Dystroglycan: an extracellular matrix receptor linked to the cytoskeleton

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Dystroglycan provides a crucial linkage between the cytoskeleton and the basement membrane for skeletal muscle cells. Disruption of this linkage leads to various forms of muscular dystrophy. Significant recent advances in understanding the structure and function of dystroglycan include detailed *in vitro* and *in vivo* analyses of its binding partners in muscle, an examination of its function at the neuromuscular junction, and emerging evidence of its roles in nonmuscle tissues.

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Abbreviations

AChR	acetylcholine receptor
DGC	dystrophin–glycoprotein complex
ECM	extracellular matrix
GAG	glycosaminoglycan
MASC	myotube-associated specificity component
MuSK	muscle-specific kinase
NMJ	neuromuscular junction

Introduction

Dystroglycan was originally identified in skeletal muscle as a component of the dystrophin–glycoprotein complex (DGC)—a collection of tightly associated proteins that anchors dystrophin to the sarcolemma [1,2]. Molecular cloning of the dystroglycan gene revealed that dystroglycan consists of a 156 kDa extracellular laminin-binding subunit (α-dystroglycan) plus a 43 kDa transmembrane subunit (β-dystroglycan) [3]. Now, dystroglycan is thought to form a continuous link between laminin-2, in the extracellular matrix (ECM), and dystrophin, in the cytoskeleton [4,5••]. Current thinking holds that this dystroglycan-mediated connection between the ECM and the cytoskeleton contributes to the structural integrity of the sarcolemma. Disruption of the DGC appears to underlie many forms of muscular dystrophy [6]. Recent studies have shown that dystroglycan is an agrin receptor, suggesting that it may also play a role in nerve–muscle synapse formation [7–10]. Dystroglycan is expressed in a number of nonmuscle tissues, indicating that its function is not restricted to muscle [3,11]. In this review, we will examine the recent advances in understanding dystroglycan structure and function both in muscle, where it has been most extensively studied, and in nonmuscle tissues, where exciting new avenues of research are opening up.

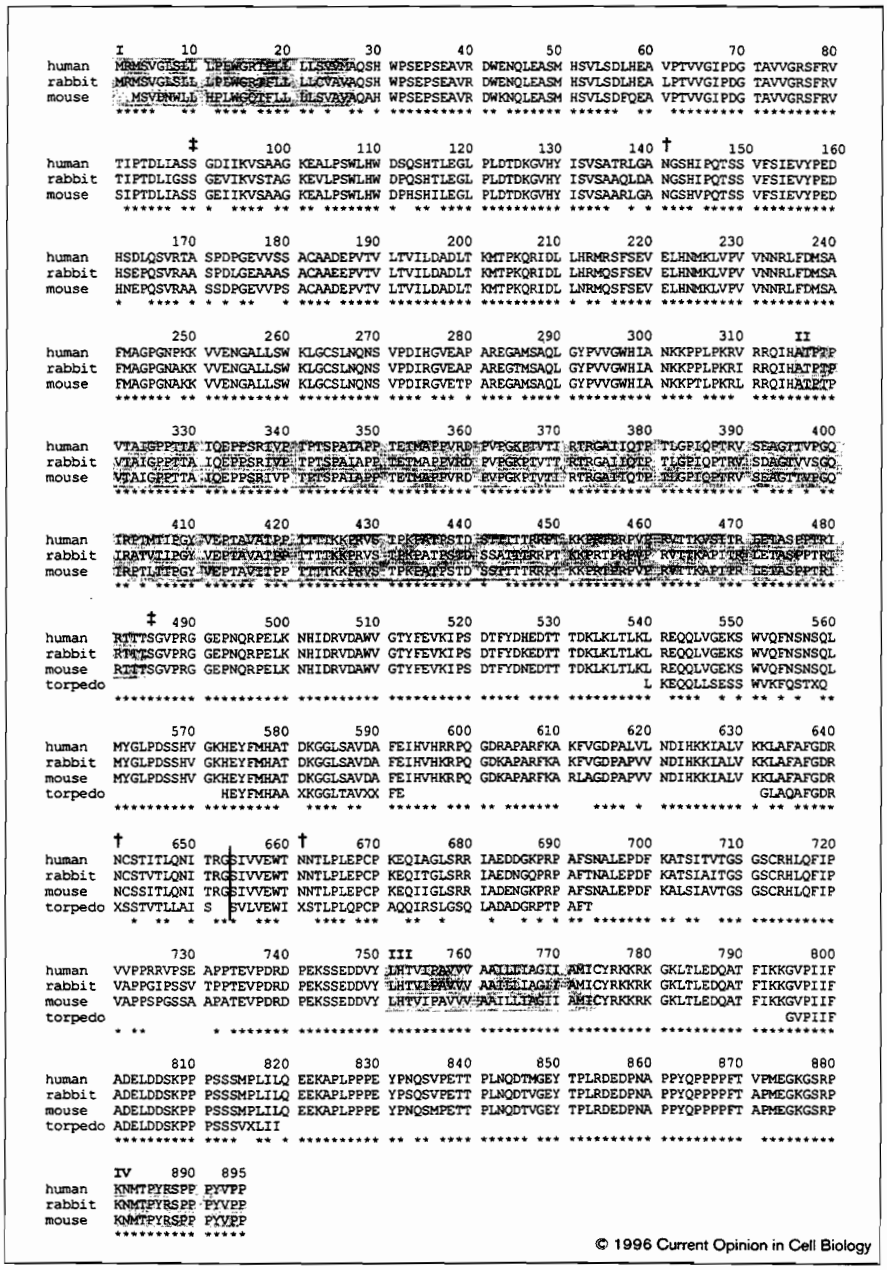
Dystroglycan structure

The primary sequences of dystroglycan from several organisms are presented in Figure 1. Comparison of the available sequences reveals remarkable structural conservation among rather evolutionarily divergent organisms. Dystroglycan exists as a noncovalently linked complex of α- and β-glycoprotein subunits [7,12], which arise from a single mRNA transcript encoded by a single gene [3,11,13]. In humans, this gene maps to chromosome 3p21, a region thought to contain a tumor suppressor gene involved in several types of cancer [11]. α- and β-dystroglycan subunits are probably post-translationally processed forms of a precursor polypeptide. Recent studies have identified Ser654 as the amino terminus of β-dystroglycan [14•,15].

Although the primary sequence of α-dystroglycan predicts a 72 kDa polypeptide, α-dystroglycan isolated from different tissue sources shows considerable size heterogeneity. For example, α-dystroglycan isolated from mammalian skeletal muscle and brain migrates in SDS-polyacrylamide gels as diffuse bands of around 156 kDa and 120 kDa, respectively [3], whereas that from *Torpedo californica* electroplax is ~190 kDa in size [7]. α-dystroglycan contains both Asn- and Ser/Thr-linked carbohydrates [16] and deglycosylation abolishes its laminin-binding activity *in vitro* [17,18]. The possibility that nearly two thirds of the weight of α-dystroglycan might be carbohydrate led to initial speculation that it could be a proteoglycan. In support of this idea, C2C12 muscle cell lines deficient in glycosaminoglycan (GAG) biosynthesis express a lower molecular weight form of α-dystroglycan with impaired agrin-binding properties [8–10]. Smalheiser and Kim [14•] have recently re-examined the carbohydrate modifications of α-dystroglycan. Their work suggests that brain-derived α-dystroglycan possesses a mucin-like carbohydrate structure. One way to reconcile these different ideas is to suggest that α-dystroglycan has both proteoglycan and mucin-like characteristics and that these properties might be modulated in a functionally important, tissue-specific manner. The current confusion over the nature of dystroglycan's carbohydrate moieties emphasizes the need for more detailed studies.

A high resolution understanding of dystroglycan structure does not yet exist, but we might have had our first glimpse at α-dystroglycan during this past year. Brancaccio *et al.* [19•] reported a 'dumbbell' shape for chicken cardiac α-dystroglycan using rotary shadowed electron microscopy.

Figure 1



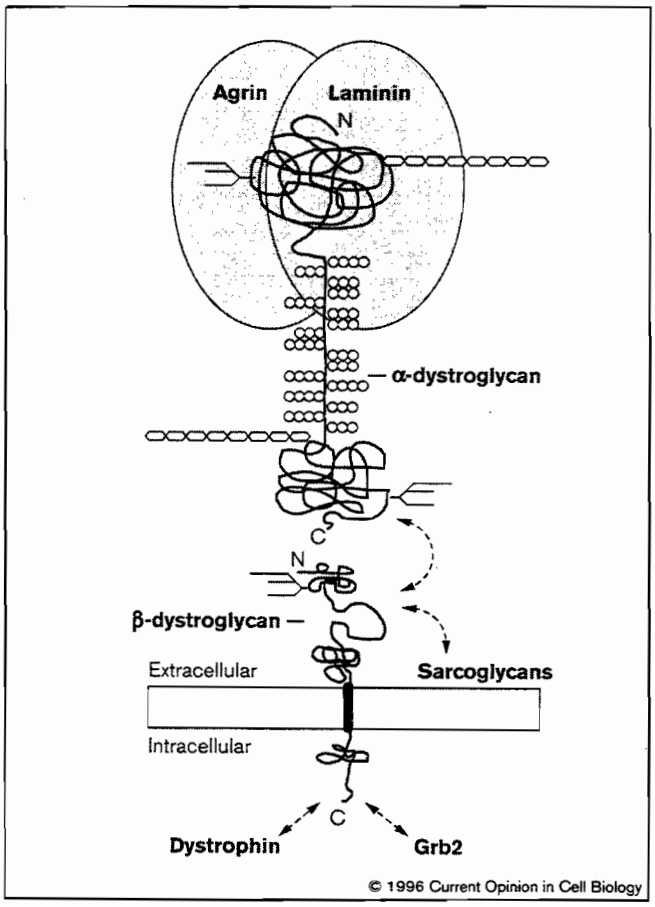
Dystroglycan amino acid sequences. Complete primary sequences of dystroglycan from human [11], rabbit [3] and mouse ([13]; J Lee, KP Campbell, unpublished data), and several peptide sequences from dystroglycan of *Torpedo californica* (torpedo) [15], are shown (the single-letter amino acid code is used). * denotes a residue that is identical among all of the sequences. Shaded regions indicate the following: I, the signal peptide; II, the mucin-like region; III, the transmembrane domain; and IV, the dystrophin-binding site. † marks the positions of putative N-linked carbohydrates conserved in all of the available sequences. Further evidence for the existence of these modifications at Asn641 and Asn661 comes from the fact that peptide sequencing over these residues is blocked [15]. ‡ shows the positions of potential GAG addition sites. The bold vertical line separates α- and β-dystroglycan.

They, too, interpreted this to be consistent with a rod-like mucin domain in the central region of α-dystroglycan. Although it is not yet clear if these first images represent a native dystroglycan structure, an extended conformation for α-dystroglycan is also suggested by other recent experimental findings [12,20•]. A current model highlighting known or suspected structural features of dystroglycan is presented in Figure 2.

Function at the sarcolemma

Dystroglycan is localized throughout the sarcolemma in skeletal muscle, including in the post-synaptic membrane at the neuromuscular junction (NMJ) [2]. *In vitro*, α-dystroglycan binds to laminin-2 and agrin (both of which are components of the basal lamina surrounding muscle fibers) in a calcium-dependent, heparin-inhibitable manner [7,17,21,22•]. Recent efforts have more closely examined the heparin sensitivity of the binding of α-dystroglycan to its ligands. Pall *et al.* [23•] found that heparin substantially blocks the binding of skeletal muscle α-dystroglycan to laminin-1, but not to laminin-2. In contrast, binding of brain- and peripheral nerve derived α-dystroglycan to both laminin isoforms is inhibited by heparin [21,23•]. Heparin inhibition of agrin binding to dystroglycan depends on the presence of an alternatively spliced insert in agrin [24••,25•]. Taken together, these

Figure 2



Schematic representation of dystroglycan structure. This is a view of skeletal muscle dystroglycan, summarizing currently available information. At the top, agrin and laminin bind to a similar, though unknown, site on α-dystroglycan [9,22•]. α-dystroglycan is shown with an extended structure [12,20•] which is probably due to the presence of a mucin-like domain in its central region [14•,19•]. Putative N-linked carbohydrates are marked by branches, O-linked mucin-type sugars by connected circles, and potential GAG chains by connected hexagons. β-dystroglycan begins at Ser654 [14•,15] and is noncovalently associated with α-dystroglycan [12]. Dotted lines with arrowheads denote molecular associations. The sarcoglycan complex interacts with the dystroglycan complex, but whether it binds to α-dystroglycan, β-dystroglycan, or both is not known (this uncertainty is not shown). The cytoplasmic domain of β-dystroglycan interacts with dystrophin and Grb2 [4,5••,33••,36•]. The carboxy-terminal 15 amino acids of β-dystroglycan constitute the dystrophin-binding site [5••]. N, amino terminus; C, carboxyl terminus.

results suggest that α-dystroglycan binding to extracellular ligands might be modulated by GAG chains present on other proteoglycans or on dystroglycan itself.

Dystroglycan is likely to interact with the sarcoglycans—fellow transmembrane components of the DGC. Two groups independently showed that the DGC can be separated into three subcomplexes which include the dystroglycan complex and the sarcoglycan complex [12,26]. The sarcoglycan complex is composed of what are now known as α-, β- and γ-sarcoglycan [12,26]. The nature of the interaction between the dystroglycan and sarcoglycan complexes is not yet known. However, we can offer some speculation based on recent results. Mutations in each of the sarcoglycans result in distinct forms of limb-girdle muscular dystrophy [27–30]. Mutational inactivation of one sarcoglycan results in the concomitant absence of the other sarcoglycans from the sarcolemma. Although, by immunostaining, dystroglycan appears to be present in the sarcolemma of biopsy specimens taken from patients with limb-girdle muscular dystrophies, it might not be entirely functional there. As the dystroglycan and sarcoglycan complexes are tightly associated within the DGC—it takes relatively harsh biochemical conditions to separate them—it seems reasonable to propose that loss of the sarcoglycan complex could have effects on the dystroglycan complex. In support of this notion, earlier studies in the cardiomyopathic hamster suggest that an intact sarcoglycan complex is required for the stable presence of α-dystroglycan in the sarcolemma [31].

A better understanding of the structure–function relationship between β-dystroglycan and its intracellular binding partners now exists. Experience with Duchenne muscular dystrophy patients indicated that mutations in the carboxy-terminal domain of dystrophin correlated with severe forms of the disease [32]. Recently, a series of *in vitro* experiments has identified a β-dystroglycan-binding site on dystrophin in the carboxy-terminal domain of dystrophin between amino acids 3054 and 3447 [4,5••]. These data fit nicely with experimental evidence obtained *in vivo*. Rafael *et al.* [33••] found that this same region on dystrophin is critical for both restoration of DGC localization to the sarcolemma and rescue of the dystrophic phenotype in dystrophin-deficient mice. Interestingly, the region of dystrophin that contains the β-dystroglycan-binding site also contains a WW/WWP domain [34]. This motif probably mediates the interaction between dystrophin and its proline-rich binding site on β-dystroglycan [5••,35]. In fact, the entire cytoplasmic domain of β-dystroglycan contains many proline residues (see Fig. 1). This led Yang *et al.* [36•] to test whether this domain contained an Src homology (SH)3-binding site. They found that Grb2, an SH2/SH3 adapter protein, bound to the cytoplasmic domain of β-dystroglycan. It is still too early to judge the significance of this finding, but it could be the first tantalizing evidence that dystroglycan is capable of directly mediating signal transduction events.

What is the role of dystroglycan in muscular dystrophy? Although mutations in a growing number of molecules that contact or surround dystroglycan in the DGC lead to muscular dystrophy, to date no form of muscular dystrophy has been linked to dystroglycan itself. In Duchenne muscular dystrophy, loss of dystrophin leads to a reduction in the amount of dystroglycan present in the sarcolemma [2,3,37–39]. Similar findings have now been extended to a study of toxin-induced muscle degeneration/regeneration [40]. As mentioned above, in

limb-girdle muscular dystrophies there may also be a loss of dystroglycan function in the sarcolemma. Moreover, in one form of human congenital muscular dystrophy and a mouse model for this disease, there is a deficiency of laminin-2, an extracellular matrix ligand for dystroglycan [41–43]. Therefore, the presence of dystroglycan in the sarcolemma and its interaction with laminin seem critical for muscle fiber viability. This interaction could form a structural link to stabilize the sarcolemma, as has been suggested [16]. In fact, it is probably necessary to maintain this linkage from the ECM all the way to the actin cytoskeleton [44,45]. Alternatively, or in addition to, a structural role, dystroglycan might mediate some sort of cell survival signal that depends on cell–ECM interaction. Impairment of this type of signal could lead to cell death as it does in other systems (see [46] for an example). The latter hypothesis would take on extra significance if a true signaling capacity is established for dystroglycan.

Function at the neuromuscular junction

A spate of papers in mid-1994 suggested the exciting possibility that dystroglycan plays an essential role in synapse formation at the NMJ [7–10]. As this early work has been reviewed extensively elsewhere [47,48], we will focus here on several subsequent developments. There is now general agreement that α-dystroglycan is an abundant agrin receptor at the NMJ. However, dystroglycan binds to inactive muscle agrin isoforms as well as, or better than, it binds to active neural agrin isoforms [10,24••,49•]. Moreover, in a system that models NMJ formation, the domain of agrin that mediates dystroglycan binding (the laminin-like G1 domain) is physically separable from the part that induces acetylcholine receptor (AChR) clusters in cultured myotubes [24••,50•]. These results substantially weaken the case for dystroglycan as the agrin signaling receptor. Now, the identity of that protein may be known. The muscle-specific kinase (MuSK) seems to have the wherewithal to be the signal transducing component of the agrin receptor [51,52,53••]. However, it apparently lacks the ability to bind agrin by itself [53••]. Analogies of this situation exist in other systems where accessory proteins are necessary for ligand binding or presentation to the signaling receptor. This idea and other data led Glass *et al.* [53••] to posit a myotube-associated specificity component (MASC) that binds agrin and MuSK into a receptor complex. As α-dystroglycan is an agrin-binding protein at the NMJ, could it be MASC? The answer is probably not. One issue is the myotube specificity of MASC. Dystroglycan is known to be expressed in a wide variety of cell types, although it might be more abundant on the surface of myotubes than it is on myoblasts [54]. It is also possible that dystroglycan is modified to a MASC-competent form during muscle differentiation. Perhaps the best evidence against dystroglycan being MASC comes from Gesemann *et al.* [24••] who showed that an agrin fragment which lacked the high-affinity α-dystroglycan-binding region still bound to myotube membranes. A definitive identification of MASC is awaited.

Where, then, is dystroglycan's seat at the NMJ table? It seems reasonable to suggest a structural role in mediating the connection between the postsynaptic membrane and the basal lamina. Perhaps it forms a stable ternary complex, via agrin, with the signaling receptor complex. Other evidence that dystroglycan plays a structural role in AChR cluster formation comes from inside the cell. Apel *et al.* [55•] showed that dystroglycan codistributes with rapsyn-induced AChR clusters in quail fibroblasts. Although details of this interaction are missing, rapsyn could be the AChR's link, through dystroglycan, to the cytoskeleton — mice lacking rapsyn fail to form postsynaptic clusters of AChR and dystroglycan [56•]. Such a molecular assembly could be the scaffold upon which AChR clusters are built. This idea is in accord with the findings of Cohen *et al.* [57•] and others [58,59] who propose a diffusion trap model for AChR clustering.

Functions in nonmuscle cells

As mentioned, dystroglycan is expressed in a variety of nonmuscle cell types. By way of contrast to muscle, far less is known about dystroglycan function in other tissues. A key question that arises is whether a DGC-like complex forms in other cells too. α-dystroglycan probably binds to the G domains of laminins-1 and -2 and agrin [24••,50•,60]. This raises the possibility that it interacts with other G-domain-containing proteins such as other laminin isoforms and the neurexins. Many of these potential ligands for α-dystroglycan are codistributed in the basement membranes of various tissues. *In vitro*, laminin-1 and -2 can compete with agrin for binding to α-dystroglycan [9,22•], arguing that they bind to a similar site. As a carbohydrate moiety of α-dystroglycan may be involved in binding to its extracellular ligands [17,18], there is the suggestion of multivalent binding to these ligands. β-dystroglycan also seems capable of interacting with a number of binding partners. Its binding site on dystrophin is present in alternatively spliced isoforms of dystrophin that are expressed in nonmuscle tissues, and is well conserved in utrophin, a widely expressed dystrophin homolog. Experiments show that dystroglycan is capable of interacting with dystrophin and its isoforms present in brain extracts [5••], and utrophin cofractionates with dystroglycan from several cell types [61,62]. Northern analysis indicates that the sarcoglycans, which are predominantly expressed in skeletal and cardiac muscle, might also be expressed at lower levels in other tissues [27–30]. Given these initial results and the extensively overlapping distribution of dystroglycan and its binding partners, it seems likely that different types of dystroglycan complexes can form in different tissues. Furthermore, different types of dystroglycan complexes may form within the same tissue. For instance, in the NMJ utrophin might replace dystrophin at the crests of the junctional folds [63,64].

One nonmuscle tissue in which dystroglycan may be playing an important role is the kidney. Durbeej *et al.* [65•]

showed that antibody perturbation of the dystroglycan–laminin interaction disrupts kidney epithelial morphogenesis (Ekblom, this issue, pp 700–706). Neural tissue is another rich source of dystroglycan. Recent work has described the localization of dystroglycan mRNA and protein in various regions and cell types within the central nervous system [13,14•,66]. The function of dystroglycan in the brain is not yet clear, although dystroglycan does localize to the glial–vascular interface, suggesting a role in maintenance of the blood–brain barrier ([66]; M Jucker, personal communication). In peripheral nerves, dystroglycan is a laminin-binding protein localized to the Schwann cell outer membrane [18,21,22•,67]. In the *dy* mouse—a model for congenital muscular dystrophy in humans that shows a deficiency in laminin α2 chain expression—there are defects in peripheral nerve myelination, implicating dystroglycan in myelinogenesis [68].

With the diverse tissue distribution of dystroglycan in adult organisms apparent, Schofield *et al.* [69] examined dystroglycan expression during mouse embryogenesis. They found that dystroglycan mRNA is expressed throughout the embryo as early as embryonic day 9.5. Evidence for dystroglycan expression at earlier embryonic stages comes from the dystroglycan knockout mice which die at around embryonic day 6.5, long before any muscle development occurs (R Williamson *et al.*, unpublished data). This phenotype indicates that dystroglycan plays important roles in developmental processes and may explain why dystroglycan loss-of-function mutations have not been identified in muscular dystrophies.

Conclusions

Although initially discovered in muscle, dystroglycan can generally be regarded as a distinct type of ECM receptor that is present in many, if not most, cell types. It will continue to be instructive to compare and contrast dystroglycan with other ECM receptors such as the integrins. A clear goal of future studies will be to further define dystroglycan's molecular interactions in muscle and other tissues. Among the key questions to be addressed are: does α-dystroglycan interact selectively or promiscuously with extracellular ligands that might be codistributed in the ECM? Could specificity for these interactions be determined by modifications to dystroglycan itself or by its association with other proteins such as the sarcoglycans? It stands to reason that distinct dystroglycan complexes could have distinct functions.

What are the functions of dystroglycan? Its apparent central involvement in a variety of muscular dystrophies argues in favor of a role for it in the maintenance of cellular integrity. Perhaps dystroglycan accomplishes this simply by being a molecular link between the ECM and the cytoskeleton. A more subtle variation on this theme is that dystroglycan acts as a hitching post for the organization of other molecules, both inside and outside of the cell. This ability of dystroglycan may be important for its role in the establishment and maintenance of complex molecular assemblies like the DGC and the NMJ. The ability of dystroglycan to spatially organize other molecules might also reflect a signal transduction capacity. Future efforts will be aimed at understanding these and other possible roles for dystroglycan in adult and developing organisms.

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