Dystrophin, the protein product of the Duchenne muscular dystrophy (DMD) gene, is associated with a large oligomeric complex of sarcolemmal glycoproteins, including dystroglycan which provides a linkage to the extracellular matrix component, laminin. In patients with DMD, the absence of dystrophin leads to the loss in all of the dystrophin-associated proteins, causing the disruption of the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix. This may render the sarcolemma vulnerable to physical stress. These recent developments in the research concerning the function of the dystrophin–glycoprotein complex pave a way for the better understanding of the pathogenesis of muscular dystrophies. © 1994 John Wiley & Sons, Inc.

Key words: Duchenne muscular dystrophy • dystrophin–glycoprotein complex • dystrophin-associated proteins • dystroglycan • sarcolemma

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DYSTROPHIN–GLYCOProTEIN COMPLEX: ITS ROLE IN THE MOLECULAR PATHOGENESIS OF MUSCULAR DYSTROPHIES

KIICHIRO MATSUMURA, MD, and KEVIN P. CAMPBELL, PhD

Duchenne muscular dystrophy (DMD) is one of the most severe and common neuromuscular diseases. Although extensive research efforts have been directed toward the elucidation of the mechanism causing muscle degeneration in this devastating disease, it was not until 1986 when the causative gene was finally identified. Over the last several years, biochemical investigation of dystrophin, the protein product of the DMD gene, has led to the identification of a large oligomeric complex of novel sarcolemmal glycoproteins associated with dystrophin, including dystroglycan which binds the extracellular matrix component, laminin. In this article, we review these recent developments in the field of skeletal muscle dystrophy research and discuss the involvement of the dystrophin-associated proteins in the molecular mechanism leading to muscle cell necrosis in DMD.

DYSTROPHIN

The entire coding sequence of dystrophin was published in 1988. Dystrophin was predicted to be a rod-shaped cytoskeletal protein of 427 kd, composed of four structural domains: (1) the amino-terminal domain with high homology to actin binding regions of such actin binding proteins as α-actinin, β-spectrin, and Dictyostelium actin-binding protein 120; (2) a series of 24 repeats of 109-amino acids in the form of a triple helix; (3) a cysteine-rich domain homologous to the carboxyl-terminal domain of Dictyostelium α-actinin; and (4) the carboxyl-terminal domain with no homology to the previously described sequences at that time.

Antibodies against fusion proteins or synthetic peptides were soon produced and used for the initial identification of this predicted protein. Immunohistochemical and immunoelectron microscopic analyses localized dystrophin to the cytoplasmic face of normal skeletal and cardiac sarcolemma, and immunoblot analysis detected a protein with a molecular mass of 400 kd in normal skeletal and cardiac muscles. Dystrophin was absent in the skeletal and cardiac muscles of DMD patients.

Dystrophin was initially reported to constitute only 0.002% of the total skeletal muscle protein, raising a doubt about the possibility that dystrophin could play a major structural role in skeletal muscle.
tightly associated with membrane glycoproteins. Further investigation revealed that dystrophin is associated with a large oligomeric complex of novel sarcolemmal proteins comprised of a 156-kd glycoprotein (156DAG), a 59-kd protein (59DAP), a 50-kd glycoprotein which was originally called SL50 (50DAG), a 43-kd glycoprotein (43DAG), a 35-kd glycoprotein (35DAG), and a 25-kd protein (25DAP) (Fig. 1a). Tight association of these proteins in the complex was demonstrated by: (1) copurification; (2) cosedimentation on sucrose density gradient; (3) coimmunoprecipitation; (4) stoichiometric ratio; (5) colocalization to the sarcolemma (Fig. 1b); and (6) crosslinking. Dystrophin and the dystrophin-associated proteins (DAPs) also colocalize to the sarcolemma of intrafusal muscle fibers, and to the neuromuscular and myotenon junctions, two specialized regions of the sarcolemma where dense dystrophin-staining is observed (Matsumura and Campbell, unpublished results).

Extensive biochemical analysis of the dystrophin–glycoprotein complex (DGC) indicates the following: (1) the 156DAG is an extracellular protein extractable from the membranes by pH 12 treatment; (2) the 50DAG, 43DAG, 35DAG, and 25DAP are transmembrane proteins; and (3) the 59DAP is a cytoplasmic and probably cytoskeletal protein, extractable from the membranes by pH 11.

**FIGURE 1A.** Components of the dystrophin–glycoprotein complex separated on 3–12% SDS-PAGE. The 156DAG is not stained well with Coomassie blue due to heavy glycosylation.

ULTRASTRUCTURAL analysis has demonstrated that dystrophin is a rod-shaped molecule, as predicted from the primary sequence. Recently, the amino-terminal domain of dystrophin was expressed as a fusion protein and shown to associate with F-actin by cosedimentation analysis. Two putative actin-binding sites were identified by proton NMR spectroscopy of synthetic peptides corresponding to defined regions of the amino-terminal domain of dystrophin. Since dystrophin is localized to the cytoplasmic face of the sarcolemma, it is presumed to interact with cytoskeletal actin such as γ-actin rather than α-actin of thin filaments in muscle cells. Morphological studies indicate that dystrophin does not distribute uniformly along the sarcolemma but is highly enriched in costameres where the Z bands are presumed to be attached to the overlying sarcolemma.

**FIGURE 1B.** Immunohistochemical analysis of the components of the dystrophin–glycoprotein complex in normal skeletal muscle. Immunostaining for dystrophin (a), 156DAG (b), 59DAP (c), 50DAG (d), 43DAG (e), and 35DAG (f) is shown (modified from ref. 55).
treatment, like dystrophin. Coseedimentation analysis demonstrates the interaction of the DGC with F-actin (Ervasi and Campbell, in press).

Which domain of dystrophin interacts with the glycoprotein complex? The C-terminal domains (cysteine-rich and carboxyl-terminal domains) were originally suggested to interact with the DAPs because of the following observations: (1) the lack of significant homology between the carboxyl-terminal domain and proteins of known function except dystrophin-related protein (now called utrophin), an autosomal homologue of dystrophin; (2) the conservation of the C-terminal domains of dystrophin among different species; (3) the clinical observation that the phenotype of the patients with deletions in the C-terminal domains is severe; and (4) the results of immunogold labeling studies. More recently, the results of limited calpain digestion of the DGC demonstrated that the DAPs-binding site was confined to the cysteine-rich and the first half of the carboxyl-terminal domains. On the other hand, dystrophin lacking the C-terminal domains was reported to be localized properly to the sarcolemmal region in unique patients with DMD. This led to a speculation that the C-terminal domains are not essential for the interaction of dystrophin with the sarcolemma. However, the possibility that truncated dystrophin with an intact amino-terminal domain may properly localize to the sarcolemmal region by associating with other sarcolemmal cytoskeletal components such as γ-actin, even when it is not associated with the DAPs, was not addressed. This question will be discussed further in the section "DMD Patients Lacking the C-Terminal Domains of Dystrophin."

Recently, dystrophin was reported to be associated with a postsynaptic protein with molecular
mass of 58 kd in Torpedo electric tissue, which is derived embryologically from immature striated muscle and retains many similarities to mammalian skeletal muscle. Another postsynaptic protein with molecular mass of 87 kd, which shares homology with the C-terminal domains of dystrophin, was also shown to be associated with this 58-kd protein. Immunohistochemical analysis indicates the presence of a mammalian skeletal muscle protein which shares immunological homology with the 58-kd Torpedo protein.

PRIMARY STRUCTURE OF DYSTROGLYCAN (43DAG/156DAG)

In order to understand the function of the DAPs, the primary structure of each component had to be clarified. A single cDNA encoding two of the DAPs, the 43DAG and 156DAG, was isolated and characterized. Posttranslational processing of a 97-kd precursor protein translated from a 5.8-kb mRNA results in these two proteins. Consistent with the aforementioned biochemical data, the carboxy-terminal portion of the precursor protein processed into the 43DAG has three potential N-glycosylation sites, a single potential transmembrane domain and a 120-amino-acid-long cytoplasmic tail. The amino-terminal portion of the precursor protein corresponding to the 56-kd core protein of the 156DAG has no transmembrane domain but one potential N-glycosylation site and many potential O-glycosylation sites. Carbohydrate moieties constitute almost two-thirds of the molecular mass, suggesting that the 156DAG may be a proteoglycan. Heavy glycosylation is presumed to explain the high resistance of the 156DAG to proteolysis. Based on the glycosylated nature and the association with dystrophin, the 43DAG/156DAG was named dystroglycan.

LAMININ-BINDING PROPERTIES OF DYSTROGLYCAN: DGC IS A TRANSSSARCOLEMAL LINKER BETWEEN THE SUBSARCOLEMMAL CYTOSKELETON AND THE EXTRACELLULAR MATRIX

The 156-kd dystroglycan has been shown to bind the extracellular matrix component, laminin. This binding is inhibited by high salt, divalent cation chelating agent (EDTA) or heparin (Ervasti and Campbell, in press). The 156-kd dystroglycan is a highly specific laminin receptor: it does not bind other well-characterized extracellular matrix components such as fibronectin, collagen I, collagen IV, entactin, or heparan sulphate proteoglycan (Ervasti and Campbell, in press). Dystrophin, all of the DAPs, and laminin colocalize to the sarcolemma in skeletal muscle, and to the sarcolemma and transverse tubules in cardiac muscle. These findings indicate that the DGC is a transsarcomemal linker between the subsarcomemal cytoskeleton and the extracellular matrix (Fig. 1c). The DGC is expected to provide a structural support to the sarcolemma and, indeed, could be a unique plasma membrane-supporting mechanism which has developed in striated muscle, a tissue which undergoes both extreme contraction and stretch. In addition to this structural role, the DGC may have far more diverse biological functions such as signal transduction and regulation of the intracellular calcium concentration.

Does the DGC or a homologous complex exist in nonmuscle tissues and, if so, what is its function? Northern blot analysis has demonstrated that dystroglycan mRNA is expressed not only in skeletal, cardiac, and smooth muscles but also in nonmuscle tissues such as brain, lung, liver, and kidney which do not express dystrophin to any significant extent. A cell surface laminin-binding protein with molecular mass of 120 kd was purified from brain and shown to have the primary sequence identical with the 156-kd dystroglycan. The difference in size of the 156-kd dystroglycan from skeletal muscle and brain suggests a different level of glycosylation of the protein between these two tissues, which could reflect different functions of the same gene product in different tissues. Difference in size of the 156-kd dystroglycan is also found among skeletal muscle, diaphragm, cardiac muscle, smooth muscle, lung, and kidney (Ibrahimov-Beskrovnaya and Campbell, personal communication).

At present, the cellular distribution of dystroglycan in nonmuscle tissues is not known. It is also unclear if dystroglycan is associated with dystrophin or dystrophin isoforms/homologues in these tissues. Recently, a novel DMD gene product with molecular mass of 71 kd was identified in nonmuscle tissues, including brain, lung, liver, and kidney. Another DMD gene product with molecular mass of 116 kd was identified in the peripheral nerve. Since these two proteins share homologous C-terminal domains with full-size dystrophin, they could associate with the DAPs in nonmuscle tissues.

The primary structure, function, and tissue distribution of the DAPs other than dystroglycan are unknown. However, partial amino acid sequence analysis indicates that each of the DAPs is a novel protein. Characterization of all of the DAPs at both molecular biological and biochemical levels is es-
sential for the better understanding of not only the structural organization and function of the DGC in skeletal muscle but also the identification of the homologous complexes in nonmuscle tissues.

THE ROLE OF THE DGC IN THE MOLECULAR PATHOGENESIS OF THE mdx MOUSE

The elucidation of the precise mechanism by which the absence of dystrophin leads to muscle cell necrosis is a prerequisite for the development of effective therapies for DMD, the ultimate goal of DMD research. In this respect, the dystrophin-deficient mdx mouse is a good animal model for biochemical investigations.

Immunohistochemistry revealed that all of the DAPs were drastically reduced in the sarcolemma of mdx mice. Immunoblot analysis showed approximately 80–90% reduction in all of the DAPs in mdx skeletal muscle membranes compared with normal membranes. These results were independent of the age of the animals and the severity of degeneration of individual muscle fibers, indicating that the loss of the DAPs is a direct consequence of the absence of dystrophin and not due to the nonspecific secondary effects of muscle fiber degeneration. This hypothesis is also supported by the finding that all of the DAPs are well preserved in dy/dy mice which have normal expression of dystrophin but have severe dystrophy.

Is the synthesis of the DAPs reduced or is the degradation increased in mdx mice? Northern blot analysis revealed the normal production of the dystroglycan mRNA in mdx skeletal muscle. This suggests that the DAPs are synthesized but may not be properly assembled and/or integrated into the sarcolemma or may be degraded in the absence of dystrophin.

What is the status of the residual 10–20% of the DAPs in mdx skeletal muscle? The results of sucrose density gradient centrifugation and immunoprecipitation experiments suggest the presence of four subfractions of the DAPs in mdx skeletal muscle: (1) a complex of the 156DAG and 43DAG; (2) a complex of the 50DAG and 35DAG; (3) unassociated 59DAP; and (4) the DAPs associated with utrophin (discussed later). Since the DAPs associated with utrophin constitute less than 20–30% of the residual DAPs, most of the residual DAPs do not serve a function in the linkage of the subsarcolemmal cytoskeleton to the extracellular matrix. This suggests that the actual disruption of this linkage in mdx skeletal muscle is far more severe than what one expects from the level of the DAPs in the sarcolemma as revealed by immunohistochemistry.

MOLECULAR PATHOGENESIS OF DMD AND RELATED DISEASES

DMD. The structural organization of the DGC (Fig. 1c) suggested that the absence of dystrophin may disrupt the linkage of the DAPs to the subsarcolemmal actin–cytoskeleton in DMD skeletal muscle. This could lead to the dysfunction of the DGC and/or the loss of the DAPs in the sarcolemma, in analogy to other diseases involving cytoskeletal proteins, such as hereditary elliptocytosis, in which the deficiency in one component of the membrane cytoskeleton leads to the loss of the other components.

Immunohistochemical analysis revealed a drastic reduction in all of the DAPs in DMD patients of various ages (Fig. 2). The loss of the DAPs is considered a direct consequence of the absence of dystrophin and not due to the nonspecific secondary effects of muscle degeneration, based on the following observations: (1) all of the DAPs are preserved in a variety of other neuromuscular diseases where muscle fiber necrosis and degeneration occur; (2) the loss of the DAPs is common in all DMD patients, irrespective of age; (3) the loss of the DAPs is found in all muscle fibers, independent of the severity of degeneration; (4) the abundance of many other glycoproteins is not affected in DMD muscle; and (5) other proteins including the membrane cytoskeletal protein spectrin remain well preserved in DMD.

Based on these results and the structural organization of the DGC, we proposed that the disruption of the DGC could play a key role in the cascade of events leading to muscle cell necrosis in DMD. The absence of dystrophin causes the disruption of the linkage of the DAPs to the subsarcolemmal actin–cytoskeleton, which leads to a drastic reduction in all of the DAPs. The resulting disruption of the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix may lead to sarcolemmal instability and eventually to muscle cell necrosis. This may be the case, especially during muscle contraction which may cause physical breaks or tears of the sarcolemma. This hypothesis is quite consistent with the reported morphological abnormalities in the sarcolemma of DMD patients.

DMD Patients Lacking the C-Terminal Domains of Dystrophin. Dystrophin lacking the C-terminal domains was reported to be localized properly to the sarcolemma in unique patients afflicted with DMD. Despite the proper intracellular localization of truncated dystrophin, the phenotype of these patients was quite severe. This indicated
that the C-terminal domains were likely to be very important in the function of dystrophin.\textsuperscript{11,30,34,76}

Recently, the status of the DAPs was studied in similar patients. Immunohistochemistry revealed that all of the DAPs were drastically reduced in the sarcolemma even though dystrophin lacking the C-terminal domains was properly localized to the sarcolemmal region.\textsuperscript{38} The results suggest that the DAPs-binding site is missing in these patients, and thus, are consistent with the observation that the C-terminal domains are essential for interaction with the DAPs.\textsuperscript{93} The loss of the DAPs in the sarcolemma causing the disruption of the linkage between the subsarcolemmal cytoskeleton and extracellular matrix is presumed to be the cause of the severe phenotype of these patients.\textsuperscript{38}

**Symptomatic DMD Carriers.** Dystrophin deficiency is found in some muscle fibers and is speculated to cause muscle fiber degeneration in symptomatic DMD carriers.\textsuperscript{3,59} To test this hypothesis, it was important to know the status of the DAPs in these individuals. Immunohistochemistry showed that all of the DAPs were lost in the sarcolemma of dystrophin-deficient muscle fibers, while they were well preserved in dystrophin-positive fibers, in symptomatic DMD carriers (Fig. 9) (Sewry et al., manuscript in preparation).\textsuperscript{57} This indicates that the linkage between the subsarcolemmal cytoskeleton and the extracellular matrix is disrupted in dystrophin-deficient fibers.\textsuperscript{57} Thus, the same sarcolemmal instability as in the case of DMD may be responsible for the muscle fiber degeneration in symptomatic DMD carriers.

**Becker Muscular Dystrophy (BMD).** Immunohistochemistry has shown reduced and/or patchy dystrophin staining along the sarcolemma, and immunoblot analysis has detected dystrophin of abnormal size and/or reduced quantity.\textsuperscript{5,6,33} How-
milder than in typical DMD patients or the DMD patients lacking the C-terminal domains of dystrophin, indicating that the rod domain is not crucial for the interaction with the DAPs. This suggests that in-frame mutations of the dystrophin gene having no effects on the interaction with the DAPs will not result in a significant loss of the DAPs and/or the disruption of the linkage to the extracellular matrix. However, dystrophin with defects in the rod domain may not have a normal function or may be unstable, and this may lead to a mild reduction in the density of the DGC. This could explain the mild phenotype of these BMD patients. In patients with mutations in the amino-terminal domain of dystrophin, on the other hand, the anchorage of the DGC to the subsarcolemmal actin-cytoskeleton may be disrupted due to the loss or defects of the actin-binding activity of dystrophin. Analysis of the DAPs in these patients would be interesting.

**Autosomal Muscular Dystrophies with DMD-Like Phenotype.** Recent discoveries about the structural organization of the DGC raised a possibility that a primary defect of a DAP could be the cause of autosomal muscular dystrophy. So far, two autosomal diseases have been reported to show abnormalities of the DAPs.

*Severe Childhood Autosomal-Recessive Muscular Dystrophy (SCARMD).* Specific deficiency of the 50DAG was demonstrated in the patients afflicted with a severe childhood autosomal recessive form of muscular dystrophy which is prevalent in North Africa (Fig. 4). Patients with SCARMD present with DMD-like symptoms despite the normal expression of dystrophin. Since the 50DAG deficiency is common to both DMD and SCARMD, it is presumed to be playing an important role in the molecular pathogenesis leading to muscle cell necrosis in these two diseases. In contrast to DMD, where the absence of dystrophin causes a secondary reduction in all of the DAPs and the disruption of the DGC, the deficiency of the 50DAG may cause a dysfunction of the DGC in SCARMD. Although the deficiency of the 50DAG characterizes the early stages of this disease, other components of the DGC could also be affected in the advanced stages.

The initial identification of the 50DAG deficiency was made in 1 Lebanese and 3 Algerian patients. Why is SCARMD prevalent in North Africa? Although the high rate of consanguinity in this region could be the cause, this disease may be specific to Arab populations. Recently, two studies...
were carried out to know if this disease exists in the non-Arab populations. In a study of European patients afflicted with severe childhood muscular dystrophy, 1 Italian, 1 Greek, and 3 French patients were found to be deficient in the 50DAG despite the near-normal presence of dystrophin and the other DAPs (Fardeau et al., submitted). In a Brazilian study, 4 negroid patients with the 50DAG deficiency were identified (Zatz et al., submitted). Thus, SCARMD exists in various populations. All of these non-Arab patients had been diagnosed as DMD/BMD on clinical grounds until the immunohistochemical test revealed the deficiency of the 50DAG instead of dystrophin. Interestingly, consanguinity was negative in all of the European patients. This suggests that the high rate of consanguinity may be the cause of high prevalence of SCARMD in North Africa, even though this disease may not be specific to Arab populations. Whether the 50DAG deficiency exists in the North American or Asian populations remains to be investigated.

With increasing numbers of patients identified, a clearer picture is emerging for the phenotype of 50DAG deficiency. It resembles the phenotype of severe BMD or so-called outliers in many respects. It can be summarized as the following: (1) both sexes are affected equally; (2) weakness of the lower extremities begins between 5 and 10 years of age; (3) calf hypertrophy is common in the early stages; (4) some patients become wheelchair bound as early as 10 years of age; (5) the serum CK value is elevated to 50 times the normal upper limit in the early stages; (6) electromyography and muscle histology reveal myopathic changes which resemble but are milder than those of DMD; (7) early death due to cardiomyopathy can occur; (8) severity of the symptoms vary greatly among both unrelated and related patients; and (9) mental retardation is absent.
The primary defect causing the deficiency of the 50DAG in SCARMD is unknown. It could be due to a primary defect in the structure or expression of the gene for this protein or a secondary effect of an unknown primary defect. Molecular biological and linkage analysis will be needed for the elucidation of the primary cause of SCARMD. Recently, the defective gene responsible for Tunisian autosomal recessive Duchenne-like muscular dystrophy (DLMD) was mapped to the pericentromeric region of chromosome 13q by linkage analysis. It is crucial to clarify the relationship between the DLMD gene and the 50DAG.

Fukuyama-Type Congenital Muscular Dystrophy (FCMD). FCMD is a severe autosomal recessive muscular dystrophy prevalent in Japan. The phenotype of FCMD consists of muscular dystrophy and brain anomaly. In most cases, dystrophin is expressed at near-normal level in this disease. Recently, the abnormal expression of the DAPs was reported in FCMD skeletal muscle. The DAPs staining was reduced in the sarcolemma in a number of muscle fibers despite the near-normal expression of dystrophin (Fig. 5). Muscle fibers with abnormally intense staining of the sarcolemma or with diffuse cytoplasmic staining were also observed.

Genetic observations have suggested a possible interaction between dystrophin and the putative FCMD gene product. Based on the phenotype, the FCMD gene product is expected to be expressed in both muscle and brain. Interestingly, the abnormality of the expression of the 43-kd dystroglycan was prominent in FCMD muscle (Fig. 5). Since dystroglycan is expressed in both muscle and brain, these findings suggest the dystroglycan gene as a candidate gene for FCMD mutations.

**FIGURE 5.** Immunohistochemical analysis of the components of the dystrophin–glycoprotein complex in skeletal muscle from a FCMD patient. Immunostaining for dystrophin (a), 156DAG (b), 59DAP (c), 50DAG (d), 43DAG (e), and 35DAG (f) is shown (modified from ref. 55). Although dystrophin is well preserved, the dystrophin-associated proteins are generally reduced in the sarcolemma. Muscle fibers with abnormally intense staining of the sarcolemma or diffuse cytoplasmic staining for the dystrophin-associated proteins are also observed.
DIAGNOSIS OF MUSCULAR DYSTROPHIES

The findings described above indicate that the status of the DAPs in the sarcolemma shows a good correlation with the severity of the clinical symptoms in certain muscular dystrophies, including DMD, BMD, DMD carriers, SCARMD, and possibly FCMD (Table 1). Thus, the immunochemical analysis of the DAPs, in addition to dystrophin, may be effective for the accurate diagnoses of these diseases. This is especially the case for the diagnosis of male sporadic patients who are affected with severe muscular dystrophy and have reduced amount of normal-sized dystrophin in the immunoblot analysis. According to the standard diagnostic criteria, they would be diagnosed as severe BMD/outlier. However, a fraction of these patients could be afflicted with SCARMD instead, since the phenotype of SCARMD is very close to that of severe BMD/outlier and dystrophin could be reduced in the advanced stages of SCARMD. This indicates that the immunochemical analysis of the 50DAG is necessary for the differential diagnosis of these patients.

So far immunohistochemical abnormalities of the DAPs have not been found in the following diseases: limb-girdle muscular dystrophy, myotonic dystrophy, facioscapulohumeral muscular dystrophy, oculopharyngeal muscular dystrophy, non–Fukuyama-type congenital muscular dystrophy, and spinal muscular atrophy (Matsumura et al., submitted). This does not necessarily exclude a possibility of dysfunction(s) in the components of the DAPs undetected by the current immunohistochemical methods.

### Table 1. Correlation between the phenotype and the status of the expression of dystrophin and the dystrophin-associated proteins (DAPs).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Dystrophin</th>
<th>DAPs</th>
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<tr>
<td>Normal</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DMD</td>
<td>–</td>
<td>Severe reduction</td>
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<tr>
<td>DMD (Lacking C-terminal domains)</td>
<td>–</td>
<td>Severe reduction</td>
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<tr>
<td>Symptomatic BMD carrier</td>
<td>Mosaic Reduced and/or patchy (intact C-terminal domains)</td>
<td>Mosaic Reduced and/or patchy</td>
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<tr>
<td>SCARMD</td>
<td>+</td>
<td>50DAG deficiency</td>
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<tr>
<td>FCMD</td>
<td>+</td>
<td>Abnormal expression</td>
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UTROPHIN–GLYCOPROTEIN COMPLEX

Urophin is an autosomal homologue of dystrophin. While urophin is ubiquitously expressed, it is localized exclusively to the neuromuscular junction in adult skeletal muscle. Urophin is associated with the DAPs or their homologues in skeletal muscle, suggesting that the urophin–glycoprotein complex could be playing an important role in the formation and maintenance of the neuromuscular junction.

In contrast to normal muscle, urophin appears to spread out of the neuromuscular junction and be expressed throughout the sarcolemma in muscle from DMD patients and mdx mice. In mdx mice, this phenomenon seems most prominent in the small-caliber skeletal and cardiac muscles which are relatively free from degeneration. Furthermore, in these muscles of mdx mice, the dystrophin/urophin-associated proteins are well preserved in the sarcolemma compared to the large skeletal muscles such as quadriceps muscle. In the large skeletal muscles of mdx mice, less than 20–30% of the residual DAPs are associated with urophin. Since the residual DAPs in these muscles of mdx mice are equivalent to only 10–20% of the normal level, the DAPs potentially serving as a link between the subsarcolemmal cytoskeleton and extracellular matrix is presumed to be as low as 2–6% of the normal level in these muscles of mdx mice. Thus, the upregulation of urophin could have compensatory effects for dystrophin deficiency, but the level of upregulation in the large skeletal muscles of mdx mice might not be high enough to fully compensate for the absence of dystrophin.

THERAPEUTIC IMPLICATIONS OF THE DAPs FOR DMD

The finding that all of the DAPs, including the laminin-binding dystroglycan and the 50DAG, whose deficiency alone causes severe muscular dystrophy, are lost in the DMD sarcolemma raised a serious question concerning the efficacy of the potential therapies for DMD, such as myoblast transfer therapy or dystrophin gene therapy. The success of such therapies will depend not only on the replacement of dystrophin but also on the restoration and stabilization of the DAPs in the sarcolemma. Since dystrophin can properly localize to the sarcolemmal region without interaction with the DAPs as described above, localization of dystrophin to the sarcolemmal region after these therapies does not necessarily mean that all of the DAPs are restored in the sarcolemma.
Recently the status of the DAPs was analyzed in mdx mice transgenic for the full-size dystrophin gene. All of the DAPs were restored in the sarcolemma of dystrophin-positive muscle fibers, while the DAPs remained reduced in dystrophin-negative fibers, in transgenic mdx mice. Both immunohistochemical and immunoblot analyses demonstrated a good correlation between the level of restoration of the DAPs and the expression of dystrophin (Fig. 6). The results suggest that the gene transfer therapy of dystrophin could be effective in restoring all components of the DGC and, presumably, in correcting the molecular defects. Immunohistochemical analysis of the status of the DAPs in the sarcolemma will be useful for the evaluation of these potential therapies.

Another intriguing therapeutic approach for DMD is the utilization of a protein which could substitute for dystrophin in skeletal muscle. One such candidate is utrophin as described above. If the expression of utrophin could be upregulated by a genetic or pharmacological manipulation in DMD muscle, it could be beneficial for the prevention of muscle degeneration. A similar therapeutic approach was reported for the β-globin diseases. Butyrate, a natural fatty acid which is known to stimulate the synthesis of the fetal isoform of globin (γ-globin), was intravenously administered to patients with β-globinopathies. Both the proportion of reticulocytes producing hemoglobin F and the level of γ-globin mRNA increased in response. These findings suggest that pharmacological agents which stimulate the expression of utrophin could have potential therapeutic value for DMD.

REFERENCES


56. Matsunuma K, Lee CC, Caskey CT, Campbell KP: Restora


75. Ragot T, Vincent N, Chafey P, Vigne E, Giljenkrahn H,


