Molecular Basis of Three Muscular Dystrophies: Duchenne, Becker, and Congenital

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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 of dystrophin, expressed in muscle and brain, consists of four structurally distinct domains (the amino-terminal actin-binding 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 gmd 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 that association of dystrophin with sarcomembranous glycoproteins and suggested that dystrophin was involved in the anchoring of sarcomembranous proteins to the underlying cytoskeleton (Campbell and Kahl, 1989). Subsequently, dystrophin-glycoprotein complex was purified by sucrose gradient centrifugation in the form of a large (~18S) complex and was shown to contain several novel sarcomembranous protein and glycoprotein components (Ervasti et al., 1990; Yoshida-and Ozawa, 1990) (Table 1).

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 sarcomembranous 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 DAG) (Ervasti et al., 1990; Yoshida and Ozawa, 1990; Ibraghimov-Beskrovnaya et al., 1992; Suzuki et al., 1994). Finally, dystrophin links the subsarcomembranous 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 DAG) also directly associates with the carboxy-terminal domain of dystrophin (Suzuki et al., 1994).
Table 1. Components of the Dystrophin-Glycoprotein Complex

<table>
<thead>
<tr>
<th>Location</th>
<th>Protein</th>
<th>Other Names(s)</th>
<th>Size(kDa)</th>
<th>Gene Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraceullar Matrix</td>
<td>Laminin a2 chain</td>
<td>Merosin</td>
<td>400</td>
<td>6p22-23</td>
<td>Basal lamina component that binds α-dystroglycan</td>
</tr>
<tr>
<td>Sarcolemma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracellular</td>
<td>α-Dystroglycan</td>
<td></td>
<td>156</td>
<td>3p21</td>
<td>Binds laminin-2 and is involved in linkage of dystrophin to laminin-2</td>
</tr>
<tr>
<td>Transmembrane</td>
<td>β-Dystroglycan</td>
<td></td>
<td>43</td>
<td>3p21</td>
<td>Binds dystrophin and is involved in linkage to laminin-2</td>
</tr>
<tr>
<td></td>
<td>Adhalin</td>
<td></td>
<td>50</td>
<td>17q21</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>Unknown</td>
<td>May form a subcomplex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td>α-Syntrophin</td>
<td></td>
<td>59</td>
<td>20q11</td>
<td>Binds dystrophin/utrophin</td>
</tr>
<tr>
<td>Cytoskeleton</td>
<td>Dystrophin</td>
<td></td>
<td>427</td>
<td>Xp21</td>
<td>Membrane cytoskeletal protein linking transmembrane glycoprotein complex to F-actin</td>
</tr>
</tbody>
</table>

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.

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 (Petrof et 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
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-Beskrovnaya et 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 et al., 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 dystrophin 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<sup>dy</sup> 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

<table>
<thead>
<tr>
<th>Muscular Dystrophy</th>
<th>Symbol</th>
<th>MIM1</th>
<th>Gene Location</th>
<th>Animal Model</th>
<th>Protein Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-linked recessive</td>
<td>DMD/BMD</td>
<td>310200</td>
<td>Xp21</td>
<td>mdx mouse</td>
<td>Dystrophin</td>
</tr>
<tr>
<td></td>
<td>Emery-Dreifuss</td>
<td>310300</td>
<td>Xq28</td>
<td>-</td>
<td>Emerin</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>SCARMD</td>
<td>253600</td>
<td>15q</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Autosomal recessive</td>
<td>SCARMD</td>
<td>253700</td>
<td>13q12</td>
<td>BI O 14.6 hamster?</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>LGMD2D</td>
<td>253700</td>
<td>17q21</td>
<td>-</td>
<td>Adhalin</td>
</tr>
<tr>
<td>Congenital (merosin deficient)</td>
<td>CMD</td>
<td>156225</td>
<td>6q2</td>
<td>dy and dy2/ mice</td>
<td>Merosin?</td>
</tr>
<tr>
<td>Fukuyama-type congenital</td>
<td>FCMD</td>
<td>253800</td>
<td>9q31-33</td>
<td>-</td>
<td>Unknown</td>
</tr>
<tr>
<td>Autosomal Dominant</td>
<td>Limb-girdle</td>
<td>LGMD1A</td>
<td>159000</td>
<td>myd mouse?</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Limb-girdle</td>
<td>LGMD1A</td>
<td>159000</td>
<td>-</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

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

* 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 dy2/ 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.

References

Hillaire, D., Leclerc, A., Faure, S., Topaloglu, H., Chinnakulchhai, Guil...


