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Review

Increasing complexity of the dystrophin-associated protein complex

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ABSTRACT **Duchenne muscular dystrophy is a severe X chromosome-linked, muscle-wasting disease caused by lack of the protein dystrophin. The exact function of dystrophin remains to be determined. However, analysis of its interaction with a large oligomeric protein complex at the sarcolemma and the identification of a structurally related protein, utrophin, is leading to the characterization of candidate genes for other neuromuscular disorders.**

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy, affecting 1 in 3,300 boys. It leads to severe muscle wasting and eventual death in the late teens or early twenties due to cardiac or respiratory failure. Becker muscular dystrophy (BMD) is an allelic disorder that is less common (1 in 30,000 boys) and less severe, with a later onset and much longer survival rate. Both disorders are caused by mutations within the dystrophin gene, resulting in a lack of dystrophin (usually DMD) or the expression of mutant forms of dystrophin (usually BMD). Dystrophin is a member of a growing family of related proteins, including the autosomally encoded protein utrophin. There have been several recent reviews covering the structure and potential functions of dystrophin and utrophin, and the reader is referred to these for detailed references (1–3). This review focuses on the proteins with which dystrophin and utrophin interact.

Dystrophin and Utrophin Genes: A Brief Overview

The dystrophin gene is the largest gene so far identified in humans, covering >2.5 megabases and containing 79 exons. The unusually high mutation rate at the DMD locus may, in part, be accounted for by the large size of the gene. The corresponding 14-kb dystrophin mRNA is expressed predominantly in skeletal, cardiac, and smooth muscle; lower levels appear in brain. Transcription of dystrophin in different tissues is regulated from either the brain promoter (predominantly active in neuronal cells) or muscle promoter (differentiated myogenic cells and primary glial cells) giving rise to different first exons. A third promoter between the muscle promoter and the second exon of dystrophin regulates expression in cerebellar Purkinje neurons. Alternatively spliced isoforms originating from the carboxyl-terminal coding region of dystrophin have also been described. The significance of these isoforms at the RNA and protein level has not been elucidated.

Dystrophin is a 427-kDa protein localized to the cytoplasmic face of the sarcolemma, enriched at myotendinous junctions and the postsynaptic membrane of the neuromuscular junction (NMJ). Dystrophin colocalizes with β-spectrin and vinculin in three distinct domains at the sarcolemma (overlaying both I bands and M lines), lying as an array of thick bands localizing at the sites of attachment of the sarcomeres to the muscle plasma membrane. The carboxyl-terminal region of dystrophin is bound to the protoplasmic half of the plasmalemma. Thus dystrophin forms an intricate part of the muscle cytoskeleton and may function to link the normal contractile apparatus to the sarcolemma. In the brain, dystrophin is also localized at the postsynaptic regions of some neurons.

The amino terminus of dystrophin has been shown *in vitro* to contain a functional actin-binding domain. Deletions within this region of the dystrophin gene result in a range of phenotypes from mild to severe, suggesting that the ability to bind actin is an important, but not an absolute, requirement for dystrophin function. The central rod domain consists of a number of α-helical coiled-coil repeats that are similar to spectrin and probably give the molecule a flexible rod-shaped structure. In-frame deletions within this domain usually give rise to intermediate or milder BMD. The carboxyl-terminal domain has no homology with any other identified sequences apart from the related protein utrophin. Mutations in the cysteine-rich domain and first half of the carboxyl-terminal domain almost always result in severe DMD phenotypes, indicating their importance for the normal function of dystrophin. Loss of the last 200 amino acids of dystrophin appears to produce an intermediate DMD/BMD phenotype. The potential functions of the dystrophin carboxyl terminus are discussed later.

Three shorter transcripts, expressed from two different promoters, encode proteins that have a majority of sequence identical to that of the cysteine-rich and carboxyl terminus of dystrophin. Apo-dystrophin-1 (Dp71) and apo-dystrophin-3 are regulated by a promoter situated between exons 62 and 63 of the dystrophin gene and are expressed in nonmuscle tissues, including brain, lung, liver, and kidney. Apo-dystrophin-1 transcripts are only detectable in fetal and newborn muscle. Muscle samples taken after 15 days postnatally have no apo-dystrophin-1 transcript as determined by reverse transcription-PCR. In rat brain, apo-dystrophin-1 transcripts continue increasing until they reach a maximum after ≈1 mo. Thus apo-dystrophin-1 appears to be developmentally regulated in muscle and brain (4). Dp116 (apo-dystrophin-2) is regulated by a promoter situated between exons 55 and 56 of the dystrophin gene and is expressed in peripheral nerve. In contrast to dystrophin, neither apo-dystrophin-1 nor apo-dystrophin-2 is expressed before organogenesis in the developing mouse embryo (5). Apo-dystrophin-1 is first detected at 11.5 days in the ventral midline of the hindbrain. Later expression of apo-dystrophin-1 occurs in central nervous system glia, teeth primordia, and in the liver. Apo-dystrophin-2 appears to be a rare transcript during development but is detected at low levels in the region of the caudate putamen in newborn mouse brain (5).

Utrophin is a 395-kDa protein encoded by a large (1 Mb) multi-exonic gene located on chromosome 6q24 (6). The amino acid sequence shows that utrophin has a strong similarity to dystrophin. Utrophin has an amino-terminal actin-binding domain (S. J. Winder, L. Hemmings, S. K. Maciver, S. J. Bolton, J.M.T., K.E.D., D. R. Critchley, and J. Kendrick-Jones, personal communication), a repeated coiled-coil rod domain, and a cysteine-rich carboxyl-terminal domain. In normal adult muscle, utrophin is localized at the membrane of the NMJ. High-resolution analysis of rat NMJs suggests that utrophin is colocalized with the acetylcholine receptors (AChRs) at the crests of the junctional folds (7) and may play a role in

Abbreviations: DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy; NMJ, neuromuscular junction; DAP, dystrophin-associated protein; DAG, dystrophin-associated glycoprotein; AChR, acetylcholine receptor; SCARMD, severe childhood autosomal-recessive muscular dystrophy.

the clustering of AChRs (8). In fetal muscle development, utrophin is expressed before dystrophin. Higher levels of utrophin mRNA have been detected in human and mouse fetal tissue compared with adult. In normal human fetal muscle, utrophin is localized to the sarcolemma and NMJs, showing maximum expression at 17–18 weeks of gestation (9). When dystrophin is expressed, utrophin is gradually depleted from the sarcolemma to be replaced by dystrophin, leaving the NMJs as the only site of utrophin localization (9). It is not known whether utrophin has a functional role while located at the fetal sarcolemma—i.e., the fetal form of dystrophin—or is simply in the process of migrating with the developing AChR clusters before formation of the mature NMJs. In contrast to dystrophin, utrophin is detectable in most tissues—particularly the lung, blood vessels, and nerves. Utrophin is enriched in the vascular regions of the brain—for instance, the choroid plexus and at the astrocytic foot processes of the blood–brain barrier.

The Dystrophin-Associated Protein Complex in Muscle

In skeletal muscle, previous analysis of the functional domains of dystrophin identified a carboxyl-terminal domain that bound to a large oligomeric complex of six novel proteins localized to the sarcolemma. These sarcolemmal proteins were identified almost simultaneously by Campbell *et al.* (10, 11) and Yoshida and Ozawa (12). Tight association of these proteins was demonstrated by copurification from rabbit muscle membranes, cosedimentation on sucrose density centrifugation, immunoprecipitation, colocalization to the sarcolemma, and protein crosslinking experiments. Table 1 details the names of the dystrophin-associated glycoproteins (DAGs) and dystrophin-associated proteins (DAPs), their molecular weights, and location within muscle. Fig. 1 depicts diagrammatically the location of the DAP complex and other associated proteins localized to the sarcolemma (described later).

The 43DAG (A3) and 156DAG are derived from a single 97-kDa precursor protein translated from a 3.8-kb transcript (15). The 43-156DAG transcript is expressed not only in skeletal, cardiac, and smooth muscle but also in other tissues—namely, brain, lung, liver, and kidney. Cleavage and posttranslational modification of the carboxyl-terminal portion gives rise to the 43DAG. The 43DAG has sites for glycosylation, a transmembrane domain, and a cytoplasmic tail (15). On acrylamide gels, the 43DAG (A3) is a component of a doublet, A3a and A3b (12, 16). The 43DAG is the same as A3a, as determined by peptide sequence analysis of the purified gel band. A3b is distinct from A3a, as it has a different proteolytic cleavage pattern (14) and is unable to bind dystrophin (16). The 56-kDa amino-terminal core protein once glycosylated at many of the potential O-glycosylation sites gives rise to the fully processed 156DAG (15). Recently a protein of 120 kDa called laminin-binding protein 120 (LBP120) has been isolated from the brain and shown to have peptide sequences identical to 156DAG (17). The size difference between the two proteins is probably due to different glycosylation. Because of their posttranslational modification and their interaction with dystrophin, these proteins have now been renamed α-(156DAG) and β-(43DAG) dystroglycan (15).

The 59-kDa membrane-bound protein named 59DAP or A1 has been independently cloned from a number of species, and recent evidence shows this is a family of related genes, some of which bind to dystrophin. They have been renamed syntrophins (syntrophos from Greek, meaning companion). These constitute an increasingly complex family of proteins and are discussed later.

The 50-kDa glycoprotein (50DAG/A2) has been cloned from rabbit skeletal muscle (18). The protein consists of a short signal sequence, one transmembrane domain, and two potential sites for N-linked glycosylation. In contrast to dystroglycan, expression of the 50DAG is specific to skeletal, cardiac, and smooth muscle (18, 19). This protein has been renamed adhalin (from the Arabic word for muscle, adhal).

At present the cloning of the 35DAG (A4) and 25DAP (A5) has not been reported, so their potential functions have yet to be elucidated. However, 35DAG expression appears restricted to striated muscle (19). A 94-kDa protein, A0, which also copurifies with the DAP complex, has been identified (12). Circumstantial evidence suggests that A0 may be related to the *Torpedo* 87-kDa postsynaptic protein (discussed later).

Recently, the purified DAP complex purified from rabbit skeletal muscle has been divided into three subcomplexes based upon detergent solubilization using 1-octyl β-D-glucoside (14). The groups consisted of α- and β-dystroglycan (dystroglycan complex), adhalin, 35DAG and A3b (sarcoglycan complex), and a complex of dystrophin, syntrophin, and A0. The restricted expression patterns of adhalin and 35DAG suggests that the sarcoglycan complex may be specific to striated muscle.

Emergence of the Syntrophin Family of Proteins

In 1987, Froehner *et al.* (20) described a 58-kDa protein that was associated with the cytoplasmic side of the AChRs in *Torpedo* electroplaques. Analysis of adult rat muscle using a monoclonal antibody raised against this 58-kDa protein strongly stained the NMJ but also, to a lesser extent, stained the sarcolemma (20). In 1990, initial analysis of rabbit 59DAP/A1 by one-dimensional PAGE demonstrated that the protein separated as a triplet of bands thought to be a result of differing posttranslational modifications or possi-

Table 1. Nomenclature of the dystrophin-associated proteins

Campbell *et al.* (10, 11)		Yoshida and Ozawa (12)				
Name	Size,* kDa	Name	Size,* kDa	Renamed	Sarcolemmal localization	Non-DAP binding
156DAG	156			α-Dystroglycan	Extracellular	Merosin, agrin, laminin
59DAP	59					
59-1DAP		α-A1	60	α1-Syntrophin		
		β-A1	64	β1-Syntrophin β2-Syntrophin	Cytoplasmic	Dystrophin, apo-dystrophins, utrophin
50DAG	50	A2	52	Adhalin	Transmembrane	
43DAG	43	A3a	43	β-Dystroglycan	Transmembrane	Dystrophin, utrophin, apo-dystrophins
		A3b				
35DAG	35	A4	36		Transmembrane	
25DAP	25	A5	24		Transmembrane	
		A0	94		Cytoplasmic	Dystrophin, utrophin,† apo-dystrophins†

*Estimated size of the protein.
†Binding not shown experimentally. Assumed because of sequence similarity to dystrophin carboxyl terminus.

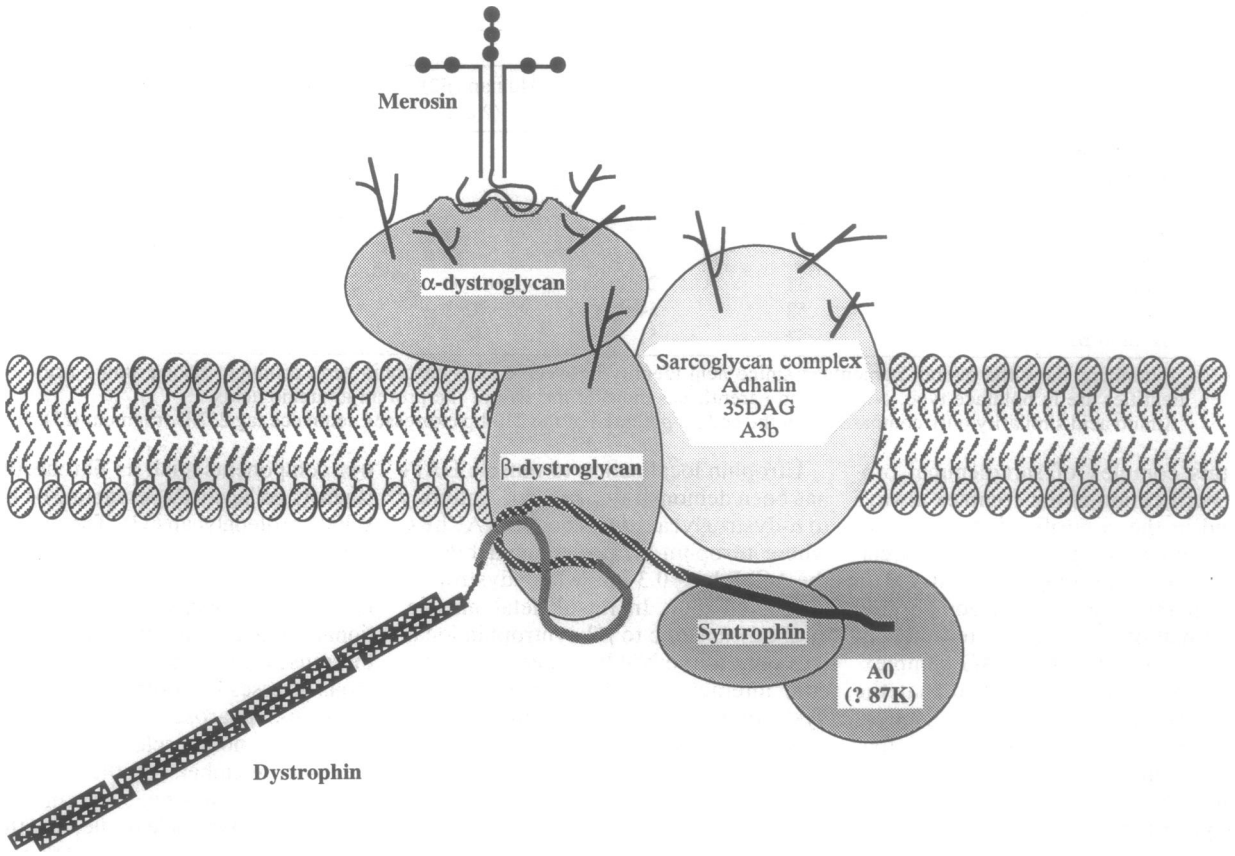

FIG. 1. Schematic representation of the dystrophin-associated protein complex embedded in the sarcolemma. This is based on the model of Suzuki *et al.* (13) and the incorporation of the dystroglycan subcomplexes—i.e., dystroglycan, sarcoglycan, and dystrophin/syntrophin/A0 (14). The dystrophin rod domain is represented by the rectangles, the cysteine-rich domain is represented by the shaded line, the first half of the carboxyl terminus is represented by the hatched line, and the latter half of the carboxyl terminus is represented by the black line.

bly different isoforms (12, 21). Recent analysis of 59DAP/A1 by two-dimensional gel electrophoresis resolved the proteins into two major groups—namely, the β-A1 group which was larger (64 kDa) and more basic than the α-A1 group (60 kDa) (16). In retrospect, the 58-kDa *Torpedo* protein and the 59DAP/A1 proteins all originate from the same family of genes, the syntrophins.

Syntrophin genes of *Torpedo*, mouse, rabbit, and human have recently been cloned. In the mouse, two related proteins were shown to be derived from different genes. These were named syntrophin-1 and syntrophin-2 (22). Expression of the 2.2-kb mouse syntrophin-1 transcript was highest in striated muscle, lower levels were found in brain, and expression was barely detectable in the other tissues tested. There are multiple syntrophin-2 transcripts (10 kb, 5.0 kb, and 2.2 kb) expressed in all tissues tested with lowest levels in skeletal muscle. However, the relative expression of each of these transcripts varies from tissue to tissue. For example, the 2.2-kb transcript was predominant in testes, the 5.0-kb transcript was predominant in lung, and the 10.0-kb transcript was predominant in brain (22). An antibody against *Torpedo* syntrophin (20) reacts with both of the mouse syntrophin species (22). Recently, using peptide antibodies specific to mouse syntrophin-1 or mouse syntrophin-2, syntrophin-1 was localized to rat muscle sarcolemma and AChRs, whereas syntrophin-2 was localized only at the AChRs (23).

The cDNA coding for 59-1DAP has been cloned from rabbit skeletal muscle and shown to have 94% amino acid identity to mouse syntrophin-1, suggesting that this is the rabbit homolog of syntrophin-1 (24). The 2.4-kb transcript has an almost identical expression pattern as mouse syntrophin-1. An antibody to rabbit syntrophin-1 detects only the lowest band of the 59DAP/A1 triplet of proteins seen by one-dimensional PAGE (24).

Finally, a third syntrophin gene has been identified from human tissue (25). Purification and partial peptide sequencing of the human α- and β-A1 proteins (separated by two-dimensional PAGE) confirmed that α-A1 is the human homolog of mouse syntrophin-1. A cDNA containing the β-A1 peptide sequences was isolated encoding a protein of 58.7 kDa, which is related to but distinct from mouse syntrophin-1 and -2. There appear to be multiple transcripts (7.8, 7.2, 6.0, 3.8, and 2.4 kb) expressed from the β-A1 gene, which are detected in various amounts in all tissues tested. A search of sequence data bases using the human β-A1 cDNA sequence identified a skeletal muscle-expressed sequence tag (EST25263) that had strong homology to mouse syntrophin-2 (25).

It has been agreed by the laboratories that have adopted the syntrophin nomenclature to rename the syntrophins on the basis of their amino acid similarity and their acidic (α, pI ≈6.7) or basic (β, pI ≈9+) nature (23). Thus mouse syntrophin-1, rabbit 59-1DAP, and *Torpedo* syntrophin are now named α1-syntrophins. Mouse syntrophin-2 and the human gene encoding the EST25263 fragment are β2-syntrophins. Finally the human β-A1 is a member of the β1-syntrophins. Table 2 shows the amino acid similarity between the cloned syntrophin species. The overall amino acid similarity of *Torpedo* syntrophin is in the order of 50% when compared with either the α1-syntrophins or the β-syntrophins. It will be of interest to see if *Torpedo* has only one syntrophin, suggesting that the other syntrophin genes have evolved from a single ancestor.

Association of Other Proteins with Muscle DAPs

As the name DAP suggests, dystrophin was the first protein shown to interact

Table 2. Similarity of syntrophins

	α1-Syntrophin			β-Syntrophin		
	Rabbit (506 aa)	Mouse (504 aa)	*Torpedo* (489 aa)	Human (β2)* (? aa)	Mouse (β2) (440 aa)	Human (β1) (538 aa)
α1-Syntrophin						
Rabbit	+++					
Mouse	**94**	+++				
Torpedo	59	59	+++			
β-Syntrophin						
Human β2	31	31	25	+++		
Mouse β2	53	52	54	**82**	+++	
Human β1	52	52	51	48	59	+++

Numbers show the overall percentage amino acid identity between each syntrophin gene product. Strong amino acid identities are in boldface type. Numbers in parentheses indicate the size of the protein in amino acids.
*Amino acid similarity only compared with the coding potential of the 210-bp expressed sequence tag 25263 fragment (25).

with this complex of sarcolemmal proteins. Purification of the complex always resulted in the copurification of dystrophin, demonstrating their tight association. Suzuki *et al.* (26) demonstrated *in vitro* that the cysteine-rich domain and the first half of the carboxyl terminus of dystrophin bound to the DAG complex. More recent evidence showed that the β-dystroglycan binds to this region of dystrophin but also demonstrated that syntrophin and another protein of 87 kDa (A0) probably bind to the latter half of the carboxyl terminus (13). *In vitro* studies have shown that β-dystroglycan is bound directly to a purified dystrophin fusion protein consisting of only the cysteine-rich domain of dystrophin (residues 3080–3265). However, binding to a fusion protein also containing the first half of the carboxyl terminus (residues 3266–3442) contributes to a more stable interaction. Interestingly, a protein consisting of only the carboxyl terminus of dystrophin was unable to bind β-dystroglycan, suggesting that this region is unable to bind directly but may be important for maintaining the correct conformational binding structure (13). Immunogold labeling of muscle localized β-dystroglycan to the plasma membrane at the same site as the dystrophin carboxyl terminus (27).

The latter half of the carboxyl terminus of dystrophin has a leucine-zipper motif (1), which may play a role in the binding of syntrophin and/or the 87-kDa protein (13). One interpretation of the literature (although not formerly proven) is that this 87-kDa protein may be the same as that labeled A0 (12, 13), which in turn may be the same as the 87-kDa cytoplasmic peripheral membrane protein identified in *Torpedo* (28). This is a good candidate because in *Torpedo*, 87-kDa protein expression is restricted to the electric organ, brain, and skeletal muscle (28) and, most importantly, is copurified as a complex containing dystrophin and syntrophin (29). Sequence analysis of the 87-kDa protein reveals homology to the carboxyl-terminal region of dystrophin, including the putative leucine zipper (1, 29).

Utrophin localization to muscle DAPs has been demonstrated *in vivo*. Antibody to α-dystroglycan stains both the AChRs, where utrophin is located, and beyond the AChR by ≈0.3 μm, where dystrophin is located (30). In rat skeletal muscle, antibody specific to β2-syntrophin localizes only to the NMJs, suggesting a specific interaction with utrophin (23). In dystrophin-deficient *mdx* mouse skeletal muscle, α-dystroglycan and syntrophin strongly stain only the NMJ (23, 29, 31). Utrophin association with the DAP complex has also been shown *in vitro* by copurification of a DAP/utrophin complex from *mdx* skeletal muscle (31). The binding of utrophin to components of the DAP complex is not surprising since the sequence comparison of utrophin reveals an 88% amino acid similarity to the β-dystroglycan-binding domain of dystrophin (32). Utrophin also has the same leucine zipper motif in the latter half of the carboxyl terminus as dystrophin and the 87-kDa *Torpedo* protein (1).

It is not known what DAP complex is present at the fetal muscle sarcolemma before dystrophin expression. Expression and formation of the DAP complex may simply mimic the expression pattern of dystrophin. However, given the overall domain similarity between dystrophin and utrophin, it would be reasonable to hypothesize that utrophin may be the fetal form of dystrophin and function similarly. Dystrophin may be more specialized and adapted to dealing with the much greater stresses of skeletal and cardiac muscle development after birth. However, until this time, utrophin could give some form of structural rigidity to the developing myotubes.

Laminins are a family of basement-membrane glycoproteins. The α-dystroglycan binds to laminin and merosin (M-laminin) situated in the extracellular matrix (33). Laminin-Sepharose, as well as purifying α-dystroglycan, also copurified all the other members of the DAP complex (33). The binding of α-dystroglycan to laminin is specific—probably at one of the heparin-binding domains, with no demonstrable binding of other extracellular matrix components such as fibronectin, collagen I, collagen IV, entactin, or heparin sulfate proteoglycan (33). The major effect of binding laminin to α-dystroglycan would be to link the sarcolemma to the extracellular matrix, potentially providing an additional strengthening mechanism to allow the muscle fibers to withstand the enormous stresses of contraction and stretch. Laminin-Sepharose also bound α-dystroglycan from nonmuscle tissues. However, the other members of the muscle DAP complex were lacking, suggesting they do not exist in nonmuscle tissue, that they are antigenically dissimilar, or that they simply do not bind together as tightly as the muscle complex (33).

Agrin is an extracellular matrix protein that appears to be able to direct the accumulation of AChRs at developing and regenerating NMJs. Independent evidence from three different groups suggests that α-dystroglycan is an agrin receptor (34–36). Bowe *et al.* (34) purified a complex from *Torpedo* electric organ postsynaptic membranes capable of binding agrin and showed that the complex consisted of two proteins (190 kDa and 50 kDa). Partial peptide analysis of the two proteins demonstrated that they had very strong homology to α- and β-dystroglycan. Campanelli *et al.* (35) and Gee *et al.* (36) demonstrated that α-dystroglycan could bind agrin *in vitro* by different methods and that the binding was calcium and heparin dependent (35). Analysis of mouse C2 myotubes by immunofluorescence demonstrated that agrin, utrophin, and α-dystroglycan were concentrated at the AChR clusters (35, 36).

Thus, in muscle the DAP complex appears to have two very important roles. (*i*) The complex (possibly containing α1-syntrophin) links the sarcolemma to the extracellular matrix via merosin and the internal cytoskeleton via dystrophin in a regular array along the length of the myotubes to give the myotubes strength (Fig. 1). (*ii*) The DAP complex (containing β2-syntrophin) is involved in the formation/stabilization of AChR clusters linking agrin in the extracellular matrix and utrophin within the cell to establish the junctional

regions between the motor neuron and muscle (Fig. 2).

Association of Other Proteins with Nonmuscle DAPs

It is very probable that utrophin also binds to a related complex in nondystrophin-expressing cell types. The presence of α-dystroglycan expression in nonmuscle tissues (15) and the identification of a smaller (120 kDa) brain α-dystroglycan isoform (17) suggests that this protein may have other binding partners. In mouse, β2-syntrophin expression occurs in all tissues examined (22, 24), mimicking utrophin expression and suggesting a higher specificity for binding utrophin. Specific antibodies only detect β2-syntrophin in rat kidney and not in α1-syntrophin (23). In peripheral nerves found among muscle fibers of normal and DMD muscle, utrophin and β-dystroglycan are clearly detected, but no 50DAG is detected (37). The peripheral nerves must contain a DAP complex deficient in 50DAG. It is possible that in nonmuscle cells there is no sarcoglycan complex or there is a related sarcoglycan complex. Further analysis of A3b and 35DAG is required to answer these questions.

Possibly the DAP complex in nonmuscle cell types could consist of only the α- and β-dystroglycan, β2-syntrophin, and possibly A0. From the proposed DAP binding complex of Ervasti and Campbell (21) and Suzuki *et al.* (13), this would be the minimum complex required to link laminin (in the extracellular matrix) to actin via utrophin. As actin and laminin isoforms are found in all cells, this could provide a scaffold to link stably these intra- and extracellular proteins and thus be a mechanism to give rigidity to all cells.

Syntrophin preparations from various rat tissues copurify a protein of 71 kDa, which reacted with an antibody raised against the unique amino terminus of apodystrophin-1 (Dp71) (38).

Potential for Disease Caused by Defects in the DAPs or Associated Proteins

In DMD the levels of all the DAPs are reduced (11, 37, 39), although the dystroglycan mRNA (and presumably the other DAP mRNAs) is expressed at normal levels (15). Patients with mutations in the carboxyl terminus of dystrophin always result in a severe phenotype with a reduction in the DAPs (40). In some cases these patients express a truncated form of dystrophin that appears to localize correctly to the sarcolemma—presumably due to an intact amino-terminal actin-binding domain (41). Dystrophin mutations originating within the actin-binding domain giving rise to an in-frame fusion protein result in variable phenotypes, suggesting that the presence of a functional dystrophin carboxyl terminus is capable of stabilizing a proportion of the DAP complexes (42).

In BMD there is a general mild reduction in the DAPs—probably because most mutations involve in-frame deletions of the rod domain, leaving the actin-binding and DAP-binding regions intact (43). These truncated forms of dystrophin must be partially effective in both organizing the DAP complex into the correct conformation and linking with the cytoskeletal actin. Interestingly, symptomatic DMD carriers have significantly reduced DAPs in dystrophin-deficient regenerating fibers, whereas they were expressed at normal levels in the normal dystrophin-positive fibers (44, 45).

All of these results have led to the notion that dystrophin localizes or stabilizes the DAP complex. Without dystrophin the complex is unable to organize properly, and consequently the linkage between the extracellular matrix and the subsarcolemma is disrupted; this eventually leads either to the gross muscle necrosis seen in DMD or to the less severe phenotype in BMD, where expression of a partially functional dystrophin results in a partial retention of the DAPs.

Further analysis of autosomal muscular dystrophies has strengthened this hypothesis. Deficiency in only adhalin (50DAG) is observed in patients suffering from severe childhood autosomal-recessive muscular dystrophy (SCARMD) (46–48). SCARMD has been mapped to 13q12 by linkage analysis of 16 Tunisian pedigrees (49, 50). However analysis of three Brazilian SCARMD families was unable to demonstrate linkage to 13q, yet affected individuals were deficient in adhalin (51). Until the chromosomal localization of the adhalin gene is determined, it is impossible to suggest which, if any, of the autosomal-recessive muscular dystrophies are caused by adhalin mutations. The autosomally recessive, dystrophic hamster (NSJ-*my*/*my*) may turn out to be a model for SCARMD. Analysis of the DAPs shows a specific decrease in the levels of adhalin and 35DAG and, to a lesser extent, β-dystroglycan (52, 53). In these animals there is widespread muscle fiber necrosis and active regeneration. In Fukuyama-type congenital muscular dystrophy abnormal staining of the

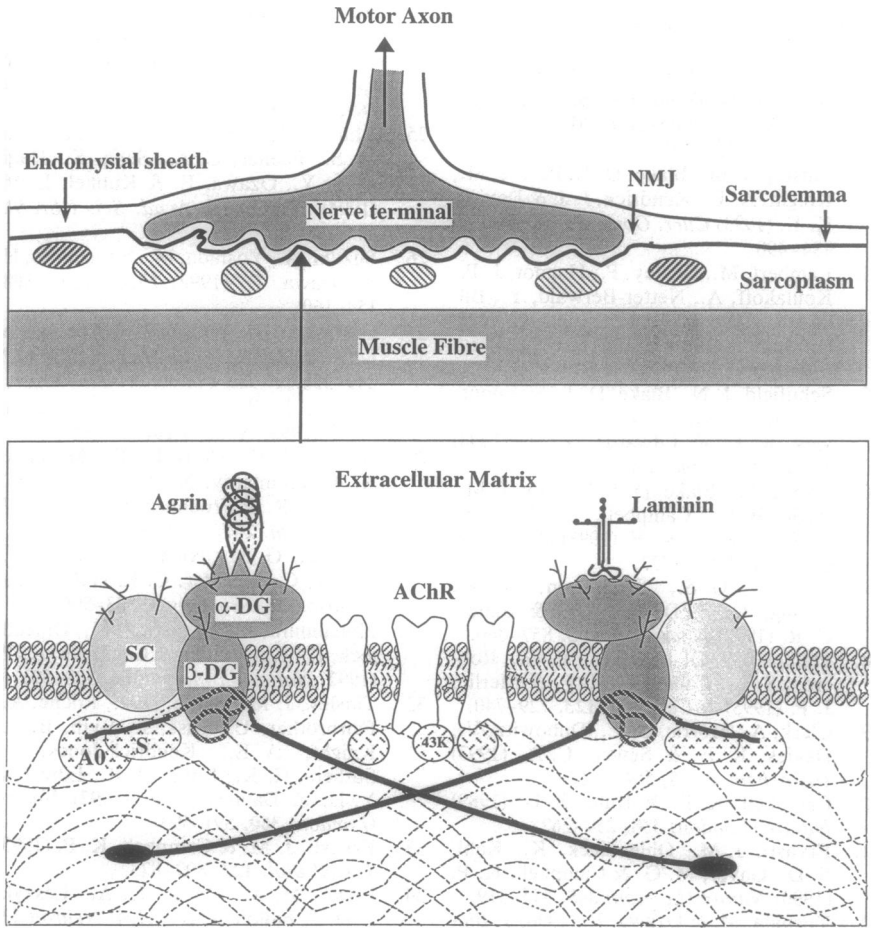

FIG. 2. Schematic representation of a muscle nerve terminal. The lower part of the diagram represents the proteins associated with an AChR at the crests of the junctional folds, linked by utrophin to the actin cytoskeleton. α-DG, α-dystroglycan; β-DG, β-dystroglycan; SC, sarcoglycan complex; S, β2-syntrophin; 43K, 43-kDa AChR-associated protein.

Table 3. DAP gene location and potential disease association

Gene	Chromosome location		Human		Mouse	
	Human	Mouse	Disease	Locus	Mutant	Locus
Dystroglycan	3p21	9			*du/du, tip/tip*	9
Adhalin			SCARMD	13q12	NSJ-*my/my* (hamster)	
β2-syntrophin	16*					
β1-syntrophin	8q23					
Merosin	6q22-23	10	CMD	6q2	*dy/dy*	10
Dystrophin	Xp21	X	DMD/BMD	Xp21	*mdx*	X
Utrophin	6q24	10				
			FCMD	9q31-33		

FCMD, Fukuyama-type muscular dystrophy; CMD, congenital muscular dystrophy.
*Tentative gene localization.

DAPs is seen, particularly of β-dystroglycan (43DAG), in some muscle fibers (54, 55). However abnormal β-dystroglycan staining can only be a secondary effect, as the genetic defect for Fukuyama-type muscular dystrophy has been mapped to 9q31-33, whereas the dystroglycan gene is located at 3p21 (56) (Table 3). In both these human diseases, dystrophin expression is normal, but symptoms are akin to severe BMD.

Decreased laminin expression has been demonstrated in a number of muscular dystrophies (57). Recently, analysis of the severely dystrophic, autosomally recessive *dy/dy* mouse demonstrated a complete lack of merosin and vastly reduced levels of merosin mRNA in skeletal muscle, cardiac muscle, and peripheral nerve. The heterozygous *dy/+* (nondystrophic) had normal levels of merosin. Ultrastructural analysis of the basement membrane showed it was unusually fragmented or even absent in the dystrophic mice. The human chromosomal localization of merosin is 6q22-23. Recently merosin-negative congenital muscular dystrophy has been mapped to 6q2 (58). This region is syntenic with mouse chromosome 10 at the position where the *dy* locus maps. These results suggest that a defect in merosin expression may be directly responsible for the muscle necrosis and resulting muscular dystrophy seen in these mice (59–61). Two autosomal recessive mouse mutants, ducky and tippy, map to chromosome 9. Both mouse mutants have problems walking, and *tip/tip* mice also have neurological defects. This region is syntenic with human 3p21-pter, which contains the dystroglycan gene, suggesting that mutations in this gene may cause muscle and/or neurological disorders (56).

The above results demonstrate the importance of the DAP complex in muscle function and integrity and suggest that any deficiency of this complex either directly, or indirectly, results in muscular dystrophy and possibly other neurological disorders (Table 3). At present the genomic localizations of the other members of the DAPs are not known; their role as candidate genes has not yet been fully explored. Given the wide diversity of cell types in which members of the DAP complex are expressed, it is also possible that other nonmuscle disorders will be caused by aberrant expression of this complex family of interacting membrane proteins.

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