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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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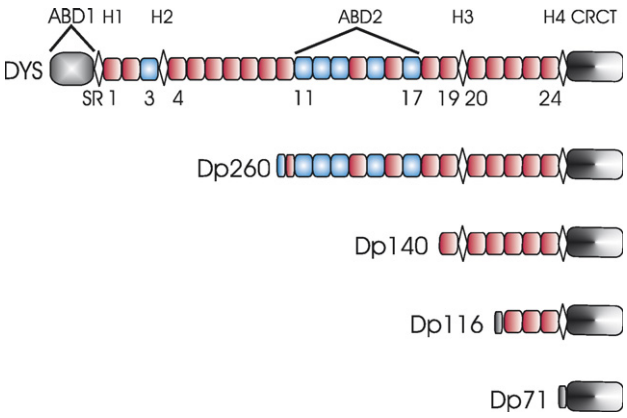

Fig. 1. Protein products of the DMD gene. Shown is a schematic diagram illustrating the domain structure of the protein isoforms encoded by the DMD gene. Dystrophin (DYS) contains an amino-terminal actin binding domain (ABD1) consisting of tandem CH domains, a spectrin-like triple-helical repeat (SR) 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