

Dystroglycan in development and disease

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Our understanding of the structure and function of dystroglycan, a cell surface laminin/agrin receptor, has increased dramatically over the past two years. Structural studies, analysis of its binding partners, and targeted gene disruption have all contributed to the elucidation of the biological role of dystroglycan in development and disease. It is now apparent that dystroglycan plays a critical role in the pathogenesis of several muscular dystrophies and serves as a receptor for a human pathogen as well as being involved in early development, organ morphogenesis, and synaptogenesis.

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Abbreviations

AChR	acetylcholine receptor
DGC	dystrophin–glycoprotein complex
ECM	extracellular matrix
FCMD	Fukuyama-type congenital muscular dystrophy
Gal	galactose
GlcNAc	<i>N</i> -acetyl glucosamine
Man	mannose
NMJ	neuromuscular junction
OPL	outer plexiform layer
Sia	sialic acid

Introduction

α -dystroglycan is a highly glycosylated peripheral membrane protein associated with the membrane spanning protein β -dystroglycan. These two proteins were originally isolated from skeletal muscle as components of the dystrophin–glycoprotein complex (DGC). Together with dystrophin they are dramatically reduced in the sarcolemma of patients with Duchenne muscular dystrophy and also in dystrophin deficient *mdx* mice [1–3]. In skeletal muscle α -dystroglycan binds to the extracellular matrix (ECM) component laminin $\alpha 2$ chain [4], whereas the intracellular domain of β -dystroglycan binds to the cytoskeletal protein dystrophin [5]. Thus, dystroglycan is believed to act as a transmembrane link between the ECM and the cytoskeleton and this linkage seems to be crucial for maintaining normal muscle function [6,7,8*].

Dystroglycan is not restricted to muscle but is widely expressed in many cell types [3,9–14,15**,16*]; moreover, α -dystroglycan also binds to laminin 1 and agrin [3,17,18*]. β -dystroglycan, in turn, also binds to the dystrophin

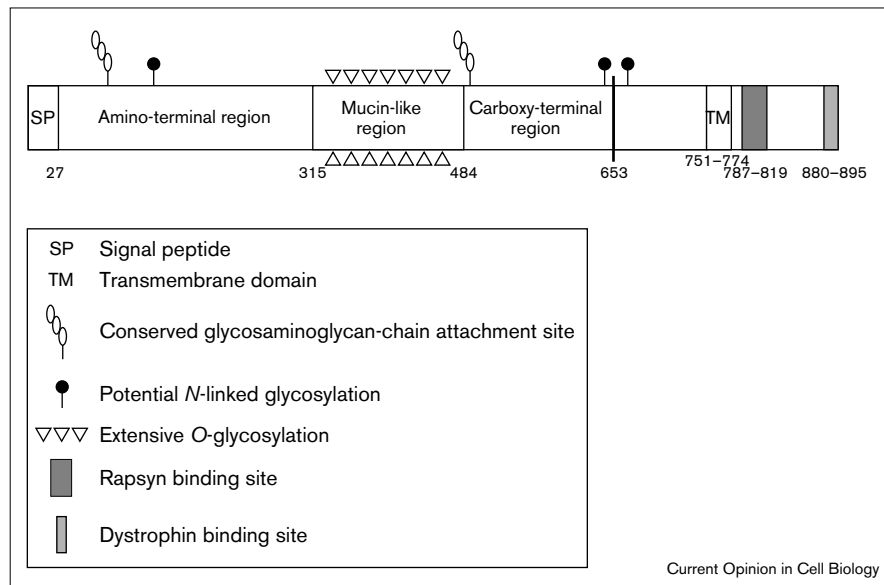
isoforms Dp71 and Dp260 [5] and has been shown to be associated with utrophin, the autosomal homologue of dystrophin [19]. Thus, different dystroglycan complexes may form in different tissues implying that dystroglycan may have important roles outside muscle. Indeed, besides being involved in various types of muscular dystrophy [7,8*,20], dystroglycan has been implicated in early mouse development [15**], epithelial morphogenesis [10], cell adhesion [21*], myelination [13] and synaptogenesis [18*,22*,23]. In addition, a novel function for dystroglycan as a receptor for bacterial invasion has recently been shown [24]. In this review we will discuss the most recent advances in our understanding of dystroglycan structure and function in both muscle and nonmuscle tissues from the past two years.

Dystroglycan structure

Full-length or partial dystroglycan cDNA clones exist for several vertebrate species including human [25], rabbit [3], mouse [9,12,26], rat, cow, pig (Genbank accession numbers, AA819114, AA819455 AA859828, AA899842, AB009079, F14847), and *Torpedo californica* [27]. The corresponding amino acid sequences are strikingly similar between the different species. In particular, there is a 100% conservation of the carboxy-terminal dystrophin-binding site [5] and a 93% conservation of the central Pro–Thr rich region [26] (see Figure 1). Dystroglycan sequences have also been identified in *Drosophila* (S Baumgartner, personal communication). The dystroglycan propeptide is post-translationally modified, giving rise to the α and β subunits [3]. β -dystroglycan has a molecular weight of 43 kDa in most tissues [3,16*]. The primary protein sequence predicts a molecular weight of 72 kDa for α -dystroglycan; however, α -dystroglycan in mammalian skeletal muscle is 156 kDa [28], 140 kDa in cardiac muscle [29*], and 120 kDa in brain and peripheral nerves [17,30]. Therefore, α -dystroglycan must undergo significant post-translational modifications. Previous studies suggest that α -dystroglycan resembles mucin proteins [31,32], and the susceptibility of α -dystroglycan to *O*-sialoglycopeptidase supports the hypothesis that α -dystroglycan is a sialylated mucin-type glycoprotein [32]. Recently it was demonstrated that exhaustive sialidase treatment of α -dystroglycan or the addition of sialic acid to the incubation medium diminished the laminin binding activity of α -dystroglycan. These data suggest that the sialic acid residues of α -dystroglycan are essential for laminin binding [33]. In a study by Chiba *et al.* [34*] the structures of the sialylated *O*-linked oligosaccharides of bovine peripheral nerve α -dystroglycan were analyzed. A novel *O*-linked mannose-type oligosaccharide, Sia α 2–3Gal β 1–4GlcNAc β 1–2Man–Ser/Thr was shown to constitute 66% of the sialylated *O*-linked sugar chains. Furthermore, a laminin binding inhibition study suggested

Figure 1

Dystroglycan domain organization. The amino-terminal domain of α -dystroglycan folds autonomously, has a globular shape and is connected to the globular carboxyl terminus via the extended mucin-like region [31,35^{*}]. Known binding partners for α -dystroglycan include laminins and agrin [3,4,17,18^{*},30,42,58^{*}]. High affinity binding of dystroglycan to laminin is probably mediated by the extensive *O*-glycosylation in the mucin-like region [34^{*}]. Positions of *N*-linked carbohydrates and potential GAG addition sites are indicated. The bold vertical line separates α - and β -dystroglycan [27,32]. Known binding partners for β -dystroglycan include dystrophin and several smaller dystrophin isoforms [5], rapsyn [53] and also the adapter protein Grb2 (not shown in the figure), which is believed to interact with proline-rich regions in the carboxyl terminus of β -dystroglycan [59].



that the sialyl *N*-acetylglucosamine moiety of this sugar chain (Sia α 2-3Gal β 1-4GlcNAc) is involved in the interaction of α -dystroglycan with laminin. The same *O*-mannosyl glycan has been found in rabbit skeletal muscle α -dystroglycan (Sasaki *et al.*, unpublished data), indicating that this unique *O*-linked carbohydrate is present on α -dystroglycan from different tissues and species. Ervasti *et al.* [29^{*}] proposed a novel sialic acid modification of skeletal muscle α -dystroglycan, SiaX α 2-6GalNAc, to indicate a sialic acid with an unidentified substitution. In this study, on the other hand, they could not show an effect of sialidase digestion on the laminin binding activity of α -dystroglycan from rabbit sciatic nerve or bovine brain. Hence, further studies may more clearly define the carbohydrate structures important for α -dystroglycan binding to laminin.

The three dimensional structure of α -dystroglycan is still largely unknown, but Brancaccio and coworkers have begun to analyze this question [31,35^{*}]. Few similarities between dystroglycan and other proteins have been found that might guide this analysis. By using primary sequence analysis and electron microscopy, α -dystroglycan was shown to have a dumbbell-like shape due to the presence of a central elongated, highly glycosylated, mucin-like region connecting two globules [31]. The amino-terminal region is oriented towards the extracellular space, but recombinant α -dystroglycan amino-terminal domain does not bind laminin or agrin, although it could have yet unidentified binding partners [35^{*}].

Tissue distribution of dystroglycan complexes

Skeletal muscle

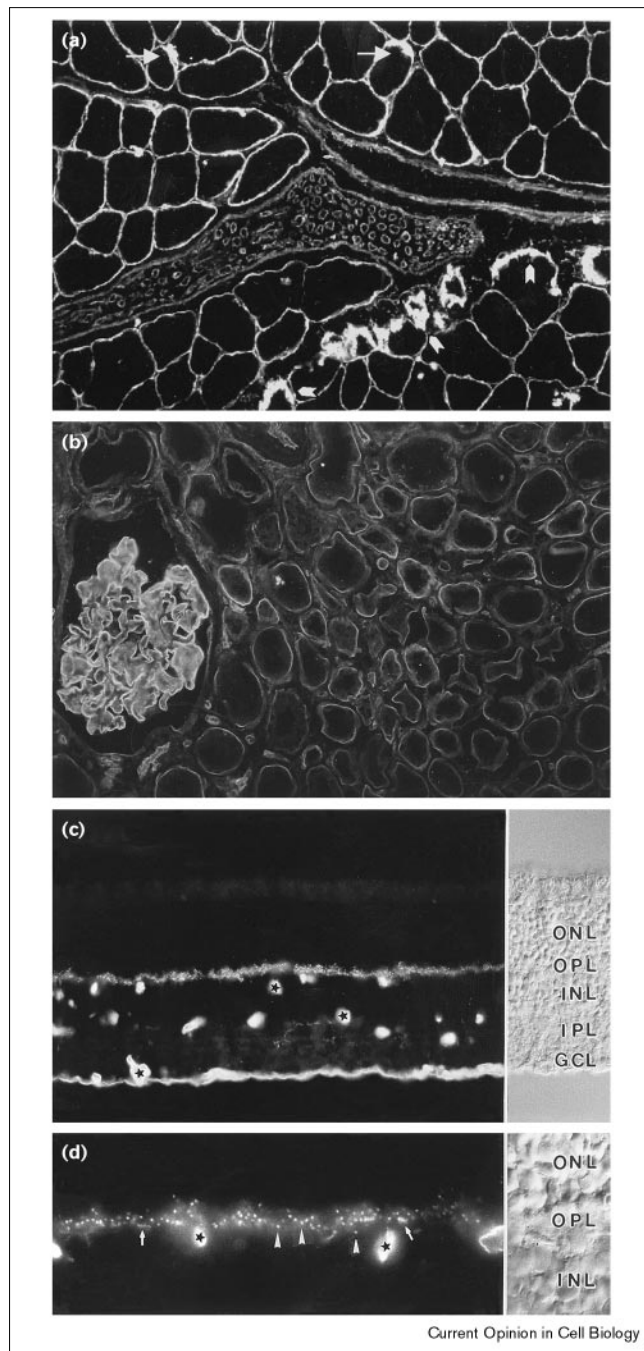
The DGC in skeletal muscle is comprised of dystrophin, dystroglycan, the syntrophin complex, the sarcoglycan complex (α , β , γ and δ sarcoglycans) [8^{*}] and the recently identified 25 kDa component termed sarcospan [36].

Sarcospan has four transmembrane domains and is a member of the tetraspan superfamily of proteins, which are thought to associate with other molecules, including integrins, to facilitate transmembrane protein interactions [37]. The DGC in skeletal muscle is expressed throughout the sarcolemma, at the neuromuscular junction (NMJ) [28] and at the myotendinous junction (see Figure 2). Skeletal muscle dystroglycan binds to the basement membrane component laminin 2. Interestingly, α -dystroglycan binding to the laminin α 2 chain is heparin insensitive whereas α -dystroglycan binding to laminin α 1 chain is heparin sensitive. Thus, α -dystroglycan can discriminate biochemically between different laminins [38,39]. One possibility is that heparan sulfate proteoglycans specifically modulate the interaction of α -dystroglycan with ECM molecules [38]. It is noteworthy that agrin, which is a heparan sulfate proteoglycan, was recently shown to bind to laminins [40]. Hence, agrin is a good candidate for such a modulatory factor. At the NMJ dystroglycan is also associated with utrophin [41], agrin [18^{*}] and may also bind laminin 4 (α 2 β 2 γ 1). The role of dystroglycan in various muscular dystrophies and synaptogenesis will be discussed in more detail below.

Peripheral nerves

In the peripheral nervous system, α -dystroglycan is expressed in the Schwann cell membrane together with Dp116 and utrophin. The precise composition of the DGC in peripheral nerve is unclear but α -sarcoglycan is absent [13]. Both the laminin α 2 chain and agrin are expressed in the basement membrane that surrounds the Schwann cells and Yamada *et al.* [42] have shown that α -dystroglycan is a receptor for both these ECM components. In the *dy* mouse, that shows a deficiency in laminin α 2 chain expression, there are myelination defects, implicating dystroglycan in myelination [13].

Figure 2



Immunohistochemical localization of dystroglycan in skeletal muscle, kidney and retina. **(a)** In skeletal muscle dystroglycan is expressed throughout the sarcolemma: at the neuromuscular junction (arrows); at the myotendinous junction (arrowheads); and in peripheral nerves. **(b)** In the adult kidney dystroglycan is confined to the basal side of most epithelial cells. Prominent staining is also detected in the glomerular basement membrane region. **(c)** In the retina, a clear concentration of β -dystroglycan immunoreactivity can be observed in the OPL, in the blood vessels (*), and in the inner limiting membrane. **(d)** Magnification of the OPL to illustrate the concentration of immunoreactivity in terminals of rods (arrowheads) and cones (arrows). Nomarski pictures are shown on the right side of **(c)** and **(d)** to reveal various retinal layers. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

Central nervous system

Dystroglycan is also expressed in the brain in many different cell types [9,11] (Table 1). For example, in the cerebellum dystroglycan is expressed in Purkinje cells and binds the laminin $\alpha 2$ chain at the glial-vascular interface, suggesting a role for dystroglycan in maintaining the blood-brain barrier [43]. At many sites, dystroglycan colocalizes with utrophin, dystrophin, or Dp71 [11,44]. It is interesting to note that mice deficient for Dp71 have reduced levels of dystroglycan in the brain, indicating that Dp71 may play a role in the function or organization of dystroglycan in the brain [45].

Table 1

Patterns of dystroglycan expression.

Tissue	Reference	Tissue	Reference
Muscle			
Skeletal	[28]	Respiratory system	
Smooth	[16*]	Lung	[3,10]
Cardiac	[3]	Trachea	[16*]
NMJ	[28]	Kidney	
MTJ	[*]	Tubules	[10,16*]
CNS			
Pyramidal neurons	[9]	GBM	[16*]
Dentate gyrus	[9]	Reproductive system	
Olfactory bulb	[9]	Uterus	[16*]
Purkinje neurons	[9]	Mammary gland	[16*]
Thalamus	[9]	Testis	[16*]
Choroid plexus	[9,10]	Prostate	[*]
Astrocytes	[43]	Skin	
Retina	[46-48]	Epidermis	[16*]
PNS			
Schwann cells	[13]	Hair follicles	[16*]
Digestive system			
Tooth	[10]	Sweat gland	[16*]
Salivary gland	[16*]	Lymphatic tissue	
Pancreas	[16*]	Spleen	[*]
Small intestine	[16*]	Thymus	[*]
Liver	[16*]	Lymph node	[*]
Early mouse embryo			
		Reichert's membrane	[15**]
		BM between VE and E	[15**]
		Decidual cells	[12,15**]

Published data on expression patterns of mammalian dystroglycan are summarized (unpublished data from our laboratory is indicated with [*]). Both mRNA and protein data from various stages of development are included. BM, basement membrane; CNS, central nervous system; E, ectoderm; GBM, glomerular basement membrane; MTJ, myotendinous junction; PNS, peripheral nervous system; VE, visceral endoderm.

Retina

The localization of dystroglycan has been extensively studied in the retina [14]. There is now convincing evidence that β -dystroglycan is concentrated in the extensions of photoreceptor terminals protruding into the outer plexiform layer (OPL), but it appears to be absent from the synaptic regions [46,47] (Figure 2). Dp260 is an isoform of dystrophin specifically expressed in the OPL of the retina. Dp260 may be necessary for localization of β -dystroglycan in the OPL, suggesting a requirement of Dp260 in the formation of a dystroglycan complex in the

retina [48]. Duchenne muscular dystrophy patients have alterations in their electroretinogram but it is not clear how mutations in dystrophin cause altered synaptic transmission within the OPL [14]. In the inner limiting membrane β -dystroglycan is associated with membranous structures bound to the basement membrane and is further associated with retinal blood vessels [46–48] (Figure 2).

Epithelial cells

Recent studies have revealed abundant dystroglycan expression on the basal side of most epithelial cells facing basement membranes in both embryonic and mature tissues [10,16•] (see also Table 1, Figure 2). In addition, there are reports of dystroglycan expression at sites of cell–cell contacts both *in vitro* and *in vivo* [16•,49]. Neither α , β , γ nor δ sarcoglycans appear to be focally expressed on epithelial cells [M Durbeej, unpublished observations]. However, ϵ -sarcoglycan, a homologue of α -sarcoglycan, was recently identified and shown to be widely expressed in nonmuscle tissues [50], but whether ϵ -sarcoglycan and dystroglycan form complexes on epithelial cells remains to be determined. The intracellular binding partners of β -dystroglycan in epithelial cells have not been identified but may include utrophin and/or the shorter dystrophin isoforms Dp71 and Dp140 [51].

Early embryonic tissues

Dystroglycan is expressed in the mouse embryo as early as embryonic day 5.5, where it is expressed in apposition to two embryo-derived basement membrane structures: Reichert's membrane and the basement membrane separating the visceral endoderm and ectoderm (Table 1). There is also a less pronounced pericellular dystroglycan expression on most embryonic cells [15••]; moreover, immediately after mouse embryo implantation dystroglycan is highly upregulated in maternal decidual cells [12,15••]. In addition to the pericellular staining seen in the mouse embryo [15••] and various cell types and organs [16•,49], this is one site where dystroglycan expression is not associated with a true basement membrane, though in the decidual tissue it is associated with basement membrane molecules [12].

Role of dystroglycan in development Synaptogenesis

At the NMJ, α -dystroglycan was initially suggested to be the agrin receptor mediating acetylcholine receptor (AChR) clustering and subsequent formation of postsynaptic specializations. Agrin has been shown to bind to α -dystroglycan with high affinity but the domain of agrin involved in α -dystroglycan binding is distinct from the part of the agrin molecule that induces AChR clustering. Thus, the role of α -dystroglycan in initial agrin signaling is unclear. Instead, the muscle-specific receptor tyrosine kinase (MuSK) complex represents at least a part of the signaling pathway involved in AChR clustering [18•]. Apel *et al.* [52•] point to a model in which rapsyn, a post-synaptic peripheral membrane protein, has indirect or direct

interactions with MuSK. Furthermore, rapsyn is required in an early step of MuSK signaling leading to AChR clustering. Cartaud and colleagues [53] recently showed evidence for *in situ* and *in vitro* association between β -dystroglycan and rapsyn. Thus, it is possible that dystroglycan could be part of the MuSK signaling pathway via its interaction with rapsyn. Another possibility is that rapsyn and dystroglycan are involved in the alternative pathway for AChR clustering that is discussed below.

Sugiyama *et al.* [54•] elegantly showed that there is an alternative pathway for AChR clustering during synaptogenesis. They showed that laminin 1 specifically induces AChR clustering via a MuSK independent pathway. Laminin 1 had previously been shown to induce clustering of dystroglycan on embryonic muscle cells. The laminin 1 fragment E3 (comprised of the carboxy-terminal 395 amino acid residues of the laminin α 1 chain), which binds dystroglycan, inhibits laminin induced clustering but does not itself cluster dystroglycan. These data suggest that other portions are also required for dystroglycan clustering activity [55•]. Notably, Montanaro *et al.* [22•] have implicated α -dystroglycan as the receptor that mediates MuSK independent aggregation. Jacobson *et al.* [23] showed that muscle cell lines with reduced amounts of α -dystroglycan exhibit seemingly normal signaling through the MuSK receptor and yet form significantly fewer AChR clusters compared to wild-type cells. These data indicate that α -dystroglycan could be required at some other stage of the clustering pathway. One possibility is that α -dystroglycan acts downstream of MuSK, and might be involved in the consolidation of microclusters, or growth and maintenance of AChR aggregates.

Epithelial morphogenesis

The developing kidney was the first nonmuscle organ in which dystroglycan was shown to have a crucial role. Antibody perturbation experiments suggested that dystroglycan might be involved in kidney epithelial morphogenesis [10]. Likewise, antibody perturbation experiments suggest a role for dystroglycan in salivary gland morphogenesis (M Durbeej, KP Campbell, P Ekblom, unpublished data). Dystroglycan has been shown by several groups to bind to the E3 fragment of laminin 1, [17,31] which also has been implicated in branching epithelial morphogenesis and basement membrane formation of the kidney and salivary gland [56,57]. Hence, these early *in vitro* data point towards a role for dystroglycan as a laminin receptor involved in epithelial cell development and basement membrane formation.

Recently it was shown that an agrin isoform, which is inactive in AChR aggregation, is expressed in several nonmuscle tissues including kidney. Moreover, this agrin isoform was also identified as a high-affinity binding-partner of dystroglycan [58•]. Taken together, it is possible that dystroglycan interactions with laminins and agrin are important for the maintenance of tissue integrity.

Early mouse development

The results described above indicated that dystroglycan might have important, broad ranging developmental roles. This was emphasized recently by the analysis of the dystroglycan knockout mouse [15**]. The dystroglycan null mutant fails to progress beyond the early egg cylinder stage of development, around embryonic day 5.5. This early lethality appears to stem from a specific perturbation of Reichert's membrane, an early basement membrane produced by extra-embryonic tissues. In the mutant, the normally continuous arrangement of basement membrane proteins, laminin and collagen IV, is grossly fragmented. The barrier normally presented by Reichert's membrane is broken down, allowing for the infusion of maternal blood into the yolk sac cavity; however, the parietal endoderm cells, which synthesize Reichert's membrane, seem unaffected in these mutants. Together the data indicate that dystroglycan plays a role in the formation of basement membranes. This could explain the mechanism by which dystroglycan is involved in epithelial morphogenesis and formation of the neuromuscular synapse — two processes intimately connected with the formation of basement membranes. Whether dystroglycan's role in the formation of basement membranes is primarily structural, or whether in addition it is involved in mediating signal transduction events, remains to be elucidated. Indeed, the signal transduction molecule Grb2 binds to β -dystroglycan and so could directly link dystroglycan to signaling events [59].

Role of dystroglycan in human diseases

Above we have outlined dystroglycan's involvement in a diverse array of processes in both developing and adult tissues. It is reasonable to speculate that disruption of dystroglycan function could have pathological implications for a number of diseases. Currently, there is no firm genetic data linking the dystroglycan gene to human disease. Given the phenotype of the dystroglycan knockout mice, null mutations in the dystroglycan gene would probably result in embryonic lethality. It is worth noting, however, that the human dystroglycan gene maps to chromosome 3p21, a locus that is involved in tumor suppression [25]. Therefore, one exciting task will be to analyze the role of dystroglycan in cancer. Although there is as yet no direct evidence of genetic involvement, dystroglycan has been implicated in two severe human diseases, namely various forms of muscular dystrophies and more recently, leprosy.

Muscular dystrophy

The involvement of the DGC in Duchenne muscular dystrophy is well established. Absence of dystrophin leads to a drastic reduction of the DGC components, including dystroglycan, in the sarcolemma of Duchenne muscular dystrophy patients [1] and *mdx* mice [2]. Also, mutations in the *LAMA2* gene encoding the laminin $\alpha 2$ chain, which is the extracellular ligand for α -dystroglycan in skeletal muscle, have been identified in a subset of congenital muscular dystrophies [8*]. There is now emerging evidence that dystroglycan may be involved in the

pathogenesis of several limb-girdle muscular dystrophies. Earlier studies have suggested that an intact sarcoglycan complex is required for the stable presence of α -dystroglycan in the sarcolemma of the dystrophic and cardiomyopathic BIO 14.6 hamster [60,61] that lacks the sarcoglycan complex due a mutation in the δ -sarcoglycan gene [62–64]. In support of this notion, Holt *et al.* [65**] recently demonstrated a rescue of the entire sarcoglycan complex, as well as a restored stable association of α -dystroglycan with the sarcolemma in the BIO 14.6 hamster, using δ -sarcoglycan gene transfer. Also, mice deficient in α -sarcoglycan, which develop a progressive muscular dystrophy, have impaired localization of α -dystroglycan to the sarcolemma [66*]. Moreover, a limb-girdle muscular dystrophy patient, homozygous for a Thr151→Arg missense mutation in the β -sarcoglycan gene, has a reduction of sarcolemmal α -dystroglycan staining [67].

Recently, the Fukuyama-type congenital muscular dystrophy (FCMD) gene was identified, which is thought to encode an extracellular protein [68]. Interestingly, abnormalities in the basal lamina of FCMD muscle have been detected as well as a decreased immunostaining of β -dystroglycan [69] and the laminin $\alpha 2$ chain [70]. It is thus tempting to speculate that dystroglycan could play a role in pathogenesis of FCMD: besides muscular dystrophy patients with this disease show a neuronal migration disorder.

Taken together, mutations in dystrophin or the sarcoglycans result in severe muscular dystrophies with impaired dystroglycan expression. Thus, proper localization of dystroglycan in the sarcolemma appears to be important for muscle integrity. It is possible that dystroglycan mechanically stabilizes the sarcolemma by linking the ECM to the cytoskeleton. Alternatively, or in addition to this role, dystroglycan could play an important part in maintaining the muscle cell basement membrane consistent with its developmental role in the formation of basement membranes.

Leprosy

Very recently, a novel function of α -dystroglycan as a receptor for a human pathogen has been described by Rambukkana *et al.* [24]. Initial studies suggested that the G (globular)-domain of the laminin $\alpha 2$ chain, expressed in the basement membrane of the Schwann cells, serves as a target for *Mycobacterium leprae* (the causative organism of leprosy) [71*]. Subsequent studies [24] revealed that α -dystroglycan is a Schwann cell receptor for *M. leprae* and it appears that *M. leprae* only binds to α -dystroglycan in the presence of the G-domain of the laminin $\alpha 2$ chain. The data suggest a mechanism by which *M. leprae* invades Schwann cells by exploiting the interaction between laminin 2 and α -dystroglycan in the peripheral nervous system.

Conclusion

Recent published reports have documented significant roles of dystroglycan in development and disease.

Importantly, it is now evident that dystroglycan is a ubiquitous laminin/agrin receptor expressed in a wide variety of cell types and often in association with basement membranes. Future studies are likely to include further investigations into the role of dystroglycan in basement membrane formation, in order to explain the mechanism by which dystroglycan is involved in developmental processes and pathogenesis of human disease.

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