The Dystrophin Glycoprotein Complex: Signaling Strength and Integrity for the Sarcolemma

Karen A. Lapidos, Rahul Kakkar, Elizabeth M. McNally

Abstract—The dystrophin glycoprotein complex (DGC) is a specialization of cardiac and skeletal muscle membrane. This large multicomponent complex has both mechanical stabilizing and signaling roles in mediating interactions between the cytoskeleton, membrane, and extracellular matrix. Dystrophin, the protein product of the Duchenne and X-linked dilated cardiomyopathy locus, links cytoskeletal and membrane elements. Mutations in additional DGC genes, the sarcoglycans, also lead to cardiomyopathy and muscular dystrophy. Animal models of DGC mutants have shown that destabilization of the DGC leads to membrane fragility and loss of membrane integrity, resulting in degeneration of skeletal muscle and cardiomyocytes. Vascular reactivity is altered in response to primary degeneration in striated myocytes and arises from a vascular smooth muscle cell–extrinsic mechanism. (Circ Res. 2004;94:1023-1031.)

Key Words: dystrophin ■ sarcoglycan ■ membrane ■ cardiomyocyte ■ skeletal muscle

Striated muscle contraction requires precise and coordinated activity of both the nervous and somatic systems, from excitation of individual myofibers at the neuromuscular junction to the ATP-regulated power stroke of myosin. Muscle contraction in both heart and skeletal muscle results in cellular deformation and shortening. Throughout this process, the contractile machinery inside the myofibers must remain intimately connected with the membrane and extracellular matrix. Without this association, movement would be improperly transmitted and myocytes would risk damage to their membranes. One function of the dystrophin glycoprotein complex (DGC) is to provide a strong mechanical link from the intracellular cytoskeleton to the extracellular matrix. The DGC is composed of transmembrane, cytoplasmic, and extracellular proteins. With the emergence of data on the numerous and diverse components of the complex and how they interact with one another, it has become increasingly clear that the DGC holds both structural and signal transduction properties. Known components of the DGC include dystrophin, sarcoglycans, dystroglycan, dystrobrevins, syntrophins, sarcospan, caveolin-3, and NO synthase (Figure 1). These proteins are found in differing combinations depending on muscle type (Table).

Dystrophin
Mutations in the X-linked dystrophin gene produce Duchenne muscular dystrophy (DMD), the milder Becker muscular dystrophy (BMD), and X-linked dilated cardiomyopathy (XLDCM). Cardiomyopathy is frequent in both DMD and BMD; subjects with XLDCM differ in that they have little to no skeletal muscle disease. The large size of the dystrophin gene contributes significantly to its high rate of spontaneous mutation. The 79 exons that encode the 14-kb cDNA span over 2.4 MB of chromosome Xp21 (Figure 2). Several amino-terminally truncated forms of dystrophin are generated by the use of internal tissue-specific promoters. The

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internal promoters drive expression of mRNAs encoding smaller dystrophin products with a distinct tissue-specific distribution. The small 71-kDa carboxyl-terminal form, referred to as Dp71, is broadly expressed. Mutations that preferentially affect dystrophin mRNA expression in cardiac but not skeletal muscle provide one molecular explanation for cardiomyopathy without skeletal muscle involvement, as seen in XLDCM.4-5 However, not all cases of XLDCM can be explained in this manner. For example, point mutants have been described in XLDCM, suggesting that the function of dystrophin may differ between cardiac and skeletal muscle.6,7 Supporting this, DGC proteins are present in T-tubule invaginations that project internally into cardiomyocytes. In skeletal muscle, the DGC is restricted to the plasma membrane, with no internal pattern.8,9 Therefore, this differential cellular localization may indicate additional functions for the DGC in cardiomyocytes. Alternatively, protective mechanisms may be variably present that keep cardiac muscle from undergoing degeneration at the same rate as skeletal muscle.

**Dystrophin Has Four Functional Domains**

The amino-terminus of dystrophin contains a calponin-like actin binding domain with homology to other actin binding proteins, such as \( \beta \)-spectrin and \( \alpha \)-actinin. The amino-terminal actin binding domain is responsible for anchoring dystrophin to cytoskeletal, filamentous \( \gamma \)-actin.8 The central rod domain of dystrophin consists of 24 spectrin-like repeats. Like other spectrin repeats, three helix bundles align to form each repeat unit and provide structural stiffness. Flexibility of the rod region is thought to derive from breaks in the spectrin repeat pattern at four hinge regions. Within the rod region, spectrin repeats 11 through 17 constitute a second site for binding \( \gamma \)-actin, and this site differs considerably in the dystrophin homolog utrophin.9 At the carboxyl-terminus, the cysteine-rich region interacts with the intracellular portion of the transmembrane protein \( \beta \)-dystroglycan and anchors dystrophin to the sarcolemma. The extreme carboxyl-terminal region is \( \alpha \)-helical in nature and mediates its interaction with the syntrophins.10-14

In the absence of dystrophin, the transmembrane DGC elements are unstable and are reduced at the sarcolemma. Skeletal and cardiac muscle that lacks full-length dystrophin and the DGC is abnormally susceptible to damage from contraction. In skeletal muscle, enhanced myofiber damage occurs with eccentric contraction. Contraction of a lengthened muscle lacking dystrophin rapidly loses peak force with rapid, successive contraction.15 In the heart, aortic banding experiments performed on the dystrophin-deficient *mdx* mouse similarly result in accelerated cardiac damage.16 These studies demonstrate the essential role of dystrophin and the DGC in protecting the plasma membrane against contraction-induced damage.

The *mdx* mouse is a model for DMD that carries a naturally occurring point mutation in the dystrophin gene that results in

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**Figure 1.** The DGC is composed of dystrophin (blue), an elongated cytoskeletal protein that links to cytoplasmic \( \gamma \)-actin and the transmembrane components of the DGC. Dystrophin binds to the tail of \( \beta \)-dystroglycan (orange). Dystroglycan is composed of 2 subunits, \( \alpha \) and \( \beta \), each produced from the same gene. Dystroglycan binds to the extracellular matrix protein laminin-\( \alpha \). The sarcoglycan complex (blue-green) is composed of multiple subunits. Mutations in the genes encoding \( \alpha \), \( \beta \), \( \gamma \), and \( \delta \)-sarcoglycan lead to a similar phenotype as dystrophin mutations and include cardiomyopathy and muscular dystrophy in humans and mice. Additional subcomplexes in the DGC in skeletal muscle include cardiomyopathy and muscular dystrophy in skel-

**Figure 2.** In its full-length form, dystrophin expression is driven by 3 different promoters active in cardiac muscle, skeletal muscle, and the brain to generate a 14-kb cDNA encoding a 427-kDa protein. The full-length form includes a classical actin binding domain (ABD) at its amino-terminus. The rod region of dystrophin is composed of 24 spectrin repeats. These repeats are interrupted at 4 points (ovals) to create flexible hinge regions in the rod. Unlike other spectrin repeat–containing proteins, dystrophin is thought to exist as a monomer. A second actin binding domain has been identified in the rod region in spectrin repeats 11 through 17. The carboxyl-terminus of dystrophin includes the cysteine-rich (CR) and carboxyl-terminal (CT) domains that interact with the remainder of the DGC. There are 4 additional promoters within the dystrophin gene that drive expression of the dystrophin short forms that lack the amino-terminal actin binding domain. Dp280 is highly expressed in the retina, whereas Dp140 and Dp116 are expressed in the nervous system. Dp71 is widely expressed in nearly all cell types.
a premature stop codon. There is a complete loss of dystrophin protein expression, with the exception of rare revertant fibers that arise from exon skipping to restore dystrophin and DGC protein expression. Compared with human DMD, the phenotype of the mdx mouse follows a less severe course, especially with respect to cardiomyopathy. Creatine phosphokinase levels are elevated, and the membrane is abnormally permeable to vital tracers such as Evans blue dye (EBD). At the molecular level, the loss of dystrophin results in destabilization of the rest of the DGC, including the sarcoglycan complex. Thus, the mechanical link between the sarcolemma and the extracellular matrix is compromised, providing a mechanism for the plasma membrane leakiness. Intracellular levels of Ca$^{2+}$ are generally elevated.

Antibodies to specific regions of dystrophin have been useful to identify that disrupted dystrophin is a feature of the decompensated cardiomyopathic heart. Antibodies directed against epitopes at dystrophin’s amino-terminus have a greatly reduced staining pattern in core samples taken from patients with heart failure undergoing ventricular assist device implantation. In this setting, epitopes recognized in dystrophin’s rod and carboxyl-terminus appear intact and normally localized. Moreover, improved remodeling provided by afterload reduction from ventricular assist device implantation is associated with restoration of the normal dystrophin pattern at the sarcolemma of cardiomyocytes. Disrupted dystrophin and its role in remodeling was noted in cardiomyopathic hearts of diverse etiologies. Dystrophin disruption is also important in viral cardiomyopathy. The enteroviral protease 2A specifically cleaves dystrophin in its rod portion, contributing to cardiomyocyte degeneration in Coxsackie virus infection. Interestingly, dystrophin deficiency also enhances enteroviral infection, because the DGC may play a role in propagating infection. Therefore, defects in dystrophin contribute to nongenetic forms of cardiomyopathy.

### The Sarcoglycan Complex and Sarcospan

The six sarcoglycans, α, β, δ, ε, γ, and the recently identified ζ, copurify as a complex within the DGC (Figure 3). All are transmembrane-spanning glycoproteins with at least one glycosylation site. α- and ε-sarcoglycan share high homology and are both type I transmembrane proteins. γ-, δ-, and ζ-sarcoglycan are also highly related, and these three subunits likely resulted from multiple gene duplication events, because lower organisms have a single (γ/δ/ζ) sarcoglycan-like gene. These sarcoglycan subunits show weak homology to β-sarcoglycan and are all type II transmembrane proteins. Although the precise function of the complex has remained somewhat elusive, investigations with δ-sarcoglycan–null and γ-sarcoglycan–null mice have helped define the role of the sarcoglycan complex. In the absence of δ-sarcoglycan, the remaining sarcoglycans cannot assemble in the endoplasmic reticulum. In contrast, with the loss of γ-sarcoglycan, assembly of α-, β-, and δ-sarcoglycan can still be detected but is greatly reduced. In sarcoglycan-null muscle, dystrophin remains localized at the plasma membrane and does not require sarcoglycan for normal distribution. However, the

![Figure 3. Sarcoglycan complex. There are 6 sarcoglycans, α through ζ. α- and ε-sarcoglycan are type I transmembrane protein with hydrophobic signal sequences at the amino-terminus. These 2 genes have an identical intron-exon structure, and the proteins are ~60% related. α-Sarcoglycan is found only in cardiac and skeletal muscle, whereas ε-sarcoglycan is expressed outside of muscle. γ-, δ-, and ζ-sarcoglycan have an identical intron-exon structure and are ~70% related at the amino acid level. β-sarcoglycan, like γ-, δ-, and ζ-sarcoglycan, is also a type II transmembrane protein, but it is only weakly homologous to these sarcoglycans. Most homology is found in the conserved cysteine residues found at the extreme carboxyl-terminus (black bars). In cardiac and skeletal muscle, the major sarcoglycan complex is composed of α-, β-, γ-, and δ-sarcoglycan. In vascular smooth muscle, the major sarcoglycan is composed of ε-, β-, δ-, and ζ-sarcoglycan.](image-url)
that such focal necrosis was produced from vascular spasm.\textsuperscript{39}
Supporting this, mice null for δ-sarcoglycan or β-sarcoglycan display a disrupted vascular smooth muscle sarcoglycan complex and evidence for vascular spasm.\textsuperscript{27,40} Transgenic rescue of cardiomyocyte δ-sarcoglycan expression corrects not only cardiomyopathy but also vascular spasm.\textsuperscript{41} These studies demonstrate that a cardiomyocyte-intrinsic defect is responsible for cardiomyopathy in DGC mutant hearts. Additionally, these studies underscore a paradigm where cardiomyocyte degeneration leads to vascular spasm. Vascular spasm in these models is pathogenic, because reduction of vascular spasm limits cardiomyopathy progression.\textsuperscript{38,42}

Sarcospan is a member of the tetraspanin family that associates tightly with the DGC and sarcospan.\textsuperscript{14,43,44} Sarcospan is a highly hydrophobic protein whose amino and carboxyl-termini each face the cytoplasm. In addition, the presence of the sarcoglycan complex is required for the stability of sarcospan at the plasma membrane. Sarcospan-null mice maintain the proper assembly of the entire DGC, showing normal muscle function and histology, serum creatine kinase levels, and impermeability of muscle fibers to EBD.\textsuperscript{45,46} The tetraspanin family is highly diverse, so additional sarcospan-like proteins may accommodate the loss of sarcospan. In other tissues, tetraspanins have been implicated in mediating integrin-signaling responses.\textsuperscript{47}

**Dystroglycan**

Dystroglycan forms an essential core of the DGC as it connects the cytoskeletal components of the DGC to the extracellular matrix.\textsuperscript{48} Dystroglycan is produced from a single gene and is posttranslationally cleaved to produce α and β subunits. β-Dystroglycan is a single-pass transmembrane protein, and its carboxyl-terminus interacts with the cysteine-rich domain of dystrophin. β-Dystroglycan also binds to Grb2, providing a known signaling pathway for β-dystroglycan.\textsuperscript{49} Caveolin-3 also interacts with β-dystroglycan, and it may compete for the same binding site as dystrophin.\textsuperscript{50} The amino-terminus of β-dystroglycan interacts with its extracellular binding partner α-dystroglycan. α-Dystroglycan forms an important connection to the extracellular matrix through its interactions with the α2 chain of laminin 2. Thus, dystroglycan forges a link between the sarcolemma and the extracellular milieu.

Dystroglycan is found in nearly all cell types and is also expressed highly during development, although its posttranslational modifications may differ with cell and tissue type. Unlike the sarcoglycan subunits, dystroglycan exhibits O-linked glycosylation, and genetic studies have now implicated a novel class of genes in the posttranslational processing of α-dystroglycan.\textsuperscript{51} Mutations in these genes lead to muscular dystrophy, abnormal central and peripheral nervous system function, as well as cardiomyopathy. Of these novel genes, the fukutin-related protein gene leads to a form of muscular dystrophy that is highly associated with cardiomyopathy.\textsuperscript{52} In common with these disorders is aberrant processing of α-dystroglycan.

**Syntrophin**

Syntrophin, first identified as a 58-KDa protein in the electric tissue of *Torpedo*, interacts directly with the carboxyl-terminus of both full-length and truncated forms of dystrophin.\textsuperscript{53,54} Three syntrophin forms exist, and each isoform contains two pleckstrin homology domains, a PH1 domain with an internal PDZ domain and a PH2 domain. Syntrophin binds to PIP2, neuronal NO synthase (nNOS), calmodulin, and Grb2.\textsuperscript{55–57} Recently, a link between syntrophin, the DGC, and downstream signaling was identified through Rac1 interactions. A putative signaling pathway was identified in which syntrophin acts as an adaptor for the signaling complex Grb2-Sos1.\textsuperscript{59}

**α-Dystrobrevin**

α-Dystrobrevin shares significant homology with the cysteine-rich and carboxyl-terminal domains of dystrophin, although it lacks dystrophin’s actin binding and rod domains.\textsuperscript{60} Three isoforms of α-dystrobrevin, derived from alternative splicing, are components of skeletal muscle DGC. Important motifs present in the longest isoform, α-dystrobrevin-1, include the Ca\textsuperscript{2+}-binding EF hand, zinc finger ZZ-domain, coiled-coil domain, and a tyrosine kinase substrate domain. The coiled-coil domains directly interact with dystrophin, and an upstream syntrophin binding site allows α-dystrobrevin to interact with the syntrophins.\textsuperscript{61} α-Dystrobrevin-null mice show a complete absence of nNOS at the sarcolemma despite the presence of other DGC components, such as β-dystroglycan, α- and β-sarcoglycan, dystrophin, and syntrophin.\textsuperscript{62} nNOS is responsible for increasing cyclic GMP levels to reduce vasoconstriction of smooth muscle. α-Dystrobrevin-null mice were unable to increase cyclic GMP levels on stimulation, suggesting that nNOS may be a downstream signaling mediator of α-dystrobrevin.\textsuperscript{63} These mice display mild skeletal and cardiac muscle disease. A mutation in α-dystrobrevin has been described in human left ventricular noncompaction with congenital heart disease.\textsuperscript{64} The mutation, found in exon 3, resulted in an amino acid change from proline to leucine and is predicted to alter the EF hand domain.\textsuperscript{64}

**Caveolin and NO Synthase**

Caveolin-3 is expressed only in striated muscle.\textsuperscript{65} Like other caveolin forms, caveolin-3 can oligomerize to form caveolae, small membrane invaginations that participate in membrane organization and uptake of small solutes. Caveolae are thought to be docking sites for signaling proteins, and it was recently shown that caveolin-3 is critical for the localization of src.\textsuperscript{66} The absence of caveolin-3 increases myofiber apoptosis.\textsuperscript{66} Caveolin binds directly to nNOS. In dystrophin-deficient skeletal muscle, nNOS is displaced from the plasma membrane, and this disruption mediates abnormal exercise-induced vasoconstriction.\textsuperscript{67,68} Moreover, muscle that expresses α-syntrophin deleted for the nNOS binding site has impaired vasoconstriction, confirming that nNOS membrane localization with the DGC is critical for vasoconstriction.\textsuperscript{69} The role of NOS may differ between the cardiac and skeletal muscle DGC. In cardiac muscle, it is endothelial NO synthase that participates in a complex with γ-sarcoglycan and δ-sarcoglycan.\textsuperscript{70}
Costameric Arrangement of the DGC

In skeletal and cardiac muscle membranes, the DGC is concentrated over costameres.71,72 Costameres are transverse, rib-like structures that overlie the Z lines of the sarcomere (Figure 4). Focal adhesion proteins such as vinculin, α-actinin, β1 integrin, and β-spectrin are also costameric, but the exact relationship between these focal adhesion components and the DGC is not known. Costameres are thought to transmit mechanical force from the sarcomere to the sarcolemma, the extracellular matrix, and even surrounding fibers and require both outside-in and inside-out signaling.73 Dys-patization, the extracellular matrix, and even surrounding fibers transmit mechanical force from the sarcomere to the sarco-

Figure 4. DGC is found at the plasma membrane. A, Staining for 2 different sarcoglycan proteins, β-sarcoglycan (anti-β-sg) and δ-sarcoglycan (anti-δ-sg). Cross sections of cardiomyocytes are shown, and sarcoglycan subunits, like other DGC proteins, are found at the membrane. Mice lacking γ-sarcoglycan (gsg-) show decreased membrane staining for the remaining sarcoglycan proteins, including β-sarcoglycan and δ-sarcoglycan. Bar=20 μm. B, Normal costameric pattern for β-sarcoglycan (left) and δ-sarcoglycan (right) in skeletal muscle. Costameres overlie the Z line in cardiac and skeletal muscle.

Gene Expression Studies: Pathogenic Pathways Uncovered

Similar to the biochemical and cell biological studies of the DGC, high-density DNA microarray technology has been applied to DGC mutant muscle to ascertain its common and unique features. Gene expression profiles of human and mouse muscle mutant for dystrophin or α-sarcoglycan documented net positive gene expression, consistent with an overwhelming inflammatory response in dystrophic muscle.74 The disruption of the costameric arrangement may be the major initiating factor in the loss of membrane permeability that is a feature of both cardiac and skeletal muscle lacking sarcoglycan or dystrophin (Figure 5). The major integrin complex of cardiac and skeletal muscle includes β10 integrin and is also concentrated at costameres.75 Direct interaction between integrins and the DGC has not been shown, but immunoprecipitation studies suggest bidirec-

Figure 5. Disruption of the sarcoglycan complex leads to membrane permeability defects in skeletal muscle (A) and heart (B). Shown is a γ-sarcoglycan-null heart stained for dystrophin (green) to outline cardiomyocytes. The vital tracer EBD was injected before euthanasia and is seen as red fluorescence inside myofibers. EBD uptake is focal in mutant muscle and heart and is not seen in normal hearts (not shown). Mice lacking dystrophin or sarcoglycan display these permeability defects.

Interestingly, the costameric organization of dystrophin-positive myofibers is compromised in BMD.74 The disruption of the costameric arrangement may be the major initiating factor in the loss of membrane permeability that is a feature of both cardiac and skeletal muscle lacking sarcoglycan or dystrophin (Figure 5). The major integrin complex of cardiac and skeletal muscle includes β10 integrin and is also concentrated at costameres.75 Direct interaction between integrins and the DGC has not been shown, but immunoprecipitation studies suggest bidirec-

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studies of dystrophic muscle. Many aspects of the 56-day-old mdx skeletal muscle expression profile display striking resemblance to the profile of regenerating skeletal muscle. The relative lack of inflammatory infiltrate coupled with the lack of an aggressive regenerative program are two of the important features that differ between the heart and skeletal muscle of DGC mutants.

A comparison of DMD and α-sarcoglycan mutant muscle gene expression profiles was used to identify common features. Like the profiles from murine tissue, the net change in gene expression in these studies was positive. However, the number of differentially expressed genes associated with an inflammatory response was negligible. Genes associated with metabolism, energetics, and mitochondrial function were universally decreased, which the authors suggested may represent a metabolic crisis within human dystrophin-deficient skeletal muscle. Overall, gene expression profiles of dystrophin mutant versus α-sarcoglycan mutant muscle biopsies were remarkably similar. Of the 20 genes identified as differentially regulated between these genetically distinct disorders, many were found differentially regulated in other microarray studies of Duchenne versus control skeletal muscle. Serum amyloid protein A, Her3, osteopontin, and c-fos were uniquely called within the α-sarcoglycan group and not seen in other human studies. The significance of this observation is unclear but may reflect some of the unique differences conferred by individual sarcoglycan gene disruption.

Therapeutic Restoration of DGC: Stem Cell Transplantation

DGC mutants provide important models in which to test gene and stem cell therapies. Stem cell therapy relies on isogenic stem cells as a vehicle to deliver a normal allele to a mutant organism. As it applies to the muscular dystrophies, the ideal stem cell or its progeny would home to and engraft in regions of muscle damage, fuse to preexisting myofibers, differentiate to restore the missing protein, and, finally, improve muscle function. Over the past several years, there has been an evolution in this field in terms of the refinement of stem cell populations as well as a diversity of delivery methods. Primary myoblasts isolated from the muscle of neonatal mice and transplanted into mdx mice were the first successful stem cell transplants in the field. Although these results were promising, the search for more pluripotent, ancestral progenitors led to the isolation of additional stem cell populations from distinct tissue compartments, such as bone marrow and stroma, muscle, skin, and heart. Isolated from fetal, neonatal, or adult mice and using Hoechst dye exclusion or preplating techniques, stem cells have been frequently transplanted into the mdx mouse, because the ability to restore dystrophin protein expression provides a useful marker of successful transplantation.

Side population (SP) cells can be isolated from adult bone marrow and skeletal muscle by the ability to rapidly efflux Hoechst dye 33342. Cell surface markers on SP cells vary depending on their tissue of origin. Hematopoietic SPs are positive for c-kit, CD43, and CD45, whereas muscle-derived SPs are negative for these antigens. Despite these distinctions, systemic transplantation of male hematopoietic SPs or muscle-derived SPs into lethally irradiated female mdx resulted in restoration of limited dystrophin protein expression in skeletal muscle, and up to 0.5% of myofibers in the recipients were both donor-derived and expressed dystrophin. More recently, SPs have been isolated from the skin of adult mice. When normal male skin-derived SP cells were injected into the tail vein of nonirradiated female mdx mice, up to 0.2% of myofibers contained a donor-derived Y chromosome and dystrophin protein expression. Other SPs have been isolated from numerous tissues, including mammary epithelial cells, liver, and heart. The multidrug resistance pump ABCG2 has been implicated in the SP cell’s ability to extrude toxic dyes such as Hoechst 33342.

Muscle-derived stem cells can be isolated from neonatal mouse muscle using a preplate technique in which nonadherent cells are enriched. Clones of these muscle-derived stem cells from mdx mice have been retrotransduced with a human mini-dystrophin gene expressing dystrophin and the stem cell markers CD34 and Sca-1. Transduced cells injected directly into the gastrocnemius muscle of mdx mice produced a low level of human dystrophin in recipient mice. Embryonic cells have also been tested in mouse DGC models. Mesangioblasts are vessel-associated stem cells isolated from fetal mice that can differentiate into most cell types of the mesoderm. Male mesangioblasts transplanted into female α-sarcoglycan–null mice by a single femoral artery injection successfully engrafted and restored sarcoglycan protein expression and resulted in improved histopathology and decreased uptake of a vital dye in the treated leg. Stem cell transplantation in DGC mutant recipients provides an important forum in which to test the capabilities for both embryonic and adult stem cells. Future studies will be facilitated by the availability of these models to test stem cell regeneration for cardiomyopathy and in larger models such as the dystrophin-deficient canine model of DMD.

Therapeutic Restoration of DGC: Viral-Based Gene Therapy

Both high-capacity adenoviral and adeno-associated viral strategies have been successfully used to restore the DGC in skeletal muscle. The large size of dystrophin full-length cDNA (14 kb) combined with the 8-kb DNA cloning capacity of adenoassociated vectors allowed investigators to test several truncated but not full-length forms of dystrophin in gene therapy approaches. These investigations showed promising results but only temporary dystrophin expression because of a virally induced immune response. Adenoviral vectors have been altered to increase cloning capacity and decrease the immune response. Full-length mouse dystrophin cDNA driven by the muscle creatine kinase promoter in a gutted adenovirus vector produced significant dystrophin expression, improved force production, a decrease in susceptibility to contraction-induced injury, and a low-level immune response.

Recently, Yue et al reported using adenovirus-mediated therapy and a novel neonatal cardiac gene transfer technique to test expression of a micro-dystrophin gene in the hearts of mdx mice. A specialized cold chamber was used to anesthetize the mice and provide cold shock for the delivery of virus
directly into the cardiac cavity. Shown by a human-specific antibody, the micro-dystrophin gene driven by the CMV promoter induced expression of dystrophin, β-sarcoglycan, and β-dystroglycan in cardiomyocytes. The expression of dystrophin also correlated with impermeability of that fiber to EBD after β-isoproterenol challenge. The BIO14.6 Syrian hamster model of cardiomyopathy arises from a large deletion in the δ-sarcoglycan gene. Given the larger size of this animal model, viral gene therapy approaches have been more extensively used to demonstrate the success of gene therapy in cardiomyopathic hearts.106–111

**Summary**

The DGC is a critical complex for the maintenance of normal cardiac and skeletal muscle, although the function in these two muscle types may differ slightly. Overall, many of the main components, including dystrophin, dystroglycan, sarcoglycans, and syntrophins, may be similar in heart and skeletal muscle. However, some differences in the more peripheral aspects of the DGC may differ between these muscle types. Current efforts are directed at understanding the tissue-specific expression and function of the DGC and include studying the DGC complex in smooth, including vascular, muscle. The DGC is best thought of as a mechanosignaling complex with dual mechanical and nonmechanical membrane stabilizing functions. This dual functionality can also be ascribed to the sarcoglycan complex, because this complex itself displays both mechanical and signaling roles. The importance of this complex to human disease is now well established. DGC mutants provide excellent models in which to test both cell-based and viral-based gene therapy as well as models in which to study the pathogenesis of skeletal and cardiac power dysfunction.

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**References**


89. Lapidos et al Dystrophin Glycoprotein Complex 1031


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