Dystrophin and the membrane skeleton

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Recent studies have confirmed several predictions concerning the structure and possible function of dystrophin, including a direct interaction with F-actin and an indirect interaction with laminin via linkage through a transmembrane protein complex. The results of the past year support a role for dystrophin in linking the actin cytoskeleton with the extracellular matrix in striated muscle, but they have not explained its function in other tissues.

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Introduction

In the process of dissecting Duchenne muscular dystrophy, scientists have identified novel constituents of the membrane cytoskeleton which are vital to normal cell function. Identification and characterization of the Duchenne muscular dystrophy gene has rapidly led to understanding how defects in the gene correlate with the absence or abnormality of the protein product, named dystrophin (reviewed in [1]). These findings have led to intense efforts to develop gene transfer therapies as a method of dystrophin replacement in dystrophic muscle [2••,3••]. However, until recently, speculation on the function of dystrophin has largely been based on its predicted primary structure as expressed in skeletal muscle. Here, we review recent studies of dystrophin which examined its structure and cellular location in various tissues as well as its interactions with other cell constituents. These data reasonably support a role for skeletal muscle dystrophin in stabilizing the sarcolemmal membrane. They further suggest that, like ankyrin [4], different domains of dystrophin may serve as modules for differential protein recognition, to allow modification of dystrophin function in non-muscle tissues.

The structure of dystrophin

Dystrophin is a large protein of 427 kD. Based on its deduced primary structure, dystrophin was originally predicted to consist of four distinct regions, dominated by a large rod-shaped domain composed of 24 spectrin-like repeats with an overall length of 125 nm [5]. The large rod-shaped domain of dystrophin is flanked on its amino terminus by 240 amino acids with high homology to the actin-binding domains of α-actinin, spectrin and Dictyostelium actin binding protein 120 [5–7]. Immediately carboxyl-terminal to the rod-shaped domain of dystrophin is a cysteine-rich region with significant homology to a domain of Dictyostelium α-actinin that contains two Ca\(^{2+}\)-binding sites. However, this putative Ca\(^{2+}\)-binding domain is thought to be non-functional in skeletal muscle dystrophin [1]. The last carboxyl-terminal 420 amino acids comprise the fourth distinct domain of dystrophin and exhibit no homology with any known sequence.

Recent characterization of the Duchenne muscular dystrophy gene has revealed a number of dystrophin isoforms which differ from the structure described above. In addition to alternatively spliced carboxyl-terminal isoforms [8], distinct isoforms of dystrophin lacking the amino-terminal and large rod-shaped domains have been reported [9••,10••,11]. These novel dystrophin isoforms are expressed at high levels in some non-muscle tissues [10••,11,12] in contrast to full-length dystrophin, which is expressed predominantly in muscle and at much lower levels in brain [1]. An autosomal homologue of the Duchenne muscular dystrophy gene has also been identified [13]. Information available thus far indicates that the autosomal gene product, known as dystrophin-related protein [14], is 80% homologous with the cysteine-rich and carboxyl-terminal domains of skeletal muscle dystrophin [13]. Dystrophin-related protein is of similar size to dystrophin [14,15] and ubiquitously expressed [16]. Taken together, these results suggest that dystrophin is a diverse family of proteins expressed in all tissues.

The cellular location of dystrophin

Numerous immunocytochemical studies have localized skeletal muscle dystrophin to the cytoplasmic face of the sarcolemma, including the neuromuscular and myotendinous junctions [17••,18••]. In comparison, with other sarcolemmal proteins, the relative abundance of dystrophin (5%) is similar to the density of spectrin in brain membranes, suggesting that dystrophin is a major structural element of the subsarcolemmal cytoskeletal network [19•]. These results seem to imply that skeletal muscle dystrophin is involved in stabilizing the plasma membrane.
from structural distortion during contraction. However, recent immunohistochemical studies have demonstrated that the uniform distribution for dystrophin in skeletal muscle does not hold true in other tissues. In cardiac and smooth muscle, dystrophin appears to be absent from the regions of the membrane which overlay adherens junctions [17•]. The localization of dystrophin to post-synaptic membrane specializations in the mammalian central nervous system [20] and its polarized distribution in the Torpedo electric organ membrane [21•,22,25] suggest that dystrophin may fulfill different roles in non-muscle tissues. In addition, dystrophin-related protein is largely restricted to myotendinous and neuromuscular junctions in skeletal muscle [15,24,25], further demonstrating that proteins in the dystrophin family play varied roles even within the same cell.

Membrane interactions of dystrophin

Extraction of dystrophin from membranes requires detergents or treatment with strong alkali (pH 11) [19•], suggesting that its mechanism of interaction with the membrane is different than that for spectrin or α-actinin, which are readily extracted by relatively mild changes in ionic environment. Recently, it was demonstrated that skeletal muscle dystrophin is tightly associated with an oligomeric complex of six sarcolemmal proteins, four of which are glycosylated [26–28]. Further characterization of the purified dystrophin-glycoprotein complex suggested that dystrophin and a 59 kDa associated protein are cytoskeletal elements tightly linked to a highly glycosylated, 156 kDa extracellular glycoprotein through a complex of 50, 43, 35 and 25 kDa transmembrane proteins [29,30•]. Cloning of the 43 kDa transmembrane component of the dystrophin-glycoprotein complex revealed that it is part of a larger precursor polypeptide which is post-translationally processed into both the 43 kDa and the 156 kDa dystrophin-associated glycoproteins [31••]. The sequence of the 156 kDa dystrophin-associated glycoprotein, dystroglycan, suggests that the core protein is 57 kDa in size with a signal sequence but no transmembrane domain [31••]. This prediction supports the biochemical data [30••] which indicate that the 156 kDa dystrophin-associated glycoprotein is extensively glycosylated and extracellular. Although they share no sequence homology, the processing of the 43/156 dystrophin-associated glycoprotein precursor is similar to that of the human immunodeficiency virus type 1 (HIV-1) envelope protein. The HIV-1 envelope protein is translated as a 160 kDa glycoprotein and subsequently cleaved to 120 kDa (50% carbohydrate) extracellular and 41 kDa transmembrane glycoproteins [32]. Interestingly, cell necrosis as a result of HIV-1 infection involves membrane auto-fusion or the formation of giant multinucleate cells (an integral step in skeletal muscle differentiation), and both of these processes are mediated by the HIV-1 envelope proteins [32].

In support of its proposed extracellular location [30••], dystroglycan was shown to specifically bind laminin [31••], suggesting that one function of the skeletal muscle dystrophin-glycoprotein complex is to link the sarcolemmal membrane with the extracellular matrix (Fig. 1). In Duchenne muscular dystrophy, the loss of this linkage resulting from the deficiency of the dystrophin-associated proteins [33•,34•] could make muscle fibers susceptible to necrosis by rendering the sarcolemmal membrane more prone to injury [35•,36•], or by altering specific calcium regulatory mechanisms [37•]. The specific deficiency of the 50 kDa dystrophin-associated glycoprotein in skeletal muscle biopsies from patients with a form of autosomal-recessive muscular dystrophy [38•] further suggests that this linkage can also be disrupted independently of dystrophin.

The domains of dystrophin which interact with the plasma membrane and the dystrophin-associated proteins have recently been identified. The lack of significant homology with proteins of known function led to speculation that the last carboxyl-terminal 420 amino acids may be involved in the interaction of dystrophin with the sarcolemmal membrane [5]. Immunogold labeling studies [39] with an antibody against the extreme carboxyl terminus of dystrophin indicate that the carboxyl-terminal domain is closely apposed or inserted into the plasma membrane of skeletal muscle. Recently, Suzuki et al. [40•] demonstrated that a dystrophin fragment corresponding to the cysteine-rich domain and the first half of the carboxyl-terminal domain remained bound to the glycoprotein complex after limited calpain digestion of the skeletal muscle dystrophin-glycoprotein complex [40•]. Furthermore, the cysteine-rich and carboxyl-terminal domains of Torpedo electric organ dystrophin lack only the carboxyl-terminal 15 amino acids present on the predominant skeletal muscle isoform of dystrophin [21•], yet Torpedo dystrophin does not appear to associate with any glycosylated constituents [42•]. On the other hand, patients clinically diagnosed with Duchenne muscular dystrophy have recently been shown to express a truncated dystrophin lacking the cysteine-rich and carboxyl-terminal domains which properly localizes to the sarcolemmal membrane [42–44]. While the severity of the patients' clinical features illustrates the essential role of these domains for normal dystrophin function, the localization results suggest that the cysteine-rich and carboxyl-terminal domains are not solely responsible for targeting dystrophin to the sarcolemmal membrane. Implication of the four spectrin-like repeats of α-actinin in binding the β1 integrin subunit led to the suggestion that the multiple repeat domain of dystrophin may interact with the sarcolemma through an integrin [45]. While α7β1 integrin has been observed to co-distribute with dystrophin in chick embryonic myotubes [46], there is currently no evidence for a direct interaction between integrin and dystrophin. Furthermore, expression of a dystrophin construct lacking 16 of 24 multiple repeats of the rod-like domain of dystrophin results in correct localization of the truncated dystrophin to the sarcolemmal membrane [35•]. Alternatively, dystrophin may also receive localization cues through interactions of the amino-terminal domain with the membrane cytoskeleton. The latter hypothesis is attractive in light of recent
demonstrations of F-actin binding to skeletal muscle dystrophin (see below) and implication of the actin-based cytoskeleton in the establishment and maintenance of discrete domains of specific plasma membrane proteins [47].

Dystrophin as an F-actin binding protein

Until recently, the role for dystrophin as an actin-binding protein has largely been speculated from the sequence similarities of its amino-terminal domain with well characterized actin-binding proteins [5-7]. Hemmings et al. [58••] demonstrated that a chimera composed of the first 233 amino acids of dystrophin and the last 645 amino acids of smooth muscle α-actinin localized to actin-containing structures when expressed in COS cells. In addition, bacterially expressed fusion proteins corresponding to the putative actin-binding domains of dystrophin have been shown to co-sediment with F-actin [48••,49•]. More recently, the skeletal muscle dystrophin–glycoprotein complex was also observed to co-sediment with F-actin (JM Ervasti, KP Campbell, unpublished data). Nuclear magnetic resonance experiments with synthetic peptides corresponding to defined regions of the amino-terminal domain of dystrophin provide evidence for two actin-binding sites on dystrophin located at amino acids 17-26 and 128-136 [50]. While these results provide support for a direct interaction between dystrophin and actin in skeletal muscle, they raise the issue of what actin-based structures skeletal muscle dystrophin may bind to in vivo. Dystrophin does not appear to be directly associated with the myofibrillar actin filaments [51,52]. However, peripheral actin filaments emanating from the Z-lines and M-lines of skeletal muscle myofibers have recently been identified [53], while γ-actin [54] and dystrophin [18••] are two of several cytoskeletal proteins which exhibit discrete, lattice-like organizations comprising a longitudinal element and transverse elements coincident with the I bands and M-lines. The low abundance of γ-actin in adult skeletal muscle would also favor its interaction with dystrophin from a stoichiometric point of view. In addition, the recent identification of actin-related proteins in other cell types [55,56] raises the possibility for discovery of a novel actin-like protein in skeletal muscle which specifically binds dystrophin.

Is actin binding a universal function of dystrophin? Probably not in light of the observation that dystrophin accumulates in domains of the *Torpedo* electric organ membrane which are devoid of actin [23]. It is even more difficult to reconcile actin binding as a general function of dystrophin with recent reports of alternative dystrophin gene products, which completely lack the amino-terminal actin-binding domain, yet are the predominant isoforms in some non-muscle tissues [10••,12]. Perhaps the cytoskeletal interactions of dystrophin are governed by important sequence elements located in its amino-terminal and rod-like domains, and it is the variation [13,21•] or absence [5••,10••,11] of these elements which dictate the specificity of interaction between dystrophin isoforms and different cytoskeletal proteins. Such specificity of interaction could explain the observed differences in the cellular location of dystrophin [20,21•,22,23]. Once the complete sequences are available for dystrophin-related protein [13] and *Torpedo* dystrophin [21•], the amino-terminal and rod-like domains of these two proteins can be compared with skeletal muscle dystrophin, possibly to identify sequence elements which may confer cytoskeletal specificity.
Conclusions

The findings of the past year suggest that the function of dystrophin in skeletal muscle is to link the actin-based subsarcolemmal cytoskeleton with the extracellular matrix by way of a transmembrane complex of glycoproteins. While providing a plausible explanation for the (dis)function of dystrophin in skeletal muscle, research over the past year has also revealed the potential for a diverse family of dystrophins of varied function. Whatever the outcome, future studies of dystrophin and its related and associated proteins are certain to advance and enrich our basic understanding of the membrane skeleton.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest 
•• of outstanding interest


In addition to describing dystrophin distribution in mouse skeletal, cardiac and smooth muscle, this study also reports the absence of dystrophin from peripheral nerve. However, the antibodies used are directed towards epitopes missing from the dystrophin gene product lacking the amino-terminal and rod-like domains described in [9, 10, 11] that was found in nerve cells. The distict distributions for dystrophin and vinculin observed in smooth muscle would tend to argue against the direct interaction between these two proteins implied by the results presented in [18].


The first observation of a non-homogeneous distribution of dystrophin in skeletal muscle. Dystrophin, β-spectrin and vinculin are observed to co-distribute in a discrete network in skeletal muscle. Dystrophin is not necessary for the maintenance of this network because the spectrin lattice is present in dystrophin-deficient mdx mouse muscle, which can
also be taken as additional evidence that the cytoskeletal interactions of dystrophin are important in determining its cellular location.


This report demonstrates that dystrophin is sufficiently abundant in sarcolemmal membranes to support its hypothesized role as a structural element in skeletal muscle.


Demonstration that Torpedo dystrophin cDNA shows higher sequence homology with a skeletal muscle isoform of dystrophin than with dystrophin-related protein. However, Torpedo dystrophin shows a restricted localization in electric organ reminiscent of dystrophin-related protein in muscle. See [22,23].


30. Ervasti JM, Campbell KP: Membrane Organization of the Dystrophin–Glycoprotein Complex. Cell 1991, 66:1121–1131. Demonstration that the 50, 43, 35 and 25 kD dystrophin associated proteins are integral membrane proteins. Surprisingly, the 156 kD dystrophin-associated glycoprotein (dystroglycan) was demonstrated to be a peripheral membrane protein. The extensive glycosylation and protease resistance of dystroglycan led to the proposal that it was extracellular and suggested that dystrophin was in communication with the extracellular matrix. A more complete justification of the model illustrated in Fig. 1 is also presented.


Describes the cloning and sequencing of cDNA encoding the 43 kD dystrophin associated glycoprotein, which was also demonstrated to encode the core protein of dystroglycan. Both proteins are ubiquitously expressed and, in contrast to dystrophic muscle [26,33,34], their abundance in non-muscle tissues appears to be normal. This study also demonstrates that dystroglycan binds laminin in vitro.


This study extends the findings in [26] by demonstrating that the 50, 43, and 35 kD dystrophin-associated proteins are 80–90% deficient in mdx mouse skeletal muscle.


Analysis of the dystrophin–glycoprotein complex in Duchenne muscular dystrophy and a number of other neuromuscular diseases.


Presents evidence in support of a membrane-stabilizing role for dystrophin.


Describes evidence in support of the hypothesis that the absence of dystrophin lowers the threshold for work-induced injury to skeletal muscle.


The authors present evidence in support of the hypothesis that elevated intracellular Ca2+ in dystrophic muscle is the result of abnormal calcium-selective leak channels present in the sarcolemmal membrane. Reference is also made to reports describing abnormalities in mechanosensitive calcium channels which have been observed in dystrophic muscle.


The first evidence to suggest that the absence of a dystrophin associated glycoprotein as the cause of a Duchenne-like muscular dystrophy in which dystrophin is normal.


Site-specific antibodies and microsequencing are used to identify the region of dystrophin which remains associated with the glycoprotein complex after limited pronase digestion. As noted by the authors, this study does not exclude the possibility that other domains of dystrophin are also involved in membrane association.


Affinity purification of dystrophin from detergent-solubilized membranes of Torpedo electric organ resulted in the co-purification of a
58 kD peripheral membrane protein. The near-universal abundance of this protein in mice mouse skeletal muscle suggests that it is different from the 59 kD component of the dystrophin-glycoprotein complex which is reduced by 90% in dystrophic muscle.


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The authors note that the dystrophin fusion protein interacts specifically with F- and not G-actin, and estimate the dissociation constant (Kd) for dystrophin fusion protein binding to F-actin to be 44 \( \mu \)mol/L, which is about two orders of magnitude lower than the measured affinity of dimeric \( \alpha \)-actinin for F-actin. It is possible that dimeric dystrophin may bind F-actin with higher affinity or one of the dystrophin-associated proteins may modulate the interaction of dystrophin with F-actin. See [48**].


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