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Review

Dystrophin, its interactions with other proteins, and implications for muscular dystrophy

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Abstract

Duchenne muscular dystrophy is the most prevalent and severe form of human muscular dystrophy. Investigations into the molecular basis for Duchenne muscular dystrophy were greatly facilitated by seminal studies in the 1980s that identified the defective gene and its major protein product, dystrophin. Biochemical studies revealed its tight association with a multi-subunit complex, the so-named dystrophin–glycoprotein complex. Since its description, the dystrophin–glycoprotein complex has emerged as an important structural unit of muscle and also as a critical nexus for understanding a diverse array of muscular dystrophies arising from defects in several distinct genes. The dystrophin homologue utrophin can compensate at the cell/tissue level for dystrophin deficiency, but functions through distinct molecular mechanisms of protein–protein interaction.

Keywords: Dystrophin; Utrophin; Actin; Dystroglycan; Sarcoglycan; Syntrophin; Dystrobrevin; Costamere; Muscular dystrophy

1. Dystrophin

Dystrophin is the 427 kDa protein product of the gene defective in Duchenne muscular dystrophy [1,2]. Dystrophin is comprised of four major domains [2], three of which are homologous to domains present in several actin binding cytoskeletal proteins including α-actinin and β-spectrin (Fig. 1). The amino terminal domain contains a pair of calponin homology (CH) modules that together form a functional actin binding domain in dystrophin and related proteins. The largest domain of dystrophin consists of 24 triple helical spectrin like repeats interspersed with 4 putative hinge domains [3] that together are thought to give dystrophin an elongated and flexible rod shape. The third domain of dystrophin, initially named the cysteine-rich domain, encodes two EF hand-like modules [2] bounded by WW [4] and ZZ [5] modules. Finally, the carboxy-terminal domain is unique to dystrophin and its closest homologues utrophin [6] and the dystrobrevins [7]. To date, crystal structures have been solved for the tandem CH domains (ABD1) of dystrophin [8] and utrophin [9] and also for sequence encoding the WW and EF hand modules of the cysteine-rich domain of dystrophin [10], which represents less than 14% sequence coverage for the entire protein. In addition to three promoters that regulate expression of full-length dystrophin in a tissue-specific fashion, the DMD gene also contains four internal promoters that drive expression of distinct, serially truncated proteins (Fig. 1) in non-muscle tissues [11].

Dystrophin is localized to the cytoplasmic face of the muscle cell plasma membrane, or sarcolemma [12], and particularly within a cytoskeletal lattice termed costameres [13,14]. Through an extensive network of interacting proteins [15] costameres physically couple the sarcolemma with the Z disk of force-generating myofibrils (Fig. 2). The absence of dystrophin in humans and the *mdx* mouse leads to costamere disorganization [13,16–19], sarcolemmal fragility [20–24], muscle weakness [25,26] and necrosis [27]. Sarcolemmal fragility, muscle weakness and necrosis are all exacerbated by mechanical stress, improved by muscle immobilization, and corrected in the *mdx* mouse by transgenic expression of full-length dystrophin [22,23,26,28–34]. Taken together, these studies provide compelling evidence that dystrophin stabilizes the sarcolemma against mechanical forces experienced during muscle contraction or stretch.

Identification of the dystrophin domains important for its function has been elegantly advanced through the characterization

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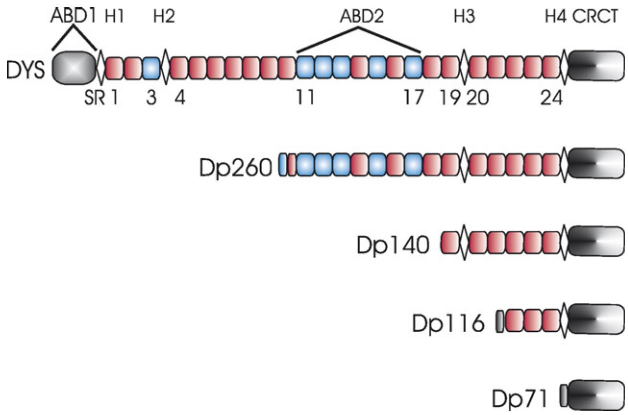

Fig. 1. Protein products of the DMD gene. Shown is a schematic diagram illustrating the domain structure of the protein isoforms encoded by the DMD gene. Dystrophin (DYS) contains an amino-terminal actin binding domain (ABD1) consisting of tandem CH domains, a spectrin-like triple-helical repeat (SR) domain with 4 putative hinge modules (H1–H4) interspersed throughout its length, a cysteine-rich (CR) domain critical for binding β-dystroglycan, and a carboxy-terminal domain (CT) important for binding syntrophins and α-dystrobrevin-2. Acidic spectrin repeats are colored red, basic repeats colored blue, and a cluster of basic repeats form a second independent actin binding domain (ABD2). Alternate promoters drive the expression of four truncated non-muscle isoforms, Dp260, Dp140, Dp116, and Dp71 each with unique amino-terminal sequences and the indicated domains in common with full-length dystrophin.

of transgenic *mdx* mice expressing dystrophin constructs bearing deletions in different domains. The severe phenotype of *mdx* mice expressing a dystrophin deleted in the cysteine-rich domain [35] suggested it is necessary for dystrophin function. Expression of Dp71 also resulted in a severe phenotype [36,37], thus indicating that the cysteine-rich domain was not sufficient for dystrophin function. Intriguingly, transgenic *mdx* mice expressing dystrophin constructs deleted for the amino-terminal tandem CH domain or carboxy-terminal domain presented with a very mild or no phenotype suggesting neither is essential for dystrophin function [38,39]. Specific deletion of the large rod domain was well tolerated to the extent that only 4 of 24 spectrin repeats were necessary to largely retain function [40]. In contrast, substitution with the 4 homologous spectrin repeats of α-actinin-2 was not tolerated [41]. Finally, co-expression of Dp71 and the cysteine-rich domain deleted construct failed to rescue the dystrophic phenotypes of *mdx* muscle [42]. These studies demonstrated that the cysteine-rich domain present in cis with either the amino-terminal domain or portions of the rod domain are minimally required for dystrophin function.

2. The dystrophin–glycoprotein complex

Shortly after identification of the DMD gene and dystrophin, it was shown that dystrophin could be dramatically enriched from detergent-solubilized skeletal muscle membranes using wheat germ agglutinin chromatography [43]. The dystrophin-enriched fraction was further purified by serial anion exchange chromatography and sucrose gradient centrifugation to identify 10 tightly associated proteins of 156 kDa, 88 kDa, a triplet of 59 kDa, 50 kDa, a doublet of 43 kDa, a singlet of 35 kDa present at a molar ratio of 2:1 relative to dystrophin, and 25 kDa [44]. The 156, 50, 43, and 35 kDa proteins were shown to be glycosylated with the 156 kDa protein so extensively glycosylated that it stained poorly with Coomassie blue [44]. Since these proteins co-localized with dystrophin at the sarcolemma, co-purified with dystrophin in stoichiometric amounts through several purification steps, and were diminished in biopsies from DMD patients and muscle of the dystrophin-deficient *mdx* mouse [44,45], it was concluded that dystrophin functioned as part of a larger, hetero-oligomeric glycoprotein complex (Fig. 2) that may serve to stabilize the sarcolemma against the repetitive stress imposed during muscle contraction. Dystrophin and its tightly associated proteins were collectively named the dystrophin–glycoprotein complex.

The genes encoding all core components of the dystrophin–glycoprotein complex have been characterized and their interactions with dystrophin and each other better defined (Fig. 2). The 156 kDa and one of the 43 kDa dystrophin-associated glycoproteins are encoded by a single transcript and the propeptide is proteolytically processed into extracellular 156 kDa and single-pass transmembrane 43 kDa subunits which remain non-covalently associated [46]. Based on the extensive glycosylation of the 156 kDa subunit [45] and tight association of both proteins with dystrophin [44,45], the 156 kDa and 43 kDa subunits were renamed α- and β-dystroglycan, respectively. Using limited proteolysis, wheat germ agglutinin chromatography and an array of site-specific dystrophin antibodies, Ozawa and colleagues demonstrated that the cysteine-rich and first half of the C-terminal domains of dystrophin were important for its binding to the glycoprotein complex [47]. By blot overlay assay, they further showed that β-dystroglycan, and the 88 kDa and 59 kDa dystrophin-associated proteins directly bound the cysteine-rich and/or C-terminal domains of dystrophin [48]. Several biochemical studies have since refined the sites of molecular contact between dystrophin and β-dystroglycan [49–52] with the most recent work demonstrating that the WW, EF hand and ZZ domains are all required for dystrophin binding to β-dystroglycan [53]. Interestingly, a DMD-causing missense mutation (C3340Y) results in loss of β-dystroglycan binding activity [53], which reinforces the importance of dystrophin/β-dystroglycan interaction in normal muscle function. While no human muscle disease has been linked with mutations in the dystroglycan gene, its protein products are clearly essential to the function of the dystrophin–glycoprotein complex because muscle-specific ablation of dystroglycan in mice causes muscular dystrophy [54,55].

Elucidation of the genes encoding isoforms of the 88 kDa and 59 kDa dystrophin-associated proteins (named dystrobrevins and syntrophins, respectively) greatly benefited from comparative investigations into the molecular composition of the mammalian neuromuscular junction and electric organ of *Torpedo californica* that preceeded the discovery of dystrophin [7,56,57]. Dystrobrevins and syntrophins are cytoplasmic proteins that bind directly to each other and to sequences within the carboxy-terminal domain of dystrophin [57]. While syntrophins are thought to function as modular adaptors that anchor ion channels and signaling molecules to the

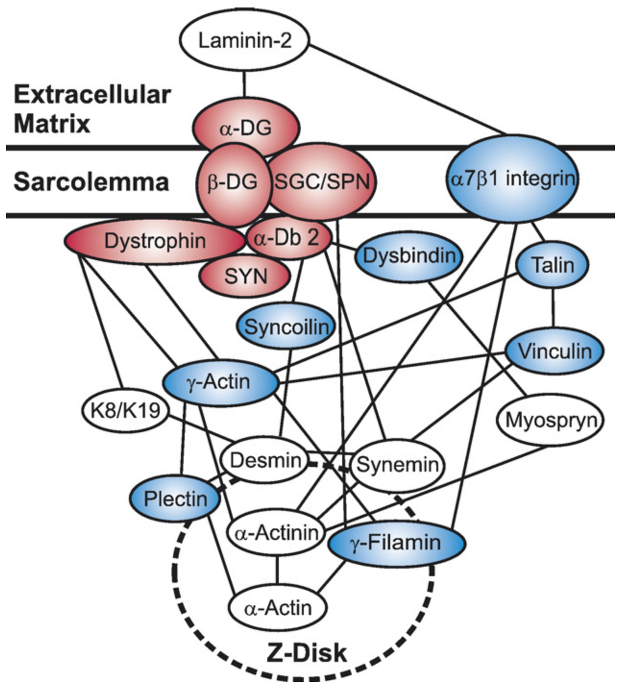

Fig. 2. The dystrophin–glycoprotein complex network. Shown in red are the constituents of the core dystrophin–glycoprotein complex, which co-purify as a highly stable complex from skeletal muscle and which show greatly decreased abundance in dystrophin-deficient muscle. α-Dystroglycan and β-dystroglycan (α-DG, β-DG); the sarcoglycan complex (SGC); sarcospan (SPN); α-dystrobrevin-2 (α-Db 2); syntrophin (SYN). Also shown are structural proteins that interact directly with components of the dystrophin–glycoprotein complex, their direct binding partners, and their location within striated muscle cells. Cytokeratins 8 and 19 (K8/K19). Proteins highlighted in blue are present at increased levels when dystrophin is absent.

dystrophin–glycoprotein complex, no myopathy is associated with syntrophin ablation in mice [58–60]. In contrast, knockout of α-dystrobrevin results in a progressive myopathy [61], suggesting an important role in dystrophin–glycoprotein complex function.

Distinct but related genes encode the 50 kDa subunit [62], the second 43 kDa protein [63], and two different 35 kDa proteins [64,65] of the dystrophin–glycoprotein complex, which were renamed α-, β-, γ- and δ-sarcoglycan. The sarcoglycans are all single pass transmembrane proteins that co-assemble into a stable tetrameric complex [66]. While its function is not fully understood, the sarcoglycan complex appears to strengthen interaction of β-dystroglycan with α-dystroglycan and dystrophin [66]. Importantly, mutations in individual sarcoglycan genes lead to loss of the entire sarcoglycan complex resulting in forms of limb-girdle muscular dystrophy in humans and progressive muscular dystrophy when knocked out in mice [66]. The 25 kDa dystrophin associated protein, named sarcospan [67], is also stably associated with the sarcoglycan complex. However, no human myopathy has been linked to mutations in the sarcospan gene and ablation of sarcospan in mice caused no muscle phenotype [68]. Finally, α-dystrobrevin has been shown to directly interact with the sarcoglycan complex [69], which raises the possibility that the myopathy accompanying α-dystrobrevin ablation may arise from destabilization of an indirect linkage between dystrophin and the sarcoglycan complex.

3. Molecular partners of the dystrophin–glycoprotein complex

A screen of known extracellular matrix molecules for skeletal muscle α-dystroglycan binding activity identified laminin as the first extracellular ligand for α-dystroglycan [46,70]. Laminin-Sepharose pull-down of the entire dystrophin complex definitively demonstrated that α-dystroglycan was a stoichiometric component of the complex [70]. Agrins, neurexins and perlecan all contain modules homologous to the α-dystroglycan binding G-domain of laminin [71], and all have been shown to bind α-dystroglycan with high affinity [72,73]. Like laminins, these proteins all bind to α-dystroglycan in a manner dependent on its oligosaccharide modifications [72,73]. In contrast, the chondroitin sulfate chains of the proteoglycan biglycan have been shown to mediate its binding to the core protein of α-dystroglycan [72,73]. While the physiologic significance of α-dystroglycan binding to such a wide variety of extracellular matrix molecules is not clear, the functional role of the dystroglycan complex may depend on which extracellular ligand is locally available. O-linked oligosaccharides of unknown structure are clearly important for α-dystroglycan binding to extracellular ligands as well as its function in vivo because mutations in glycosyltransferases that post-translationally modify α-dystroglycan result in hypoglycosylation, loss of extracellular ligand binding, and several forms of congenital muscular dystrophy in humans and mice [72,73].

As noted earlier, sequence similarity of the dystrophin amino-terminal domain with the tandem CH actin binding domains of β-spectrin and α-actinin suggested that it may bind actin filaments. Recombinant proteins encoding the first 246 amino acids of dystrophin or the first 261 amino acids of its homologue utrophin have been shown by several groups to bind actin filaments in vitro with a K_d of ~ 12 μM and 1:1 stoichiometry (summarized in [74]). Electron microscopy and image reconstruction analysis suggested substantial plasticity in the modes of actin filament binding displayed by the utrophin amino-terminal tandem CH domain [75–78]. However, all of these studies assumed that the actin binding function of dystrophin and utrophin is restricted to the amino-terminal, tandem CH domain, which exhibits 25-fold lower affinity for actin compared to purified dystrophin–glycoprotein complex [79]. Furthermore, the stoichiometry of dystrophin–glycoprotein complex binding to actin filaments (1 dystrophin/24 actin monomers) demonstrated a more extensive lateral association between dystrophin and actin than could be explained by the amino-terminal domain alone [79]. Limited proteolysis experiments led to the identification of a second actin binding site (ABD2, Fig. 1) situated in the middle third of the dystrophin rod domain [79]. Five of seven spectrin repeats in the second site were rich in basic amino acid residues (Fig. 1) and the cluster of basic repeats was shown to independently bind acidic actin filaments through electrostatic attraction [80,81]. Moreover, the dystrophin–glycoprotein complex was shown to slow depolymerization of actin filaments in vitro but neither the amino-terminal, nor middle rod domain alone or present in trans had any effect on actin filament depolymerization

[79,82]. Although separated by ~1200 amino acids, the two sites were proposed to act in concert to effect an extended lateral association that could account for the measured 1:24 stoichiometry of binding [79,82]. The redundancy of two actin binding domains also provided a molecular basis to explain why neither the amino-terminal nor middle rod domain are essential for dystrophin function [38,40], yet expression of the cysteine-rich and carboxy-terminal domains alone (and recovery of dystrophin-associated proteins) was insufficient to correct the dystrophic phenotype [36,37].

While dystrophin exhibits no preferential binding to cytoplasmic actin over sarcomeric α-actin in vitro [83], it co-immunoprecipitated with cytoplasmic γ-actin [84] even though this isoform represents only 1/4000th of the total actin expressed in adult skeletal muscle [85]. Immunofluorescence analysis of mechanically peeled sarcolemma demonstrated that dystrophin is tightly attached to the sarcolemma [14] and was closely co-localized with cytoplasmic γ-actin filaments [86]. Importantly, γ-actin filaments were absent from all sarcolemma peeled from muscle fibers of the dystrophin-deficient *mdx* mouse [86]. Transgenic expression of utrophin [87], or dystrophin constructs retaining the β-dystroglycan binding site plus either the amino-terminal [85] or middle rod actin binding domain [88] was sufficient to restore coupling between the sarcolemma and γ-actin in *mdx* muscle. These data demonstrate that dystrophin functions to mechanically anchor cytoplasmic γ-actin filaments of the cortical cytoskeleton to the sarcolemma (Fig. 2) and that utrophin can compensate in this role when dystrophin is absent.

Two-hybrid screens using α-dystrobrevin as bait have identified several novel interacting proteins [89–91]. Two of these proteins, synemin [91] and syncoilin [89,92], are structurally related to intermediate filament proteins and interact with the intermediate filament protein desmin (Fig. 2). Synemin also directly binds to α-actinin [93] and vinculin [94] to provide additional mechanical linkages between the dystrophin–glycoprotein complex and muscle cytoskeleton (Fig. 2). Mice null for either α-dystrobrevin [61] or desmin [95,96] exhibit skeletal and cardiomyopathy, which suggests that mechanical coupling of the dystrophin–glycoprotein complex to the intermediate filament cytoskeleton is necessary for normal muscle function. Curiously, the skeletal myopathies of α-dystrobrevin null and desmin null mice manifests in the absence of sarcolemmal fragility [61,95,96] and the sarcolemma of desmin null muscle is actually protected from stress-induced injury [97,98]. Reiterative two-hybrid screens with a third α-dystrobrevin binding protein, dysbindin [90], led to the identification of a novel 413 kDa muscle protein named myospryn [99]. Interestingly, the myospryn gene was recently identified as a downstream target for the MEF2A transcription factor and myospryn protein binds directly to α-actinin-2 [100]. Two hybrid screens also led to the identification of γ-filamin as a sarcoglycan interacting protein [101]. Like dystrophin, filamin contains an amino-terminal tandem CH actin binding domain, but in combination with a large number of Ig motifs instead of spectrin repeats [102]. Thus, the α-dystrobrevin/dysbindin/α-actinin-2 and sarcoglycan/γ-filamin/actin axes provide additional structural linkages between the sarcolemmal dystrophin–glycoprotein complex and myofibrillar apparatus (Fig. 2). Finally, dystrophin has been shown to co-immunoprecipitate with cytokeratins 8 and 19 [84] through a direct interaction of dystrophin's amino-terminal tandem CH domain with cytokeratin 19 [103]. Thus, many of the proteins found to interact with the dystrophin–glycoprotein complex couple it with other structural elements of muscle (Fig. 2) suggesting it plays an essential structural/mechanical role in striated muscle.

4. Costamere remodeling in dystrophin-deficient muscle

Mounting evidence suggests that dystrophin-deficient muscle may sense the underlying structural defect in sarcolemmal integrity and partially adapts through activation of a compensatory cytoskeletal remodeling program. Cytoplasmic γ-actin protein levels are elevated 10-fold within dystrophin-deficient *mdx* muscle fibers (Fig. 2) while its mRNA is increased 2-fold [85]. In contrast, cytoplasmic β-actin mRNA and protein were elevated only 2-fold in *mdx* muscle and β-actin was undetectable within muscle fibers (L.M. Hanft and J.M. Ervasti, unpublished results). Besides γ-actin, several costameric actin binding proteins are also upregulated in *mdx* muscle (Fig. 2) including γ-filamin [101], the cytolinker plectin [104], talin and vinculin [105]. Furthermore, dysbindin [90] and syncoilin [89] expression are increased in dystrophin-deficient muscle, as is α7β1 integrin [106,107], which can form a parallel mechanical linkage between laminin-2, the sarcolemma, and the myofibrillar Z disk (Fig. 2). While normally expressed at very low levels in normal postnatal muscle (Table 1), utrophin shows increased expression in dystrophin-deficient muscle and is targeted to costameres [86,87]. Thus, it seems likely that dystrophin-deficient muscle attempts to compensate for the absence of dystrophin through upregulation of available structural proteins. In fact, transgenic overexpression of either utrophin [108] or α7 integrin [109] has been shown to further compensate for dystrophin deficiency. Thus, many of the proteins upregulated in dystrophin's absence are capable of forming parallel mechanical links between the sarcolemma and myofibrillar apparatus. As such, these findings further reinforce an important mechanical function for the dystrophin–glycoprotein complex.

5. Dystrophin versus utrophin

Utrophin is a widely expressed autosomal gene product [6] with significant homology to dystrophin (Fig. 3). Utrophin is distributed throughout the sarcolemma in fetal and regenerating muscle, but is down-regulated at birth and restricted to the myotendinous and neuromuscular junctions in normal adult muscle [11]. Because utrophin and dystrophin bind the same complement of proteins [110,111], it was hypothesized that utrophin may be capable of compensating for dystrophin deficiency. Indeed, continued utrophin expression in adult *mdx* mice partially attenuates the phenotype associated with dystrophin deficiency as mice lacking both proteins exhibit a severe phenotype more similar to that seen in DMD patients [112,113]. Moreover, transgenic overexpression of full length utrophin rescued all known phenotypic parameters of dystrophin deficiency in *mdx* mice [87,108]. Based on the original quantitative estimate of dystrophin abundance in normal muscle [1] and the measured abundance of utrophin in a line of transgenic

Table 1
Utrophin abundance in WT, *mdx*, and transgenically rescued *mdx* muscle

Line	% Total protein [a]	% WT DYS=0.002 [b]	% WT DYS=0.026 [c]
WT	0.0006	30	2
mdx	0.0013	65	5
Fiona/mdx	0.014	700	54

[a] Values in this column taken from [87].
[b] Value for dystrophin abundance (% of total muscle protein) from [1].
[c] Value for dystrophin abundance (% of total muscle protein) from [114].

mice fully corrected for the *mdx* phenotype [87], it is widely perceived that 7-fold higher levels of utrophin may be necessary to compensate for dystrophin deficiency (Table 1). However, new quantitative measurements using full-length recombinant mouse dystrophin as standard [114] indicate that the abundance of dystrophin in normal muscle is 13-fold higher (Table 1) than previously reported [1]. Thus, utrophin upregulation can fully rescue all known parameters of the *mdx* phenotype [108] even when expressed at one-half the measured abundance of dystrophin in normal muscle (Table 1).

While the studies of dystrophin-deficient *mdx* and transgenic mice provide compelling evidence that utrophin over-expression can compensate for the absence of dystrophin, biochemical data suggest that utrophin differs from dystrophin in its mode of binding to actin filaments and β-dystroglycan. Utrophin lacks the cluster of basic, actin binding spectrin repeats present in the middle rod domain of dystrophin [81]. However, full-length recombinant utrophin bound actin filaments with high affinity and a stoichiometry of 1 utrophin per 14 actin monomers [87], implying a stronger and more extensive lateral association with actin filaments than anticipated from studies with isolated utrophin fragments [74,81]. Interestingly, utrophin constructs encoding the amino-terminal tandem CH domain plus 10 spectrin repeats bound actin filaments with the same properties as full-length utrophin, while constructs encoding the tandem CH domain plus 9, 6, 3 or no spectrin repeats each bound actin filaments with progressively lower affinity and stoichiometry [115]. Thus, the amino-terminal CH domain and first 10 spectrin repeats encode the complete actin binding domain of utrophin (Fig. 3), which may provide a molecular explanation for the greater effectiveness of full-length utrophin in rescuing dystrophin-deficient muscle [108] compared to a utrophin mini-gene deleted for spectrin repeats 4–19 [116,117].

Full-length recombinant dystrophin bound actin filaments with properties [114] remarkably similar to those previously measured for purified dystrophin–glycoprotein complex [79], suggesting that neither α1-syntrophin [118], nor any other dystrophin-associated protein contributes to dystrophin–glycoprotein complex binding to actin filaments. In direct comparison, dystrophin and utrophin differed in their extent of lateral association with actin filaments, in sensitivity of actin binding to increasing ionic strength, and in the spectrin repeat modules necessary for actin filament binding [114]. In stark contrast to utrophin, spectrin repeats 1–10 of dystrophin play no direct role in actin binding other than to link the two distinct and spatially separated actin binding modules residing within the amino-terminal CH domain and spectrin repeats 11-17 (Fig. 3). Furthermore, their modes of contact differ to the extent that dystrophin and utrophin do not compete for binding sites on actin filaments [114].

Given their similar affinities for actin filaments [114] and efficacies in maintaining normal muscle function in vivo [87,114], dystrophin and utrophin are likely to bind β-dystroglycan with similar affinities. Ozawa and colleagues have recently reported that utrophin was less effective than dystrophin in competitively inhibiting dystrophin binding to β-dystroglycan[53]. However, my group's quantitative comparison of actin binding properties shows that dystrophin and utrophin can bind a common molecular partner with similar affinities yet not compete due to distinct modes of contact [114]. Following this theme, mutagenesis experiments performed by Ozawa's group demonstrated that dystrophin and utrophin also exhibit different modes of contact with β-dystroglycan [53].

6. Unsolved mysteries

Based on its association with several proteins implicated in signal transduction, the dystrophin–glycoprotein complex is also hypothesized to play a role in cellular signaling [119,120]. For example, α-syntrophin interacts with neuronal nitric oxide synthase [121], which in turn regulates vasodilation during exercise [122,123]. MAP kinase signaling is also altered in dystrophic muscle [124–126]. However, it remains to be demonstrated that the dystrophin–glycoprotein complex actively participates in a signal transduction pathway or that altered signaling initiates the pathologies observed in dystrophic muscle. In fact, a recent study aimed at revealing putative signaling functions for the dystrophin–glycoprotein complex instead concluded that mechanical destabilization is the primary cause of muscle necrosis in dystrophin-deficient muscle [127].

The Lisanti laboratory recently reported that the proteasome inhibitor MG-132 rescued dystrophin expression in *mdx* muscle

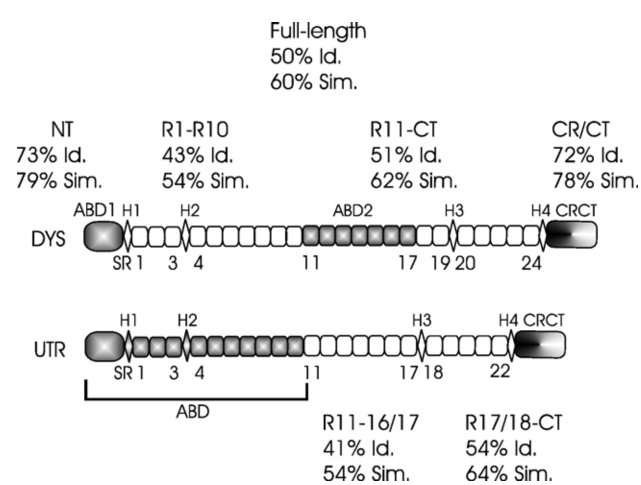

Fig. 3. Dystrophin and utrophin are the same only different. Shown is a schematic diagram comparing the domain structures and sequence identity/similarity of murine dystrophin and utrophin. Shaded regions are important for binding actin filaments and β-dystroglycan. Utrophin lacks modules corresponding to spectrin repeats 15 and 19 in dystrophin and also the cluster of basic actin binding spectrin repeats present in dystrophin (ABD2). Spectrin repeats 1–10 of utrophin are important in actin filament binding, but the corresponding repeats of dystrophin play no direct role in actin binding.

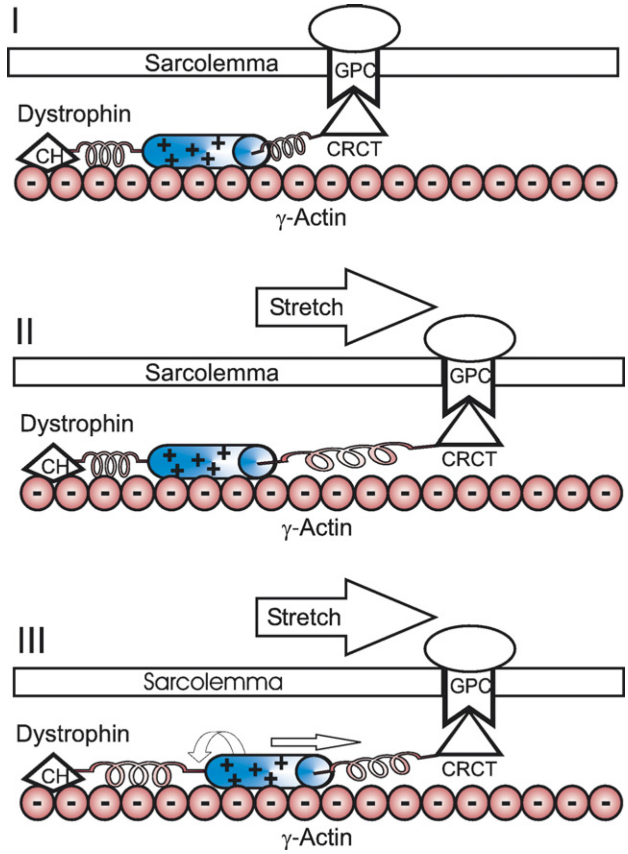

Fig. 4. Dystrophin as a molecular shock absorber. Shown is a hypothetical model for how dystrophin may function to dampen elastic extension during muscle stretch. (I) relaxed muscle. (II) Muscle stretch imposes forces that uncoil spring-like elements within repeats 1–10 and 18–24. (III) Electrostatic interaction of basic actin binding repeats 11–17 with acidic actin filaments dampens extension of the spring-like elements. The "non-specific" electrostatic interaction between the basic spectrin repeats and actin filaments is optimal because it does not require a specific orientation for interaction and would allow sliding between dystrophin and actin. As muscle rapidly shortens during contraction, the electrostatic interaction of the basic actin binding repeats with acidic actin filaments would also serve to dampen elastic recoil.

and restored the dystrophin–glycoprotein complex to the sarcolemma [128]. Despite compelling evidence that both actin filament and β-dystroglycan binding activities are necessary for normal dystrophin function, the dystrophin molecule credited with effecting these outcomes incredibly lacked the carboxy-terminal two thirds of sequence, including the cysteine-rich domain important for dystrophin binding to β-dystroglycan. Alternatively, it seems more logical that MG-132 inhibition of protein degradation caused increased utrophin levels as is the case in a mechanistically diverse array of therapeutic strategies [129–132].

The accumulated data [87,108,114] strongly suggest that utrophin can functionally compensate for dystrophin-deficiency in vivo. However, the clear differences between dystrophin and utrophin with respect to molecular mechanisms of actin filament [87,114,115] and β-dystroglycan binding [53] support the possibility of distinct in vivo functions that remain to be identified. We recently hypothesized [114] that the cluster of basic actin binding spectrin repeats could act as the long speculated molecular "shock absorber" to dampen elastic extension and recoil during rapid changes in muscle length (Fig. 4). In this role, the "non-specific" electrostatic binding of the dystrophin middle rod domain to actin filaments would be attractive because it would be less affected by changes in conformation and/or binding interface orientation that may occur with mechanical distortion. The availability of full-length dystrophin through expression in the baculovirus system [114] makes immediately possible studies to test such mechanical hypotheses at the level of single molecules [133,134] while tests in vivo promise to be more daunting. In contrast to the two site design of dystrophin, the actin binding interface of utrophin functions as a single contiguous unit [115] and probably lacks the capacity to function as a molecular shock absorber. Instead, utrophin may normally function to stabilize newly polymerized actin filaments during costamere development. It is also interesting to speculate that their non-competitive binding [114] would allow utrophin and dystrophin to simultaneously bind and stabilize costameric filaments during the downregulation of utrophin and upregulation of dystrophin that occurs shortly after birth.

The large size and multi-domain structure of dystrophin and utrophin suggest that additional interacting proteins remain to be identified. In support of this hypothesis, Chamberlain and colleagues [88] demonstrated that Dp260 (Fig. 1) restored costameric actin on mechanically peeled sarcolemma and sarcolemmal integrity when transgenically expressed in *mdx* muscle. However, muscle weakness and necrosis were not markedly improved. Because Dp260 lacks the amino-terminal actin binding domain and spectrin repeats 1–10, but retains the basic middle rod actin binding site (Fig. 1), it is possible that sequences absent from Dp260 encode binding sites for unidentified dystrophin-interacting proteins. Furthermore, dystrophin and utrophin exhibit marked differences in amino acid sequence, particularly within the large rod domain (Fig. 3). Thus, it is possible that proteins with unique specificity for dystrophin or utrophin remain to be identified. Toward addressing these possibilities, the availability of biochemical amounts of recombinant dystrophin and utrophin should prove valuable.

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