Dystroglycan: from biosynthesis to pathogenesis of human disease

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Summary

α- and β-dystroglycan constitute a membrane-spanning complex that connects the extracellular matrix to the cytoskeleton. Although a structural role for dystroglycan had been identified, biochemical and genetic discoveries have recently highlighted the significance of post-translational processing for dystroglycan function. Glycosylation is the crucial modification that modulates the function of dystroglycan as a receptor for extracellular binding partners. It has become clear that perturbation of dystroglycan glycosylation is the central event in the pathogenesis of several complex disorders, and recent advances suggest that glycosylation could be modulated to ameliorate the pathological features. Our increased understanding of the mechanisms of interaction of dystroglycan with its ligands has become an essential tool in deciphering the biological processes related to the human diseases in which the proteins are implicated.

Key words: Dystroglycan, Extracellular matrix, Glycosylation

Introduction

Dystroglycan was originally isolated from skeletal muscle as an integral membrane component of the dystrophin-glycoprotein complex (DGC), a multimeric transmembrane protein complex first isolated from skeletal muscle membranes (Ervasti and Campbell, 1991; Ibraghimov-Beskrovnaya et al., 1992). The exact function of the entire DGC is not completely determined but evidence indicates that it confers structural stability to the sarcolemma during contraction (Petrof et al., 1993). In fact, mutations in components of this complex render muscle fibers more susceptible to damage and lead to various types of muscle disorder such as Duchenne muscular dystrophy and limb-girdle muscular dystrophies (Cohn and Campbell, 2000; Straub and Campbell, 1997). Within the DGC, dystroglycan spans the sarcolemma and interacts directly with subsarcolemmal proteins and components of the extracellular matrix (ECM), providing a physical link between the subsarcolemmal cytoskeleton and the basement membrane. Dystroglycan is also expressed in many other cell types and it plays important roles outside skeletal muscle (Durbeej et al., 1998; Ibraghimov-Beskrovnaya et al., 1992). It has been implicated in early mouse development (Williamson et al., 1997), structure and function of the central nervous system (Moore et al., 2002), myelination and nodal architecture of peripheral nerves (Saito et al., 2003), epithelial morphogenesis (Durbeej and Ekblom, 1997; Durbeej et al., 2001), cell adhesion (Matsumura et al., 1997), synaptogenesis (Jacobson et al., 1998; Montanaro et al., 1998) and signaling (Langenbach and Rando, 2002; Spence et al., 2004).

Although no mutations in the dystroglycan gene have been identified in any human disorder, recent advances have highlighted the importance of post-translational processing for the interaction of dystroglycan with its ligands and have provided insights into its involvement in the pathogenesis of complex diseases. α-Dystroglycan is also the cellular receptor for arenaviruses and *Mycobacterium leprae*, whose entry could be affected by its processing (Cao et al., 1998; Rambukkana et al., 1998). In addition, perturbations of dystroglycan processing are associated with severe congenital disorders and cancer progression (Muntoni et al., 2004; Singh et al., 2004). Here, we review current understanding of dystroglycan modifications and discuss new aspects of dystroglycan function revealed by the analysis of humans and mice that have defects in dystroglycan glycosylation.

Dystroglycan structure

Dystroglycan genes from several species have been cloned, including human (Ibraghimov-Beskrovnaya et al., 1993), rabbit (Ibraghimov-Beskrovnaya et al., 1992), mouse (Gorecki et al., 1994), *Torpedo* (Bowe et al., 1994), *Drosophila* (Deng et al., 2003), zebrafish (Parsons et al., 2002) and *Caenorhabditis elegans* (Grisoni et al., 2002). The corresponding amino acid sequence is highly conserved in vertebrates. The gene *DAG1* has been mapped to human chromosome 3p21 and mouse chromosome 9 (Gorecki et al., 1994; Ibraghimov-Beskrovnaya et al., 1993). The coding sequence is organized into two exons, separated by a large intron. The derived 5.8 kb transcript contains an 895-residue open reading frame and can be detected on northern blots in a variety of fetal and adult tissues, most abundantly in skeletal muscle and heart (Ibraghimov-Beskrovnaya et al., 1993).

Dystroglycan consists of two subunits (α and β), which are translated from a single mRNA as a propeptide that is proteolytically cleaved into two noncovalently associated proteins (Holt et al., 2000; Ibraghimov-Beskrovnaya et al., 1992) (Fig. 1). The first 29 amino acids of the translated

propeptide are predominantly hydrophobic and represent a signal peptide. Post-translational processing through cleavage at Ser654 yields the two mature proteins α-dystroglycan and β-dystroglycan. The significance of this cleavage is unknown and the amino acid sequence around the cleavage site is not conserved between vertebrates and invertebrates. However, overexpression of a propeptide mutated at the cleavage site (DG_{S654A}) inhibits this post-translational processing and leads to dystrophic changes in a transgenic mouse model (Jayasinha et al., 2003). This suggests that dystroglycan cleavage is essential for normal muscle function.

The 43 kDa β-dystroglycan protein contains a single transmembrane domain (amino acids 751-774 of the precursor), one potential N-linked glycosylation site, and a 121-residue C-terminal cytoplasmic tail that is enriched in proline. α-Dystroglycan is an extracellular protein that contains three potential N-linked glycosylation sites. The mature protein has a central, highly *O*-glycosylated, mucin domain that connects the globular N- and C-terminal domains (Brancaccio et al., 1995; Brancaccio et al., 1997). The N-terminal domain appears to be commonly processed by convertase-like activity between Arg312 and Gln313. However, it is not clear whether this occurs extracellularly or during the trafficking of dystroglycan to the plasma membrane (Kanagawa et al., 2004). Although the predicted molecular mass of the processed core peptide is ~40 kDa, the size of α-dystroglycan detected by immunoblot ranges widely from 120 kDa (in brain) to 156 kDa (in skeletal muscle) (Ibraghimov-Beskrovnaya et al., 1992; Ibraghimov-Beskrovnaya et al., 1993; Leschziner et al., 2000; Yamada et al., 1994). Because the removal of N-linked glycans alters the molecular weight of α-dystroglycan by only 4 kDa (Ervasti and Campbell, 1991), the variability in molecular mass is probably a result of tissue-, developmental- and species-specific *O*-glycosylation within the mucin domain (Table 1).

Extensive treatment of α-dystroglycan with *N*-glycanases does not have any impact on its activity as an ECM receptor, indicating that N-linked sugars are not required for ligand binding (Ervasti and Campbell, 1993). By contrast, full chemical deglycosylation of α-dystroglycan results in the complete loss of ligand-binding activity (Ervasti and Campbell, 1993). Thus, the sugar chains on the mucin-like domain are believed to mediate these interactions. Antibody- and lectin-binding studies indicate several glycostructures might decorate this region, and the susceptibility of α-dystroglycan to *O*-sialoglycopeptidase supports the hypothesis that α-dystroglycan is a sialylated mucin-type glycoprotein (Ervasti et al., 1997; Smalheiser and Kim, 1995).

Structural analysis of the sialylated O-linked oligosaccharides of bovine peripheral nerve and rabbit skeletal muscle dystroglycan revealed a high abundance of the uncommon *O*-mannosyl oligosaccharide Neu5Ac(α2-3)Gal(β1-4)GlcNAc(β1-2)Man-Ser/Thr (Chiba et al., 1997; Sasaki et al., 1998). Binding inhibition studies suggest that this tetrasaccharide is involved in the interaction between dystroglycan and extracellular ligands such as laminin. However, recent studies show that digestion of α-dystroglycan with a combination of enzymes that degrade this tetrasaccharide enhances the binding to laminin 1, indicating that other sugar moieties might contribute to the ligand-binding activities of α-dystroglycan (Combs and Ervasti, 2005). In addition, structural and functional analyses have recently shown that not only the first half of the mucin-like domain but also the N-terminal domain of the protein is essential for the synthesis of functional α-dystroglycan as a laminin receptor. In fact, although the N-terminal domain is cleaved in the mature protein, there is evidence that a putative glycosyltransferase, LARGE (for 'like-acetylglucosaminyltransferase'), uses this domain to recognize dystroglycan as a substrate (see below) (Fujimura et al., 2005; Kanagawa et al., 2004).

Table 1. Apparent molecular mass of α-dystroglycan*

Tissue/organ	α-DG (kDa)	Glyco-modification (kDa)
Skeletal muscle	156	116
Heart	140	100
Smooth muscle	120	80
Nerve	120	80
Brain	120	80
Kidney	100-156	60-116
Epithelia	156	116

*The size of α-dystroglycan (α-DG) varies in different tissues owing to specific O-linked glycosylation. Numbers in the third column represent the contribution of O-linked glycosylation on the ~40 kDa protein backbone to the total molecular mass.

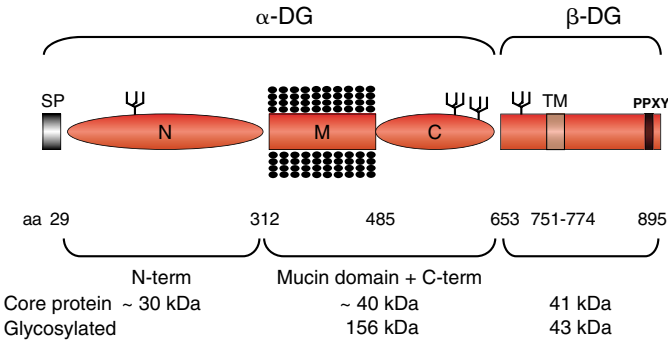

Fig. 1. Dystroglycan domain organization. In the mature α-dystroglycan (α-DG), the N-terminal domain is cleaved by convertase-like activity between Arg312 and Gln313. Circles indicate O-linked sugar chains. Branches indicate N-linked sugar chains. SP, signal peptide; TM, transmembrane domain; PPXY, dystrophin-binding site. Molecular masses at the bottom refer to the protein mass in the absence (core) and presence of post-translational glycosylation.

Dystroglycan interactions

Not only are α- and β-dystroglycan tightly connected with each other, but they also associate with numerous other proteins (Fig. 2). In skeletal muscle, dystroglycan is an integral part of the DGC. The core skeletal muscle DGC also contains dystrophin, the sarcoglycans [α-SG, β-SG, γ-SG and δ-SG (Ervasti and Campbell, 1991)], sarcospan (Crosbie et al., 1997) and the syntrophins (Yang et al., 1994). In addition, several extra- and intracellular proteins are less tightly associated with the DGC, such as nitric oxide synthase [nNOS (Brenman et al., 1995)], dystrobrevin (Grady et al., 1999; Yoshida et al., 2000) and laminin 2 (Henry and Campbell, 1996). In conjunction with a possible role in cell signaling (Grady et al., 1997), the

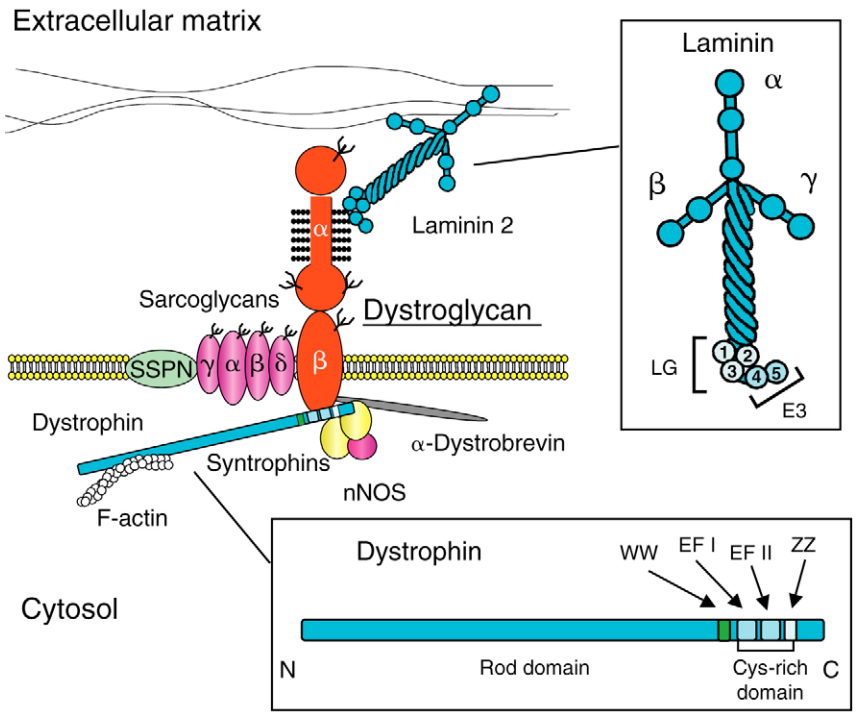

Fig. 2. DGC and dystroglycan ligands. Schematic representation of dystroglycan within the DGC. Structural domains of dystroglycan-binding proteins are represented in the insets. Domains of dystrophin described in the text are represented. A similar domain structure is found in utrophin. α-Dystrobrevin lacks a WW domain but contains the EF-hand and ZZ domains. Laminins are composed of three distinct chains termed α, β and γ. To date, 5 α, 4 β and 3 γ laminin chains have been identified that can combine to form 15 different isoforms (Hallmann et al., 2005). In the C-terminal ends of the α chains, there are globular G domains (LG 1-5) composed of five similar modules. Dystroglycan binds with high affinity to the LG domains of laminin 1 (α1β1γ1) and 2 (α2β1γ1) α chains (Talts et al., 1999). Antibodies against the C-terminal LG4-LG5 pair of the α1 chain (E3 fragment) have been widely used for organ morphogenesis studies.

DGC components appear to strengthen the interaction between α- and β-dystroglycan. In fact, studies in animal models of muscular dystrophy have shown that the dystroglycan complex is not tightly anchored at the sarcolemma of dystrophin- and sarcoglycan-deficient mice (Chamberlain et al., 1997; Duclos et al., 1998; Durbeej et al., 2000).

β-Dystroglycan connects intracellularly to dystrophin, which binds to the actin cytoskeleton, and extracellularly to α-dystroglycan. The C-terminus of β-dystroglycan contains a PPXY motif that binds to a WW-like domain containing two highly conserved tryptophan residues 21 residues apart in the cysteine-rich domain and the first half of the C-terminal domain of dystrophin (Figs 1 and 2). This link is stabilized by two Ca^{2+}-binding EF-hand domains in dystrophin (Ervasti and Campbell, 1991; Jung et al., 1995; Rentschler et al., 1999). In tissues other than striated muscle, β-dystroglycan binds to alternative proteins encoded at the dystrophin locus, such as Dp260, Dp140, Dp116 and Dp71 (Finn and Ohlendieck, 1998; Jung et al., 1995; Saito et al., 1999), or utrophin, which is an autosome-encoded homolog of dystrophin (Chung and Campanelli, 1999; Matsumura et al., 1992).

Recent studies suggest that the presence in both dystrophin and utrophin of a ZZ domain, which contains cysteine residues that might participate in Zn^{2+} binding, and EF-hand-like structures are also essential to reinforce their binding to β-dystroglycan (Ishikawa-Sakurai et al., 2004). In addition, α-dystrobrevin, a more distantly related dystrophin-family member that lacks a WW domain but contains the EF-hand domain, binds to β-dystroglycan (Chung and Campanelli, 1999). Caveolin-3, a muscle-specific member of a family of proteins localized at caveolae (small invaginations of the plasma membrane) (Galbiati et al., 2001), might also compete with dystrophin for binding to the PPXY motif of β-dystroglycan (Sotgia et al., 2000). Other molecules that bind to the cytoplasmic tail of β-dystroglycan include the signaling molecule Grb2, components of the ERK-MAP kinase cascade including MEK and ERK, and rapsyn, a postsynaptic protein involved in the clustering of acetylcholine receptor (Cartaud et al., 1998; Spence et al., 2004; Yang et al., 1995). β-Dystroglycan could therefore be involved in signal transduction pathways and in the maintenance of the neuromuscular junction.

α-Dystroglycan binds to several extracellular ligands, including laminin, agrin and perlecan in muscle (Bowe et al., 1994; Campanelli et al., 1994; Gee et al., 1994; Sugiyama et al., 1994), and neurexin in brain (Sugita et al., 2001). Each of these has laminin G (LG)-like domains that mediate their high-affinity Ca^{2+}-dependent binding to α-dystroglycan (Fig. 2) (Hohenester et al., 1999; Tisi et al., 2000). Recent reports have demonstrated that this interaction is strictly dependent on the glycosylation status of α-dystroglycan (Michele et al., 2002; Michele and Campbell, 2003; Muntoni et al., 2004). By contrast, biglycan, a proteoglycan found in the ECM, binds to the protein core of the C-terminal domain of α-dystroglycan in a fashion that is dependent on its chondroitin sulfate side chains (Bowe et al., 2000).

The broad range of extracellular ligand partners indicates that dystroglycan has an important role as a receptor and is involved in the assembly and maintenance of basement membranes. Dystroglycan is expressed at high levels in developing and adult tissues, typically in cell types facing basement membranes. In mice, disruption of the *Dag1* gene results in embryonic lethality around embryonic day 5.5, owing to structural and functional perturbations of a basement membrane (Reichert's membrane) that forms early in the rodent embryo and separates the embryo from the maternal circulation (Williamson et al., 1997). However, another basement membrane between the visceral endoderm and ectoderm appears to persist in *Dag1*-null embryos, perhaps arguing against a general role for dystroglycan in basement

membrane assembly (Henry and Campbell, 1998; Li et al., 2002; Williamson et al., 1997). Indeed, although dystroglycan is essential for muscle function and stability, the ultrastructure of basement membranes appears unaffected in mice lacking dystroglycan in skeletal muscle (Cohn et al., 2002; Cote et al., 1999). Nevertheless, mice with a targeted disruption of dystroglycan in astrocytes show discontinuous pial basement membranes (Moore et al., 2002). Furthermore, dystroglycan is expressed abundantly on the basal side of most epithelial cells (Durbeej et al., 1995), and antibody perturbation experiments and treatment of cultured organs with the C-terminal region of the laminin α1 polypeptide (E3 fragment) (Fig. 2), which binds α-dystroglycan with high affinity, indicate that it might be involved in branching morphogenesis of kidney, lung and salivary glands (Durbeej et al., 1995; Durbeej et al., 2001).

Given its role in the organization and assembly of the basement membrane, loss of dystroglycan could be associated with epithelial cancer progression (Henry et al., 2001; Muschler et al., 2002; Sgambato et al., 2003). Indeed, loss of α-dystroglycan expression in prostate and breast cancer cells correlates with a higher grade of malignancy (Henry et al., 2001). Similar loss of α-dystroglycan has been observed in skin and squamous carcinoma cells (Herzog, 2004; Jing et al., 2004). Interestingly, β-dystroglycan expression is preserved in these cells, although an anomalous 31 kDa form is often observed (Losasso et al., 2000). *DAG1* is normally expressed in these cells, and the loss of α-dystroglycan is a result of protease-dependent shedding from the cell surface (Singh et al., 2004). The glycosylation of α-dystroglycan is altered in invasive carcinoma cells, which causes loss of binding to laminin 1 (Singh et al., 2004). Thus, aberrant post-translational modifications of α-dystroglycan perturb the interaction between cells and the ECM, and contribute to the devastating disease progression.

Glycosylation is also likely to be important in the binding of viruses and bacteria to α-dystroglycan. It has been shown that *M. leprae* requires laminin 2 as a cofactor for binding to α-dystroglycan and infecting Schwann cells (Rambukkana et al., 1997; Rambukkana et al., 1998). α-Dystroglycan also serves as a receptor for the lymphocytic choriomeningitis virus (LCMV) and the Lassa fever virus (LFV) (Cao et al., 1998; Kunz et al., 2001). A fusion protein comprising the C-terminus of α-dystroglycan and the transmembrane domain of the platelet-derived growth factor (PDGF) receptor can act as a viral receptor, indicating that β-dystroglycan does not act as a cofactor for virus entry (Kunz et al., 2003). The fact that *M. leprae* and viruses bind to the native protein but not to recombinant forms indicates that glycosylation is likely to be essential in infection (Cao et al., 1998; Rambukkana et al., 1998). Specifically, *O*-mannosylation and post-translational modification of α-dystroglycan by the putative glycosyltransferase LARGE is crucial for its action as a cellular receptor for viruses (Imperiali et al., 2005; Kunz et al., 2005).

Perturbation of α-dystroglycan glycosylation

The enzymatic pathway for dystroglycan glycosylation and the exact function of the carbohydrate chains in ligand binding remain obscure. Perturbation of the synthesis of the *O*-mannosyl tetrasaccharide mentioned above leads to hypoglycosylation of α-dystroglycan and abolishes ligand-binding activity (Barresi et al., 2004; Michele et al., 2002; Willer et al., 2003). Further insights into the role of this oligosaccharide have been gained from genetic and biochemical analyses of individuals who have primary defects in known or putative glycosyltransferases.

Defects in glycosylation pathways cause numerous human diseases, such as the well-characterized congenital disorders of glycosylation (CDGs), which is a group of multisystemic syndromes that particularly affect the central nervous system (Jaeken and Carchon, 2004). CDGs identified to date affect *N*-glycan assembly (CDG type I) or processing (CDG type II). By contrast, defects in glycosyltransferases involved in the post-translational processing of α-dystroglycan affect *O*-glycosylation pathways. These lead to devastating effects on muscle fiber integrity and neuronal migration (Muntoni et al., 2004). The human disorders muscle-eye-brain disease (MEB), Walker-Warburg syndrome (WWS), Fukuyama congenital muscular dystrophy (FCMD), and congenital muscular dystrophy 1C and 1D (MDC1C and MDC1D) are caused by mutations in proteins that may either directly or indirectly glycosylate α-dystroglycan (Fig. 3). Mutations in the gene encoding protein *O*-mannosyltransferase 1 (POMT1) have been identified in some cases of WWS (Beltran-Valero De Bernabe et al., 2002; Currier et al., 2005), as well as in a novel form of recessive limb girdle muscular dystrophy with mild mental retardation and abnormal α-dystroglycan glycosylation (Balci et al., 2005). POMT1 catalyzes the first step in the assembly of the *O*-mannose-linked glycan moiety of α-dystroglycan (Jurado et al., 1999). Mutations in the gene encoding POMT2, a homolog of POMT1, can also cause WWS, since these enzymes work as a complex to add a mannose residue directly to the polypeptide backbone (Manya et al., 2004; van Reeuwijk et al., 2005; Willer et al., 2002). The gene defective in MEB encodes the second enzyme in the *O*-mannosylation pathway, protein *O*-mannosyl β-1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1). This is a type II membrane protein similar to other Golgi glycosyltransferases that adds an *N*-acetylglucosamine (GlcNAc) residue through β1,2 linkage to the mannose group using uridine 5′-diphosphate (UDP)-GlcNAc as a donor substrate (Yoshida et al., 2001; Zhang et al., 2002).

Individuals who have FCMD, MDC1C and MDC1D have mutations in putative glycosyltransferases. FCMD is caused by mutations in the gene encoding fukutin (Kobayashi et al., 1998). The function of fukutin is unknown, although it shows sequence similarity to the *fringe*-like family of enzymes that modify glycolipids and glycoproteins (Aravind and Koonin, 1999). In vitro studies using tagged fukutin constructs have shown that it colocalizes with Golgi markers (Esapa et al., 2002; Matsumoto et al., 2004). A homolog of fukutin, fukutin-related protein (FKRP), is mutated in MDC1C and in the milder allelic variant limb girdle muscular dystrophy 2I (LGMD2I) (Brockington et al., 2001a; Brockington et al., 2001b). The specific function of FKRP has also not been established. Longman and colleagues have identified a patient with MDC1D and heterozygous mutations in the *LARGE* gene (Longman et al., 2003a). The ortholog of this gene is affected in the transgenic enervated (*enr*) mouse and the myodystrophy mouse (Large^{myd}), which is a spontaneous mutant that presents pathological features similar to those of congenital muscular dystrophy patients (Grewal et al., 2001; Holzfeind et al., 2002; Levedakou et al., 2005; Michele et al., 2002).

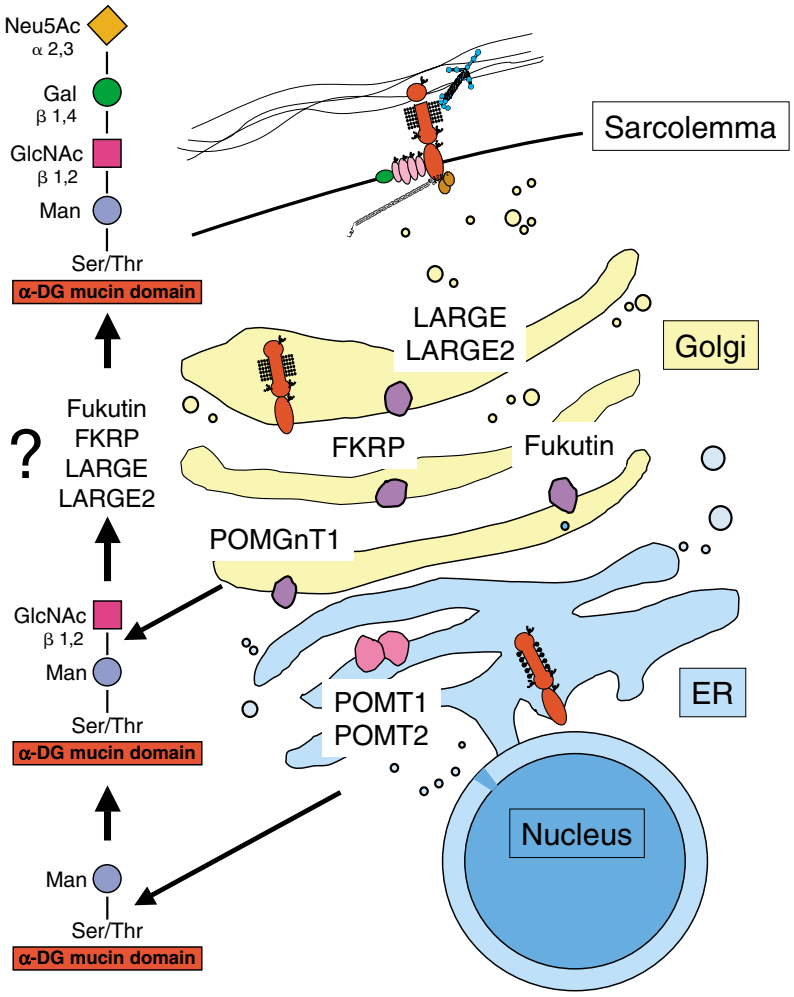

Fig. 3. Glycosylation of α-dystroglycan. Localization of glycosyltransferases involved in the *O*-glycosylation pathway of α-dystroglycan within the cellular organelles. Known steps of the glycosylation pathway are represented on the left.

The finding that α-dystroglycan glycosylation is perturbed in these forms of muscular dystrophy and in the mouse models is based on loss of immunoreactivity to one or both monoclonal antibodies IIH6 and VIA4-1, which are specific for glyco-epitopes of α-dystroglycan (Brockington et al., 2002; Grewal et al., 2001; Hayashi et al., 2001; Kano et al., 2002; Michele et al., 2002). Interestingly, the IIH6 antibody inhibits laminin 1 binding, which suggests that the same glyco-epitopes are required for antibody and ligand binding (Ervasti and Campbell, 1993). Hypoglycosylated α-dystroglycan can also be identified in these patients by a polyclonal antibody, GT20ADG, which was raised against the entire DGC and purified against a fusion protein comprising hypoglycosylated full-length α-dystroglycan and human Fc that was transiently expressed in HEK-293 cells (Barresi et al., 2004; Kunz et al., 2001; Michele et al., 2002).

Mutations in the genes encoding LARGE, POMGnT1, POMT1 and fukutin lead to expression of α-dystroglycan species that have lower molecular masses (~90 kDa) and altered capacity for binding to laminin, agrin and neurexin (Kim et al., 2004; Michele et al., 2002). The defects in α-dystroglycan glycosylation appear to cause perturbation of the basement membrane and might be the molecular basis for these muscle and central nervous system pathologies (Ishii et al., 1997; Sabatelli et al., 2003; Vajsar et al., 2000). Indeed, the pathological muscle phenotype of chimeric mice or muscle-specific mutants proves that dystroglycan has a central role in the maintenance of muscle fiber integrity (Cohn et al., 2002; Cote et al., 1999). Consistent with this idea are the findings that the brain isoform of α-dystroglycan (originally called cranin) binds to laminin 1 and laminin 2, and that mouse models in which the gene encoding dystroglycan is deleted in the central nervous system develop a pathological phenotype closely resembling the abnormalities observed in patients (Moore et al., 2002; Smalheiser and Schwartz, 1987; Tian et al., 1996).

Interestingly, patients with mutations in the *FKRP* gene show a variable reduction in α-dystroglycan glycosylation rather than a complete absence (Mercuri et al., 2003). The presence of partially functional species of α-dystroglycan correlates with the milder phenotype of these patients. This suggests that the residual activity of the mutated glycosyltransferase limits the severity of the pathological phenotype (Esapa et al., 2004). In addition, residual POMGnT1 activity has been shown in patients with MEB (Zhang et al., 2003). Whereas most of the FCMD patients carry a 3 kb retrotransposon insertion in the 3′ non-coding region of the gene encoding fukutin, individuals homozygous for this insertion show a milder phenotype than compound heterozygotes carrying the insertion in combination with a missense or nonsense mutation on the other allele, and patients with homozygous nonsense modifications (Beltran-Valero de Bernabe et al., 2003; Toda et al., 2000). Similarly, deletions in the murine *Fukutin* and *POMT1* genes are lethal, although these embryos survive a few days longer than the *Dag1*-null mutants (Kurahashi et al., 2005; Takeda et al., 2003; Willer et al., 2004). This suggests that residual sugar moieties on α-dystroglycan might partially support the development of basement membranes. Taken together, these data indicate that, similarly to loss of dystroglycan itself, complete depletion of proteins that are involved in the post-translational processing of α-dystroglycan is not compatible with survival. However, Large^{myd} mice escape this embryonic lethality, which might indicate the presence of a developmentally regulated LARGE activity or other compensatory mechanisms.

Given that the glycosylation pathways of α-dystroglycan are not fully understood yet, mutations in other enzymes involved might be responsible for several reported muscle pathologies (Longman et al., 2003b). Recently, perturbations in α-dystroglycan glycosylation have been reported in patients that have hereditary inclusion-body myopathy (HIBM), a neuromuscular disorder associated with mutations in the gene that encodes UDP-*N*-acetylglucosamine-2-epimerase/*N*-acetylmannosamine kinase [*GNE* (Huizing et al., 2004)]. This bifunctional enzyme catalyzes the first two steps of sialic acid

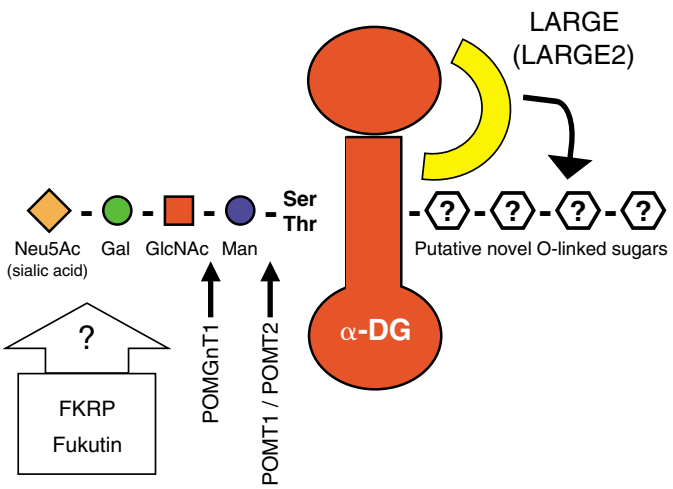

Fig. 4. Known and putative glycosyltransferases involved in the *O*-glycosylation pathway of α-dystroglycan. The function of fukutin and FKRP is still unknown. Although it has not been excluded that LARGE and LARGE2 are involved in *O*-mannosylation of α-dystroglycan, it is very likely that overexpression of these putative enzymes stimulates the synthesis of novel and still unknown O-linked sugar chains on the mucin domain of α-dystroglycan by direct binding of LARGE to the N-terminal and the first half of the mucin domain.

biosynthesis (Hinderlich et al., 1997). In muscle biopsies of four HIBM patients, the immunolabeling of α-dystroglycan with VIA4-1 and IIH6 antibodies is greatly reduced, but labeling by an antibody to the core α-dystroglycan protein is normal (Huizing et al., 2004). Although these findings resemble those described for other congenital muscular dystrophies, this putative partial hyposialylation of α-dystroglycan does not affect its laminin-binding properties and thus it probably does not play a key pathogenic role in HIBM (Broccolini et al., 2005).

Modifications and enhancement of α-dystroglycan glycosylation

LARGE

Recent studies have shown that increased expression of LARGE affects the post-translational modification of α-dystroglycan by inducing the synthesis of species that are enriched in glycans (⩾200 kDa) and have higher affinity for extracellular ligands (Barresi et al., 2004; Brockington et al., 2005). The putative LARGE glycosyltransferase contains a transmembrane domain followed by a coiled-coil domain and two DxD-containing catalytic domains homologous to the known bacterial α-glycosyltransferase and mammalian β-1,3-*N*-acetylglucosaminyltransferase (Grewal et al., 2001; Peyrard et al., 1999). The DxD motif is a short conserved motif that might play a carbohydrate-binding role in glycosyltransferases. To date, the glycosyltransferase activity of LARGE and its direct effect on dystroglycan processing remain to be established. Nevertheless, in vivo and in vitro studies using adenoviral gene transfer into mouse skeletal muscle, as well as into human myoblasts and fibroblasts, suggest that the concentration of LARGE is a limiting factor in the glycosylation of α-dystroglycan (Barresi et al., 2004).

Enzymes that appear to be related to LARGE catalyze modifications unrelated to the known *O*-mannosyl moiety. Moreover, several reports strongly indicate that LARGE might be involved in the synthesis of sugar chains other than the tetrasaccharide that is defective in patients with congenital muscular dystrophies (Barresi et al., 2004; Fujimura et al., 2005; Patnaik and Stanley, 2005). In fact, overexpression of LARGE generates highly glycosylated α-dystroglycan even in cells from individuals who have defects in synthesis of *O*-mannosyl sugar chains (i.e. WWS and MEB) (Fig. 4), whereas overexpression of other glycosyltransferases involved in these disorders (i.e. POMGnT1, FKRP) does not generate similar enhancements in glycosylation (Barresi et al., 2004; Esapa et al., 2002).

To date, attempts to identify the glycan epitopes produced by LARGE have not been successful. Studies in Chinese hamster ovary (CHO) cells that have mutations in various glycosyltransferases have provided evidence that LARGE does not transfer sugars residues to lactose, fucose or sialic acid, or induce the transfer of these sugars to α-dystroglycan (Patnaik and Stanley, 2005). Enzymatic deglycosylation treatments have excluded N-linked sugars or glycosaminoglycan (GAG) as the acceptors in LARGE-induced hyperglycosylation (Barresi et al., 2004; Fujimura et al., 2005). Reactivity for glycosylated α-dystroglycan disappears after alkaline *O*-deglycosylation, which indicates these novel glycans are O-linked. However, as enzymes able to detach these glycans have yet to be identified, the composition of this sugar chain is still unknown (Barresi et al., 2004). Recent lectin-binding analyses indicate the presence of terminal α-linked GlcNAc, which cannot be enzymatically removed (Combs and Ervasti, 2005). Given that one of the putative catalytic domains of LARGE is similar to *N*-acetylglucosaminyltransferase, it is tempting to speculate that LARGE adds this sugar residue to α-dystroglycan. However, further investigations are necessary to verify this.

The recently identified *LARGE*-relative *GYLTL1B*, also known as *LARGE2*, stimulates α-dystroglycan hyperglycosylation even more effectively than LARGE and recognizes the N-terminal domain of α-dystroglycan equally well (Brockington et al., 2005; Fujimura et al., 2005). This reveals flexibility in the glycosylation pathway of α-dystroglycan in skeletal muscle and introduces new perspectives for the development of therapies in muscular dystrophies with perturbed glycosylation.

Other modifications

Adjustments of glycosylation in skeletal muscle might also be beneficial for muscular dystrophy in which the genetic defect does not primarily affect a glycosylation pathway. Nguyen et al. achieved upregulation of utrophin, and redistribution of DGC components along myofibers, in the absence of dystrophin by overexpressing the GalNAc transferase, which makes synaptic cytotoxic T cell (CT) carbohydrate antigen GalNAc-β1,4[NeuAc-α2,3]Gal-β1(3GalNAc or 4GlcNAc-R) in mdx mice (Nguyen et al., 2002). Although α-dystroglycan became glycosylated with the CT carbohydrate antigen, it is not clear how this modification contributed to the overall improvement of the muscle phenotype of the mdx-CT mice (Nguyen et al., 2002; Xia et al., 2002). Nevertheless, synaptic CT GalNAc transferase might therefore be considered as a target for therapeutic intervention in some forms of muscular dystrophy.

Conclusions

Recent genetic and biochemical findings have significantly improved our understanding of the pathways involved in the biosynthesis and post-translational modification of dystroglycan, and it is now clear glycosylation is crucial for dystroglycan function. Functionally abnormal α-dystroglycan is likely to be the major cause of disease in muscular dystrophies resulting from glycosyltransferase deficiencies. The knowledge that α-dystroglycan glycosylation is a flexible pathway has potential application in the development of therapies. We must still make further efforts to understand the mechanism of post-translational processing of dystroglycan. However, the finding that manipulation of α-dystroglycan glycosylation may be achieved by modulation of the expression or activity of endogenous proteins not only provides new avenues of therapeutic potential for disorders resulting from functionally abnormal α-dystroglycan but also offers additional clues to the biosynthesis of functional dystroglycan.

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