Dystroglycan inside and out
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Dystroglycan connects the extracellular matrix and cytoskeleton. Key findings in the past year indicate that dystroglycan interacts with a wider repertoire of extracellular ligands than originally appreciated, that dystroglycan plays a critical role in organizing extracellular matrix molecules on the cell surface and in basement membranes, and that at least two human pathogens utilize dystroglycan to gain access to host cells. Together, these advances begin to help elucidate important biological roles for dystroglycan in development and disease.

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Abbreviations
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AChR	acetylcholine receptor
CNS	central nervous system
HSPG	heparan sulphate proteoglycan
LG domain	laminin globular domain
MuSK	muscle-specific kinase

Introduction
Dystroglycan was originally isolated from skeletal muscle as an integral membrane component of the dystrophin–glycoprotein complex [1]. When the gene encoding dystroglycan was cloned in 1992, expression-pattern analysis suggested roles for dystroglycan beyond muscle [2]. Indeed, it is now apparent that dystroglycan does have important roles outside of muscle, which cover a broad range of biological inquiry. Generally, dystroglycan provides a transmembrane linkage between the extracellular matrix and the cytoskeleton [3]. In this review, we focus on advances made in the past year exploring the molecular associations of dystroglycan both inside and outside of the cell (see a guide to these in Figure 1); how this information has helped further elucidate dystroglycan function; and how understanding dystroglycan function might lead to a deeper understanding of several human diseases.

Interaction of β-dystroglycan with molecules inside the cell
To date, only one highly conserved dystroglycan gene has been discovered in mammals and several dystroglycan orthologues have emerged from invertebrate databases ([2]; S Baumgartner, J Kramer, personal communication). This gene, which encodes a single mRNA species, is post-translationally cleaved into α and β polypeptide subunits

present in mature dystroglycan — a processing event that still remains largely undefined. α-dystroglycan is an extracellular peripheral membrane glycoprotein, bristling with O-linked carbohydrates, which is noncovalently attached to the membrane-spanning β-dystroglycan [4]. β-dystroglycan, in turn, binds to a variety of cytoplasmic molecules. The best understood examples are its interactions with dystrophin and related molecules. Within the context of the dystrophin–glycoprotein complex in skeletal muscle, the carboxy-terminal 15 amino-acid residues of β-dystroglycan bind tightly to a region of dystrophin containing WW (Trp–Trp) domain and Ca^{2+}-binding motifs [5,6].

Figure 1

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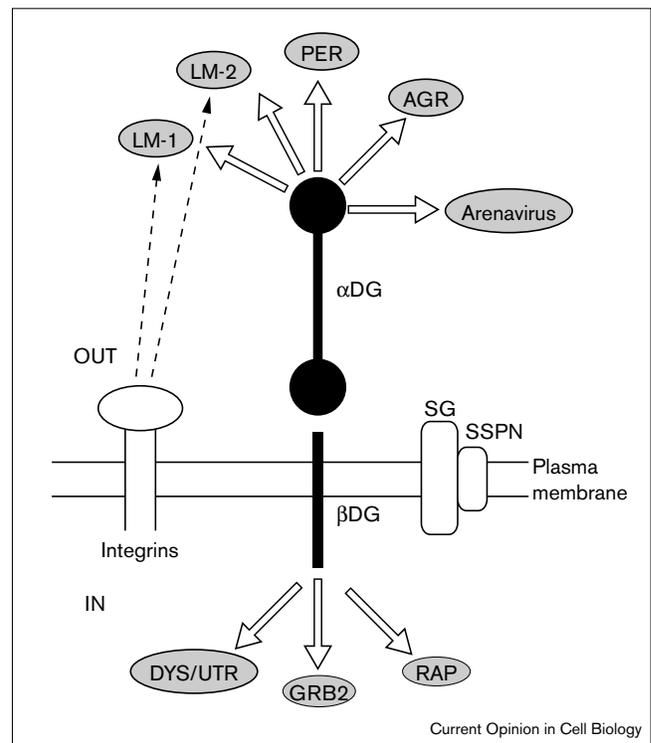
A guide to the dystroglycan intermolecular interactions discussed in this review. Outside of the cell (OUT) α-dystroglycan (block dumbbell) binds directly to the following extracellular matrix molecules: laminin-1 (LM-1), laminin-2 (LM-2), perlecan (PER), and agrin (AGR). Several arenaviruses also bind directly to α-dystroglycan. Inside of the cell (IN), the cytoplasmic domain of β-dystroglycan (black rectangle) binds to cytoskeletal molecules including members of the dystrophin family (DYS) and perhaps utrophin (UTR), the signaling adapter molecule grb2 (GRB2), and rapsyn (RAP), which is involved in the formation of the neuromuscular junction. Other transmembrane molecules might interact with dystroglycan. Evidence indicates that the sarcoglycan complex (SG) and perhaps sarcospan (SSPN) help to stabilize the association of α-dystroglycan with the cell surface. Integrins are not yet known to interact directly with dystroglycan, but they do bind (dotted arrows) to members of the laminin family and can be co-localized with dystroglycan in certain cell types.

Outside of muscle and brain, full-length dystrophin is not expressed but alternative products from the dystrophin locus containing the dystroglycan interaction domain are; as is utrophin, which bears a conserved dystroglycan interaction domain. Saito *et al.* [7•] has provided some insight to this linkage in Schwann cells. In those cells, Dp116, a truncated version of dystrophin, interacts with β-dystroglycan. Along with Dp71 [5], Dp116 appears to be an important dystrophin-isoform-binding partner for dystroglycan in the central nervous system (CNS) [8]. Compared with dystrophin from skeletal muscle, the connection between Dp116 and β-dystroglycan is apparently more tenuous. These authors speculate that this could be due to the lack of a detectable sarcoglycan complex in Schwann cells. It was previously suggested that the sarcoglycan complex would serve to stabilize dystroglycan associations by studies showing destabilization of dystroglycan interactions in skeletal muscle of sarcoglycan-deficient animal models [9,10]. How sarcoglycans stabilize dystroglycan binding interactions is not known, but recent evidence suggests that δ-sarcoglycan is in close molecular proximity to α–β-dystroglycan [11]. Furthermore, how dystroglycan function might be affected by sarcoglycans or isoforms of dystrophin (and vice versa) is unknown, but the tissue specific expression patterns of these molecules vis-à-vis dystroglycan suggests a possible regulatory role. Clearly, defining the makeup of dystroglycan complexes in different tissues is a critical task.

Other molecules that bind to the cytoplasmic tail of β-dystroglycan include Grb2 and rapsyn. In the case of Grb2, this interaction has been known for some time but its biological significance has remained elusive [12]. Cavaldesi *et al.* [13] has recently shown that a complex isolated from bovine brain synaptosomes by laminin chromatography contained dystroglycan, Grb2 and focal adhesion kinase. Although it is not yet clear whether focal adhesion kinase and dystroglycan are connected through Grb2 — possibly through Src-homology (SH)2 and SH3 mediated interactions respectively — this result, nevertheless, further fuels speculation that dystroglycan could be involved in signal transduction pathways.

Rapsyn plays an important role in clustering acetylcholine receptors (AChRs) during the formation of the neuromuscular synapse [14]. It is now apparent in electrocytes of *Torpedo californica* that rapsyn binds directly to the juxtamembrane portion of the cytoplasmic domain, a site distinct from the dystrophin binding site [15•]. This interaction may serve to consolidate or stabilize AchR clusters [16]. Initially, because of dystroglycan's interaction with agrin, rapsyn was thought to play an instructive role in AChR clustering [17,18]. Subsequently though, those initial hypotheses were modified to include other roles for dystroglycan in this process. Indeed, it now seems unlikely that dystroglycan participates in the initial signaling events necessary for AChR clustering through the agrin–MuSK (muscle spindle kinase) pathway [19]. Dystroglycan antisense-inhibited myotubes, though defective in AChR cluster formation, show normal MuSK and AChR phosphorylation patterns [20]. Further suggestion of a maintenance role for dystroglycan in AChR clustering is provided by the observation that following axotomy (severing a nerve), dystroglycan immunoreactivity disappears before that of the AChR clusters as the synapses fall apart [21].

A growing list of extracellular ligands for dystroglycan

α-dystroglycan was originally shown to bind laminin-1 [2]. This high affinity interaction occurs through the carboxy-terminal region of the laminin α1 polypeptide — the so-called E3 domain [22]. This domain contains two ~190 amino acid laminin G (LG) modules (α1LG4 and α1LG5). Andac *et al.* [23••] showed that chicken lung α-dystroglycan preferentially interacts with the α1LG4 module. This same module demonstrated a heparin-binding site consistent with heparin inhibition of laminin-1 binding [24]. Point mutations revealed that a series of basic residues mediated dystroglycan binding and the heparin site constituted a subset of these residues. Surprisingly, Talts *et al.* [25••] has shown that α-dystroglycan actually exhibits slightly greater affinity to the LG1–3 modules of the laminin α2 chain than its LG4–5 modules. These homologous modules would be present in the E8 fragment of laminin-1, which does not appreciably bind to α-dystroglycan [22]. Moreover, unlike the α1 chain, the α2LG5 domain bound heparin more strongly than the α2LG4 domain. Of course, one should be cautious in using the activities of recombinant expressed fragments and proteolytic cleavage products of laminins to draw firm conclusions about the binding sites in the native protein. Nevertheless, a picture is emerging from this work and earlier studies [19] that not all LG domains are created equal with respect to their interactions with α-dystroglycan, and that co-operative interactions among LG modules might be required for expression of full dystroglycan binding activity. This could have important implications for future work aimed at determining whether dystroglycan interacts with other proteins containing LG domains, even other members of the laminin family.

An example of an LG-domain-containing protein that binds to dystroglycan is perlecan. Perlecan is a heparansulphate proteoglycan (HSPG) found ubiquitously throughout basement membranes in tissues. It was demonstrated earlier that binding occurred between α-dystroglycan and agrin, an HSPG involved in the formation of the neuromuscular junction [17,18]. Peng *et al.* [26•] noticed that perlecan co-clustered with dystroglycan and the AChR on the surface of muscle cells and that perlecan could be co-immunoprecipitated with anti-dystroglycan antibodies. Talts *et al.* [25••] then showed direct binding between dystroglycan and the LG domains in perlecan. Somewhat like laminin-2, perlecan binding was Ca^{2+}-dependent but only modestly sensitive to heparin treatment. Perlecan and laminins-1/2 share overlapping

binding sites on α-dystroglycan. In their assays, however, Talts and colleagues found that the affinity of the recombinant G-domain fragment of perlecan for α-dystroglycan exceeded that of α1LG4–5 by a considerable degree. This raises the intriguing possibility that perlecan might outcompete laminin binding to dystroglycan *in vivo*. The biological significance of the dystroglycan–perlecan interaction is still unclear. One role may be for dystroglycan, through perlecan, to anchor acetylcholinesterase at the neuromuscular junction [27]; however, given the broad distribution of both dystroglycan and perlecan around muscle fibers, more biological information will be necessary to specify this function.

Together these data show that α-dystroglycan is capable of binding to LG modules in the context of a number of extracellular matrix molecules. Many of these proteins are co-distributed with dystroglycan in various tissues. Although these G-domain proteins can compete with one another for binding to α-dystroglycan, suggesting they utilize a similar binding site, the valency of these interactions is unclear. This raises the question of whether, steric environment permitting, dystroglycan might, look like a Christmas tree ornamented with different matrix proteins or whether mechanisms permit selectivity among the mix of potential ligands. Differential glycosylation of α-dystroglycan could, in principle, specify ligand affinity, although to date no clear evidence for this has emerged. McDearmon and colleagues [28••] present an alternative hypothesis. Following up on previous work, they show that the binding of dystroglycan to laminin purified directly from skeletal muscle (laminin-2) is insensitive to heparin inhibition. This contrasts with the heparin-sensitive dystroglycan binding of laminin-1 [24]. Interestingly, laminin purified from the muscle of a mouse with a genetic deficiency in laminin-2 exhibited heparin-sensitive dystroglycan binding. In this case, the laminin in question is not laminin-1, but probably laminin-8. This underscores the necessity to clearly define dystroglycan's interaction profile with the entire laminin family. These results are also in concordance with those described above indicating that the heparin binding sites on the laminin-1 and laminin-2 G-domains are distinct. So HSPGs, like agrin and perlecan, might act to influence their own binding to α-dystroglycan or to that of other extracellular matrix ligands perhaps conferring critical biological specificity to these interactions.

Dystroglycan and assembly of the extracellular matrix

What is dystroglycan doing in its associations with the diverse set of extracellular matrix proteins mentioned above? One possibility is that dystroglycan could act as an adhesion molecule to anchor cells to the extracellular matrix. Surprisingly, little evidence has emerged to support this idea; however, Shimizu *et al.* [29•] now report that dystroglycan could mediate endothelial cell adhesion to laminin-1. Paradoxically, both this study and another

demonstrating cell adhesive properties of dystroglycan [30] also showed that a fraction of α-dystroglycan is shed into the culture medium and not retained on the cell surface. On the face of it, this would seem rather curious behavior for a cell adhesion molecule but could also reflect some sort of mechanism for regulating cell adhesion. This study also showed that a bacterially expressed recombinant fragment of the laminin α5 chain, expected to be the native ligand for dystroglycan in endothelial cells, bound to α-dystroglycan in blot overlay assays. In marked contrast, Ferletta and Ekblom [31•] show that dystroglycan-function-blocking antibodies do not perturb the adhesion of two different epithelial cell lines to purified laminin-10/11 containing the laminin α5 subunit. Thus, dystroglycan's ability to serve as an adhesion receptor to individual matrix proteins could display important cell-type specificity.

Recent studies have also indicated other roles for dystroglycan's interactions with extracellular matrix proteins. The phenotype of the dystroglycan-null mouse indicated that dystroglycan is required for the formation of a particular basement membrane — Reichert's membrane — during early embryonic development [32]. Subsequently, we showed that dystroglycan is required for the formation of the sub-endodermal basement membrane in embryoid bodies [33••]. In this same study, we employed genetically defined lines of embryonic stem cells to show that dystroglycan is required for the organization of laminin-1 on the cell surface, indicating that this cellular activity of dystroglycan might underlie its requirement for basement membrane assembly at the tissue level of organization.

At least two important questions have emerged from this study. First, 'Does dystroglycan play a general role in basement membrane assembly in all of the tissues in which it is found?'. Second, 'What is the mechanistic relationship between dystroglycan's cell surface matrix organizing capacity and basement membrane assembly?'. With reference to the first question, dystroglycan could provide an attractive means for specifying where and when basement membranes are formed out of secreted extracellular matrix proteins in developing tissues. Dystroglycan is certainly expressed in many cell types associated with basement membranes in developing and adult tissues [34]; moreover, previous studies implicated dystroglycan in kidney epithelial morphogenesis, a phenotype that could be consistent with a role for dystroglycan in basement membrane assembly [35]. Another basement membrane between the visceral endoderm and ectoderm appears to persist in dystroglycan-null embryos, however, perhaps arguing against a general role for dystroglycan in basement membrane assembly [32]. It should also be noted that in both Reichert's membrane and the sub-endodermal basement membrane of embryoid bodies, laminin-1 is the predominant, if not exclusive, laminin isoform present (M Henry, unpublished data), whereas other basement membranes have different and complex mixtures of laminin hererotrimers.

With reference to the second question it has previously been established that dystroglycan is required for the clustering of agrin [17,18], laminin-1 [36] and perhaps perlecan on the surfaces of skeletal muscle cells. This has traditionally been interpreted as being a reflection of dystroglycan's role in the formation of the neuromuscular junction as AChRs are often found as components of those clusters. The dystroglycan mediated clustering of these matrix proteins, however, might be more reflective of a general matrix assembly role for dystroglycan, which occurs in a specialized form at the neuromuscular junction. Colognato *et al.* [37••] have shown that indeed dystroglycan can mediate the formation of a receptor–cytoskeleton network that is broadly distributed over the entire surface of the muscle cell suggesting that this cytoskeleton–receptor–matrix configuration is more similar to the extrasynaptic membrane/basal lamina than the neuromuscular junction. They show that both dystroglycan binding and laminin polymerization are required for the formation of this network. Dystroglycan might simply serve to increase the local concentration of laminin at the cell surface, thus facilitating the laminin self-assembly reaction, and/or it might bind laminin-1 and elicit changes within the cell that are important for network formation. Colognato *et al.* also show that the actin cytoskeleton and tyrosine phosphorylation are required for network formation.

Dissecting dystroglycan-mediated extracellular matrix assembly mechanisms in embryonic stem cells and myotubes is just beginning. Understanding how a large number of different matrix proteins, some of which are capable of binding to dystroglycan, come together to form structurally and functionally diverse basement membranes will undoubtedly be a complex affair. This process is very likely to involve other cell surface matrix receptors such as the integrins [37••]. Indeed, integrins have already demonstrated their ability to assemble the extracellular matrix, particularly fibronectin fibrils [38,39]. The α3 integrin subunit and the β1 integrin family have also been implicated in the formation of the laminin-based matrix in basement membranes [40–42]; therefore it will be important to understand how dystroglycan and integrins might conspire in parallel and complementary pathways of extracellular matrix assembly.

Dystroglycan and diseases

Dystroglycan has recently emerged as an important mediator of microbial pathogenesis. Following on previous observations that *Mycobacterium leprae* bound to laminin-2, Rambukkana and colleagues [43,44••] have shown that the bacterium binds to α-dystroglycan within the context of the laminin α2 domains. This might help explain how these bacteria gain entry into Schwann cells and cause the debilitating symptoms of leprosy. Bacteria are not the only pathogens that have taken advantage of dystroglycan. Cao *et al.* [45••] showed that certain members of the arenavirus family bind directly α-dystroglycan and that this event is required for infection. This study has tremendous impor-

tance for understanding how several fatal hemorrhagic fevers, like Lassa fever, can rapidly attack many different tissues in its victims.

Dystroglycan has long been associated with muscular dystrophies, but its precise role in those diseases has remained difficult to understand. Conventional wisdom is that dystroglycan, within the context of the dystrophin–glycoprotein complex, connects the extracellular matrix and cortical cytoskeleton of muscle cells in a way that is important for protecting the integrity of the sarcolemma from shear forces exerted by muscle usage [4]. Some evidence has emerged in support of this model [46,47]. Now, Williams and Bloch [48] have shown that in dystrophin-deficient muscle, there are abnormalities in the distribution of cortical membrane–cytoskeletal molecules, including β-dystroglycan, that could translate into mechanical deficiencies in the sarcolemma.

In some forms of muscular dystrophy, mutations in the genes encoding dystrophin (Duchenne and Becker muscular dystrophy) or sarcoglycan (limb girdle muscular dystrophies type 2) can lead to a secondary decrease in dystroglycan from the sarcolemma [1,2,9,10,49,50] and also in brain [51]. This could have qualitative (dystroglycan is missing key interactions with other proteins) or quantitative (a reduced amount of dystroglycan) consequences for dystroglycan function. Brown and colleagues [52•] present evidence that supports an alternative hypothesis for dystroglycan's role in muscular dystrophy. Antagonizing dystroglycan function with inhibitory antibodies in primary muscle cultures results in myotubes that are decreased in size, show myofibril disorganization, and a loss of spontaneous contractile activity compared to controls. They interpret these findings to suggest that dystroglycan may participate with laminin in a signaling pathway important for skeletal muscle maturation and maintenance. This finding is interesting in the light of earlier studies showing that laminin-2 promoted trophic effects critical for myotube survival [53].

Yet another possibility for dystroglycan's role in muscular dystrophy has been suggested by the recent studies described above demonstrating its role in extracellular matrix organization. Reduction of dystroglycan in skeletal muscle could result in perturbations in matrix organization in a way that promotes disease. So far, there is only limited evidence for defective matrix organization in Duchenne dystrophy [54], although it has been difficult to determine to what extent these changes reflect the ongoing regenerative processes in dystrophic muscle; however, *dy* mice, with a mutation in the laminin α2 gene encoding dystroglycan's ligand, show gross abnormalities in muscle basement membrane organization [55]. Perhaps then, derangement of the extracellular matrix, either by a direct mutational event in a matrix protein or indirectly by loss of a cell surface receptor critical for appropriate matrix organization, could substantially contribute to muscular dystrophy.

Conclusions

This has been an exciting year for dystroglycan. The elucidation of its role in organizing the extracellular matrix sets the stage for many more experiments to understand the mechanism of this process. Tissue-specific disruption of dystroglycan function should begin to test whether dystroglycan has a general role in matrix organization in the tissues in which it is found. If so, understanding this role of dystroglycan could have important implications for better understanding tissue morphogenesis and could be relevant to efforts aimed at engineering tissues *in vitro*. It will also be fascinating to learn more about how pathogens utilize dystroglycan and whether dystroglycan would prove to be a useful target for therapeutic intervention in leprosy and certain viral hemorrhagic fevers.

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