

## $\alpha$ -Actinin-2 Is a New Component of the Dystrophin–Glycoprotein Complex<sup>1</sup>

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**The human skeletal muscle yeast two-hybrid cDNA library was screened with the carboxyl-terminal region (the last 200 amino acids) of dystrophin. Two interacting clones were identified corresponding to  $\alpha$ -actinin-2 and actin. Interactions between  $\alpha$ -actinin, actin, and dystrophin were confirmed by the ligand-blotting technique, by colocalization of dystrophin and  $\alpha$ -actinin-2 to the isolated skeletal muscle sarcolemmal vesicles and to the plasma membranes isolated from C<sub>2</sub>C<sub>12</sub> myoblasts, and by indirect immunolocalization of dystrophin and  $\alpha$ -actinin-2 in skeletal muscle cells. This is the first identification of a direct interaction between  $\alpha$ -actinin, actin, and the carboxyl-terminal region of dystrophin. We propose that dystrophin forms lateral, multicontact association with actin and that binding of  $\alpha$ -actinin-2 to the carboxyl-terminus of dystrophin is the communication link between the integrins and the dystrophin/dystrophin–glycoprotein complex.** © 1999 Academic Press

**Key Words:** Duchenne muscular dystrophy; dystrophin; actinin; actin.

Duchenne muscular dystrophy (DMD)<sup>3</sup> is an X-linked, degenerative muscle disorder caused by the

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<sup>3</sup> Abbreviations used: DMD, Duchenne muscular dystrophy; DGC, dystrophin–glycoprotein complex; ECM, extracellular matrix; GST, glutathione *S*-transferase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin; EGTA,

absence of dystrophin in muscle (1–8). Dystrophin is a cytoskeletal protein that binds actin and associates with the dystrophin–glycoprotein complex (DGC) to link the cytoskeleton to the extracellular matrix (ECM) (7, 9). The DGC is made up of integral and peripheral membrane proteins organized into three distinct entities: dystroglycans, sarcoglycans, and syntrophins (6, 7, 9). Defects in dystrophin and/or other components of the DGC are responsible for several phenotypes of muscular dystrophy including DMD, Becker muscular dystrophy, and limb-girdle muscular dystrophies (1, 4).

The function of the DGC remains elusive, but the complex may play a role in maintaining the normal architecture of the muscle plasma membrane by forming a link between the subsarcolemmal cytoskeleton and the ECM. Dystrophin is one important component of the DGC. The protein is organized into distinct structural domains (5, 6, 9, 10). The N-terminus of the protein binds F-actin, followed by a long rod-like central domain composed of triple helical coiled-coil repeats similar to those found in spectrin. The carboxyl-terminal domain of dystrophin is made up of the cysteine-rich domain and the C-terminal domain. The Cys-rich domain interacts with the cytoplasmic tail of  $\beta$ -dystroglycan and also binds to the syntrophins (6). The Cys-rich domain of dystrophin is critical for interacting with the DGC *in vivo*, and disruption of this interaction renders dystrophin completely nonfunctional (7). The absence of dystrophin alters the mechanical properties of the muscle cell surface and modifies the state of ion fluxes and a change in mechanotransduction of growth signals, which ultimately lead to cell death (6). Since dystrophin anchors F-actin indirectly to the ECM, the protein may play an important

ethylene glycol bis( $\beta$ -aminoethyl ether) *N,N'*-tetraacetic acid; GST, glutathione; FITC, fluorescein isothiocyanate.

role in stabilizing muscle fibers and maintaining the integrity of the DGC.

The goal of this study was to identify new molecules associated with dystrophin. Using the yeast two-hybrid system, we screened the human skeletal muscle cDNA library with the C-terminal region of dystrophin and identified novel interactions between this region of dystrophin and  $\alpha$ -actinin-2 and actin. These interactions were confirmed by colocalization of dystrophin and  $\alpha$ -actinin-2 in muscle cells, by copurification in isolated sarcolemma from skeletal muscle and from C<sub>2</sub>C<sub>12</sub> myotubes, and by the ligand-blotting technique. This constitutes the first identification of  $\alpha$ -actinin and actin binding sites to the C-terminal region of dystrophin, supporting the hypothesis that actin molecules may lie alongside of dystrophin.  $\alpha$ -Actinin binding to dystrophin may also provide a communication link between DGC and integrins.

## EXPERIMENTAL PROCEDURES

**Plasmids and the yeast two-hybrid assay.** The human skeletal muscle matchmaker cDNA library (Clontech Laboratories) was used in this study. To construct the library, mRNA was isolated from a male skeletal muscle of thigh (*M. quadriceps*), primed with oligo(dT) and random primer followed by cloning into *EcoRI* site into the vector. The library was screened for interacting proteins with the C-terminal region of dystrophin corresponding to the last 200 amino acids of human dystrophin (designated CT-1; amino acids 3485 to 3685) (11). cDNA encoding CT-1 was obtained by PCR amplification of a human dystrophin template cDNA, using the following primers: the 5' forward primer 5'-TATATGGAATTCCTGAGCCAGCCTCGTAGTCCT-3' (nucleotides 10,661–10,681 of dystrophin cDNA) and the 3' reverse primer 5'-TGGGATCCCTAAGGACTCCATCGCTCTGC-3' (nucleotides 11,294–11,317). The PCR product was cloned in frame with the GAL4 DNA-binding domain at *BamHI* and *EcoRI* restriction sites to generate the clone pGBT9-CT-1. Screening of the human skeletal muscle cDNA library was carried out as recommended by the manufacturer. Yeast transformants were assayed by replica plating for  $\beta$ -galactosidase activity using 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside as a substrate. Plasmid DNA from positive clones was isolated and used as a template for amplification of the insert present in the library vector (pGAD10). The PCR reaction used the 5' and 3' Matchmaker insert screening primers, 5'-TACCACTACAATGGATGATG-3' and 5'-GTTGAAGTGAAGTTCGCGGG-3', under standard conditions. The nucleotide sequence of the PCR products was determined and compared for identification against Genbank-deposited sequences.

For PCR-driven synthesis of cDNA encoding the C-terminus of human skeletal muscle  $\alpha$ -actinin-2 (12) the following oligodeoxynucleotide primers were used: 5' primer 5'-ATATGAATTCAGATCTTGCTGCAGAA-3' (nucleotides 1437–1452 of full-length ACTN2 cDNA) and 3' primer 5'-ATAGAATTCAGATCGCTCTCCC-3' (nucleotides 2841–2855 of full-length ACTN2 cDNA). The C-terminus of  $\alpha$ -actinin-2 was then cloned in frame with the GAL4-DNA activation domain to generate a vector designated pGAD- $\alpha$ -actinin-C.

**Isolation of plasma membranes.** C<sub>2</sub>C<sub>12</sub> muscle cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Differentiation of myoblasts into myotubes was induced with 2% horse serum at 80% confluency. After 7 days in culture the plasma membranes was isolated on the cationic colloidal silica beads followed by a Nycodenz gradient (13). Prior to SDS-PAGE proteins were precipitated with acetone at -20°C. Sarcolemmal vesicles were

isolated by wheat germ agglutination of the heavy microsomes (14, 15).

**SDS-PAGE and immunoblotting.** Proteins were separated on 5 or 10% SDS-PAGE as described by Laemmli (16). After gel electrophoresis, the proteins were transferred to nitrocellulose membranes (17) followed by immunoblotting (11). Peroxidase-conjugated secondary antibodies were used. Immunoblots were exposed to Kodak X-ray film. The standards used were Bio-Rad molecular weight markers. The anti-dystrophin antibody was made against the C-terminal peptide of human dystrophin (11). The rabbit anti- $\alpha$ -actinin-2 antibody was raised against a unique amino terminal peptide (18) and has been previously characterized and shown to be specific for the  $\alpha$ -actinin-2 isoform (18, 19). The anti-actinin antibodies were affinity purified (18) and used at 1:200 dilution.

**Protein overlay assay and CT-1 affinity chromatography.** Purified  $\alpha$ -actinin (50  $\mu$ g) was separated on SDS-PAGE and transferred to a nitrocellulose membrane (17). The nitrocellulose blots were blocked overnight in a binding buffer containing 20 mM Hepes, pH 7.0, 2 mM MgCl<sub>2</sub>, 150 mM KCl, 0.1 mM EGTA, 0.1 mM CaCl<sub>2</sub> and 5% bovine serum albumin. Thirty micrograms of protein was added to freshly made blocking buffer and incubated with the nitrocellulose blots for 2 h at room temperature. Blots were washed with blocking buffer and incubated for 2 h with dystrophin antibody (11) at a dilution of 1:100. A secondary goat anti-rabbit peroxidase antibody was used at 1:10,000 dilution.

A GST-CT-1 affinity column was synthesized by coupling recombinant GST-CT-1 to CNBr-activated Sepharose 4B. Efficiency of coupling was greater than 95%. A precolumn was prepared by incubation of CNBr-activated Sepharose 4B with recombinant GST. The GST-CT-1 column was used to investigate interactions between CT-1 and purified  $\alpha$ -actinin-2. The purified  $\alpha$ -actinin was first applied onto a precolumn to remove proteins that nonspecifically associate with GST and the matrix. The column was washed with a solution containing 10 mM morpholinepropanesulfonic acid, pH 7.0, 100 mM KCl, 2 mM MgCl<sub>2</sub>, and 0.5 mM EGTA. Bound proteins were eluted with 750 mM KCl, concentrated using an Amicon concentrator, and separated by SDS-PAGE.

**Immunofluorescence microscopy.** Human trapezius muscle was flash-frozen in liquid-nitrogen-cooled isopentane and cut into 10- $\mu$ m sections on a Micron 505 cryostat. For double-label analysis, sheep anti-30-kDa dystrophin and rabbit  $\alpha$ -actinin-2 (4B2) antibodies (18) (both 1  $\mu$ g/ml) were visualized using FITC-labeled donkey anti-sheep IgG (1:200) and Cy3 goat anti-rabbit IgG (Jackson Immunochemicals) at 1:3000. Stained sections were analyzed using a Leica TCS-NT confocal microscope using a 100 $\times$  objective and an AOTF filter to selectively visualize each label without bleedthrough. The FITC label was excited at 488 nm and Cy3 at 586 nm. Images were collected in serial planes throughout the depth of the section at a 1024  $\times$  1024-pixel resolution. Image stacks were imported into Image Space (Molecular Dynamics, Sunnyvale, CA) for subsequent rendering. Lookthrough projections were generated for each protein and superposed for qualitative analysis.

**Miscellaneous.** The carboxyl region of dystrophin (CT-1) encoding the amino acid residues 3485–3685 was expressed as a GST fusion protein and purified using a glutathione-agarose affinity column as described previously (11).  $\alpha$ -Actinin was purified from back and thigh rabbit skeletal muscles (20). All recombinant techniques were conducted according to a standard protocol (21). Protein concentration was determined by the method of Lowry *et al.* (22).

## RESULTS

*The carboxyl-terminal region of dystrophin interacts with skeletal muscle  $\alpha$ -actinin-2 and actin in the yeast two-hybrid system.* The yeast two-hybrid screening method was used to identify proteins interacting with the most C-terminal region of dystrophin (designated

CT-1) encompassing the last 200 amino acid (11). Screening of the library resulted in identification of 16 positive clones. Two of these clones (clone 5 and clone 16) were further characterized.

Clone 5 was 1449 nucleotides in length and nucleotide sequence analysis revealed that it corresponded to the C-terminus of the human skeletal muscle  $\alpha$ -actinin-2 (nucleotides 1436–2885) (12). This region encodes the last 473 amino acids of the mature  $\alpha$ -actinin-2 and does not contain the actin-binding site of the protein (12) (Fig. 1B). Figure 1A shows that cotransformation of yeast with pGAD- $\alpha$ -actinin-C and pGBT9-CT-1 resulted in rapid (<30 min) activation of the  $\beta$ -galactosidase reporter gene. Transformation of yeast with pGAD- $\alpha$ -actinin-C, pGBT9-CT-1, or control pGAD and pGBT9 vectors did not produce any color development for at least 48 h (Fig. 1A), indicating the specificity of the interaction between  $\alpha$ -actinin and dystrophin.

Analysis of clone 16 revealed that it was identical to the human skeletal muscle actin (23). Again, activation of the  $\beta$ -galactosidase reporter gene was seen when pGAD-actin and pGBT9-CT-1 vectors were cotransformed into yeast (Fig. 1A). Development of a strong blue color for these interacting molecules required less than 2 h incubation. We concluded that using the yeast two-hybrid system we have identified new protein-protein interactions localized at the C-terminal region of dystrophin.

*In vitro* interaction between  $\alpha$ -actinin-2,  $\alpha$ -actin, and dystrophin. We employed a ligand-binding (blotting) method to further confirm the interaction between dystrophin, actin, and  $\alpha$ -actinin-2. Purified rabbit skeletal muscle  $\alpha$ -actinin-2 was separated on SDS-PAGE, transferred to nitrocellulose, and incubated with purified GST-CT-1 fusion protein (11). GST was used as a control.  $\alpha$ -Actinin-2 used in this study contained bound actin. This allowed us to investigate dystrophin- $\alpha$ -actinin and dystrophin-actin interactions in the same ligand blotting experiment. Finally, anti-dystrophin antibodies, which recognize GST-CT-1, were used to detect protein complexes containing the C-terminal region of dystrophin. Figure 2A shows that GST-CT-1 interacted with both  $\alpha$ -actinin-2 and actin under the ligand-blotting conditions. A background staining with anti-dystrophin antibodies was also observed (Fig. 2A, lane 2); however, densitometric scans revealed an approximately eightfold higher binding of GST-CT-1 to  $\alpha$ -actinin-2 than background binding.

To test for direct interaction between  $\alpha$ -actinin and the C-terminal region of dystrophin under non-denatured conditions we synthesized a GST-CT-1 affinity column using the recombinant protein. Purified  $\alpha$ -actinin (containing actin) was applied onto the GST-CT-1 column. The column was extensively washed, and bound proteins were eluted with 750 mM KCl. Figure

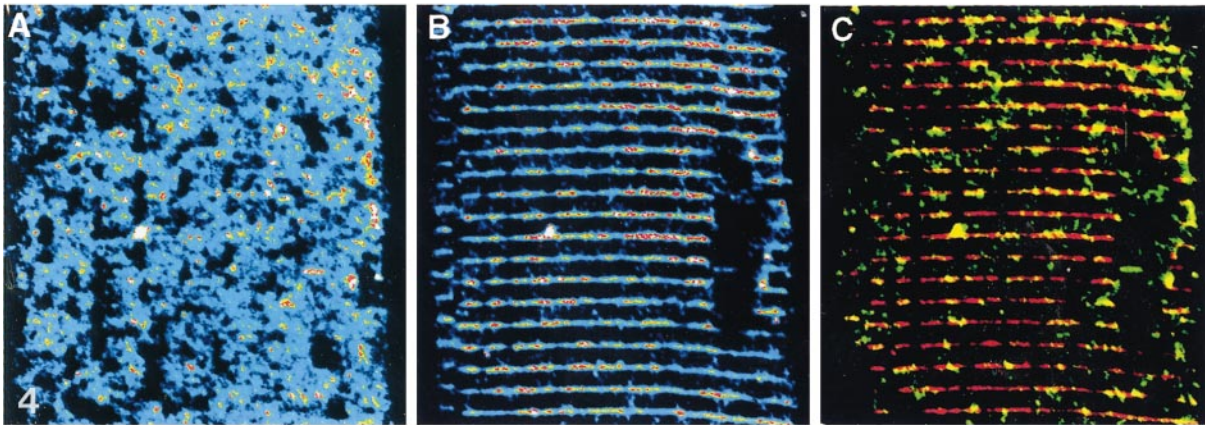
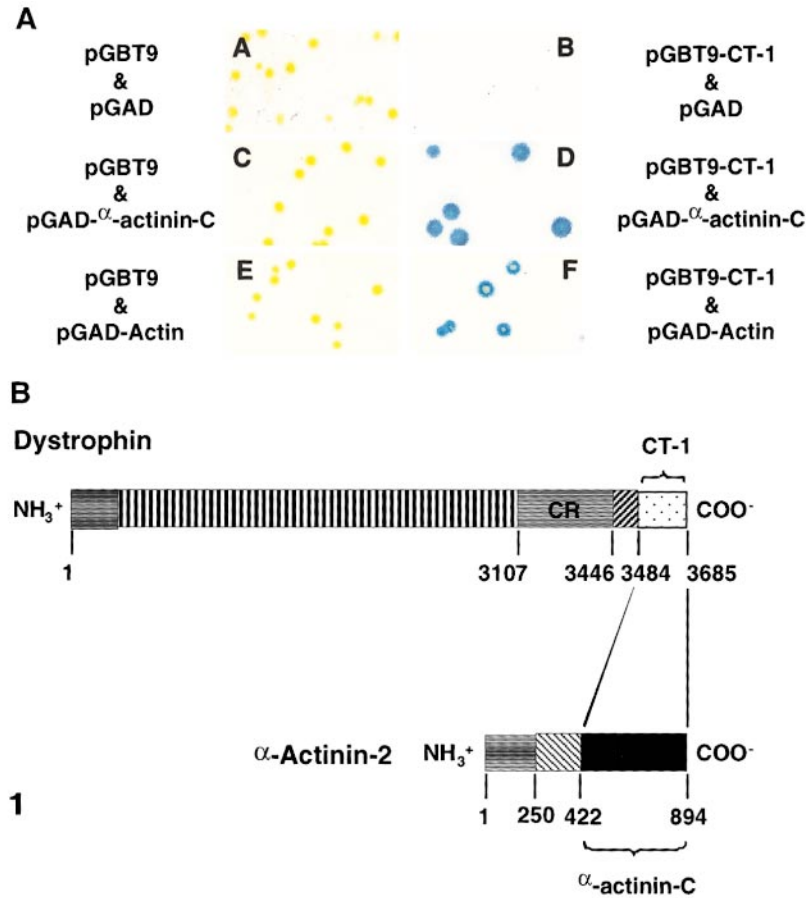
2B shows that eluted fraction contained both  $\alpha$ -actinin and actin.

*Localization of  $\alpha$ -actinin-2 to the plasma membrane of tissue culture cells and purified sarcolemma vesicles.* Having identified potential dystrophin interactions with  $\alpha$ -actinin-2 and actin we set out to determine if these proteins copurify (colocalize) with dystrophin in skeletal muscle sarcolemma and plasma membranes isolated from C<sub>2</sub>C<sub>12</sub> myoblasts in culture. First, sarcolemmal vesicles, containing dystrophin (24) were purified from rabbit skeletal muscle and tested for the presence of  $\alpha$ -actinin-2 using specific anti- $\alpha$ -actinin-2 antibodies. Figure 3A shows that a 100-kDa  $\alpha$ -actinin-2 protein band was present in the purified membrane preparation containing dystrophin (24). Second, highly purified plasma membrane preparation containing dystrophin (25) were obtained from C<sub>2</sub>C<sub>12</sub> muscle myoblasts by a method developed by Stolz and Jacobson (13). Figure 3B shows that plasma membrane vesicles purified from C<sub>2</sub>C<sub>12</sub> myoblast contained a 100-kDa  $\alpha$ -actinin-2 protein band. We conclude that dystrophin and  $\alpha$ -actinin-2 are both components of the purified skeletal muscle sarcolemma and plasma membrane of the C<sub>2</sub>C<sub>12</sub> muscle myoblasts.

*$\alpha$ -Actinin-2 and dystrophin colocalize in skeletal muscle.* Double-label immunofluorescence confocal microscopy was employed to determine whether dystrophin and  $\alpha$ -actinin-2 colocalized at the microscopic level. Sections through the plane of the plasma membrane revealed a patchy pattern of dystrophin staining where gaps represented areas above or below the plane focus (Fig. 4A). Dystrophin staining exhibits a 125-nm periodicity reminiscent of the pattern of staining of  $\alpha$ -actinin-2 (Fig. 4B). Superposition of the two images demonstrates that these represent areas of colocalization of dystrophin and  $\alpha$ -actinin-2 (Fig. 4C).

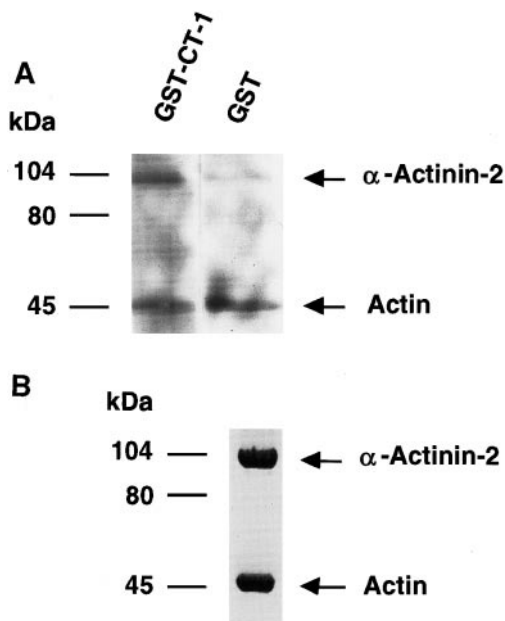
## DISCUSSION

Using the yeast two-hybrid method we identified  $\alpha$ -actinin-2 and actin as new components of the DGC. This interaction was confirmed by the ligand-blotting technique, by colocalization of dystrophin and  $\alpha$ -actinin-2 in the isolated skeletal muscle sarcolemmal vesicles and in plasma membranes isolated from C<sub>2</sub>C<sub>12</sub> myoblasts, and by indirect immunolocalization of dystrophin and  $\alpha$ -actinin-2 in skeletal muscle cells. There are two important consequences of our findings: (i) Actin molecules may have multiple attachment sites on dystrophin, both directly and indirectly (via  $\alpha$ -actinin-2), affecting the geometry and function of the DGC, and (ii)  $\alpha$ -actinin-2 binding to the C-terminus of dystrophin provides a new and important site of attachment of the DGC to other cytoskeletal proteins and a connection to adhesion complexes made up of integrin, vinculin, talin, and tensin.



**FIG. 1.** The C-terminal region of dystrophin interacts with  $\alpha$ -actinin-2 and actin in the yeast two-hybrid system. The human skeletal muscle cDNA library was screened in the yeast two-hybrid system as described under Experimental Procedures. The  $\beta$ -galactosidase filter lift assay was performed to detect positive colonies. (A) Yeast cells cotransformed with pGBT9 and pGAD control vectors. (B) Yeast cotransformed with pGBT9-CT-1 (the last 200 amino acids of the human skeletal muscle dystrophin) and pGAD. (C) Yeast cotransformed with pGBT9 and pGAD- $\alpha$ -actinin-C (the C-terminus of  $\alpha$ -actinin-2). (D) Yeast cotransformed with pGBT9-CT-1 and pGAD- $\alpha$ -actinin-C. (E) Yeast cotransformed with pGBT9 and pGAD-actin (human  $\alpha$ -actin). (F) Yeast cotransformed with pGBT9-CT-1 and pGAD-actin. (Bottom) A diagrammatic representation of dystrophin and  $\alpha$ -actinin-2. The CT-1 region of dystrophin was used for screening the yeast two-hybrid library. The  $\alpha$ -actinin-C region of  $\alpha$ -actinin-2 was identified by the yeast two-hybrid technique as interacting with the CT-1 fragment of dystrophin.

**FIG. 4.** Colocalization of dystrophin and  $\alpha$ -actinin in skeletal muscle. Confocal immunofluorescence micrographs of human trapezius muscle stained with anti-dystrophin (A) and anti- $\alpha$ -actinin-2 (B) (both pseudocolored) and combined images (C) demonstrating colocalization (yellow) where red  $\alpha$ -actinin and green dystrophin signals overlap. Elongated oval structure at right is a nucleus. Scale bar, 10  $\mu$ m.

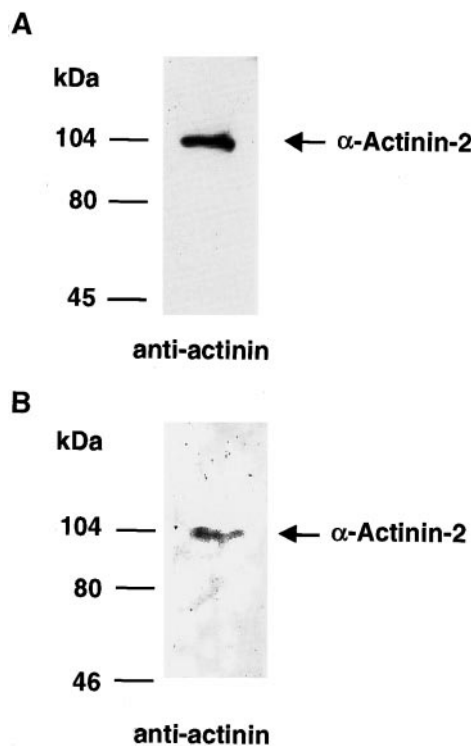


**FIG. 2.** C-terminal region of dystrophin interacts with  $\alpha$ -actinin-2 and actin *in vitro*.  $\alpha$ -Actinin, containing actin, was purified from rabbit skeletal muscle as described under Experimental Procedures. (A) Protein overlay of the purified  $\alpha$ -actinin. The proteins were separated on SDS-PAGE, transferred to nitrocellulose membranes, and probed with the purified recombinant GST-CT-1 fusion protein or with GST alone followed by anti-dystrophin antibodies to identify the GST-CT-1 binding proteins. Lane 1, ligand blotting with purified GST-CT-1; lane 2, ligand blotting with recombinant GST. (B) GST-CT-1 affinity chromatography of purified  $\alpha$ -actinin. The GST-CT-1 affinity column was synthesized by coupling recombinant GST-CT-1 to CNBr-activated Sepharose 4B. The purified  $\alpha$ -actinin was first applied onto a GST affinity column followed by a GST-CT-1 affinity column. The column was equilibrated in a solution containing 10 mM Mops, pH 7.0, 100 mM KCl, 2 mM MgCl<sub>2</sub> and 0.5 mM EGTA. Bound proteins eluted with 750 mM KCl are shown. The positions of  $\alpha$ -actinin, actin, and Bio-Rad prestained molecular mass standards are indicated.

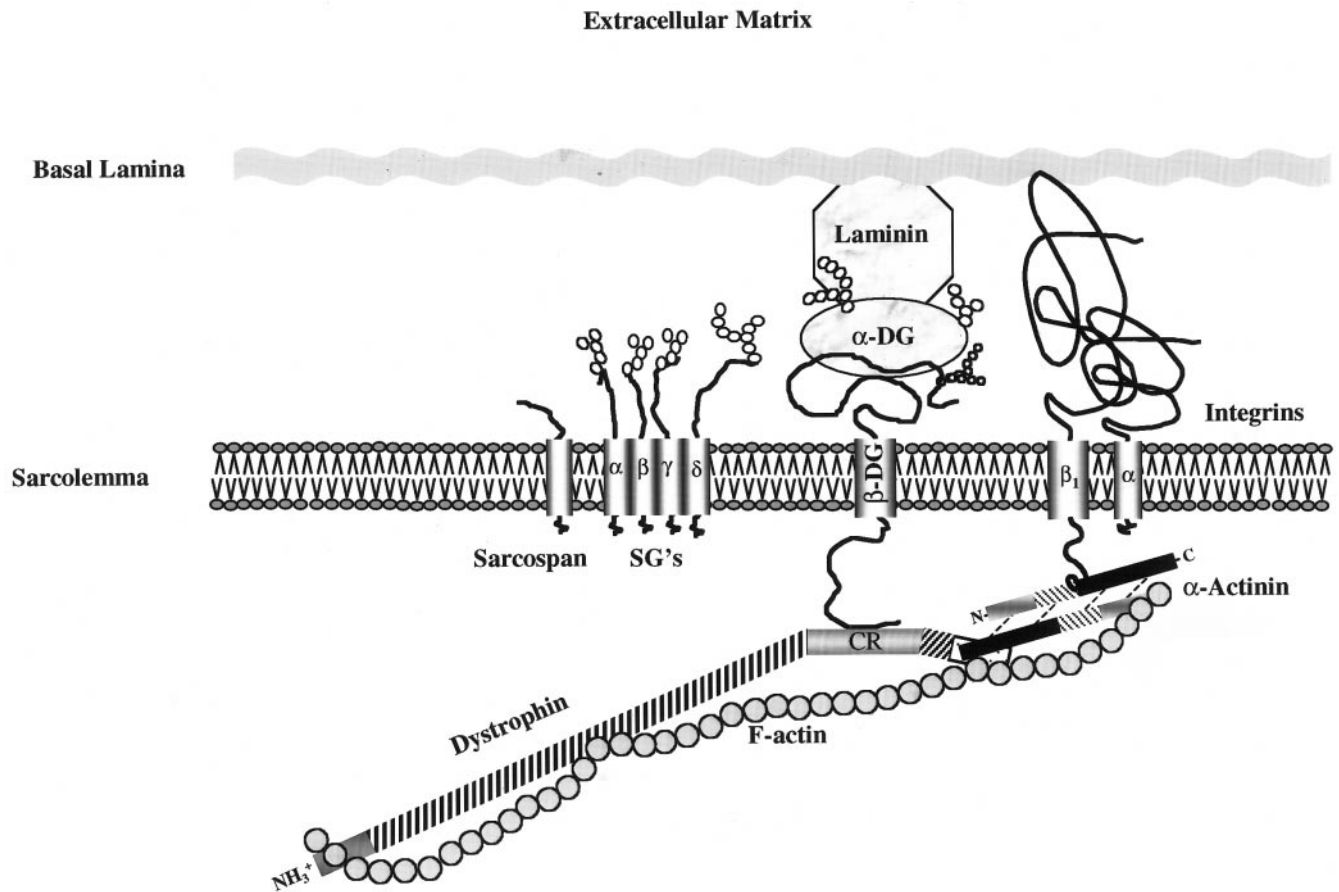
It is well established that dystrophin is a cytoskeletal protein that binds actin and interacts with a DGC to bridge the cytoskeleton, the basal lamina and the plasma membrane. Our findings add new components to the DGC complex. In Fig. 5 we propose a new model for the DGC organization that includes the  $\alpha$ -actinin-2 and actin interactions with dystrophin reported here (Fig. 5). Dystrophin is organized into distinct structural domains with the N-terminal domain binding F-actin (26). New F-actin binding sites have recently been discovered in the middle of the rod domain of dystrophin (Fig. 5) (27, 28). Here we show that the C-terminal domain of dystrophin binds  $\alpha$ -actinin and actin and, therefore, provides additional sites for F-actin attachment to the protein. We propose that dystrophin forms lateral, multicontact association with actin (Fig. 5).

What is the significance of interaction between dystrophin and  $\alpha$ -actinin-2?  $\alpha$ -Actinin interacts with the  $\beta_1$  integrin subunit to form a link between actin and

integrin (29). Figure 5 shows that binding of  $\alpha$ -actinin-2 to the C-terminus of dystrophin would, therefore, contribute a new communication link between the integrins, the DGC, and other cytoskeletal molecules at muscle sarcolemma. In skeletal muscle the  $\alpha_7\beta_1$  integrin is a primary laminin receptor linking the ECM with the cell cytoskeleton (30, 31). DMD patients and *mdx* mice exhibit increased expression of  $\alpha_7\beta_1$  integrin (32). Why is  $\alpha_7\beta_1$  upregulated in the absence of dystrophin? One major role of integrin is to maintain the muscle integrity by linking laminin with the cytoskeleton via vinculin, talin, and  $\alpha$ -actinin-2. We showed here that  $\alpha$ -actinin may play the role of a connecting molecule between the DGC and integrins. When communication between the DGC and integrin is disrupted, expression of integrin maybe upregulated to overcome the dystrophin deficiency and to help maintain muscle function and prevent necrosis. This hypothesis is further supported by a report on a bidirectional communication between the integrins and the dystrophin/DGC (33). Anti-dystrophin or anti- $\alpha$ -sarco-glycan antibodies coprecipitate integrin and other focal



**FIG. 3.**  $\alpha$ -Actinin-2 is associated with skeletal muscle sarcolemma and C<sub>2</sub>C<sub>12</sub> myoblasts plasma membrane vesicles. Rabbit skeletal muscle sarcolemma and C<sub>2</sub>C<sub>12</sub> plasma membrane vesicles were isolated as described under Experimental Procedures. Membrane proteins were separated in 10% SDS-PAGE, transferred to nitrocellulose membranes, and probed with an anti- $\alpha$ -actinin-2 antibody. (A) Rabbit skeletal muscle sarcolemmal proteins. (B) Plasma membrane vesicles isolated from the C<sub>2</sub>C<sub>12</sub> myoblasts. The positions of  $\alpha$ -actinin-2 and Bio-Rad prestained molecular mass standards are indicated.



**FIG. 5.** Model of dystrophin glycoprotein complex (DGC). A model of the DGC is presented depicting new sites of attachment for actin filaments and  $\alpha$ -actinin to the C-terminal region of dystrophin.  $\alpha$ -Actinin may exist as a homo- or heterodimer with sites interacting with dystrophin, actin, and integrin complexes. DGC is also composed of dystrophin, dystroglycan ( $\alpha$ - and  $\beta$ -dystroglycans), sarcoglycans ( $\alpha$ -SG,  $\beta$ -SG,  $\gamma$ -SG, and  $\delta$ -SG), and sarcospan. F-actin makes contacts with the N-terminus of dystrophin, with the central rod domain of the protein (27, 28), and with the C-terminal domain via  $\alpha$ -actinin (this work). Figure is not to scale.

adhesion proteins such as vinculin, talin, paxilin, and focal adhesion kinase (33). Here we provide additional lines of evidence suggesting that the dystrophin/DGC complex, via  $\alpha$ -actinin-2, interacts with the integrin adhesion system.

A common connection between all forms of muscular dystrophies is the mutation or absence of proteins in the DGC that plays an important role in maintaining the integrity of the sarcolemma membrane and myofibrils (1–10). Mutations in the  $\alpha 2$ -laminin chain gene result in congenital muscular dystrophy and decreased levels of  $\alpha 7 \beta 1$  integrin (34). The  $\alpha 7$ -deficient mice develop a similar phenotype (35), indicating a potential connection and a relationship between dystrophin/DGC and integrin. Since it is well documented that  $\alpha$ -actinin-2 plays a role in focal adhesion and it is also found concentrated at myotendinous junctions, we propose that binding of  $\alpha$ -actinin-2 to the C-terminus of dystrophin plays an important role in maintaining the communication link between dystrophin/DGC and integrin.

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## REFERENCES

1. Campbell, K. P. (1995) *Cell* **80**, 675–679.
2. Ahn, A. H., and Kunkel, L. M. (1993) *Nature Genet.* **3**, 283–291.
3. Tinsley, J. M., Blake, D. J., Zuelling, R. A., and Davies, K. E. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 8307–8313.
4. Worton, R. (1995) *Science* **270**, 755–756.
5. Ohlendieck, K. (1996) *Eur. J. Cell Biol.* **69**, 1–10.
6. Michalak, M., and Opas, M. (1997) *Curr. Opin. Neurol.* **10**, 436–442.
7. Henry, M. D., and Campbell, K. P. (1996) *Curr. Opin. Cell Biol.* **8**, 625–631.
8. Sunada, Y., and Campbell, K. P. (1995) *Curr. Opin. Neurol.* **8**, 379–384.
9. Blake, D. J., Tinsley, J. M., and Davies, K. E. (1996) *Brain Pathol.* **6**, 37–47.

10. Winder, S. J. (1996) *Biochem. Soc. Trans.* **24**, 497–501.
11. Milner, R., Busaan, J., and Michalak, M. (1992) *Biochem. J.* **288**, 1037–1044.
12. Beggs, A. H., Byers, T. J., Knoll, J.-H. M., Boyce, F. M., Bruns, G.-A. P., and Kunkel, L. M. (1992) *J. Biol. Chem.* **267**, 9281–9288.
13. Stolz, D. B., and Jacobson, B. S. (1992) *J. Cell Sci.* **103**, 39–51.
14. Charuk, J. H. M., Howlett, S., and Michalak, M. (1989) *Biochem. J.* **264**, 885–892.
15. Ohlendieck, K., Ervasti, J. M., Snook, J. B., and Campbell, K. P. (1991) *J. Cell Biol.* **112**, 135–148.
16. Laemmli, U. K. (1970) *Nature* **227**, 680–685.
17. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
18. Chan, Y.-M., Tong, H.-Q., Beggs, A. H., and Kunkel, L. M. (1998) *Biochem. Biophys. Res. Commun.* **248**, 134–139.
19. Wyszynski, M., Lin, J., Rao, A., Nigh, E., Begges, A. H., Craig, A. M., and Sheng, M. (1994) *Nature* **385**, 439–442.
20. Seraydarian, E. J., and Mommaerts, W. F. H. M. (1967) *Biochim. Biophys. Acta* **133**, 399–411.
21. Ausubel, F. M., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1989) *Current Protocols in Molecular Biology*, Greene and Wiley-Interscience, New York.
22. Lowry, D. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
23. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H., and Kedes, L. (1983) *Mol. Cell. Biol.* **3**, 787–795.
24. Walsh, M. P., Busaan, J. L., Fraser, E. D., Fu, S. F., Pato, M. D., and Michalak, M. (1995) *Biochemistry* **34**, 5561–5568.
25. Belkin, A., and BurrIDGE, K. (1995) *J. Biol. Chem.* **270**, 6328–6337.
26. Anderson, J. T., Rogers, R. P., and Jarrett, H. W. (1996) *J. Biol. Chem.* **271**, 6605–6610.
27. Rybakova, I. N., Amann, K. J., and Ervasti, J. M. (1996) *J. Cell Biol.* **135**, 661–672.
28. Rybakova, I. N., and Ervasti, J. M. (1997) *J. Biol. Chem.* **272**, 28771–28778.
29. Otley, C. A., Pavalko, F. M., and BurrIDGE, K. (1990) *J. Cell Biol.* **111**, 721–729.
30. Song, W. K., Wang, W., Sato, H., Bielser, D. A., and Kaufman, S. J. (1993) *J. Cell Sci.* **106**, 1139–1152.
31. Blaschuk, K. L., and Holland, P. C. (1994) *Dev. Biol.* **164**, 475–483.
32. Hodges, B. L., Hayashi, Y. K., Nonaka, I., Wang, W., Arahata, K., and Kaufman, S. J. (1997) *J. Cell. Sci.* **110**, 2873–2881.
33. Yashida, T., Pan, Y., Hanada, H., Iwata, Y., and Shigekawa, M. (1998) *J. Biol. Chem.* **273**, 1583–1590.
34. Xu, H., Christmas, P., Wu, X.-R., Wewer, U. M., and Engvall, E. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5527–5576.
35. Mayer, U., Saher, G., Fässler, R., Bornemann, A., Echtermeyer, F., von der Mark, H., Miosage, E., Pöschel, E., and von der Mark, K. (1997) *Nature Genet.* **17**, 318–323.