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Three Muscular Dystrophies: Loss of Cytoskeleton-Extracellular Matrix Linkage

Review

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Muscular dystrophies are a group of diseases that primarily affect skeletal muscle and are characterized by progressive muscle wasting and weakness. Although these diseases have been clinically recognized for a number of years, genetic defects in a number of muscular dystrophies have only recently been identified. One of the most important advances in understanding the molecular genetics of neuromuscular diseases has been the cloning of the gene encoding dystrophin, the protein absent in muscle of Duchenne muscular dystrophy (DMD) patients. In the last few years, the role of dystrophin in skeletal muscle has been studied, and several dystrophin-associated proteins (DAPs) have been identified. Components of the dystrophin-glycoprotein complex are now being characterized, and evidence is beginning to indicate that proteins of this complex may be responsible for other forms of muscular dystrophy. The present review focuses on the molecular basis of three muscular dystrophies (DMD, severe childhood autosomal recessive muscular dystrophy [SCARMD], and congenital muscular dystrophy [CMD]) that may be caused by disruptions in the dystrophin-glycoprotein complex, which normally links the subsarcolemmal cytoskeleton to the extracellular matrix in skeletal muscle.

DMD and Becker Muscular Dystrophy

DMD and Becker muscular dystrophy (BMD) are X-linked recessive diseases that are caused by mutations in the DMD gene (reviewed by Hoffman and Kunkel, 1989). The DMD gene is an extremely large and complex gene containing at least five promoters, which regulate the expression of three isoforms of dystrophin (~ 427 kDa) and two smaller proteins of 71 kDa (DP71) and 116 kDa (DP116) (reviewed by Ahn and Kunkel, 1993). The 427 kDa isoform of dystrophin, expressed in muscle and brain, consists of four structurally distinct domains (the amino-terminal actin-binding domain, the large spectrin-like rod domain, the cysteine-rich domain, and the unique carboxy-terminal domain). Dystrophin is localized to the sarcolemma in normal skeletal muscle, but is completely absent in muscle from DMD patients and in two animal models for DMD, *mdx* mice and *grmd* dogs. Mutant forms of dystrophin that lack the cysteine-rich and carboxy-terminal domains or the amino-terminal actin-binding domain also result in a DMD phenotype. The milder BMD phenotype generally results from in-frame mutations that result in expression of dystrophin of lower abundance, smaller size, or both. The overall importance of the amino- and carboxy-terminal (cysteine-rich and carboxyl) domains of dystrophin is supported by the existence of mild cases of BMD in which a shorter dystrophin molecule is expressed that preserves these domains.

Dystrophin-Glycoprotein Complex: A Novel Laminin Receptor Linking the Cytoskeleton and Extracellular Matrix

Based on homologies to α-actinin and spectrin and on its localization to the sarcolemma, dystrophin was proposed to be a membrane cytoskeletal protein (Hoffman and Kunkel, 1989). However, the exact function of dystrophin in skeletal muscle was not revealed by its primary structure. Initial biochemical experiments demonstrated alight association of dystrophin with sarcolemmal glycoproteins and suggested that dystrophin was involved in the anchoring of sarcolemmal proteins to the underlying cytoskeleton (Campbell and Kahl, 1989). Subsequently, adystrophin-glycoprotein complex was purified by sucrose gradient centrifugation in the form of a large (~ 18S) complex and was shown to contain several novel sarcolemmal protein and glycoprotein components (Ervasti et al., 1990; Yoshida-and Ozawa, 1990) (Table 1).

Structural and functional characterization of dystrophin and the DAPs is now giving clues to the overall membrane organization (Figure 1) and to the role of the dystrophin-glycoprotein complex in skeletal muscle (reviewed by Matsumura and Campbell, 1994; Tinsley et al., 1994). Table 1 summarizes the current knowledge of the components of the dystrophin-glycoprotein complex. Present outside of the muscle cell, α-dystroglycan (also called 156 DAG, for dystrophin-associated glycoprotein) links the sarcolemmal membrane to the extracellular matrix by binding the G domain of merosin (muscle isoform of laminin) with high affinity in a calcium-dependent manner (Ibraghimov-Beskrovnaya et al., 1992; Ervasti and Campbell, 1993; Gee et al., 1993; Sunada et al., 1994). The glycoprotein complex links α-dystroglycan to the sarcolemma and is composed of the five integral membrane proteins: adhalin (50 DAG), a 43 kDa glycoprotein doublet (β-dystroglycan and a novel 43 kDa glycoprotein [43 DAG, also known as A3b]), a 35 kDa glycoprotein (35 DAG), and a 25 kDa protein (25 DAP) (Ervasti et al., 1990; Yoshida and Ozawa, 1990; Ibraghimov-Beskrovnaya et al., 1992; Suzuki et al., 1994). Finally, dystrophin links the subsarcolemmal cytoskeleton to the sarcolemma by binding F-actin through its amino-terminal domain (Hemmings et al., 1992) and the glycoprotein complex through its carboxy-terminal domains (Suzuki et al., 1994). The syntrophin triplet (59 DAP) also directly associates with the carboxy-terminal domain of dystrophin (Suzuki et al., 1994).

The characterization of the dystrophin-glycoprotein complex has provided considerable support for the schematic model shown in Figure 2. In this model, dystrophin is the cytoskeletal link between the subsarcolemmal actin-cytoskeleton and the glycoprotein complex, and dystroglycan is the extracellular link between the glycoprotein complex and merosin. The overall organization of the complex

Table 1. Components of the Dystrophin-Glycoprotein Complex

Location	Protein	Other Names(s)	Size(kDa)	Gene Location	Function
Extraceullular Matrix	Laminin a2 chain	Merosin	400	6p22-23	Basal lamina component that binds α-dystroglycan
Sarcolemma					
Extracellular	α-Dystroglycan	156DAG	156	3p21	Binds laminin-2 and is involved in linkage of dystrophin to laminin-2
Transmembrane	β-Dystroglycan	43 DAG, A3a	43	3p21	Binds dystrophin and is involved in linkage to laminin-2
	Adhalin	50 DAG, A2, SL50	50	17q21	Unknown
	43 DAG	A3b	43	Unknown	Unknown } May form a subcomplex
	35 DAG	A4	35	Unknown	Unknown } May form a subcomplex
	25 DAP	A5	25	Unknown	Unknown } May form a subcomplex
Intracellular	α-Syntrophin	59 DAP1, Syn 1	58	20q11	Binds dystrophin/utrophin
	β1-Syntrophin	59 DAP2, Syn 2	59	16	Binds dystrophin/utrophin
	β2-Syntrophin	59 DAP3, Syn 3	60	8q23-24	Binds dystrophin/utrophin
Cytoskeleton	Dystrophin		427	Xp21	Membrane cytroskeletal protein linking transmembrance glycoprotein complex to F-actin

Reviewed by Matsumura and Campbell, 1994; Tinsley et al., 1994.

and the high density of dystrophin in the sarcolemma strongly suggest that this complex has a structural role in skeletal muscle. The linkage between the cytoskeleton and the extracellular matrix through dystroglycan likely provides an important mechanism of anchoring muscle cells to the extracellular matrix. This attachment may stabilize the membrane and protect the sarcolemma from the stresses that develop during muscle contraction.

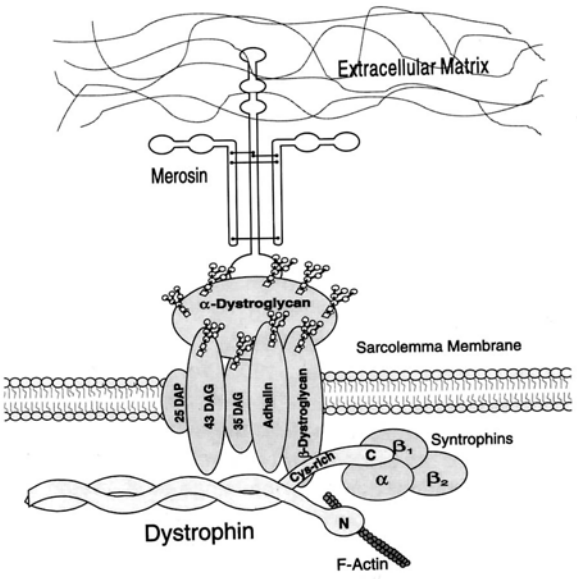

Figure 1. Membrane Organization of the Dystrophin-Glycoprotein Complex

Merosin, a component of the extracellular matrix, binds to α-dystroglycan, which is linked to the sarcolemmal glycoprotein complex that contains adhalin (50 DAG), β-dystroglycan, 43 DAG (also known as A3b), a 35 kDa glycoprotein (35 DAG), and a 25 kDa protein (25 DAP). The membrane glycoprotein complex is linked to the cysteine-rich domain of dystrophin by β-dystroglycan, and the amino-terminal (N) domain of dystrophin binds the actin-cytoskeleton. Syntrophins are bound to the carboxy-terminal (C) domain of dystrophin.

Molecular Pathogenesis of DMD

How does the absence of dystrophin lead to muscle cell necrosis in DMD? Based on current understanding of the function of the dystrophin-glycoprotein complex, the absence of dystrophin would result in the disruption of the linkage between the subsarcolemmal cytoskeleton and the glycoprotein complex in muscle of patients with DMD (Figure 2). This, in turn, may render the sarcolemma susceptible to damage from muscle contraction and thus lead to muscle cell necrosis. Support for this mechanism of pathogenesis comes from the fact that dystrophin-deficient muscle fibers of the *mdx* mouse exhibit an increased susceptibility to contraction-induced sarcolemmal rupture (Petrofet al., 1993).

Immunohistochemistry studies have also revealed a dramatic reduction in all of the DAPs in the sarcolemma of DMD muscle compared with normal muscle and muscle from a variety of other neuromuscular diseases (reviewed by Matsumura and Campbell, 1994). A similar reduction in DAPs was observed in *mdx* mice, which have a nonsense mutation in the dystrophin gene that results in the absence of dystrophin in *mdx* muscle. The specificity of the loss of the DAPs can be seen in DMD carriers, where the only fibers positive for dystrophin are also positive for the DAPs. In BMD there is a mild reduction of the DAPs, consistent with a reduced content of dystrophin. In DMD patients lacking the cysteine-rich and carboxy-terminal domains of dystrophin, all of the DAPs are drastically reduced in the sarcolemma despite proper localization of the truncated dystrophin to the sarcolemma. Therefore, the absence of dystrophin or the lack of its carboxy-terminal domains can lead to a reduction in all of the DAPs in the sarcolemma.

Autosomal Recessive Muscular Dystrophy

Studies concerning the structure and function of the dystrophin-glycoprotein complex raised the intriguing possibility that a primary deficiency in a DAP could be responsible for an autosomal recessive muscular dystrophy with

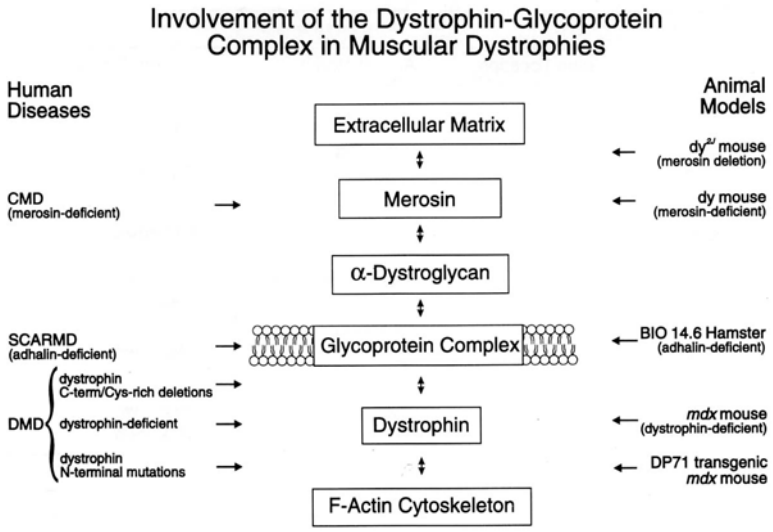

Figure 2. Involvement of the Dystrophin-Glycoprotein Complex in Various Human and Animal Muscular Dystrophies
Interactions between the various components of the dystrophin-glycoprotein complex are illustrated by double-headed arrows. Sites of disruption of the dystrophin-glycoprotein complex in various diseases are illustrated by single-headed arrows. In some cases, a genetic defect has been found in a component of the dystrophin-glycoprotein complex, whereas in others only a biochemical deficiency has been noted to date (see text for details). The BIO 14.6 cardiomyopathic hamster, not mentioned in the text, is a possible model of one form of autosomal recessive muscular dystrophy since skeletal and cardiac muscle from this hamster are deficient in adhalin; however, the underlying genetic defect is not known.

a DMD-like phenotype. Analysis of muscle from autosomal recessive muscular dystrophy revealed a specific absence of the adhalin protein in SCARMD patients (Matsumura et al., 1992). SCARMD resembles DMD or BMD, but affects females and males with equal frequency. SCARMD was first identified in Tunisia and has since been found in other populations. In several North African families, SCARMD has been linked to markers in the pericentromeric region of chromosome 13q, but the affected gene has not yet been identified (Ben Othmane et al., 1992). The adhalin gene was mapped to chromosome 17q21, excluding the gene from involvement in 13q-linked SCARMD (McNally et al., 1994; Roberds et al., 1994). However, the adhalin gene was linked to autosomal recessive muscular dystrophy in one large family, and missense mutations were identified within the gene of four affected children in this family (Roberds et al., 1994).

CMD with Merosin Deficiency

Demonstration that laminin is the native ligand for α-dystroglycan (Ibraghimov-Beskrovnayaet al., 1992) prompted investigation of whether one of the laminin subunits could be involved in other forms of autosomal recessive muscular dystrophy. A specific absence of merosin, the laminin α2 chain, was observed in 13 patients affected by a classical non-Fukuyama type of CMD (Tome etal., 1994). CMD is a heterogeneous group of severe autosomal recessive neuromuscular disease with early clinical onset. Manifestations of CMD are evident at birth or in the first few months of life and consist of muscle weakness and hypotonia, delayed motor milestones, severe and early contractures, and often joint deformities. The absence of merosin in muscle of CMD patients may lead to a disruption of the linkage between the sarcolemma membrane and the extracellular matrix. Recently, four merosin-negative CMD families have been shown by homozygosity mapping to be linked to chromosome 6q2 near the merosin gene (Hillaire et al., 1994). However, mutations in the merosin gene have yet to be identified.

An animal model for CMD with merosin deficiency is the dystrophia muscularis (*dy*) mouse, which is characterized by muscular degeneration and developmental dysmyelination of peripheral nerve. The mouse α2 chain gene *Lama2* maps to the same region of mouse chromosome 10 to which the *dy* locus has been mapped (Sunada et al., 1994). Analysis of merosin expression in dystrophic *dy* mice revealed a specific deficiency of merosin in skeletal muscle, cardiac muscle, and peripheral nerve (Arahata et al., 1993; Sunada et al., 1994; Xu et al., 1994). Recently, Xu et al. (1994) located a mutation in the merosin gene of the dy^{2J} mouse (allelic for *dy*). This mutation results in the expression of a truncated α2 chain lacking a portion of domain VI that is involved in laminin self-aggregation.

Genetic Basis of Other Muscular Dystrophies

Table 2 lists the various human muscular dystrophies and their gene locations (reviewed by McKusick, 1994). To date, ten forms of muscular dystrophy have been characterized at the chromosomal level; in three of these diseases, mutations in specific genes have been identified and the protein products are now being studied. In addition, there are several animal models that have defects in the genes responsible for the human muscular dystrophies. Emerin, the protein product of the Emery-Dreifuss muscular dystrophy gene, has only recently been characterized. Emerin is a 254 amino acid protein with one transmembrane spanning domain whose function is not known. Fukuyama-type CMD has been localized to chromosome 9q31-33, but the gene product for this form of muscular dystrophy is unknown. Two forms of autosomal recessive limb-girdle muscular dystrophy have been linked to chromosome 15q and to chromosome 2p. There are two major autosomal dominant muscular dystrophies: facioscapulohumeral and autosomal dominant limb-girdle muscular dystrophies, which have been linked to chromosome 4q35 and chromosome 5q, respectively. The *myd* mouse is a possible animal model for facioscapulohumeral dystrophy.

Table 2. Molecular Genetics of Muscular Dystrophies

Muscular Dystrophy	Symbol	MIM[a]	Gene Location	Animal Model	Protein Product
X-linked recessive					
Duchenne/Becker	DMD/BMD	310200	Xp21	*mdx* mouse *grmd* dog	Dystrophin
Emery-Dreifuss	EDMD	310300	Xq28	-	Emerin
Autosomal recessive					
Limb-girdle	LGMD1A	253600	15q	-	Unknown
	LGMD2B	253600	2p	-	Unknown
SCARMD	LGMD2C	253700	13q12	BIO 14.6 hamster?	Unknown
	LGMD2D	253700	17q21	-	Adhalin
Congenital (merosin deficient)	CMD	156225	6q2	*dy* and dy^{2J} mice	Merosin?
Fukuyama-type congenital	FCMD	253800	9q31-33	-	Unknown
Autosomal Dominant					
Facioscapulohumeral	FSHD	158900	4q35	*myd* mouse?	Unknown
Limb-girdle	LGMD1A	159000	5q	-	Unknown

Data from McKusick, 1994; Bushby and Beckmann, 1995.

[a] Reference number in McKusick, 1994.

Conclusion

The involvement of the dystrophin-glycoprotein complex in the pathogenesis of three human muscular dystrophies is illustrated in Figure 2. According to the hypothesis presented in this review, the dystrophin-glycoprotein complex is the major mechanism of attachment between the cytoskeleton and extracellular matrix in skeletal muscle. Disruption of this linkage likely leads to sarcolemmal instability and muscle cell necrosis. Biochemical deficiencies in components of the complex have been observed in three forms of muscular dystrophy: DMD (dystrophin deficient), SCARMD (adhalin deficient), and one form of CMD (merosin deficient). DMD patients are deficient in dystrophin owing to mutations in the dystrophin gene. SCARMD patients in one family were found to carry a mutation in the adhalin gene. Although the primary defect in merosin-deficient CMD patients has been gentically linked to a region of the chromosome near the merosin gene, mutations in the merosin gene have not yet been identified.

The importance of sites of interaction between individual components of the complex can be seen in rare DMD cases and in several animal models. For example, the interaction of the carboxyl terminus of dystrophin with the glycoprotein complex is essential since patients that lack this region have a DMD phenotype. The linkage of dystrophin to the actin-cytoskeleton is also crucial since patients with amino-terminal dystrophin mutations have a severe phenotype. In addition, DP71 transgenic *mdx* mice (Cox et al., 1994; Greenberg et al., 1994) have a normal glycoprotein complex, but lack the required interaction with the actin-cytoskeleton and thus have a dystrophic phenotype. The recently described dy^{2J} mouse (Xu et al., 1994), which has a deletion in merosin, reveals the importance of the interaction of merosin with the other components of the extracellular matrix. Finally, the studies of the pathogenesis of these three forms of muscular dystrophy strongly suggest that gene therapy for these diseases will only be effective if the linkage between the cytoskeleton and the extracellular matrix is restored in skeletal muscle.

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