

Dystroglycan Complexes and their Role in Muscle Development

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Abstract

Dystrophin and its associated glycoprotein complex (DAGC) effectively links the actin associated cytoskeleton of the muscle fibre with laminin in the extracellular matrix. The DAGC may be divided into two sub-complexes, namely dystroglycans and sarcoglycans, with the dystroglycan complex forming the principal linkage between the dystrophin and laminin. This arrangement is widely attributed with a role in maintaining membrane integrity during repeated cycles of contraction and relaxation, a hypothesis that is seemingly supported by observations of elevated concentrations of dystrophin at the costameres and myotendinous junctions. However, dystrophin is present in developing human muscle by 8 weeks of gestation and can be shown to co-localise with laminin from the earliest stages of secondary myotube formation. Furthermore, α -dystroglycan has now been shown to be a major binding protein of agrin and has been implicated in the formation and stabilisation of the neuromuscular junction. These observations suggest that the DAGC may prove to have an important part to play in membrane signalling events during muscle formation. In this respect the present review outlines what is currently known concerning the distribution of dystrophin and the DAGC during muscle development.

Key words: muscle development, dystroglycan, sarcoglycan, dystrophin, utrophin.

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A variety of adhesion receptors allow cells to interact with the surrounding extracellular matrix. The integrin family of receptors are well known in this regard and have been shown to mediate a wide range of responses that include cell growth, gene expression and differentiation [28]. Fundamental to the ability of these receptors to regulate such processes is their ability to form a physical linkage between the cell cytoskeleton and the extracellular matrix. In recent years the search for the underlying cause of muscular dystrophy has identified a group of proteins and glycoproteins that appear to fulfil a similar role [19, 30]. Dystrophin was the first protein of this group to be identified [26] and the transmembrane complex with which it associates is now referred to as the dystrophin associated complex (DAGC). The disruption of this complex due to mutations in one component results in muscle fibre degeneration and muscular dystrophy [63]. The most severe phenotype is shown by patients with Duchenne Muscular Dystrophy (DMD), which is caused by the absence of dystrophin itself. Despite numerous attempts to precisely define the role of dystrophin and the DAGC, their function remains unclear. One widely held hypothesis is that this arrangement functions to maintain membrane integrity

during repeated cycles of contraction and relaxation. However, recent studies of post-synaptic differentiation suggest that this complex may also have a signalling role during development. In order to examine this hypothesis further the present review outlines what is currently known concerning the expression of dystrophin and the DAGC during skeletal muscle development.

Components of the dystrophin-glycoprotein complex

Dystrophin is a 427 kDa protein that in adult muscle localises to the cytoplasmic face of the sarcolemma [61] and the post-synaptic membrane of the neuromuscular junction [10]. It is also found at much lower levels in the brain where it locates to the postsynaptic density of a subset of neurons [2]. Full length dystrophin consists of four structurally distinct domains namely (i) an N-terminal actin binding domain [14, 24, 36] (ii) a large spectrin-like rod domain [35] (iii) a cysteine-rich domain and (iv) a C terminal region with homology to only utrophin (autosomal homologue of dystrophin). The N-terminal regions of dystrophin bind to sub-sarcolemmal F-actin, whilst the cysteine rich and C terminal domains bind to the DAGC [54, 55]. As a consequence of these interactions, both the

N- and C- terminal regions of dystrophin are able to localise independently to the muscle fibre membrane [16], and they effectively link the muscle fibre cytoskeleton with the extracellular matrix [18, 30].

The DAGC may be divided into two sub-complexes, namely the dystroglycan complex which is composed of α -dystroglycan (156 kDa) and β -dystroglycan (43 kDa), and the sarcoglycan complex which is composed of α -sarcoglycan (also known as adhalin) (50 kDa), β -sarcoglycan and γ -sarcoglycan (35 kDa) (see Fig. 1). The sarcoglycan complex indirectly links to dystrophin via the dystroglycan complex, although the precise nature of the relationship between the two complexes is at present unclear.

Human dystroglycan is encoded by a single gene mapped to chromosome 3 band p21. The cDNA encodes a precursor protein of 97 kDa which is post-translationally processed into α - and β -dystroglycan [31]. α -Dystroglycan is a highly glycosylated peripheral surface proteoglycan which is able to bind to the G domain of laminin, merosin [15, 19, 30] and agrin [11, 22] in the extracellular matrix. β -dystroglycan is a transmembrane protein, the extreme C terminus of which, constitutes a unique binding site for the second half of hinge 4 and the cysteine-rich region of dystrophin [32]. α - and β -dystroglycan are tightly associated and form the principal linkage between the fibre cytoskeleton and the surrounding extracellular matrix. Dystroglycan is widely expressed in muscle and non muscle tissues. The carbohydrate moieties rather than the protein sequence differ between tissues, an example being cardiac muscle where α -dystroglycan is of a lower molecular weight compared to skeletal muscle. This variable glycosylation of the protein core has been suggested to modulate laminin binding [31].

To date no pathologies arising from the absence of dystroglycan have been reported. However, it is possible that in view of the expression of dystroglycan in non muscle cells [29, 30], its proposed role during epithelial morphogenesis [17], and the fact that the number of basement membrane components to which dystroglycan binds is still unknown, dystroglycan mutations may actually prove to be lethal during development.

By contrast a number of mutations in the sarcoglycan complex have now been shown to be the underlying cause of various forms of muscular dystrophy. For example, defects in the α -sarcoglycan gene have been associated with a form of late onset autosomal recessive muscular dystrophy (ARMD [25]), mutations in the β -sarcoglycan gene appear to be the cause of some forms of limb girdle muscular dystrophy (LGMD), and deletions in the γ -sarcoglycan gene have now been associated with severe childhood autosomal recessive muscular dystrophy (SCARM [44]). In addition a specific deficiency of merosin, the laminin M chain, results in a classical non-Japanese form of congenital muscular dystrophy [58] and dystrophinopathy in mice [53, 64].

Additional proteins that associate with the DAGC

Utrophin was first identified by its sequence homology to the C-terminus of dystrophin, and is now recognised as the autosomal homologue of dystrophin [34, 37]. It is encoded by a gene on the long arm of human chromosome 6 and mouse chromosome 10 [8]. By contrast with dystrophin, whose expression during development is largely limited to mesodermal derivatives such as cardiac and striated muscle, utrophin is abundantly expressed in neural tube, neural crest derivatives and the tendons of the digits [47]. Differences are also apparent in the adult where dystrophin is limited to predominantly skeletal, cardiac and smooth muscle with lower levels evident in the brain, and utrophin is found in a variety of tissues which include liver, kidney, muscle, testis and brain [38].

The sequence homology between utrophin and dystrophin extends throughout their entire length, but is greatest at the C-terminus where it reaches around 80% [57]. In view of this homology it is perhaps not surprising that utrophin associates with either the same or an antigenically similar DAGC [39]. Dystrophin and utrophin both complex with dystroglycan at the neuromuscular junction. However, utrophin is confined to the crests of the post-synaptic folds in close association with the AChR clusters, whereas dystrophin is found in the depths of the folds, a region that is sparse in AChRs but enriched in sodium channels [49].

The electroplaques that make up the electric organ of *Torpedo* are derived from immature striated muscle cells in the embryo and possess many of the morphological and biochemical characteristics of mammalian neuromuscular junctions. The 87 kD protein, or dystrobrevin, was first identified as a postsynaptic protein of *Torpedo* electric organ where it has been shown to associate with dystrophin and syntrophin [9]. The dystrophin-associated protein originally known as A0 now appears to be an isoform of this 87 kD protein, which shows homology with the cysteine-rich and C-terminal domains of dystrophin [67]. Whilst only one transcript is expressed in *Torpedo*, several mRNA's have recently been detected in the mouse and shown to be generated by the differential expression of multiple isoforms from a single gene locus [4]. These isoforms are predicted to interact with both dystrophin/utrophin and syntrophin at the neuromuscular junction and sarcolemma where they have been shown to localise [4].

The syntrophins are a biochemically heterogeneous group of 58 kDa intracellular membrane-associated dystrophin-binding proteins. They represent the mammalian homologue of the *Torpedo* 58 kDa postsynaptic protein, [18, 66] and directly associate as a complex with the carboxyl-terminal domain of dystrophin, probably at multiple interaction sites [54, 65]. At present, one acidic syntrophin (α 1) and two basic syntrophins (β 1 and β 2) have been identified in muscle. Comparisons of the deduced amino acid structure of the three human isoforms of syntrophin show an overall similarity. Furthermore, the de-

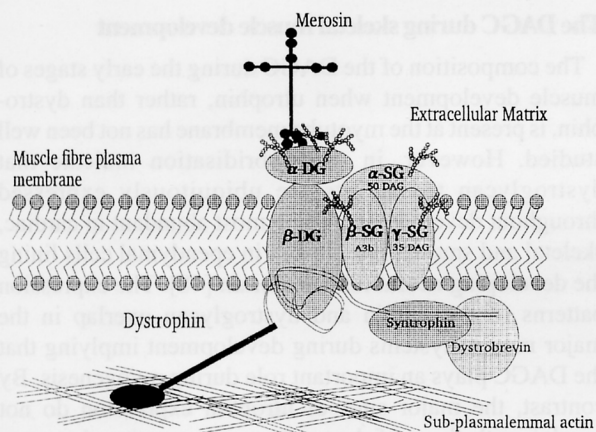


Figure 1. Diagrammatic representation of dystrophin and the dystrophin associated glycoprotein complex (DAGC). The DAGC consists of α - and β -dystroglycan (α - and β -DG) and α -, β - and γ -sarcoglycan (α -, β - γ -SG). α -dystroglycan binds to merosin in the extracellular matrix whilst dystrophin, through its binding to β -dystroglycan, links the entire complex to the actin associated cytoskeleton.

duced amino acid sequences of human $\alpha 1$ and $\beta 2$ syntrophin are nearly identical to their homologues in the mouse, indicating a strong conservation among the individual isoforms [1]. α -syntrophin is most abundant in heart and skeletal muscle locating to the sarcolemma whereas $\beta 1$ and $\beta 2$ syntrophin are expressed more widely. In muscle β -syntrophin localises to the neuromuscular synapses although the structural determinants of this specificity are at present unknown. Syntrophin does not exhibit any DAGC binding activity [65], but interacts with peptides encoding regions of dystrophin, utrophin and *Torpedo* 87 kD protein [1]. In addition it has recently been shown that nNOS (nitric oxide synthase) binds directly to $\alpha 1$ syntrophin via a PDZ motif implying that syntrophin may function as an adaptor protein linking a signalling enzyme to the sarcolemmal dystrophin complex [7]. There is at present no indication that defects in syntrophin expression lead to any form of neuromuscular disease.

The expression of utrophin and dystrophin during skeletal muscle development

Utrophin locates to the myotube membrane from before 9 weeks of gestation of human development, although at this time there is some variability both between and within individual myotubes [13]. Immunolabelling of utrophin becomes more uniform at around 17-18 weeks gestation when expression is maximal, after which there is a marked decline such that by 26 weeks sarcolemmal staining is negligible [13]. In adult muscle utrophin locates solely to the postsynaptic membrane of the neuromuscular junction, [45, 56] and the myotendinous junction, although it reappears at the sarcolemma of regenerating muscle fibres [33,

38]. The functional significance of why utrophin is replaced by dystrophin is unclear, but may relate to some form of functional adaptation at the membrane level as the fibre becomes fully active.

The distribution of dystrophin in the adult muscle fibre is similar to the so-called costameric lattice which is composed of cytoskeletal proteins such as vinculin, talin, spectrin and γ -actin. It is these proteins which, in interactions with specific cell surface glycoproteins, are thought to mediate the trans-membrane interaction of contractile elements at the level of the Z disc with the extracellular basal lamina [46]. Dystrophin can be detected in human skeletal myotubes by 8 weeks of gestation [12, 42] prior to the appearance of functional innervation. Most reports on the localisation of dystrophin during human muscle development, have noted that during the early stages of myotube formation dystrophin accumulates in the cytoplasm before becoming associated with the membrane, thereby confirming earlier work on cultured myotubes [12, 41, 43].

The initial cytoplasmic localisation of dystrophin has recently been investigated at the electron microscope level, and appears to be due to an apparent association between dystrophin and the intermediate filaments that interconnect the myofibrils, and the I band of the sarcomere [43]. This apparent co-localisation with desmin implies that dystrophin may play a role in the three dimensional organisation of the myotube cytoskeleton. Such a role would be expected to be redundant at the end of myotube maturation, and may be one explanation as to why dystrophin locates to the membrane as the myotube differentiates [43]. These observations suggest that dystrophin may fulfil several functions during myotube maturation, and the existence of several developmentally regulated isoforms that appear to differ in the C terminal region, may be an indication of this [12, 62]. However, as to whether any isoforms are specifically associated with the intermyofibrillar cytoskeleton rather than the membrane cytoskeleton is at present unclear. In this regard it is interesting to note that in cardiac muscle, approximately 35% of the 427 kDa form of dystrophin, associates with the Z disc regions of the myofibrils [40]. The existence of distinct pools of dystrophin is of considerable interest and supports the concept that dystrophin may serve diverse roles through various molecular associations.

In view of the expression of dystrophin *in vitro*, and the timing of events *in vivo*, it seems that neural factors do not play a significant part in initiating the synthesis of dystrophin. As to whether dystrophin expression is stimulated by some local biochemical or mechanical influence is uncertain, since reports of an early accumulation of dystrophin at the distal ends of the early myotubes immediately adjacent to the tendons [62] have not been confirmed by others.

By 17 weeks gestation dystrophin is membrane associated in both type I and type II fibre types, i.e. both primary and secondary myotubes [62]. At this stage myotubes are characteristically arranged in 'clusters' each of which consists of a single primary myotube surrounded by several

secondaries (see Fig. 2). Whilst dystrophin is membrane associated in both myotube generations, it appears to be absent from those areas not in direct contact with the extracellular matrix. This can be shown by double labelling muscle sections with either antibodies to dystrophin and laminin, or NCAM and laminin. Dystrophin and laminin clearly co-localise (Fig. 3a & b), as has been shown previously in cultured myotubes [15]. NCAM and laminin also co-localise except in those areas of membrane apposition between the primary and secondary myotube, where NCAM expression is evident but laminin is absent (Fig. 3c & d). In view of the exact co-localisation of dystrophin with laminin, these areas must be assumed to also be deficient in dystrophin. Since dystrophin is expressed and membrane associated in both primary and secondary myotubes at this stage, these observations cannot be related to differences in myotube maturity but rather to the formation of the basement membrane. These observations are interesting since they indicate that at this stage the dominant mechanism linking dystrophin with the membrane, is through its C terminal domains and the DAGC rather than simply through binding to sub-plasmalemmal actin.

The DAGC during skeletal muscle development

The composition of the DAGC during the early stages of muscle development when utrophin, rather than dystrophin, is present at the myotube membrane has not been well studied. However, *in situ* hybridisation indicate that dystroglycan transcripts are ubiquitously expressed throughout development, being most abundant in cardiac, skeletal and smooth muscle and in ependymal cells lining the developing neural tube and brain [48]. The expression patterns of dystrophin and dystroglycan overlap in the major muscle systems during development implying that the DAGC plays an important role during myogenesis. By contrast, the major sites of utrophin expression do not overlap with those of dystroglycan, suggesting that utrophin associates with a distinct complex at other non-muscle sites [48].

Immunocytochemical studies indicate that when dystrophin is first expressed at 8 weeks gestation β -dystroglycan is scarce [42]. This might be expected given that at this time dystrophin locates to the cytoplasm rather than the membrane. Dystrophin expression is closely paralleled by that of syntrophin [59], which seems to suggest that the sub-sarcolemmal proteins assemble first. The dystroglycan complex appears after dystrophin, and is followed by the

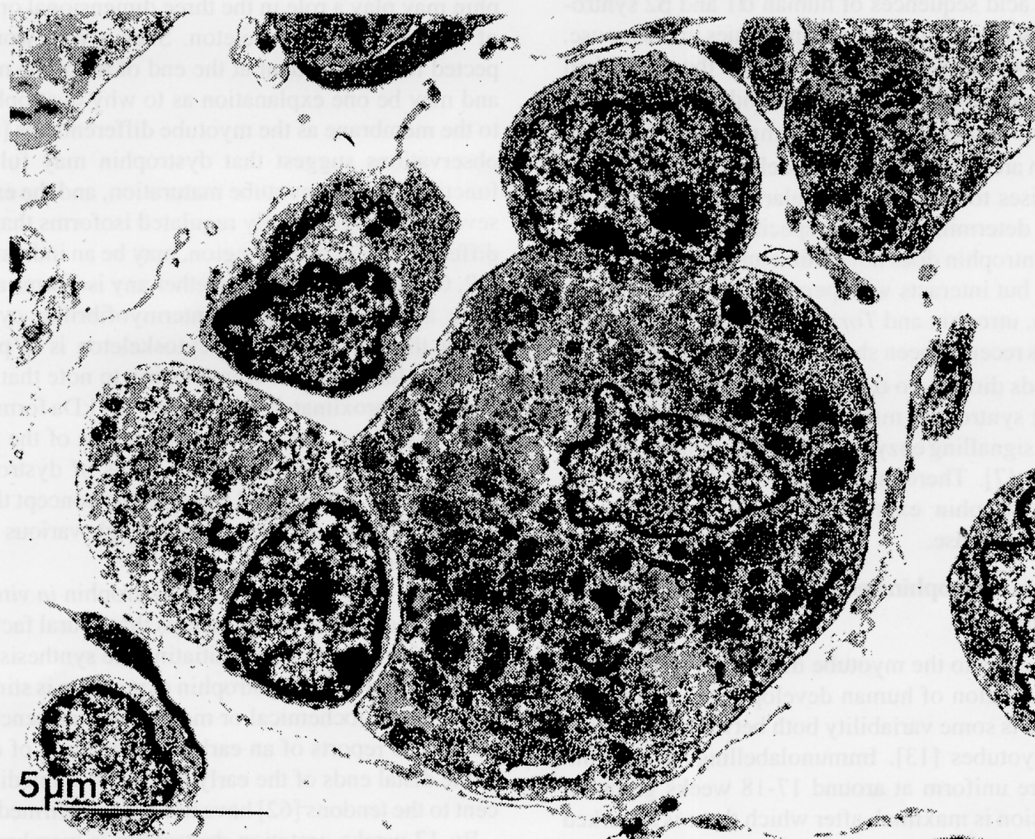


Figure 2. Electron micrograph of 16 days gestation fetal mouse muscle showing a primary myotube surrounded by two secondary myotubes all of which are enclosed within a single basement membrane.

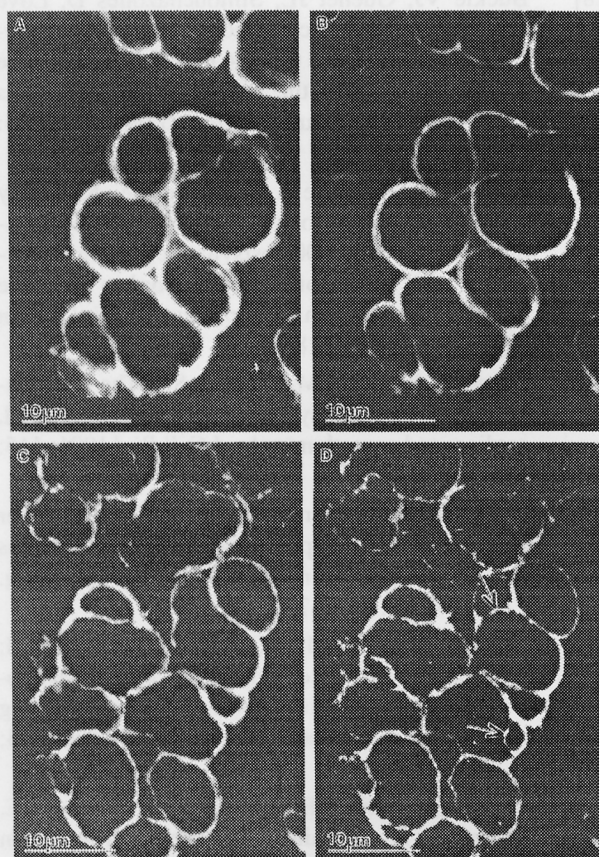


Figure 3. Immunolocalisation of dystrophin (a) and laminin (b) in a single transverse section of human fetal muscle showing the clear co-localisation of the two proteins. Immunolocalisation of laminin (c) and NCAM (d) in a single section showing the areas of membrane apposition (see arrows) that display no evidence of laminin deposition.

transmembrane α - and γ -sarcoglycan [59]. This sequence of events may be indicative of a role for the sub-sarcolemmal proteins i.e. dystrophin and syntrophin, in the assembly of the transmembrane and extracellular components of the DAGC during fetal muscle development [59].

In contrast to the embryonic chain of events studies of regenerating rat muscle show dystroglycan and adhalin to appear prior to dystrophin [60]. These observations suggest the reverse of the above i.e. that components of the extracellular matrix influence the initial distribution of dystrophin. However, regeneration takes place in the vicinity of the old (original) basal lamina tube which is a markedly different environment to that in which myogenesis takes place. For example, merosin the variant of laminin present in adult skeletal muscle fibres is composed of $\alpha 2$ - $\beta 1$ - $\gamma 1$ chains. Whilst the $\beta 1$ and $\gamma 1$ chains are evident on the surface of myotubes by 7 weeks gestation only traces of the $\alpha 2$ chain are observed at this time and all subunits are not maximally expressed until 21 weeks of gestation

[50]. Overall these observations suggest that the formation of the DAGC could be dependent on some form of reciprocal interaction between the newly formed basement membrane and the cytoskeleton of the myotube.

The DAGC and postsynaptic differentiation

During muscle development the earliest sign of postsynaptic specialisation is the aggregation of acetylcholine receptors (AChRs) on the myotube surface at the site of contact with axons of the motor neurons. This is brought about by the secretion of specific isoforms of agrin, a large protein of the extracellular matrix, that becomes incorporated into the synaptic basal membrane. Postsynaptic AChR aggregates are markedly reduced in number, size and density in muscle of agrin-deficient mutant mice supporting the hypothesis that agrin is a critical organiser of postsynaptic differentiation [20]. The mechanism by which agrin interacts with postsynaptic molecules to organise the changes in the newly formed sub-sarcolemmal cytoskeleton are not completely understood. There was consequently considerable interest when it was discovered that α -dystroglycan was a major agrin binding protein [6, 11, 22, 52], and that rapsyn could stabilise AChR clusters by linking them with the DAGC [3].

For a number of reasons including the wide distribution of α -dystroglycan in muscle and non muscle tissues, it would have been surprising if the binding of agrin to α -dystroglycan had been found to be the sole mediator of postsynaptic differentiation [5]. Indeed since these initial observations, studies have now shown that the region of the agrin molecule that binds to α -dystroglycan is not essential for AChR aggregating activity [27], and that isoforms of agrin made by muscle cells bind well to α -dystroglycan but fail to initial AChR clustering [51]. Overall these data imply that α -dystroglycan lacks the binding specificity required for a functional agrin receptor [27]. Instead recent work seems to suggest that agrin acts via a receptor complex that includes MuSK, a muscle-specific protein kinase, and a myotube-specific accessory component [23].

MuSK, although an essential component of the envisaged receptor complex, is unable to mediate the observed responses to agrin alone [23]. Given that α -dystroglycan appears to be the most abundant agrin receptor on the myotube surface, it would seem a likely candidate for the 'accessory component'. However, as mentioned above α -dystroglycan is not muscle specific. Furthermore, the dystroglycan complex does not cluster at the endplate in rapsyn-deficient mice [21] whereas several aspects of agrin/MuSK mediated signalling are intact in these mice. Nonetheless AChRs fail to cluster in rapsyn-deficient mice, raising the possibility that the dystroglycan complex may act to link MuSK activation with AChR clustering perhaps via its binding to agrin. Dystroglycan may also play a part in concentrating agrin on the muscle surface and helping present it to the MuSK receptor complex [23]. Further work in this area should prove to be particularly

profitable, since the association of a receptor tyrosine kinase with the dystroglycan complex suggests that signalling defects could in whole or in part underlie the pathology of muscular dystrophy [23].

Conclusions

Dystrophin and the DAGC link the sub-plasmalemmal cytoskeleton of the adult muscle fibre with basement membrane. This linkage is apparent from the earliest stages of secondary myotube formation when the distribution of dystrophin correlates with the deposition of laminin. Initially thought of as contributing to membrane integrity there is now increasing evidence to suggest that dystrophin and the DAGC plays an important role in extracellular matrix-mediated signal transduction and/or cytoskeletal organisation particularly in specialised areas such as the neuromuscular junction.

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References

- [1] Ahn AH, Freener CA, Gussoni E, Yoshida M, Ozawa E, Kunkel LM: The 3 human syntrophin genes are expressed in diverse tissues, have distinct chromosomal locations, and each bind to dystrophin and its relatives. *J Biol Chem* 1996; 271: 2724-2730.
- [2] Ahn AH, Kunkel LM: The structural and functional diversity of dystrophin. *Nature Genet* 1993; 3: 283-291.
- [3] Apel ED, Roberds SL, Campbell KP, Merlie JP: Rapsyn may function as a link between the acetylcholine-receptor and the agrin-binding dystrophin-associated glycoprotein complex. *Neuron* 1995; 15: 115-126.
- [4] Blake DJ, Nawrotzki R, Peters MF, Froehner SC, Davies KE: Isoform diversity of dystrobrevin, the murine 87-kDa postsynaptic protein. *J Biol Chem* 1996; 271: 7802-7810.
- [5] Bloch RJ, Randall WR: Synapse development - up the junction. *Current Biology* 1994; 4: 936-938.
- [6] Bowe MA, Deyst KA, Leszyk JD, Fallon JR: Identification and purification of an agrin receptor from torpedo postsynaptic membranes - a heteromeric complex related to the dystroglycans. *Neuron* 1994; 12: 1173-1180.
- [7] Brenman JE, Chao DS, Gee SH, Mcgee AW, Craven SE, Santillano DR, Wu ZQ, Huang F, Xia HH, Peters MF, Froehner SC, Brecht DS: Interaction of nitric-oxide synthase with the postsynaptic density protein psd-95 and alpha-1-syntrophin mediated by pdz domains. *Cell* 1996; 84: 757-767.
- [8] Buckle VJ, Guenet JL, Simonchazottes D, Love DR, Davies KE: Localization of a dystrophin-related autosomal gene to 6q24 in man, and to mouse chromosome 10 in the region of the dystrophin muscularis (dy) locus. *Human Genet* 1990; 85: 324-326.
- [9] Butler MH, Douville K, Murnane AA, Kramarcy NR, Cohen JB, Sealock R, Froehner SC: Association of the m(r)-58,000 postsynaptic protein of electric tissue with torpedo dystrophin and the m(r)-87,000 postsynaptic protein. *J Biol Chem* 1992; 267: 6213-6218.
- [10] Byers TJ, Kunkel LM, Watkins SC: The subcellular-distribution of dystrophin in mouse skeletal, cardiac, and smooth muscle. *J Cell Biol* 1991; 115: 411-421.
- [11] Campanelli JT, Roberds SL, Campbell KP, Scheller RH: Role for dystrophin-associated glycoproteins and utrophin in agrin-induced AChR clustering. *Cell* 1994; 77: 663-674.
- [12] Chevron MP, Girard F, Claustres M, Demaille J: Expression and subcellular-localization of dystrophin in skeletal, cardiac and smooth muscles during the human-development. *Neuromusc Disord* 1994; 4: 419-432.
- [13] Clerk A, Morris GE, Dubowitz V, Davies KE, Sewry CA: Dystrophin-related protein, utrophin, in normal and dystrophic human fetal skeletal-muscle. *Histochem J* 1993; 25: 554-561.
- [14] Corrado K, Mills PL, Chamberlain JS: Deletion analysis of the dystrophin-actin binding domain. *FEBS Letters* 1994; 344: 255-260.
- [15] Dickson G, Azad A, Morris GE, Simon H, Nour-sadeghi M, Walsh FS: Colocalization and molecular association of dystrophin with laminin at the surface of mouse and human myotubes. *J Cell Sci* 1992; 103: 1223-1233.
- [16] Dunkley MG, Wells KE, Piper TA, Wells DJ, Dickson G: Independent localization of dystrophin N-terminal and C-terminal regions to the sarcolemma of mdx mouse myofibers in-vivo. *J Cell Sci* 1994; 107: 1469-1475.
- [17] Durbeej M, Larsson E, Ibraghimovbeskovnaya O, Roberds SL, Campbell KP, Ekblom P: Nonmuscle alpha-dystroglycan is involved in epithelial development. *J Cell Biol* 1995; 130: 79-91.
- [18] Ervasti JM, Campbell KP: Membrane organization of the dystrophin-glycoprotein complex. *Cell* 1991; 66: 1121-1131.
- [19] Ervasti JM, Campbell KP: A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J Cell Biol* 1993; 122: 809-823.

- [20] Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP, Sanes JR: Defective Neuromuscular Synaptogenesis in Agrin-Deficient Mutant Mice. *Cell* 1996; 85: 525-535.
- [21] Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, Merlie JP: Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. *Nature* 1995; 377: 232-236.
- [22] Gee SH, Montanaro F, Lindenbaum MH, Carbonetto S: Dystroglycan- α , a dystrophin-associated glycoprotein, is a functional agrin receptor. *Cell* 1994; 77: 675-686.
- [23] Glass DJ, Bowen DC, Stitt TN, Radziejewski C, Bruno J, Ryan TE, Gies DR, Shah S, Mattsson K, Burden SJ, DiStefano PS, Valenzuela DM, DeChiara TM, Yancopoulos GD: Agrin acts via a MuSK Receptor Complex. *Cell* 1996; 85: 513-523.
- [24] Hemmings L, Kuhlman PA, Critchley DR: Analysis of the actin-binding domain of α -actinin by mutagenesis and demonstration that dystrophin contains a functionally homologous domain. *J Cell Biol* 1992; 116: 1369-1380.
- [25] Higuchi I, Yamada H, Fukunaga H, Iwaki H, Okubo R, Nakagawa M, Osame M, Roberds SL, Shimizu T, Campbell KP, Matsumura K: Abnormal expression of laminin suggests disturbance of sarcolemma extracellular-matrix interaction in Japanese patients with autosomal recessive muscular-dystrophy deficient in adhalin. *J Clin Invest* 1994; 94: 601-606.
- [26] Hoffman EP, Brown RH, Kunkel LM: Dystrophin - the protein product of the duchenne muscular-dystrophy locus. *Cell* 1987; 51: 919-928.
- [27] Hopf C, Hoch W: Agrin binding to α -dystroglycan - domains of agrin necessary to induce acetylcholine-receptor clustering are overlapping but not identical to the α -dystroglycan-binding region. *J Biol Chem* 1996; 271: 5231-5236.
- [28] Hynes RO: Integrins: versatility, modulation and signaling in cell adhesion.. *Cell* 1992; 69: 11-25.
- [29] Ibraghimovbeskrovnaya O, Campbell KP: Gene structure and polymorphism of human dystroglycan, analysis of tissue-specific isoforms. *Am J Hum Genet* 1993; 53: 696.
- [30] Ibraghimovbeskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP: Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular-matrix. *Nature* 1992; 355: 696-702.
- [31] Ibraghimovbeskrovnaya O, Milatovich A, Ozcelik T, Yang B, Koepnick K, Francke U, Campbell KP: Human dystroglycan - skeletal-muscle cDNA, genomic structure, origin of tissue-specific isoforms and chromosomal localization. *Hum Molec Genet* 1993; 2: 1651-1657.
- [32] Jung D, Yang B, Meyer J, Chamberlain JS, Campbell KP: Identification and characterization of the dystrophin anchoring site on β -dystroglycan. *J Biol Chem* 1995; 270: 27305-27310.
- [33] Karpati G, Carpenter S, Guerin C, Holland P: Localization and quantitation of the chromosome-6-encoded dystrophin-related protein (utrophin) in pathological human muscle. *Neurology* 1993; 43: A 294-A 295.
- [34] Khurana TS, Hoffman EP, Kunkel LM: Identification of a chromosome-6-encoded dystrophin-related protein. *J Biol Chem* 1990; 265: 16717-16720.
- [35] Koenig M, Kunkel LM: Detailed analysis of the repeat domain of dystrophin reveals 4 potential hinge segments that may confer flexibility. *J Biol Chem* 1990; 265: 4560-4566.
- [36] Levine BA, Moir AJG, Patchell VB, Perry SV: Binding-sites involved in the interaction of actin with the n-terminal region of dystrophin. *Febs Letters* 1992; 298: 44-48.
- [37] Love DR, Hill DF, Dickson G, Spurr NK, Byth BC, Marsden RF, Walsh FS, Edwards YH, Davies KE: An autosomal transcript in skeletal-muscle with homology to dystrophin. *Nature* 1989; 339: 55-58.
- [38] Love DR, Morris GE, Ellis JM, Fairbrother U, Marsden RF, Bloomfield JF, Edwards YH, Slater CP, Parry DJ, Davies KE: Tissue distribution of the dystrophin-related gene-product and expression in the mdx and dy mouse. *Proc Nat Acad Sci US A* 1991; 88: 3243-3247.
- [39] Matsumura K, Ervasti JM, Ohlendieck K, Kahl SD, Campbell KP: Association of dystrophin-related protein with dystrophin-associated proteins in mdx mouse muscle. *Nature* 1992; 360: 588-591.
- [40] Meng H, Leddy JJ, Frank J, Holland P, Tuana BS: The association of cardiac dystrophin with myofibrils/Z-disc regions in cardiac muscle suggests a novel role in the contractile apparatus.. *J Biol Chem* 1996; 271: 12364-12371.
- [41] Miranda AF, Bonilla E, Martucci G, Moraes CT, Hays AP, Dimauro S: Immunocytochemical study of dystrophin in muscle cultures from patients with duchenne muscular-dystrophy and unaffected control patients. *Am J Pathol* 1988; 132: 410-416.
- [42] Mora M, Diblasi C, Barresi R, Morandi L, Brambati B, Jarre L, Cornelio F: Developmental expression of dystrophin, dystrophin-associated glycoproteins and other membrane cytoskeletal proteins in human skeletal and heart-muscle. *Develop Brain Res* 1996; 91: 70-82.
- [43] Mussini I, Facciolo MC, Ennas MG, Curto M, Sogos V, Gremo F: Association of dystrophin to

- actin and intermediate filaments in immature human fetal muscle. *J Muscle Res Cell Mot* 1996; 17: 104
- [44] Noguchi S, McNally EM, Benothmane K, Hagiwara Y, Mizuno Y, Yoshida M, Yamamoto H, Bonnemann CG, Gussoni E, Denton PH, Kyriakides T, Middleton L, Hentati F, Benhamida M, Nonaka I, Vance JM, Kunkel LM, Ozawa E: Mutations in the dystrophin-associated protein gamma-sarcoglycan in chromosome-13 muscular-dystrophy. *Science* 1995; 270: 819-822.
- [45] Ohlendieck K, Ervasti JM, Matsumura K, Kahl SD, Leveille CJ, Campbell KP: Dystrophin-related protein is localized to neuromuscular-junctions of adult skeletal-muscle. *Neuron* 1991; 7: 499-508.
- [46] Porter GA, Dmytrenko GM, Winkelmann JC, Bloch RJ: Dystrophin colocalizes with beta-spectrin in distinct subsarcolemmal domains in mammalian skeletal-muscle. *J Cell Biol* 1992; 117: 997-1005.
- [47] Schofield J, Houzelstein D, Davies K, Buckingham M, Edwards YH: Expression of the dystrophin-related protein (utrophin) gene during mouse embryogenesis. *Develop Dynam* 1993; 198: 254-264.
- [48] Schofield JN, Gorecki DC, Blake DJ, Davies K, Edwards YH: Dystroglycan messenger-rna expression during normal and mdx mouse embryogenesis - a comparison with utrophin and the apo-dystrophins. *Develop Dynam* 1995; 204: 178-185.
- [49] Sealock R, Butler MH, Kramarcy NR, Gao KX, Murnane AA, Douville K, Froehner SC: Localization of dystrophin relative to acetylcholine-receptor domains in electric tissue and adult and cultured skeletal-muscle. *J Cell Biol* 1991; 113: 1133-1144.
- [50] Sewry CA, Chevallay M, Tome FMS: Expression of laminin subunits in human fetal skeletal-muscle. *Histochem J* 1995; 27: 497-504.
- [51] Slater CR: Agrin signals at the junction. *Nature* 1996; 381: 478-479.
- [52] Sugiyama J, Bowen DC, Hall ZW: Dystroglycan binds nerve and muscle agrin. *Neuron* 1994; 13: 103-115.
- [53] Sunada Y, Bernier SM, Kozak CA, Yamada Y, Campbell KP: Deficiency of merosin in dystrophic dy mice and genetic-linkage of laminin m-chain gene to dy locus. *J Biol Chem* 1994; 269: 13729-13732.
- [54] Suzuki A, Yoshida M, Hayashi K, Mizuno Y, Hagiwara Y, Ozawa E: Molecular-organization at the glycoprotein-complex-binding site of dystrophin - 3 dystrophin-associated proteins bind directly to the carboxy-terminal portion of dystrophin. *Europ J Biochem* 1994; 220: 283-292.
- [55] Suzuki A, Yoshida M, Yamamoto H, Ozawa E: Glycoprotein-binding site of dystrophin is confined to the cysteine-rich domain and the 1st-half of the carboxy-terminal domain. *Febs Letters* 1992; 308: 154-160.
- [56] Tanaka H, Ishiguro T, Eguchi C, Saito K, Ozawa E: Expression of a dystrophin-related protein associated with the skeletal-muscle cell-membrane. *Histochemistry* 1991; 96: 1-5.
- [57] Tinsley JM, Blake DJ, Roche A, Fairbrother U, Riss J, Byth BC, Knight AE, Kendrickjones J, Suthers GK, Love DR, Edwards YH, Davies KE: Primary structure of dystrophin-related protein. *Nature* 1992; 360: 591-593.
- [58] Tome FMS, Evangelista T, Leclerc A, Sunada Y, Manole E, Estournet B, Barois A, Campbell KP, Fardeau M: Congenital muscular-dystrophy with merosin deficiency. *Comptes Rendus De L Academie Des Sciences Serie Iii-Sciences De La Vie-Life Sciences* 1994; 317: 351-357.
- [59] Tome FMS, Matsumura K, Chevallay M, Campbell KP, Fardeau M: Expression of dystrophin-associated glycoproteins during human fetal muscle development - a preliminary immunocytochemical study. *Neuromusc Disord* 1994; 4: 343-348.
- [60] Vater R, Harris JB, Anderson LVB, Roberds SL, Campbell KP, Cullen MJ: The expression of dystrophin-associated glycoproteins during skeletal-muscle degeneration and regeneration - an immunofluorescence study. *J Neuropathol Exp Neurol* 1995; 54: 557-569.
- [61] Watkins SC, Hoffman EP, Slayter HS, Kunkel LM: Immunoelectron microscopic localization of dystrophin in myofibers. *Nature* 1988; 333: 863-866.
- [62] Wessels A, Ginjaar IB, Moorman AFM, Vanommen GJB: Different localization of dystrophin in developing and adult human skeletal-muscle. *Muscle & Nerve* 1991; 14: 1-7.
- [63] Worton R: Muscular-dystrophies - diseases of the dystrophin-glycoprotein complex. *Science* 1995; 270: 755-756.
- [64] Xu H, Christmas P, Wu XR, Wewer UM, Engvall E: Defective muscle basement-membrane and lack of m-laminin in the dystrophic dy/dy mouse. *Proc Nat Acad Sci US A* 1994; 91: 5572-5576.
- [65] Yang B, Jung D, Rafael JA, Chamberlain JS, Campbell KP: Identification of alpha-syntrophin binding to syntrophin triplet, dystrophin, and utrophin. *J Biol Chem* 1995; 270: 4975-4978.
- [66] Yoshida M, Ozawa E: Glycoprotein complex anchoring dystrophin to sarcolemma. *J Biochem* 1990; 108: 748-752.

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[67] Yoshida M, Yamamoto H, Noguchi S, Mizuno Y, Hagiwara Y, Ozawa E: Dystrophin-associated pro-

tein A0 is a homolog of the torpedo 87 kD protein. *Febs Letters* 1995; 367: 311-314.