Dystrophin–glycoprotein complex: molecular organization and critical roles in skeletal muscle

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Recent molecular and biochemical studies have disclosed the detailed molecular organization of the dystrophin–glycoprotein complex, which links the cytoskeleton to the extracellular matrix. Defects in several components of this complex cause different types of muscular dystrophy. This glycoprotein complex is also involved in clustering and anchoring acetylcholine receptors at the postsynaptic membrane.

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Introduction

The primary structure of dystrophin predicts a rod-shaped cytoskeletal protein. In spite of its sarcolemmal localization, dystrophin contains no transmembrane domains. Initial biochemical experiments demonstrated a tight association of dystrophin with sarcolemmal glycoproteins, which suggested that dystrophin was involved in the anchoring of sarcolemmal proteins to the underlying cytoskeleton [1]. Subsequently, a dystrophin–glycoprotein complex (DGC) was purified as a large oligomeric complex consisting of novel sarcolemmal protein or glycoprotein components [dystrophin-associated protein (DAP) or dystrophin-associated glycoprotein (DAG), respectively]: an extracellular component of 156DAG, a cytoplasmic 59DAP triplet, and five transmembrane components of 50DAG, 43DAG doublet, 35DAG, and 25DAG [2,3]. Cloning of one component of the 43DAG doublet revealed that a single messenger RNA encodes both 156DAG (α-dystroglycan) and 43DAG (β-dystroglycan) and that a large precursor polypeptide is post-translationally processed into these two glycoproteins [4]. Further characterization of other DAPs by complementary DNA cloning and biochemical analysis is now giving clues to the overall membrane organization of the DGC in skeletal muscle. This review focuses on recent progress in understanding the structure and function of the complex and its involvement in the pathogenesis of muscular dystrophies.

Syntrophin

Biochemical studies using two-dimensional gel electrophoresis revealed that the 59DAP triplet is divided into two groups by its isoelectric points: acidic and basic components [5]. On the basis of the association of the 58 kDa *Torpedo* homologue with carboxy-terminal domains of molecules of the dystrophin family [6], the 59DAP triplet was named syntrophin [7]. The recent isolation of distinct syntrophin complementary DNAs is consistent with biochemical heterogeneity and is suggestive of a syntrophin multigene family. To date, three separate genes with distinct patterns of expression have been identified [7,8•,9•]. The acidic α-syntrophin (corresponding to mouse syntrophin-1 [7], rabbit 59 DAP-1 [8•], and *Torpedo* syntrophin [7]) is expressed predominantly in skeletal and cardiac muscle. The basic β1-syntrophin isolated from a human skeletal muscle complementary DNA library is expressed in various tissues, but has relatively lower expression in brain and heart [9•]. The basic β2-syntrophin was originally identified as mouse syntrophin-2 and is also present in many tissues [7]. Different tissue distribution patterns of syntrophins suggest each syntrophin isoform may associate with different molecules of the dystrophin family. Subcellular localization determined by isoform-specific antibodies has shown that α-syntrophin is present throughout the entire sarcolemma, including junctional regions [8•,10•], whereas β2-syntrophin is localized to the neuromuscular junction [10•], suggesting

Abbreviations

AChR—acetylcholine receptor; **CMD**—congenital muscular dystrophy; **DAG**—dystrophin-associated glycoprotein; **DAP**—dystrophin-associated protein; **DGC**—dystrophin–glycoprotein complex; **LGMD**—limb-girdle muscular dystrophy; **SCARMD**—severe childhood autosomal recessive muscular dystrophy.

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that β2-syntrophin may be associated with utrophin and be involved in acetylcholine receptor (AChR) clustering. These three syntrophin proteins share 50–60% identity at the amino acid level and a common domain structure containing two pleckstrin homology domains and one discs-large homologous region domain which is inserted into the amino-terminal pleckstrin homology domain. The pleckstrin homology domain is presumed to have a function in the recognition of phosphorylated Ser or Thr residues and is found in an increasing number of intracellular signaling and cytoskeletal proteins [11]. The discs-large homologous region sequence motif of 80–90 residues was originally identified in the Dlg-R protein family which was involved in signal transduction at tight, separate, and synaptic junctions [12]. The presence of these domains implies that syntrophins may be involved in signal transduction or the membrane–cytoskeletal organization. Interestingly, brain nitric oxide synthase also contains the discs-large homologous region domain and is expressed at the sarcolemma like syntrophin [13].

Adhalin

The functional importance of the 50DAG was suggested by the identification of its deficiency in severe childhood autosomal recessive muscular dystrophy (SCARMD), which is prevalent in Arabic countries. Because of its exclusive expression in muscle tissue, the 50DAG was renamed adhalin, derived from the Arabic word 'adhal' for muscle [14]. After an initial cloning of rabbit adhalin complementary DNA [14], complete cloning of human adhalin complementary DNA was reported by two groups [15••,16•]. An open reading frame encodes a 387 amino acid protein with a predicted molecular weight of 43 255 Da. Human and rabbit adhalin proteins share 90% homology at the amino acid level and have a hydrophobic signal sequence, a single transmembrane domain, two potential *N*-linked glycosylation sites in the putative extracellular domain, and one consensus site for phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase. The extracellular domain contains four closely spaced Cys with limited homology to entactin and nerve growth factor receptor, suggesting that adhalin may serve as a receptor for an extracellular matrix protein [15••]. The expression of 1.5 kb adhalin messenger RNA is restricted to skeletal, cardiac, and selected smooth muscle. The expression of an approximately 1 kb adhalin transcript lacking the transmembrane domain has also been reported [16•]. Translation of this transcript predicts a 35 kDa product, however, a 35 kDa form of adhalin has never been detected in purified DGC using antibodies which would cross-react with both adhalin gene products.

Molecular organization of the dystrophin–glycoprotein complex

In skeletal muscle, α-dystroglycan binds laminin-2 (merosin), a tissue-specific laminin isoform which is characterized by its α2 chain [17•]. β-dystroglycan also binds directly to the Cys-rich domain of dystrophin [18••]. Thus, dystroglycan links dystrophin to laminin-2. In addition, the cytoplasmic domain of β-dystroglycan contains a phosphotyrosine consensus sequence and several proline-rich regions that could associate with Src homology 2 and 3 (SH2 and 3) domains of cytoskeletal or signaling proteins. Recently, β-dystroglycan has been shown to interact directly with the SH3 domain of Grb2, an adapter protein involved in signal transduction and cytoskeletal organization [19•]. The specific dystroglycan–Grb2 interaction may play an important role in extracellular matrix-mediated signal transduction or cytoskeletal organization, or both, in skeletal muscle.

Syntrophins also interact directly with dystrophin. Recently, three independent groups reported syntrophin-binding regions at the dystrophin carboxy-terminal domain using overlay assay or affinity precipitation with various constructs of dystrophin fusion proteins [20•–22•]. Binding sites for α- and β1-syntrophin are very close but discrete, and located tandemly around exons 73–74, which are known to be alternatively spliced to produce a dystrophin isoform in brain, heart, and smooth muscle. Mapping of syntrophin binding sites to these alternative splice-prone exons suggests that there are two functionally distinct dystrophin isoforms in the context of the ability to bind syntrophins. However, it should be noted that *in vivo* the molecular organization of syntrophin into the complex seems more complicated. Transgenic *mdx* mice expressing dystrophin lacking exons 71–74 restore all of the DAPs, including the syntrophins, and show normal muscle histology and function [23•]. This suggests that there may be more syntrophin binding sites beyond exons 71–74 as also indicated by the biochemical data showing weak syntrophin binding to a further distal carboxyl-terminal region of dystrophin [21•]. Besides the syntrophin–dystrophin/utrophin interaction, protein overlay assays have shown that α-syntrophin binds the syntrophin triplet, suggesting that syntrophins may form a subcomplex *in vivo* [22•].

Previous biochemical experiments demonstrated that syntrophins were dissociated from the complex and that transmembrane components cosedimented as a subcomplex when the DGC was alkaline-treated [24]. In addition, anti-adhalin immunoaffinity beads precipitated the 43 and 35DAG as well as adhalin from the alkaline-dissociated DGC [25]. These results suggested that these three components (possibly together with 25DAG) may form a subcomplex in the DGC. Octyl

glucoside treatment of the DGC showed the dissociation of the components into several distinct groups [26•]. This experiment suggested that three transmembrane components, adhalin, 43DAG (A3b), and 35DAG, might form a subcomplex called the 'sarcoglycan complex' which is believed to be muscle specific. The presence of the sarcoglycan complex was supported by the immunohistochemical findings that these three components were selectively lost in skeletal muscle from patients with SCARMD in whom other components were well preserved [27]. Fig. 1a shows the molecular organization of the DGC in the extrajunctional sarcolemma based on current knowledge as described above. The precise organization between the sarcoglycan complex and dystrophin or dystroglycan remains to be determined. Recently, aciculin, a 60 kDa cytoskeletal protein with high homology to phosphoglucomutase type 1, has been shown to associate with dystrophin/utrophin [28•]. The aciculin–dystrophin/utrophin interaction may provide an additional link between the DGC and the underlying cytoskeleton.

Muscular dystrophies caused by the disrupted complex

Adhalin has been shown to be deficient in SCARMD. In several North African families, SCARMD is linked to a pericentromeric region on chromosome 13q. However, the 13q locus has been excluded in other families, suggesting that adhalin deficiency is genetically heterogeneous. Last year, mutations in the adhalin gene on chromosome 17q were identified in a French family with limb-girdle muscular dystrophy (LGMD) phenotype [15••]. Since then, several different mutations, including missense, nonsense, and in-frame deletion, have been identified in an increasing number of autosomal recessive LGMD patients with varying severity of disease [29•]. Thus, primary adhalinopathy (now classified as LGMD2D) is a common cause of LGMD. Among mutations so far identified, Arg77Cys is the most frequent mutation. Homozygous patients for null mutations present the most severe phenotype.

A striking discovery in the field of muscular dystrophy in the past year was the identification of laminin α2 chain deficiency in congenital muscular dystrophy (CMD). Tomé *et al.* [30•] reported 13 occidental CMD cases characterized by the absence of laminin α2 chain in skeletal muscle basal lamina. In addition, laminin α2 chain-negative CMD was linked to chromosome 6p21 to which the α2 chain gene has been mapped [31•]. Although mutations in the α2 chain gene have not yet been identified, the data suggest primary involvement of the α2 chain as a cause of CMD. Laminin α2 chain is also deficient in the dystrophic *dy/dy* mouse, a classic murine model for muscular dystrophy [17•,32,33•]. The mouse α2 chain gene was mapped to a proximal region of chromosome 10 close to the *dy* locus [17•]. Recently, the expression of a truncated laminin α2 chain has been demonstrated in the dy^{2J}/dy^{2J} mouse, which is an allelic mutant strain of the *dy/dy* mouse [34••,35••].

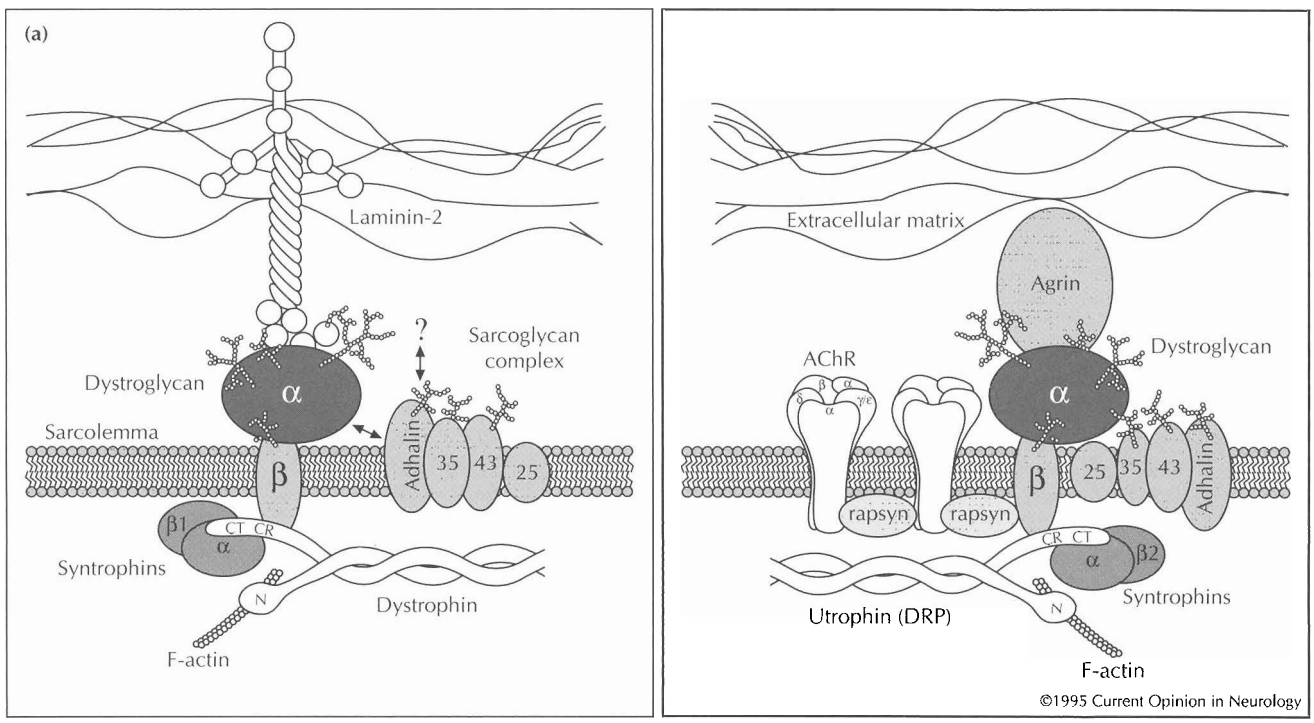

Fig. 1. The molecular organization of the dystrophin–glycoprotein complex at the **(a)** extrajunctional sarcolemma and **(b)** neuromuscular junction. Rapsyn (receptor-associated protein at the synapse) is a 43 kDa protein associated with the acetylcholine receptor (AChR). CT, carboxy terminal; CR, cysteine-rich domain; N, amino terminal.

In this mouse, a splice mutation in the α2 chain gene produces several different mutant transcripts and encodes a truncated α2 chain which lacks the amino-terminal domain VI, which is essential for self-aggregation of laminin heterotrimers. Thus, a disrupted formation of laminin-2 network in the basal lamina may lead to muscle cell degeneration. This is consistent with the hypothesis that disruption at any point of the cytoskeleton–extracellular matrix linkage via the DGC can cause muscular dystrophy.

Dystrophin–glycoprotein complex at neuromuscular junctions

Recent studies have revealed that the DGC is also important at the neuromuscular junction in clustering and anchoring of AChR. Tight colocalization of utrophin with AChR suggests its involvement in the anchoring of AChR. Utrophin has been shown to associate with DAP. Furthermore, a *Torpedo* 58 kDa protein which is complexed with the 87 kDa dystyrophin isoform at postsynaptic membranes has been shown to be a homologue of 59DAP (syntrophin). These findings suggest that utrophin together with several components of the complex may play a role in the anchoring of AChR at the postsynaptic membrane.

Synapse formation at the neuromuscular junction is characterized by AChR clustering on the postsynaptic membrane. Several lines of evidence suggest that agrin, an extracellular matrix protein produced by nerve, is the most promising candidate mediating AChR clustering [36]. Because agrin does not directly associate with AChR, it has been assumed that binding of agrin to its receptor would transmit the signal through the membrane, stimulating the formation of AChR clusters. However, the agrin receptor was not identified until last year when dystroglycan was demonstrated as an agrin-binding protein by four different groups [37••–40••]. Purification of an agrin-binding protein from *Torpedo* postsynaptic membrane by using an agrin-affinity column yielded two glycoproteins of 190 and 50 kDa. By peptide sequence analysis, the 190 kDa component, which directly associated with agrin, was identified as α-dystroglycan and the 50 kDa component β-dystroglycan [37••]. In addition, specific agrin binding to α-dystroglycan in a Ca^{2+}-dependent and heparin-inhibitory manner was demonstrated by other groups [38••–40••]. Furthermore, a monoclonal antibody specific for α-dystroglycan either completely [39••] or partially [38••] interfered with agrin-induced AChR clustering. These findings suggest that α-dystroglycan is necessary for transducing agrin signal for AChR clustering. However, one group reported no inhibition of agrin-induced clustering by an identical dystroglycan monoclonal antibody [40••]. Although the functional consequence of agrin-dystroglycan interaction remains disputed, a structural role of the glycoprotein complex in clustering and anchoring AChR is evident. Fig. 1b shows a possible molecular organization of the complex at the neuromuscular junction.

Conclusion

In the past year, much progress has been made in understanding the molecular organization of the DGC in sarcolemma through molecular cloning of syntrophins and adhalin and protein–protein interaction analysis of components. Different types of muscular dystrophy have been shown to result from primary defects in adhalin and dystrophin. Molecular cloning of other components may disclose additional types of muscular dystrophy caused by the disruption of this complex. The identification of α-dystroglycan as an agrin-binding protein may provide a basis for understanding the mechanism of synaptogenesis. Further studies should advance our understanding of the dynamic function of this complex not only in skeletal muscle but also in various nonmuscle tissues.

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