Direct Visualization of the Dystrophin Network on Skeletal Muscle Fiber Membrane

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Abstract. Dystrophin, the protein product of the Duchenne muscular dystrophy (DMD) gene locus, is expressed on the muscle fiber surface. One key to further understanding of the cellular function of dystrophin would be extended knowledge about its subcellular organization. We have shown that dystrophin molecules are not uniformly distributed over the humen, rat, and mouse skeletal muscle fiber surface using three independent methods. Incubation of single-teased muscle fibers with antibodies to dystrophin revealed a network of denser transversal rings (costameres) and finer longitudinal interconnections. Double staining of longitudinal semithin cryosections for dystrophin and α-actinin showed spatial juxtaposition of the costameres to the Z bands. Where peripheral myonuclei precluded direct contact of dystrophin to the Z bands the organization of dystrophin was altered into lacunae harboring the myonucleus. These lacunae were surrounded by a dystrophin ring and covered by a more uniform dystrophin veil. Mechanical skinning of single-teased fibers revealed tighter mechanical connection of dystrophin to the plasma membrane than to the underlying internal domain of the muscle fiber. The entire dystrophin network remained preserved in its structure on isolated muscle sarcolemma and identical in appearance to the pattern observed on teased fibers. Therefore, connection of defined areas of plasma membrane or its constituents such as ion channels to single sarcomeres might be a potential function exerted by dystrophin alone or in conjunction with other submembrane cytoskeletal proteins.

DYSTROPHIN, the protein product of the Duchenne muscular dystrophy (DMD)[1] gene locus on chromosome Xp21 is a rod like molecule with a potential capacity of self association (11, 18, 29). Extensive sequence homologies to its autosomal homologue 6q dystrophin-related protein (DRP) (21) as well as to spectrin and α-actinin suggested that these molecules may be members of a family of large structural proteins expressed in muscle tissue (18). Individuals suffering from DMD show complete lack or vast reduction of dystrophin in muscle tissue, whereas those with the allelic milder disease form, Becker muscular dystrophy (BMD), express reduced quantities of a semifunctional molecule (1, 10, 12, 27, 37). Lack of dystrophin expression also causes the myopathy in the animal model of the *mdx* mouse (4, 33). Previous immunohistochemical studies using antibodies to different portions of the dystrophin molecule have indicated that dystrophin is localized at the plasma membrane in normal skeletal muscle (3, 40). Immunoelectron microscopic localization revealed that the COOH-terminal end of dystrophin is localized at or within the plasma membrane (7) whereas the NH_2 terminus and the rod portion of the molecule are part of the subsarcolemmal cytoskeleton (5, 7, 8, 39). Ultrastructural studies using immunogold suggested a regular distribution with an average distance of ~120 nm between neighboring dystrophin molecules both on longitudinal and transversal sections (7, 8). In an effort to define the subcellular distribution of dystrophin more closely we have developed three independent methods for direct visualization of the regional dystrophin distribution on the skeletal muscle fiber membrane.

1. *Abbreviation used in this paper*: DMD, Duchenne muscular dystrophy.

Materials and Methods

Sources of Tissue

Human muscle specimens were obtained from orthopedic surgery of patients not suffering from neuromuscular diseases. DMD muscle was obtained from diagnostic biopsies of patients as described (37). In particular, muscle tissue of a DMD patient who was deleted for exons 1 to 52 as well as for the muscle and brain promoter regions and the genomic probe 754 was used as negative control for semithin sections (37). Murine (C57Bl/10/sc/sn/him) and rat (Wistar) muscle specimens were obtained from hind limb muscles (tibialis anterior and extensor digitorum longus muscle). The corresponding *mdx* (C57Bl/10/mdx/mdx/him) mouse hind limb muscles served as negative controls.

Antibodies

The following antibodies to dystrophin were used: dys 1, dys 2, and dys 3 (25, 26) (NOVOCASTRA Laboratories, Newcastle upon Tyne, UK). For the purpose of comparison it is important to note that mAb Dy8/6C5 is

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identical with the commercially available mAb dys 2, mAb Dy4/6D3 corresponds to mAb dys 1, and Dy10/12B2 corresponds to mAb dys 3 (L. V. B. Nicholson, personal communication). Further antibodies to dystrophin used in the study were 9C2, 9C5 (30), 1-2a, 10 kd, 60 kd, (11, 13, 14). mAbs to dystrophin were used on murine muscle, specimens were pretreated with 5% goat serum in PBS for 1 h to avoid unspecific background staining. Subsequently, the primary and secondary antibody solutions contained 1% goat serum, and the secondary antibodies were anti-mouse IgG raised in goat (Amersham Buchler GmbH, Braunschweig, Germany). For double staining mouse monoclonal or sheep polyclonal antibodies to dystrophin were combined with rabbit polyclonal antibodies to α-actinin (ICN Biomedicals, Costa Mesa, CA), using streptavidin Texas red (Amersham Buchler GmbH) as marker for dystrophin and streptavidin-FITC (Amersham Buchler GmbH) as marker for α-actinin. As additional control normal and DMD muscle fibers were stained with antibodies to Na-K-ATPase and insulin-receptor (Amersham Buchler GmbH).

Western Blotting

Proteins from total muscle homogenates were resolved by SDS-PAGE on linear 4–20% gradient gels using a 3.5% stacking gel and tank blotted onto nitrocellulose sheets as described (36, 37). 100 μg of protein were loaded per lane. Western blots were probed with the different antibodies as for immunofluorescence but using peroxidase/diaminobenzidine as detection system (36, 37). The amount of myosin loaded per lane was controlled by Coomassie staining of the post blot gels.

Single-teased Muscle Fibers

Single muscle fibers were teased in PBS solution in the presence of protease inhibitors (36) either before or after fixation in ethylacetimidate (Sigma Chemical GmbH, Deisenhofen, Germany) (0.3 M, 20 min). Teased fibers were placed on gelatine-coated glass slides and stained for dystrophin as described (37). All tissue specimens, semithin sections as well as teased and skinned muscle fibers were observed and photographed on a photomicroscope (III RS; Carl Zeiss; Oberkochen, Germany) equipped with epifluorescence. For double label experiments nuclear DNA was stained by ethidium bromide (Sigma Chemical GmbH, Deisenhofen, Germany), (1:10,000 vol/vol in PBS, 2 min) and detected as orange-red signal by the Texas red filter, and as a yellowish signal by the FITC filter.

Semithin Cryosections

Muscle specimens were immediately fixed in ethylacetimidate (0.3 M, 40 min) and, for human muscle only, subsequently in 4% paraformaldehyde in PBS for 20 min. Thereafter, specimens were dehydrated in sucrose (2.3 M, 2 h, containing 20% wt/vol polyvinyl pyrolidone). Semithin cryosections of 900-nm thickness were cut in a RMC MT 6,000 Ultracut at −70°C, placed on polylysine (Sigma Chemical GmbH)-coated glass slides and immunostained as single-teased fibers (cf. Fig. 1) (35).

Mechanical Skinning of Single Fibers

Mechanical skinning of single muscle fibers was performed modifying a protocol by Moisescu (23). Fixed or unfixed teased single fibers were kept in PBS solution containing protease inhibitors and pinned on wax plates under a stereo preparation-microscope (WILD). Using sharpened super fine forceps (Biology No. 5, Dumont Montignez, Switzerland) the sarcolemma was mechanically removed and transferred on gelatine-coated glass slides. Staining was performed as for single-teased fibers.

Results

Antibodies

For further characterization Western blots of muscle homogenates were probed with antibodies to dystrophin (dys 2 and dys 3) and α-actinin (Fig. 1). The mAbs dys 2 and dys 3 detected dystrophin as a single band (dys 2) or doublet (dys 3) of 400 kD in normal human rectus femoris muscle but failed to detect a band of comparable molecular weight or other immunoreactive bands in quadriceps muscle from a DMD boy with a deletion of exons 1–52 or *mdx* tibialis anterior muscle, as previously reported (26, 35). Both antibodies showed a faint crossreactive band at 130 kD in normal and DMD muscle which was more pronounced in *mdx* muscle. The identity of this protein is as yet unknown. The polyclonal antibodies to α-actinin detected a single band of 100 kD in normal human rectus femoris muscle, dystrophin-deficient Duchenne muscle and in muscle from the dystrophin-deficient *mdx* mouse (Fig. 1).

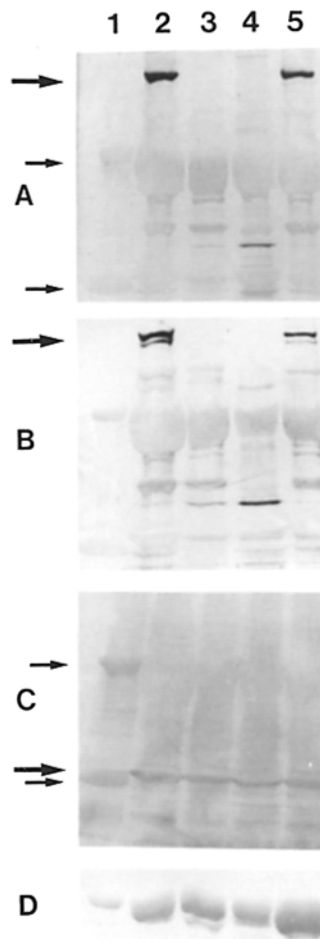

Figure 1. Three parallel Western blots of skeletal muscle homogenates with identical loading (A, B, C) were probed with mAbs dys 2 (*A*), mAbs dys 3 (*B*), and polyclonal antibodies to α-actinin (*C*). The amount of myosin loaded per lane was controlled by Coomassie staining of the post blot gels and is shown for one gel in *D*. Lanes were loaded as follows: lane *1*, molecular weight markers (the small arrows in *A* and *C* indicate myosin, a protein of 200 kD and phosphorylase (*B*), a protein of 97.4 kD); lanes *2* and *5*, normal human rectus femoris muscle; lane *3*, quadriceps femoris muscle from a DMD patient with a deletion of exons 1–52 of the dystrophin gene; lane *4*, tibialis anterior muscle from *mdx* mouse. (*A*) Dystrophin-specific mAbs dys 2 detected dystrophin (*arrow*) as a single band of 400 kD in normal (lanes *2* and *5*) but not DMD (lane *3*) or *mdx* (lane *4*) muscle. A single cross-reactive band of 130 kD was present in normal, DMD and, more pronounced, in *mdx* muscle. (*B*) The mAb dys 3 detected dystrophin (*arrow*) as a doublet with a more prominent upper and a less prominent lower band of 400 kD in normal muscle (lanes *2* and *5*) but not in DMD (lane *3*) or *mdx* (lane *4*) muscle. A cross reactive band of 130 kD was detected by dys 3 in identical position as by dys 2. (*C*) The polyclonal antibodies to α-actinin detected a single band of 100 kD (*arrow*) in normal (lanes *2* and *5*), DMD (lane *3*), and *mdx* (lane *4*) muscle. No significant cross-reactions to other proteins were observed. (*D*) Control of the myosin content of the 100 μg of muscle homogenate loaded per lane. The myosin content was slightly reduced in the *mdx* muscle specimen.

Muscle Staining

In a first approach normal human, rat, and mouse skeletal muscle specimens were carefully dissected immediately after removing and single teased muscle fibers were stained for dystrophin using a panel of eight specific antibodies. All antibodies tested revealed an identical uneven distribution of dystrophin on the muscle fiber surface. As a rule, the fiber was covered by a dystrophin network which consisted of more predominant rings, encircling the whole fiber perpen-

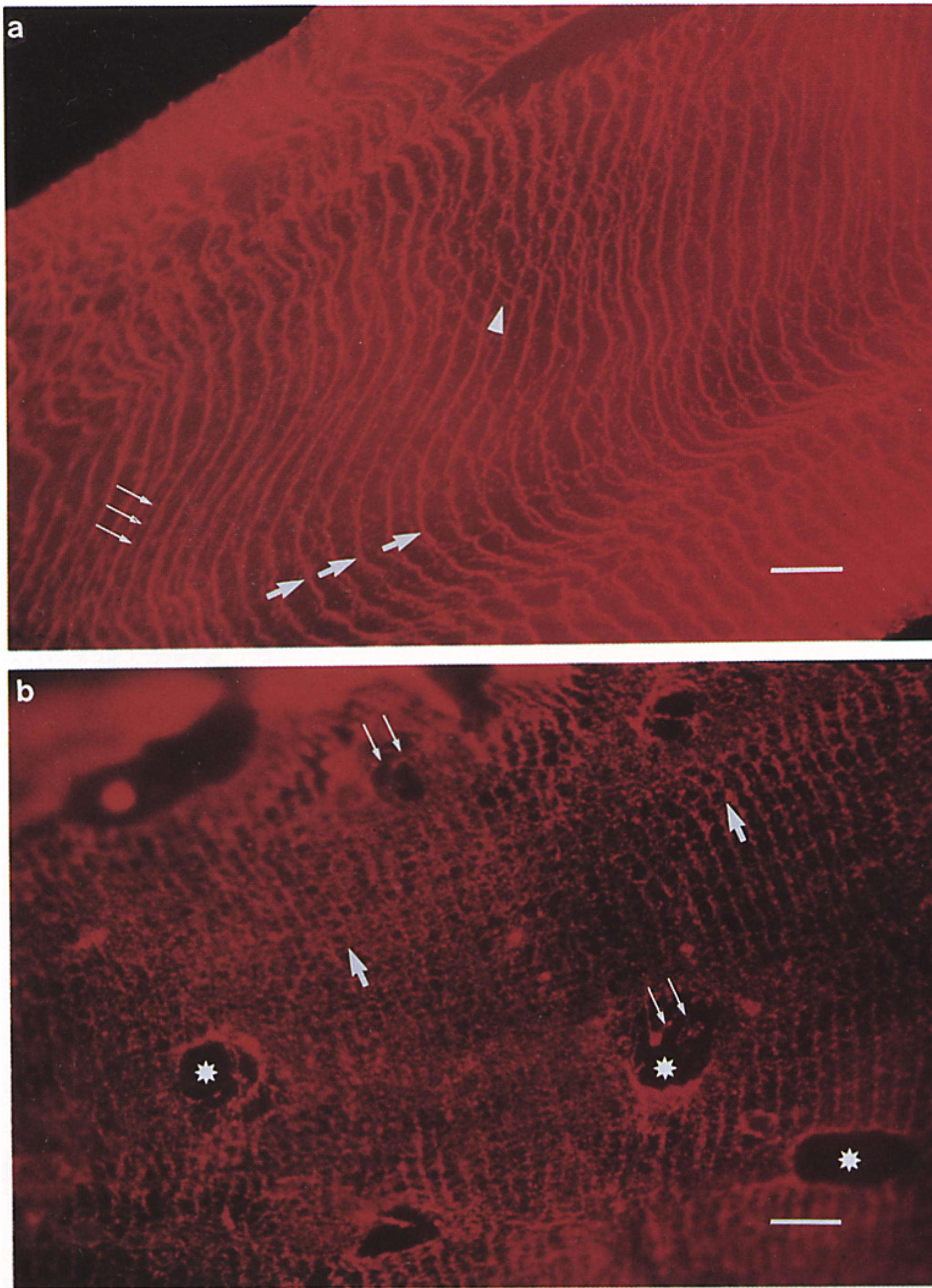

Figure 2. Organization of dystrophin on normal human skeletal muscle fiber surface. A single teased fiber of rectus femoris muscle (*a* and *b*) was labeled with mAb dys 2 anti-dystrophin from mouse followed by biotinylated goat anti-mouse antibodies and Texas red streptavidin. (*a*) On a single-teased fiber dystrophin staining pattern displayed periodic organization in costameres running transversal to the long axis of the fiber (*arrows*) and irregularly interspersed interconnections of lower intensity (*arrowhead*). Some interconnections showed longitudinal organization (*arrowhead*), and others seemed to run parallel to the costameres for a certain distance (*small arrows*). (*b*) Dystrophin staining revealed regularly organized costameres on a single-teased muscle fiber surface. Some sarcolemmal areas showed lattice-like dystrophin organization (*arrows*). In addition, lacunae (*asterisks*) were surrounded by a dystrophin ring and some were partially covered by a fine dystrophin veil (*small arrows*). Bar, 10 μm.

dicular to its longitudinal axis and at regular intervals of ~1–2 μm (Fig. 2, *a* and *b*). In analogy to the similar riblike scaffold which has been observed for the subcellular organization of vinculin, γ-actin, and spectrin in muscle tissue (6, 28) these major components of the dystrophin lattice will subsequently be termed "costameres." Using the micrometer focus the dystrophin costameres could be followed surrounding the muscle fiber in its entire circumference. The space between the dystrophin costameres was occupied by more delicate interconnections whose fine structure and orientation appeared less regular and dependent on the state of contraction of the fiber. In a more relaxed state the interconnections frequently appeared as finer rings running parallel to the costameres whereas in other places they showed longitudinal orientation (Fig. 2, *a* and *b*). Similar results were obtained using fixed and unfixed tissue with slightly better structural preservation after mild fixation (not shown). Even distribution without costameric organization was observed for Na-K-ATPase and insulin receptor on normal and DMD single-teased muscle fibers (not shown).

Because the complex organization of dystrophin with a distance of approx. 1–2 μm between the costameres on the fiber surface would not allow for any direct explanation by dystrophin dimers or tetramers (29) we sought to correlate the observed immunofluorescence pattern to the periodicity of the contractile apparatus. Double labeling of longitudinal semithin cryosections of human and mouse skeletal muscle with antibodies to dystrophin and α-actinin revealed focal

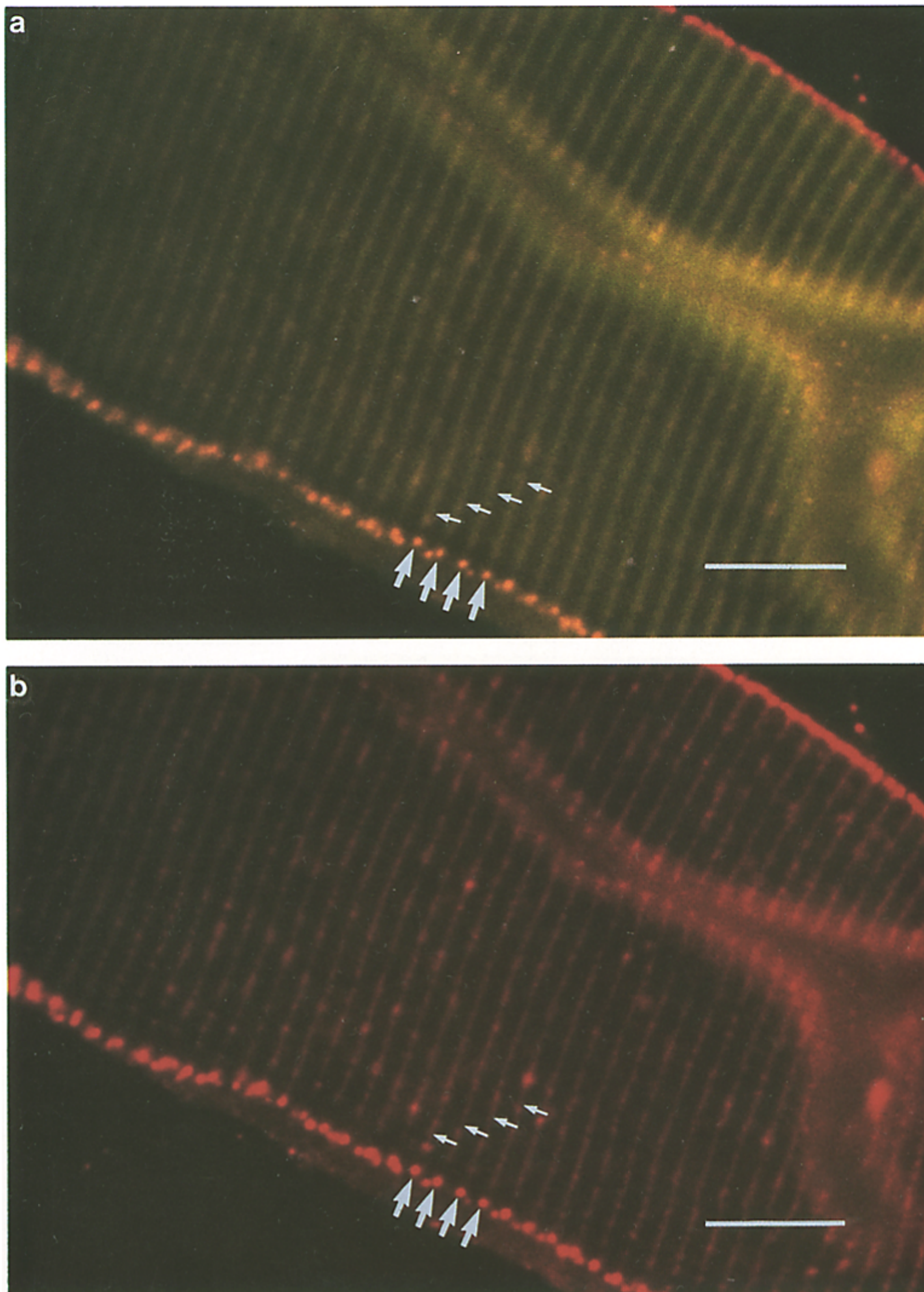

Figure 3. Semithin cryosections from normal mouse tibialis anterior muscle were labeled with polyclonal 60-kD anti-dystrophin antibodies followed by biotinylated donkey-anti-sheep antibodies and streptavidin Texas red (*a–c*) and, subsequently, with polyclonal anti-α-actinin antibodies from rabbit followed by biotinylated donkey-anti-rabbit antibodies and streptavidin FITC (*a* and *b*). (*a*) Double exposure for dystrophin (Texas red) and α-actinin (FITC, *green*) revealed one to one juxtaposition of dystrophin accumulations (*arrows*) and Z bands (*small arrows*) along the entire fiber surface. (*b*) Single exposure of the same fiber for dystrophin (*arrows*) confirmed direct juxtaposition by excluding phase shift due to change of the fluorescence filter. The Z bands were faintly labeled due to cross-reaction of these anti-dystrophin antibodies to α-actinin (13) (*small arrows*). (*c*) Semithin cryosection showing dystrophin costameres if areas of the sarcolemma were in the plane of section (*arrows*). Bar, 10 μm.

concentration of dystrophin at the region where the projected end of the Z bands would link up with the plasma membrane (Fig. 3, *a* and *b*). This finding was obtained independent from the state of myofiber contraction, so that sarcomeres of different lengths retained the juxtaposed dystrophin accumulation. This juxtaposition of the dystrophin costameres to the Z bands could be reproduced using different fixation protocols and even when unfixed fresh frozen semithin sections were stained (not shown). The costameres sometimes appeared wedge shaped, and the space between the costameres was frequently occupied by irregular dystrophin fluorescence of lower intensity, probably corresponding to the interconnections seen in single-teased fiber preparations. Interestingly, polyclonal antibodies to dystrophin known to cross react with a muscle-specific α-actinin isoform (13) confirmed the close spatial relation of the subsarcolemmal dystrophin accumulation to the projected connection of the Z bands to the plasma membrane (Fig. 3 *b*). If the plane of section happened to cut tangentially through the sarcolemma a similar dystrophin network was observed as on single-teased muscle fibers (Fig. 3 *c*).

Because this close spatial relationship between the dystrophin costameres and the Z bands suggested a functional connection between these two subcellular components we were curious to see what pattern of dystrophin organization would be found at sarcolemmal regions where peripheral myonu-

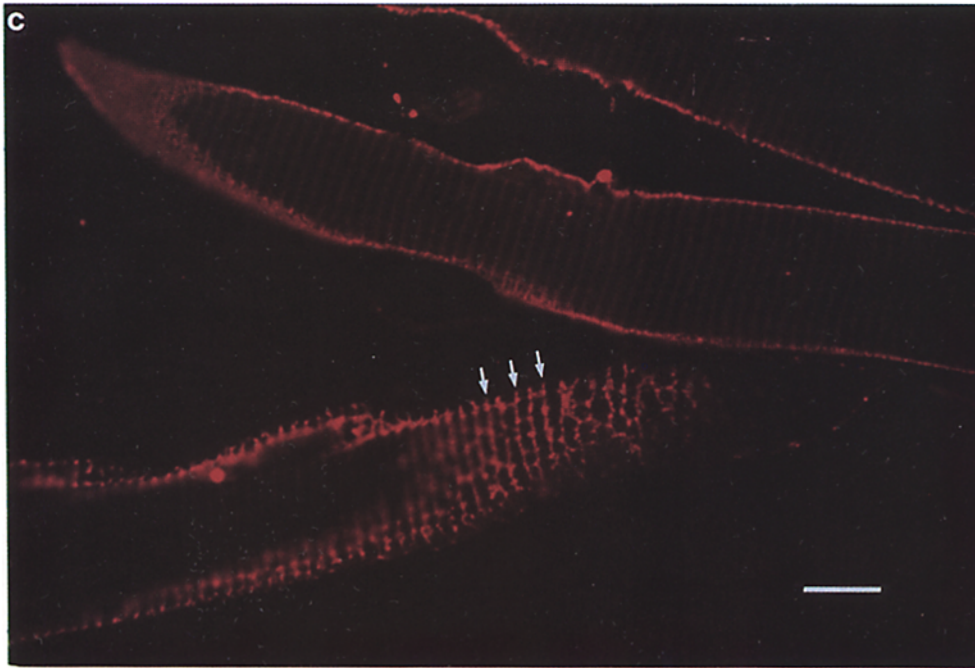

Figure 3.

clei were located between these two structures. Simultaneous staining for dystrophin and nuclear DNA by ethidium bromide demonstrated interruption of the regular dystrophin network by randomly interspersed lacunae which harbored myonuclei (Fig. 4, *a* and *b*). These lacunae appeared to be surrounded by a dystrophin ring into which the dystrophin costameres inserted at right angle. Frequently but not always the single lacuna was covered by a very fine, much less distinctly organized dystrophin veil. Probably due to mechanical preparation only fragments of dystrophin veils were retained in some lacunae and attached to the perilacunar dystrophin ring, and other lacunae lacked this cover altogether (Fig. 2 *b*). Dystrophin-deficient human muscle from previously characterized DMD patients (37) as well as muscle from *mdx* mice (4, 33) served as a negative control for teased single muscle fibers and for semithin sections. There was complete lack of staining for all antibodies to dystrophin tested (not shown).

Previous experiments using plebbing of sarcolemmal vesicles had suggested that dystrophin was more tightly associated with the plasma membrane than with the internal domain of skeletal muscle fibers (41). To test this hypothesis by using a mechanical technique without biochemical alteration of membraneous components including the subsarcolemmal dystrophin network we isolated muscle fiber membranes by mechanical skinning of single-teased fibers. If a single-teased muscle fiber was skinned no detectable fluorescence signal of dystrophin was retained on the fiber surface, indicating that at least a substantial part of the subsarcolemmal cytoskeleton was mechanically more tightly bound to the sarcolemma than to internal structures of the muscle fiber. The mechanically isolated sarcolemma of normal human, rat, and mouse muscle fibers again showed a dystrophin distribution with major transversal costameres which were interconnected by finer longitudinal bands, the network being identical in its fine topological organization to the one obtained by staining of single-teased fibers and by labeling of semithin sections (Fig. 5, *a* and *b*). Moreover, the dystrophin pattern on mechanically isolated sarcolemma was identical for NH_2-terminal, midrod and COOH-terminal antibodies to dystrophin. Lacunae which had harbored a myonucleus before skinning were detectable as holes with or without surrounding dystrophin rings on isolated sarcolemma (Fig. 5 *a*). No dystrophin was detected on the sarcolemma of DMD control muscle, where the presence of the subsarcolemmal cytoskeleton was demonstrated by antibodies to spectrin- and dystrophin-related protein (not shown).

Discussion

So far, immunoelectron microscopy had localized dystrophin to the cytoplasmic face of the sarcolemma (5, 8, 38, 39). If antibodies with specificity for different domains of dystrophin were used, the COOH-terminal part of the molecule seemed to be localized closer to or even within the plasma membrane (8). This morphological observation was in accordance to the biochemical finding that dystrophin binds via its COOH-terminal domain to integral membrane glycoproteins (9). Immunocytochemical detection of dystrophin on cryosections had shown a continuous outline of dystrophin both on longitudinal and transverse sections in a number of independent studies (1, 2, 37, 41). As a result, several hypothetical models of subcellular dystrophin distribution were based on the assumption that dystrophin was uniformly distributed over the skeletal muscle fiber surface (10, 17, 32). In keeping with this concept, the observation that muscle from patients with the allelic milder form of BMD or of DMD carriers sometimes showed a regularly "dotted" immunofluorescence pattern was attributed to focal discontinuity of the cell-surface dystrophin distribution in the presence of a semifunctional or quantitatively reduced dystrophin molecule (2, 24, 37). In contrast, all three preparation meth-

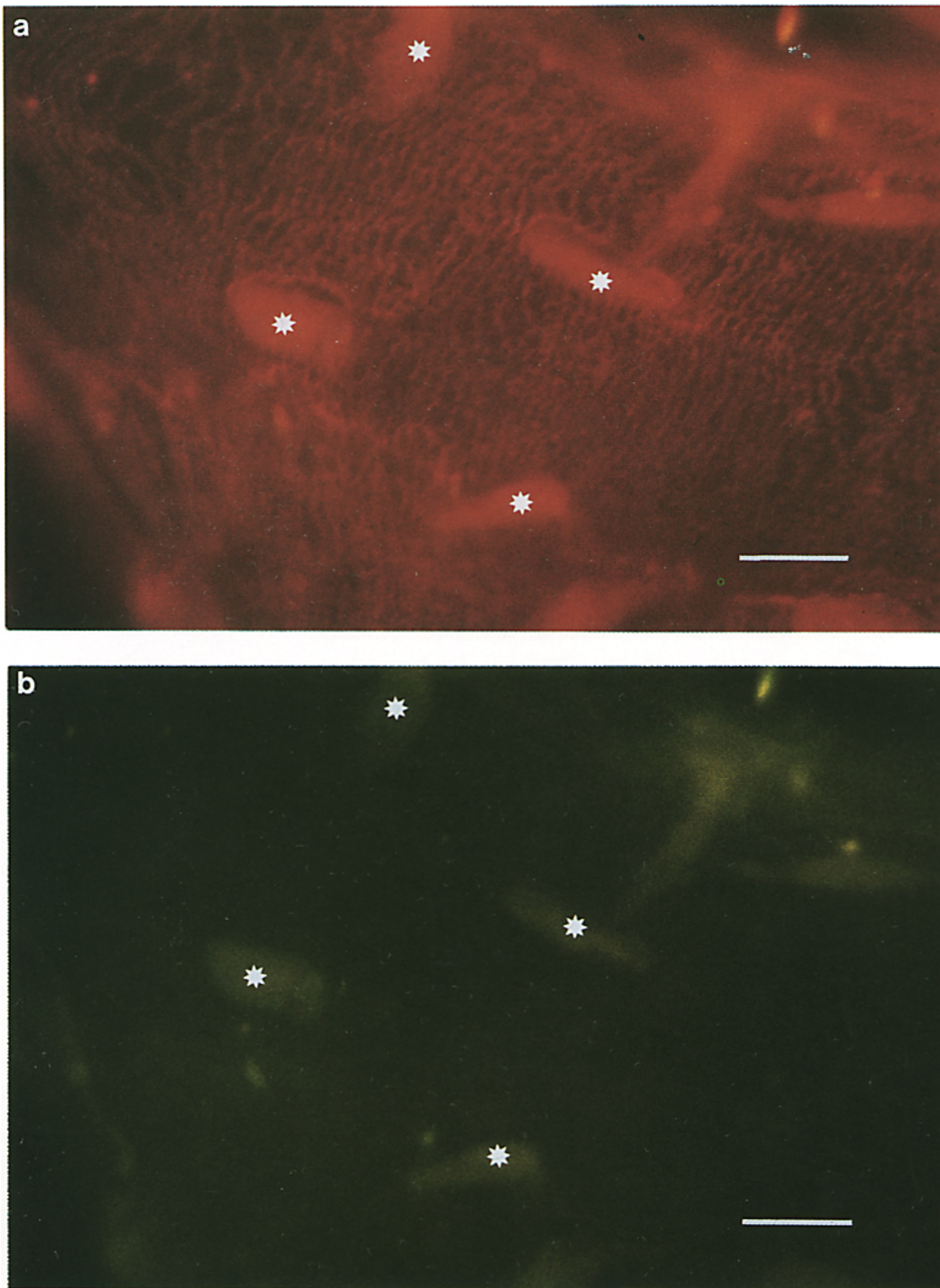

Figure 4. A single-teased fiber from normal human rectus femoris muscle was double stained for dystrophin (dys 2) and nuclear DNA (ethidium bromide). (*a*) Costameric dystrophin organization (*red*) is preserved. Lacunae are occupied by orange red fluorescent peripheral myonuclei (*asterisks*). (*b*) The same fiber analyzed by FITC filter. Peripheral myonuclei are labeled yellowish by ethidium bromide fluorescence (*asterisks*). Bar, 10 μm.

ods we used revealed a spatially organized dystrophin network on the muscle fiber surface with defined relation to the contractile apparatus. The regular "dotted" immunofluorescence pattern of dystrophin on BMD or DMD carrier muscle, therefore, probably rather corresponded to the dystrophin network as observed in our study and was laid open in BMD or DMD carrier muscle by the lower intensity of the fluorescence signal in the presence of lower dystrophin abundance. Because isolated dystrophin molecules are capable of self association as dimers, trimers, or tetramers (29) but do not form more complex structures it seems likely that the higher organization of dystrophin within the submembrane cytoskeleton in form of a network is mediated by interaction with other molecules of the submembrane cytoskeleton. Such an association is still speculative at the moment. However, a number of proteins such as vinculin, γ-actin, talin, and spectrin have been shown to constitute a submembrane cytoskeletal scaffold with costameric distribution as shown in this study for dystrophin (6, 28, 34). In connection with specific cell surface glycoproteins of similar distribution this scaffold is thought to play an important role in mechanical force transduction from the contractile apparatus to the extracellular matrix (34). Interestingly, dystrophin has previously been shown to colocalize with talin at focal adhesion sites in *Xenopus* muscle cultures (19) but no costameric distribution was observed. The actin binding sites identified in the NH_2-terminal sequence of dystrophin (20) further argue in favor of a complex interaction of the various proteins

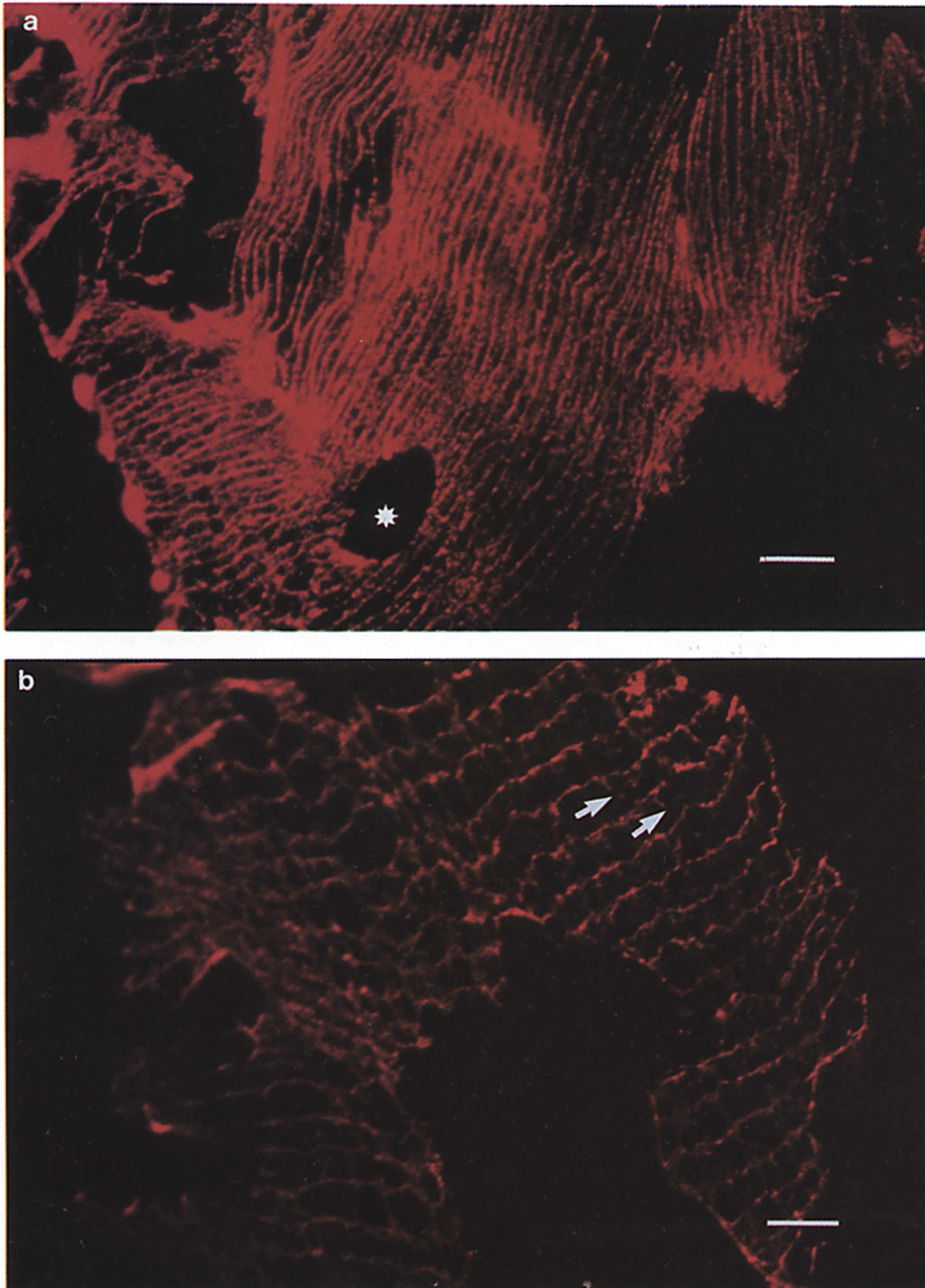

Figure 5. Isolated sarcolemma from normal human rectus femoris muscle was prepared as described in materials and methods and stained with mAb to dystrophin as for single-teased fibers (cf. Fig. 2). (*a*) The predominant pattern of dystrophin distribution with costameres and finer interconnections was retained (dys 2), including a nuclear lacuna (*asterisk*). (*b*) Due to mechanical stretching of the membrane some longitudinal interconnections (*arrows*) lose contact to the preserved costameres (dys 3). Bar, 10 μm.

which form the costameres. Finally such a complex association could also explain why in skeletal muscle of some DMD patients truncated dystrophin molecules lacking the COOH terminus can become attached to the submembrane cytoskeleton (15, 31, 37) in the absence of the glycoprotein-binding COOH terminus of the molecule (9).

The predominant dystrophin costameres were regularly found in close vicinity of the projected insertion of the Z bands into the sarcolemma. If this direct connection of the Z bands to the sarcolemma was prevented by the presence of a peripheral myonucleus the subsarcolemmal organization of dystrophin was changed which further supported the concept of a functional importance of this juxtaposition. The perinuclear lacunae within the costameric dystrophin organization have not been observed in previous descriptions of submembrane proteins with costameric organization (6, 28, 34), and longitudinal interconnections between the costameres were only mentioned for vinculin (28). However, we have evidence that at least for spectrin similar perinuclear lacunae and longitudinal interconnections between spectrin costameres are regularly found on human and mouse skeletal muscle (Bittner, R. E., and T. Voit, manuscript in preparation).

Tighter mechanical connection of dystrophin including its NH_2-terminal domain (cf. Fig. 5 *b*) to the plasma membrane than to the internal muscle fiber structures was directly proved by mechanical isolation of sarcolemma. Because, on the other hand, the dystrophin costameres were al-

ways directly juxtaposed to the Z bands independent of the state of contraction it follows that during cycles of contraction and relaxation defined areas of plasma membrane will correspond to defined sarcomeres. Lack of dystrophin has been shown to result in increased osmotic fragility of the muscle sarcolemma (22). Given these two independent observations, one could hypothesize that dystrophin plays a role in connecting defined constituents of the plasma membrane such as dystrophin-associated glycoproteins to the sarcomeres and, via these glycoproteins, even forms links to the extracellular matrix (16). Such a hypothesis does not preclude more specific interactions of dystrophin with other submembrane proteins or with other proteins within the plasma membrane (9, 10, 16). Further studies are needed to define if the molecular organization of dystrophin is the same within the costameres as within the longitudinal interconnections and especially, if any form of organization as a hexagonal grid can be observed as was suggested by the presence of hinge regions within the dystrophin molecule (17).

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References

1. Arahata, K., E. P. Hoffman, L. M. Kunkel, S. Ishiura, T. Tsukahara, T. Ishihara, N. Sunohara, I. Nonaka, E. Ozawa, and H. Sugita. 1989. Dystrophin diagnosis: Comparison of dystrophin abnormalities by immunofluorescence and immunoblot analyses. *Proc. Natl. Acad. Sci. USA.* 86:7154–7158.
2. Bonilla, E., C. E. Samitt, A. F. Miranda, A. P. Hays, A. B. S. De Oliveira, H. W. Chang, S. Servideli, E. Ricci, D. S. Younger, and S. DiMauro. 1988. Normal and dystrophin-deficient muscle fibers in carriers of the gene for Duchenne muscular dystrophy. *Am. J. Pathol.* 133:440–445.
3. Bonilla, E., C. E. Samitt, A. F. Miranda, A. P. Hays, G. Salivati, S. DiMauro, L. M. Kunkel, E. P. Hoffman, and L. P. Rowland. 1988. Duchenne muscular dystrophy: deficiency of dystrophin at the muscle cell surface. *Cell.* 54:447–452.
4. Bulfield, G., W. G. Siller, P. A. L. Wight, and K. J. Moore. 1984. X chromosome-linked muscular dystrophy (mdx) in the mouse. *Proc. Natl. Acad. Sci. USA.* 81:1189–1192.
5. Byers, T. J., L. M. Kunkel, and S. C. Watkins. 1991. The subcellular distribution of dystrophin in mouse skeletal, cardiac, and smooth muscle. *J. Cell Biol.* 115:411–421.
6. Craig, S. W., and J. V. Pardo. 1983. Gamma actin, spectrin, and intermediate filament proteins colocalize with vinculin at costameres, myofibril-to-sarcolemma attachment sites. *Cell. Motility.* 3:449–462.
7. Cullen, M. J., J. Walsh, L. V. B. Nicholson, and J. B. Harris. 1990. Ultrastructural localization of dystrophin in human muscle by using gold immunolabelling. *Proc. R. Soc. Lond. B.* 240:197–210.
8. Cullen, M. J., J. Walsh, L. V. B. Nicholson, J. B. Harris, E. E. Zubrycka-Gaarn, P. N. Ray, and R. G. Worton. 1991. Immunogold labelling of dystrophin in human muscle using an antibody to the last 17 amino acids of the C-terminus. *Neuromuscular Disorders.* 1:113–119.
9. Ervasti, J. M., and K. P. Campbell. 1991. Membrane organization of the dystrophin-glycoprotein complex. *Cell.* 66:1121–1131.
10. Hoffman, E. P., and L. M. Kunkel. 1989. Dystrophin abnormalities in Duchenne/Becker muscular dystrophy. *Neuron.* 2:1019–1029.
11. Hoffman, E. P., R. H. Brown, and L. M. Kunkel. 1987. Dystrophin: the protein product of the Duchenne muscular dystrophy. *Cell.* 51:919–928.
12. Hoffman, E. P., K. H. Fischbeck, R. H. Brown, M. Johnson, R. Medori, J. D. Loike, J. B. Harris, R. Waterston, M. Brooke, L. Specht, W. Kupsky, J. Chamberlaine, C. T. Caskey, F. Shapiro, and L. M. Kunkel. 1988. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *New Engl. J. Med.* 318:1363–1368.
13. Hoffman, E. P., S. C. Watkins, H. S. Slayter, and L. M. Kunkel. 1989. Detection of a specific isoform of alpha-actinin with antisera directed against dystrophin. *J. Cell Biol.* 108:503–510.
14. Hoffman, E. P., J. E. Morgan, S. C. Watkins, and T. A. Partridge. 1990. Somatic reversion/suppression of the mouse mdx phenotype in vivo. *J. Neurol. Sci.* 99:9–25.
15. Hoffman, E. P., C. A. Garcia, J. S. Chamberlain, C. Angelini, J. R. Lupski, and R. Fenwick. 1991. Is the carboxyl-terminus of dystrophin required for membrane association? A novel, severe case of Duchenne muscular dystrophy. *Ann. Neurol.* 30:605–610.
16. Ibraghimov-Beskrovnaya, O., J. M. Ervasti, C. J. Leveille, C. A. Slaughter, S. W. Sernett, and K. P. Campbell. 1992. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature (Lond.).* 355:696–702.
17. Koenig, M., and L. M. Kunkel. 1990. Detailed analysis of the repeat domain of dystrophin reveals four potential hinge segments that may confer flexibility. *J. Biol. Chem.* 265:4560–4566.
18. Koenig, M., A. P. Monaco, and L. M. Kunkel. 1988. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell.* 53:219–228.
19. Kramarcy, N. R., and R. Sealock. 1990. Dystrophin as a focal adhesion protein. Colocalization with talin and the Mr 48000 sarcolemmal protein in cultured Xenopus muscle. *FEBS (Fed. Eur. Biol. Soc.) Lett.* 274:171–174.
20. Levine, B. A., A. J. G. Moir, V. B. Patchell, and S. V. Perry. 1992. Binding sites involved in the interaction of actin with the N-terminal region of dystrophin. *FEBS (Fed. Eur. Biol. Soc.) Lett.* 298:44–48.
21. Love, D. R., D. F. Hill, G. Dickson, N. K. Spurr, B. C. Byth, R. F. Marsden, F. S. Walsh, Y. H. Edwards, and K. E. Davies. 1989. An autosomal transskript in skeletal muscle with homology to dystrophin. *Nature (Lond.).* 339:55–58.
22. Menke, A., and H. Jockusch. 1991. Decreased osmotic stability of dystrophin-less muscle cells from the mdx mouse. *Nature (Lond.).* 349:69–71.
23. Moisescu, D. G., and R. J. Thieleczek. 1978. Calcium and strontium concentration changes within skinned muscle preparations following a change in the external bathing solution. *J. Physiol.* 275:241–262.
24. Morandi, L., M. Mora, E. Gussoni, S. Tedeschi, and F. Cornelio. 1990. Dystrophin analysis in Duchenne and Becker muscular dystrophy carriers: correlation with intracellular calcium and albumin. *Ann. Neurol.* 28:674–679.
25. Nicholson, L.V.B., K. Davison, G. Falkous, C. Harwood, E. O'Donnell, C. R. Slater, and J. B. Harris. 1989. Dystrophin in skeletal muscle I. Western blot analysis using a monoclonal antibody. *J. Neurol. Sci.* 94:125–136.
26. Nicholson, L. V. B., M. A. Johnson, K. Davison, E. O'Donnell, G. Falkous, M. Barron, and J. B. Harris. 1992. Dystrophin or a "related protein" in Duchenne muscular distrophy? *Acta Neurol (Scand.).* 68:8–14.
27. Nicholson, L. V. B., M. A. Johnson, D. Gardner-Medwin, S. Bhattacharya, and J. B. Harris. 1990. Heterogeneity of dystrophin expression in patients with Duchenne and Becker muscular dystrophy. *Acta Neuropathol.* 80:239–250.
28. Pardo, J. V., J. D'Angelo Siliciano, and S. W. Craig. 1983. A vinculin-containing cortical lattice in skeletal muscle: Transverse lattice elements (costameres) mark sites of attachements between myofibrils and sarcolemma. *Proc. Natl. Acad. Sci. USA.* 80:1008–1012.
29. Pons, F., N. Augier, R. Heilig, J. Léger, D. Mornet, and J. J. Léger. 1990. Isolated dystrophin molecules as seen by electron microscopy. *Proc. Natl. Acad. Sci. USA.* 87:7851–7855.
30. Pons, F., N. Augier, J. O. C. Léger, A. Robert, F. M. S. Tomé, M. Fardeau, T. Voit, L. V. B. Nicholson, D. Mornet, and J. J. Léger. 1991. A homologue of dystrophin is expressed at the neuromuscular junctions of normal individuals and DMD patients, and of normal and mdx mice. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 282:161–165.
31. Récan, D., P. Chafey, F. Leturcq, J. P. Hugnot, N. Vincent, F. Tomé, H. Collin, D. Simon, P. Czernichow, L. V. B. Nicholson, M. Fardeau, J. C. Kaplan, and J. Chelly. 1992. Are cysteine-rich and COOH-terminal domains of dystrophin critical for sarcolemmal localization? *J. Clin. Invest.* 89:712–716.
32. Rojas, C. V., and E. P. Hoffman. 1991. Recent advances in dystrophin research. *Curr. Opin. Neurobiol.* 1:420–429.
33. Sicinski, P., Y. Geng, A. S. Ryder-Cook, E. Barnard, M. Darlison, and P. Barnard. 1989. The molecular basis of muscular dystrophy in the mdx mouse is a point mutation. *Science (Wash. DC).* 244:1578–1580.
34. Terracio, L., D. Gullberg, K. Rubin, S. Craig, and T. K. Borg. 1989. Expression of collagen adhesion proteins and their association with the cytoskeleton in cardiac myocytes. *Anatomical Record.* 223:62–71.
35. Voit, T., K. Haas, J. O. C. Léger, F. Pons, and J. J. Léger. 1991. Xp21 dystrophin and 6q dystrophin-related protein. Comparative immunolocalization using different antibodies. *Am. J. Pathol.* 139:969–976.
36. Voit, T., K. Patel, M. J. Dunn, V. Dubowitz, and P. N. Strong. 1989. Distribution of dystrophin, nebulin and Ricinus communis I (RCA-I)-binding glycoprotein in tissues of normal and mdx mice. *J. Neurol. Sci.* 89:199–211.
37. Voit, T., P. Stüttgen, M. Cremer, and H. H. Goebel. 1991. Dystrophin

as a diagnostic marker in Duchenne and Becker muscular dystrophy. Correlation of immunofluorescence and Western blot. *Neuropediatrics.* 22:152–162.

38. Wakayama, Y., and S. Shibuya. 1991. Gold-labelled dystrophin molecule in muscle plasmalemma of mdx control mice as seen by electron microscopy of deep etching replica. *Acta Neuropathol.* 82:178–184.
39. Watkins, S. C., E. P. Hoffman, H. S. Slayter, and L. M. Kunkel. 1988. Immunoelectron microscopic localization of dystrophin in myofibres. *Nature (Lond.).* 333:863–866.
40. Zubrzycka-Gaarn, E. E., D. E. Bulman, G. Karpati, A. H. M. Burghes, B. Belfall, H. J. Klamut, J. Talbot, R. S. Hodges, P. N. Ray, and R. G. Worton. 1988. The Duchenne muscular dystrophy gene product is localized in sarcolemma of human skeletal muscle. *Nature (Lond.).* 333: 466–469.
41. Zubryzycka-Gaarn, E. E., O. F. Hutter, G. Karpati, H. J. Klamut, D. E. Bulman, R. S. Hodges, R. G. Worton, and P. N. Ray. 1991. Dystrophin is tightly associated with the sarcolemma of mammalian skeletal muscle fibers. *Exp. Cell Res.* 192:278–288.