lin YY, muntoni F. Mutations in *B3GALNT2* cause congenital muscular dystrophy and hypoglycosylation of α-dystroglycan. Am J Hum Genet 92:354–365

Yoshida-Moriguchi T, Yu L, Stalnaker SH, Davis S, Kunz S, Madson M, Oldstone MBA, Schachter H, Wells L, Campbell KP (2010) *O*-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding. Science 327:88–92

Yoshida-Moriguchi T, Willer T, Anderson ME, Venzke D, Whyte T, Muntoni F, Lee H, Nelson SF, Yu L, Campbell KP (2013) SGK196 is a glycosylation-specific *O*-mannose kinase required for dystroglycan function. Science 341:896–899