Dystrophin serves a variety of roles at the cell membrane through its associations, and defects in the dystrophin gene can give rise to muscular dystrophy and genetic cardiomyopathy. We investigated localization of cardiac dystrophin to determine potential intracellular sites of association. Subcellular fractionation revealed that while the majority of dystrophin was associated with the sarcolemma, about 35% of the 427-kDa form of dystrophin was present in the myofibrils. The dystrophin homolog utrophin was detectable only in the sarc- 

cellular membrane and was absent from the myofibrils as were other sarcolemmal glycoproteins such as adhalin and the sodium-calcium exchanger. Extraction of myofibrils with KCl and detergents could not solubili-

The dystrophin gene encodes a 427-kDa protein in muscle and brain tissues. Absence of this full-length form of dystro-

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Alterations in components of the dystrophin-glycoprotein-lami-

nalin complex, such as the laminin-2 gene product, and a 50-kDa dystrophin-associated glycoprotein, adhalin, are asso-

ciated with muscle membrane instability and muscular dystro-

phy (11–13). A deficiency in adhalin and disruption of the mem-

brane dystrophin-glycoprotein complex has also been re-

ported in the cardiomyopathic hamster that experiences both 

hereditary cardiomyopathy and myopathy (14).

In addition to its role in membrane stability and receptor 

custering, recent studies imply that dystrophin serves a 

unique role in signal transduction by localizing nitric-oxide 

synthase to the sarcolemma (15). In order to elucidate the 

cellular roles of dystrophin, it is paramount to precisely define 

its associations. Although it is well documented that dys-

tophin localizes at the plasma membrane in a variety of cells 

(16–19), studies indicate that there may be tissue- and cell-

specific differences in dystrophin localization. For example, 

recent studies using confocal imaging (20–23) and immunogol 

labeling (23) have shown that in addition to the sarcolemma, 

a substantial pool of dystrophin also localizes at the transverse 

tubules in cardiac muscle. Furthermore, cardiac dystrophin 

was found to be distributed in three distinct subcellular pools, 

a cytoplasmic pool, a membrane-bound pool not associated 

with WGA-1-binding glycoproteins, and a membrane-bound pool 

associated with WGA-binding glycoproteins (20, 22). In view 

of the notion that dystrophin may serve diverse roles through 

its associations, here we demonstrate a selective pool of dys-

tophin molecules that associate with the myofibrils and localize 

to the Z-discs in cardiac muscle. Furthermore, the specific loss 

of myofibrillar dystrophin during progression to cardiac insuf-

ficiency in the cardiomyopathic hamster CHF 147 suggests

1 The abbreviations used are: WGA, wheat germ agglutinin; MF, myofibril; SL, sarcolemma; PAGE, polyacrylamide gel electrophoresis; DTT, dithiothreitol.

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that dystrophin may serve a unique role at the level of the sarcome in addition to its recently described membrane functions. The distinct pools of dystrophin and their selective loss may be of significance in explaining the tissue-specific pathophysiological responses in muscular dystrophy (24).

**EXPERIMENTAL PROCEDURES**

**General Conditions**—All muscle preparations were performed at 4°C, and all buffers contained the following protease inhibitors, 1 mM benzamidine, 1 mM iodoacetamide, 1 μg/ml trypsin inhibitor, 2 μg/ml leupeptin, 1 μg/ml pepstatin A, and 0.2 mM phenylmethylsulfonyl fluoride.

Isolation of Myofibrils from Cardiac and Skeletal Muscle—Myofibrils were prepared as described (25). Ventricular muscle of rabbit heart or fast-twitch skeletal muscle (4–5 g) from rabbits was homogenized in 20 volumes of buffer I (39 mM sodium borate, 25 mM KCl, 5 mM EGTA, and 1 mM dithiothreitol (DTT), pH 7.1) with the Brinkman polytron (PT-20) for 15 s, twice. The homogenate was centrifuged at 1,500 × g for 12 min and the pellet was re-extracted once more. The pellet was resuspended in 20 volumes of buffer II (39 mM sodium borate, 25 mM KCl, and 1 mM DTT, pH 7.1) and centrifuged at 1,500 × g for 12 min. This step was repeated and the pellet further extracted for 30 min with Triton X-100 buffer (39 mM sodium borate, 25 mM KCl, 1 mM DTT, and 1% Triton X-100, pH 7.1). The material was filtered and centrifuged at 1,500 × g for 12 min and the pellet re-extracted once more. The pellet was then resuspended twice with suspension buffer (10 mM Tris, 100 mM KCl, and 1 mM DTT, pH 7.1). The final pellet (myofibrils) was resuspended in low-salt buffer (10 mM Tris, 100 mM KCl, 1 mM DTT, and 20% glycerol, pH 7.1) at 2–3 mg/ml and stored at −8°C for further analysis.

Purification of sarcosomal membranes—Cardiac sarcosomal membranes from rabbit hearts were prepared by differential and discontinuous sucrose density gradient as described previously (26). The isolation of crude sarcosomal membranes from rabbit skeletal muscle was as described by Seiler and Fleischer (27).

Separation of Myofibrillar Proteins—Approximately 2.5 mg of isolated myofibrils were solubilized in 4 ml of urea buffer (0.5 M KCl, 50 mM Tris, 1 mM EGTA, and protease inhibitors, pH 8.0) and centrifuged at 100,000 × g for 1 h at 4°C. The supernatant was loaded on a 5–30% continuous sucrose density gradient and centrifuged at 200,000 × g for 24 h at 4°C. The sucrose gradient fractions were collected and concentrated using Centricon-30 tubes (Amicon) and protein composition analysis by 7.5% SDS-PAGE.

Cardiomyopathic Animals—Cardiomyopathic hamsters of the CHF-123 line (derived from the UM-X7.1 line) were obtained from Canadian Hybrid Farms (Nova Scotia, Canada). Hearts from cardiomyopathic and normal hamsters at various ages (30, 60, 120, and 180 days) were obtained and the homogenate, membrane, and myofibrillar fractions isolated as described above.

Antibodies and Western Blotting—Polyclonal antibody against dystrophin was raised in rabbits by injecting synthesized antigenic peptide corresponding to the C-terminal amino acid sequence (SSRGNTPGK) of the human dystrophin, conjugated to keyhole limpet hemocyanin as described (22). Monoclonal antibody Mancho-7 against cardiac Na+-Ca2+ exchanger was a gift from Dr. D. Parry, University of Ottawa, and anti-adhalin was provided by Dr. S. Carbonetto, McGill University. Protein samples were separated by SDS-PAGE in the presence of dithiothreitol and transferred to nitrocellulose. The nitrocellulose was blocked with 5% Blotto in Tris-buffered saline and incubated with primary antibody for 1 h. The membranes were washed three times with Tris-buffered saline-Tween (0.05%) solution and incubated with alkaline phosphatase-conjugated antibody at room temperature. After washing, the sections were incubated in goat-anti-rabbit IgG-5-m gold conjugate (Amersham Corp.). These sections were post-fixed in 2% glutaraldehyde in phosphatebuffered saline, rinsed, counterstained, coated with a thin layer of 1.5% methylcellulose, and allowed to air-dry. In order to quantify the number of gold particles over the Z-disc versus gold distributed in other areas of the myofibrils, we examined 50 micrographs from 4 papillary muscles. In total, we examined 63 μm of Z-discs and 63 μm of A band region of the contractile proteins. All electron microscopic observations were made with a JEOL 100CX.

**RESULTS**

Subcellular Fractionation Reveals Dystrophin Is Associated with the Myofibrillar Fraction—We raised antibodies to the C-terminal 17 amino acids of the skeletal dystrophin sequence. This sequence is exclusive to the 427-kDa form of dystrophin and is not present in any other protein including α-actinin (31). The antibodies were affinity purified on the peptide and specifically recognized dystrophin from skeletal muscle, heart, and brain as we described previously (20, 22, 23). We used the purified antibodies to investigate the localization of dystrophin.

Subcellular fractions from fresh rabbit hearts were isolated as described under "Experimental Procedures." SDS-PAGE analysis of the protein composition of the different fractions obtained during myofibril isolation is shown in Fig. 1A.
cardiac homogenate (lane H) was centrifuged to obtain a supernatant fraction (lane S1) and a pellet fraction (lane P1). The pellet was washed extensively with buffer I to obtain supernatant (lane S2) and pellet (lane P2). Fraction P2 was further extracted with Triton X-100 to obtain supernatant (lane S3) and pellet (lane P3). The final product represented isolated myofibrils (MF). The MF fraction consisted predominantly of myosin (200 kDa) and actin (42 kDa). Other cytoskeletal proteins, such as α-actinin (105 kDa) and desmin (55 kDa) were also present in this fraction.

The protein composition of the myofibrillar preparation was further examined by immunoblotting with various antibodies directed against known myofibrillar proteins and dystrophin. Gels with identical sample loading to that shown in Fig. 1A were electroblotted onto nitrocellulose and immunostained with the various antibodies (Fig. 1B). Anti-desmin and anti-α-actinin staining indicated that these proteins co-fractionated in a similar manner. The pellets of the washes and the isolated myofibrils stained strongly for desmin and α-actinin (Fig. 1B, panel a, lanes P1, P2, P3, and MF), whereas the supernatant fractions had no detectable desmin signal and stained only faintly for α-actinin (Fig. 1B, lanes S1, S2, and S3). Staining with anti-dystrophin antibodies was readily detectable in the cardiac homogenate, and it became stronger in the myofibrillar pellets obtained after extraction with buffer I and buffer II (Fig. 1B, panel c, lanes P1 and P2). In contrast to this, there was little dystrophin staining found in the corresponding supernatant fractions (Fig. 1B, lanes S1 and S2). Furthermore, the nonionic detergent, Triton X-100, failed to extract a significant amount of dystrophin from the pellet fraction (Fig. 1B, lane S3). After the Triton X-100 extractions the isolated myofibrils still contained a significant amount of dystrophin staining (Fig. 1B, lane MF).

To identify any cross-contamination of the myofibril fractions with the sarcolemmal membrane, we investigated the presence of the Na⁺-Ca²⁺ exchanger which is a transmembrane glycoprotein. Immunostaining with cardiac Na⁺-Ca²⁺ exchanger antibody revealed that some of this plasma membrane marker remained in the myofibril pellet during the first two washes of the isolation procedure (Fig. 1B, panel b, lanes P1 and P2). However, this residual membrane glycoprotein was completely removed from the pellet fraction by extraction with Triton X-100 (Fig. 1B, lanes S3 and P3). This immunostaining pattern for the various polypeptides was consistent in all 18 different myofibrillar preparations examined.

Proportion of Total Cardiac Dystrophin Associated with the Myofibrillar Fraction—To determine the relative amount of dystrophin present in the sarcolemmal membrane and myofibrillar fractions, the myofibril isolation protocols were modified to prepare both of these fractions from the same rabbit heart. A Coomassie Blue-stained SDS gel containing these fractions is shown in Fig. 1A. The cardiac muscle was first used to purify a crude sarcolemmal fraction, which was then subjected to discontinuous sucrose density gradient centrifugation to obtain a purified sarcolemmal fraction. The pellet obtained from the cardiac homogenate (Fig. 2A, lane P1) was used to isolate myofibrils (lane MF), whereas the supernatant was used to prepare crude sarcolemmal membranes (lane C) which were then subjected to discontinuous sucrose gradient to purify the sarcolemma (lane SL). The myofibril fraction contained mainly myosin, α-actinin, and actin (Fig. 2A, lane MF) similar to the protein composition of the myofibrillar fraction shown in Fig. 1. The crude sarcolemmal fraction contained substantially less of these myofibrillar proteins (Fig. 2A, lane C), and they were further diminished in the purified sarcolemmal fraction as judged by protein staining (Fig. 2A, lane SL).

As observed with the previous fractionation procedure, desmin and α-actinin staining was intense in the myofibrillar fraction (Fig. 2B, lane MF) when compared with the homogenate or sarcolemmal fractions. Immunostaining for the Na⁺-Ca²⁺ exchanger revealed a 120-kDa band in the crude sarcolemmal fraction (Fig. 2B, lane C) that was significantly stronger in the purified sarcolemmal fraction (Fig. 2B, lane SL). A 70-kDa band also present with the 120-kDa band in the SL fraction was most likely a degradation product of the Na⁺-Ca²⁺ exchanger (32). Na⁺-Ca²⁺ exchanger staining was undetectable in the myofibrillar fraction (Fig. 2B, lane MF). These findings indicate that the modified protocol worked well and gave a clear separation of the sarcolemmal and myofibrillar fractions.

Immunoblotting of the various fractions obtained using the modified protocols revealed the presence of the 427-kDa form of dystrophin in the purified sarcolemma (Fig. 2B, lane SL) and in the myofibrillar fraction (Fig. 2B, lane MF). Although the intensity of dystrophin staining was slightly lower in the myofibrillar fraction than in the purified sarcolemma, we reasoned that quantitatively it may represent a substantial fraction of
the total dystrophin content of cardiac muscle. Western blots of the fractions shown in Fig. 2B were therefore digitally scanned to quantitate the signal intensity of the immunostaining per lane, and this was normalized to the microgram of protein loaded per lane. These values were then multiplied by the total amount of protein present in each fraction to determine the relative amount of dystrophin, Na\(^{+}\)-Ca\(^{2+}\) exchanger, desmin, and \(\alpha\)-actinin present in the homogenate and the various fractions prepared from it. The percent of dystrophin present in the membrane and myofibrillar fractions was compared with the various markers. The results show that approximately 35% ± 5 of the total dystrophin present in the homogenate was recovered in association with the myofibrillar fraction, whereas the recovery of desmin and \(\alpha\)-actinin in this fraction was calculated to be 88% ± 17 and 80% ± 18, respectively (n = 4). The total amount of homogenate dystrophin that was recovered in the sarcolemmal fraction was approximately 55%, compared with a 90% recovery of the Na\(^{+}\)-Ca\(^{2+}\) exchanger.

Dystrophin is known to localize at the sarcolemma in association with membrane glycoproteins. We examined the glycoprotein composition of the MF and SL fractions in a WGA overlay reaction (Fig. 3a). WGA staining of glycoproteins of various molecular mass was detected in the homogenate (lane H) and crude membranes (lane C), and this intensified in the purified sarcolemmal membranes (lane SL). There was weak WGA staining detected in the pellet (lane P1) of the homogenate of glycoproteins of 160, 220, and 300 kDa, and these were seen to enrich in the myofibrils (lane MF) purified from this pellet. These glycoproteins did not appear in the soluble fraction (lane S) or the crude (lane C) and purified membranes (lane SL). Immunoblotting with an antibody against adhalin, a 50-kDa dystrophin-associated sarcolemmal glycoprotein (Fig. 3b), revealed that while this glycoprotein was detectable in the homogenate (lane H) and enriched in the sarcolemmal membrane (lane SL), it was absent from the myofibrils (lane MF).

Dystrophin Is Tightly Associated with the Myofibrillar Fraction—To determine how tightly dystrophin was associated with proteins of the myofibrillar fraction, we examined the conditions required to dissociate dystrophin from other components of this fraction. We first examined the effect of high-salt extraction. The isolated myofibrils were extracted with 0.7 M KCl for 60 min at 4°C, and the soluble proteins were separated from the insoluble material by centrifugation. The KCl wash solubilized approximately 30% of the myofibrillar proteins, and the protein composition of the soluble versus the insoluble fractions was examined in SDS gels and Western blots (Fig. 4).

The polypeptide patterns of the myofibrils, supernatant, and pellet were quite similar as seen by Coomassie Blue staining (Fig. 4a, lanes MF, S, and P). However, immunoblotting with dystrophin antibodies detected the polypeptide only in the myofibrils and the insoluble pellet and not the soluble material (Fig. 4b). Similar results were seen when detergents, such as Triton X-100, Nonidet P-40, deoxycholate, and digitonin, were used to extract the myofibrils together with KCl (data not shown).

The dissociation of dystrophin from myofibrils was further examined by solubilizing the myofibrillar fraction in 5 M urea, followed by separating the proteins in a 5–30% continuous sucrose density gradient. Protein assay indicated that more than 85% of total myofibrillar protein remained soluble in the urea buffer after centrifugation at 100,000 × g for 1 h. Western blot analysis confirmed that the dystrophin was in the soluble fraction (data not shown). The protein patterns of the various fractions obtained from the sucrose gradient fractionation are shown in Fig. 5a. Myosin, \(\alpha\)-actinin, and actin were the major components in the sucrose gradient fractions. A single myosin peak was clearly separated from a single \(\alpha\)-actinin peak. Actin appeared in two distinct peaks in the gradient. The first actin peak (Fig. 5a, Fractions 14–19) was located near the top of the sucrose gradient. This represented most of the actin present in the myofibrillar fraction. A second smaller peak was localized near the bottom of the sucrose gradient (Fig. 5a, Fractions 6–8). This peak did not precisely correspond to the myosin peak, although it substantially overlapped it. The lower actin peak did, however, precisely correspond to a single dystrophin peak in the gradient (Fig. 5, a and b, Fractions 6–8).

Comparison with Skeletal Muscle—To determine whether some cofractionation of dystrophin with the myofibrillar fraction may also occur in skeletal muscle, various subcellular fractions were prepared from rabbit fast twitch skeletal muscle. The protein patterns of the skeletal muscle fractions are shown in Fig. 6a. Skeletal myofibrils (lane MFs) contained mostly myosin, \(\alpha\)-actinin, and actin, similar to cardiac myofibrils (lane MFc). In the skeletal muscle sarcolemma (SLs) and cardiac sarcolemma (SLc) there was much less myosin, actin, and \(\alpha\)-actinin compared with the myofibrils. Immunoblotting with anti-dystrophin antibodies indicated dystrophin staining was significantly enriched in skeletal muscle sarcolemma, but in contrast to cardiac myofibrils (Fig. 6b, lane MFs) no dystrophin was detected in the myofibrils from skeletal muscle (Fig. 6b, lane MFc). Dystrophin staining was evident in the homogenates from both types of muscle (lanes Hs and Hc).

Utrophin Is Present Exclusively in the Sarcolemmal Fraction of Cardiac Muscle—The distribution of the dystrophin...
homolog utrophin in cardiac fractions was compared with that of dystrophin. Western blot analyses with utrophin antibodies revealed that utrophin staining was present exclusively in the purified sarcolemmal fraction (Fig. 7, lane SL) and was notably absent from the cardiac myofibril fraction (Fig. 7, lane MF), whereas dystrophin staining was readily detectable in the myofibrils, sarcolemma, homogenate (H), and crude membranes (C). It should be noted that overdevelopment of the reaction resulted in minor labeling of utrophin in the homogenate and crude membranes, but no utrophin was detected in myofibrils.

Immunogold Labeling Shows Dystrophin Localizes to Z-discs—To determine whether dystrophin was localized preferentially to one region within myofibrils, we examined the localization of dystrophin in intracellular regions of cardiac muscle using immunogold-labeled antibodies directed against dystrophin. Fig. 8 is a typical electron micrograph that illustrates the distribution of gold over the contractile proteins. Immunogold labeling was clearly more abundant over the Z-disc than in any other region of the myofibrils. In 50 micrographs (4 hearts, 4 sections per heart for a total of 63 μm² counted), we found a total of 373 gold particles over the Z-discs and 104 particles over other areas of the contractile proteins. We have previously documented the localization of dystrophin at the sarcolemma and transverse tubules using electron microscopy (23).

Myofibrillar Dystrophin and Cardiac Derangement in Genetic Cardiomyopathy—In order to determine a potential role for myofibrillar dystrophin in cardiac derangement, we examined the expression of dystrophin in the genetically determined cardiomyopathic hamster which experiences severe cardiac insufficiency. The time-dependent developments in cardiac derangements in the CHF 147 line (descendants of the UM-X7 line) of cardiomyopathic hamsters has been well documented (33–36). The CHF 147 strain of hamsters is particularly relevant here because these animals experience dilated cardiomyopathy as well as muscular dystrophy symptoms similar to that seen in some human cases of muscular dystrophy (37–39). This strain of hamsters exhibits the following chronology of events in the myocardium, cardiac myolysis at 60 days followed by cardiac dilation at 80–120 days and cardiac insufficiency at 150 days and in older animals. Previous studies have documented a number of ultrastructural alterations at the cardiac insufficiency stage including disorganization of the myofibrillar arrangement where the Z-discs were noted to be irregular (33–35). We examined the expression of dystrophin which may account for some of these changes in the cardiomyopathic hamster heart. Western blot analysis (Fig. 9) shows that at day 180 there was a marked decrease in the dystrophin staining in the homogenate (lane H) from cardiomyopathic hamsters (M) compared with their age-matched control (C). The membranes (lane S) isolated from the homogenates of these animals also indicated a difference in dystrophin staining between the myopathic and normal animals, although the decrease in staining intensity was not as marked as that noted in the homogenates. Dystrophin staining in the myofibrillar fraction (MF) indicated that there was a marked decrease in the myofibrillar dystrophin in the cardiomyopathic hamster heart. Quantification of the data using densitometry indicated that the total dystrophin content in the cardiomyopathic hamster heart was reduced by 63% (average n = 2) which was reflected as a drop in membrane dystrophin of 28% (average n = 2) while myofibrillar dystrophin decreased by 75% (average n = 2). We further examined the time course of dystrophin loss from the myofibrillar fraction as these animals develop cardiac insufficiency. Fig. 10A shows dystrophin staining in myofibrils isolated from cardiomyopathic hamsters (M) and age-matched controls (C) at day 30, 60, 120, and 180. The data were quantified (Fig. 10B), and it can be seen that at day 30 the dystrophin content in the myofibrils from cardiomyopathic and normal hearts was similar, and thereafter there was a progressive time-dependent decrease in dystrophin with a 75% ± 7 (n = 3) drop at day 180. The expression of desmin was noted to be similar in the two groups of animals (Fig. 10B). The time course of myofibrillar dystrophin loss closely paralleled the decrease in contractile function reported for the CHF 147 hamster heart muscle (36) while the time-dependent accumulation of myofibrillar dystrophin in the normal hamster (Fig. 10) correlated with the age-
dependent increase in contractile performance documented previously (36).

DISCUSSION

The present results demonstrate that a distinct pool of dystrophin molecules associated with the myofibrillar fraction and localized to the Z-discs in cardiac muscle. Whereas a majority of the dystrophin is localized at the surface membrane in association with glycoproteins, our results show that dystrophin at the Z-discs exists in the absence of any of these glycoproteins. Myofibrillar localization of dystrophin was exclusive to cardiac muscle, and its loss correlated with the development of cardiac insufficiency in the genetically determined cardiomyopathic hamster.

Dystrophin was tightly associated with the cardiac myofibrillar fraction that was enriched in cytoskeletal proteins such as α-actinin and desmin. While dystrophin associated with the sarcolemmal membrane was soluble in detergents such as digitonin, Nonidet P-40, Triton X-100, and deoxycholate as previously reported (22, 40–42), these treatments failed to extract dystrophin from cardiac myofibrils. High concentrations of KCl solubilized approximately 30% of the protein content of isolated cardiac myofibrils, but dystrophin was not present in the solubile fraction. Dissociation of dystrophin from other proteins in the myofibrillar fraction could only be achieved by solubilization of myofibrils in buffer containing 5 M urea followed by separation of dissolved proteins on a sucrose density gradient. The peak of myofibrillar dystrophin followed a pattern different from the myosin and α-actinin peaks, but it coincided with one of two distinct actin peaks, suggesting that myofibrillar dystrophin may be associated with actin. Dystrophin is known to bind actin via its N-terminal domain (43–45). Various isoforms of actin are expressed by mature striated muscle. In addition to muscle-specific actin present in the thin filaments, actin is also present in the Z-disc and in the cytoskeletal scaffold surrounding myofibrillar bun-
dies (46). Further work is therefore required to determine the significance of the existence of two distinct pools of actin in the myofibrillar fraction and the selective association of dystrophin with one of these pools. Given our present observation of immunogold labeling of Z-discs with dystrophin antibody in cardiac muscle, the pool of actin cofractionating with dystrophin on the sucrose gradient may represent Z-disc actin.

The tight association of cardiac dystrophin with the myofibrillar fraction suggests that dystrophin may play a unique role in the structure or function of the cardiac myofibril and that this pool of dystrophin may associate with proteins that are distinct from those of the sarcolemmal membrane dystroglycan complex. Accordingly, we found that adhalin, the 50-kDa dystrophin-associated glycoprotein, was present only in the sarcolemmal and not the myofibrillar fractions. The pool of myofibrillar dystrophin cannot therefore be associated with any of the glycoproteins found in the dystroglycan complex as is the case for a sarcolemmal pool of dystrophin. The model for the structure of the dystrophin-containing membrane cytoskeletal complex suggests that dystrophin is anchored in this complex via actin at its N-terminal domain and to one or more components of the membrane glycoprotein complex via its C-terminal domain. It will therefore be of interest to determine whether actin alone anchors dystrophin so tightly to the cardiac myofibrils or whether an additional dystrophin-binding protein is present in the Z-discs. In this regard dystrophin has been shown to localize nitric-oxide synthase to the sarcolemmal membrane and proposed to serve a role in nitric oxide signaling (15). Whether dystrophin at the Z-disc can anchor such signaling molecules at the sarcomere is now under investigation. The origin and the nature of glycoproteins of 160, 220, and 300 kDa in the myofibrillar fraction are intriguing. These glycoproteins could not be extracted with detergents and high salt. Whether dystrophin can associate with any of these glycoproteins to anchor them at the Z-disc remains to be investigated. The dystrophin-related protein, utrophin, was exclusively distributed in the sarcolemmal membrane fraction of cardiac muscle and was absent from the myofibrillar fraction. This is consistent with the immunohistochemical localization of utrophin in heart sarcolemma (47). The fact that utrophin was absent from the cardiac myofibrillar fraction argues against artificial redistribution of dystrophin during the subcellular fractionation procedure. The expression of substantial amounts of utrophin in normal adult cardiac muscle is another noteworthy difference between cardiac and skeletal muscle (29).

Our finding that about 35% of the total cardiac muscle content of the 427-kDa form of dystrophin is associated with myofibrils points to a unique role for dystrophin in the structure/function of the cardiac myofibril. It is evident that dystrophin is a component not only of the membrane cytoskeleton but also of the intracellular cytoskeletal network that organizes myofibrils at the level of the Z-disc. It is of interest that the most frequent cardiac abnormality observed in dystrophin deficiency is dilated cardiomyopathy (37–39), a condition associated with cytoskeletal disorganization as well as irregular sarcomeric and Z-disc structures (48). The cardiomyopathic hamster that exhibits autosomal recessive cardiomyopathy and experiences muscular dystrophy has been widely used as a model system. While the precise genetic defects remain to be defined, several biochemical abnormalities associated with Ca$^{2+}$ overload have been noted (49). The CHF 147 strain of hamsters experience dilated cardiomyopathy and have been shown to exhibit age-dependent morphological alterations in the sarcomeric and Z-disc arrangements that correlate with the decrease in contractile function and progression to cardiac insufficiency (33–36). Our results indicating the loss of myofibrillar dystrophin during the time course of these cardiac derangements suggest that dystrophin at the Z-disc may play an essential role in maintaining structural integrity, and its loss may contribute to the more severe cardiomyopathy compared with the milder muscular dystrophy experienced by these animals. Furthermore, the time-dependent increase in myofibrillar dystrophin paralleled the enhanced contractile performance documented for the normal hamster heart (36), implying that dystrophin serves an important role in the contractile apparatus. It should be noted that the expression of desmin was essentially unaltered during the progression of cardiac insufficiency in the CHF 147 hamster heart, and this is consistent with results obtained in human-dilated cardiomyopathy (48). The CHF 147 hamster is related to the B10 14.6 cardiomyopathic strain that has been reported to completely lack adhalin expression in both cardiac and skeletal muscle with either no change or only a slight decrease in membrane dystrophin levels (14, 50). The lack of adhalin has been suggested to result in disruption of the membrane dystrophin-glycoprotein complex in the cardiomyopathic hamster B10 14.6 strain (14, 50). We also noted the absence of adhalin in the CHF 147 hamster heart as early as day 30, prior to the onset of any symptoms (data not shown), while the membrane pool of dystrophin was reduced by only 28% at much later stages (i.e. day 180) of the disease. The expression of another membrane cytoskeletal protein, spectrin, was also unaltered in the cardiomyopathic hamster heart (50, 51). While the absence of adhalin may contribute to cardiomyopathy and myopathy in these animals, our studies show that it is the loss of dystrophin from the cardiac myofibrils that correlates with progression to cardiac insufficiency. We suggest that alterations in unique cellular pools of dystrophin in cardiac muscle may underlie some of the differential pathophysiological responses of cardiac and skeletal muscle observed in muscular dystrophy.

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