Myospryn Is a Novel Binding Partner for Dysbindin in Muscle*

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Dysbindin is a small coiled coil-containing protein that was originally identified in a yeast two-hybrid screen for proteins that interact with α- and β-dystrobrevin (1). The dystrobrevins are components of the dystrophin-glycoprotein complex (DGC)1 that bind directly to the dystrophin and syntrophin families of proteins (2, 3). Mutations in several of the components of the DGC cause different forms of muscle disease, including Duchenne muscular dystrophy (DMD), caused by the lack of dystrophin, and limb girdle muscular dystrophies, caused by mutations in the genes encoding the sarcoglycans (4). Mice carrying a targeted mutation in the gene encoding the α-dystrobrevins also have muscle disease (5). It has been proposed that α-dystrobrevin may be involved in intracellular signaling, because α-dystrobrevin-deficient mice develop a mild muscular dystrophy without disrupting the assembly of the DGC or the integrity of the sarcolemma, as is commonly seen in the other diseases involving DGC components (5, 6). One possible explanation of this phenotype is that an unknown protein linked to α-dystrobrevin or disruption of an unidentified signaling cascade may cause muscular disease in these mice.

Previously, in an attempt to elucidate this unidentified pathway, we isolated dysbindin (1). Dysbindin is a widely expressed protein that binds to both α- and β-dystrobrevin in muscle and brain, respectively. Furthermore, dysbindin is up-regulated in dystrophin-deficient muscle (1) and at cerebellar glomerular synapses of the mdx mouse model of DMD (7). Although a role for dysbindin in intracellular signaling has yet to be proven, dysbindin has recently been shown to be involved in protein trafficking and organelle biosynthesis. Dysbindin is mutated in a patient with the bleeding and pigmentation disorder Hermansky-Pudlak syndrome type 7 (HPS7) and in the sandy (sdy/sdy) mouse that is a murine model of the disease (8). These disorders are characterized by defects in the biogenesis of lysosome-related organelles, including melanosomes and platelet-dense granules (9, 10). Lysosome-related organelles are membrane-bound structures that are found in several cell types, generally have an acidic luminal pH, and contain LAMPS (lysosomal-associated membrane proteins) but lack the mannose-6-phosphate receptor (11). Dysbindin is part of a soluble 200-kDa protein complex, the biogenesis of lysosome-related organelles complex-1 (BLOC-1), and binds directly to the proteins pallidin and muted, which are encoded by genes mutated in two additional models of Hermansky-Pudlak syndrome (8, 12).

In addition to its role as dystrobrevin-binding protein and in protein trafficking, dysbindin is emerging as a major schizophrenia susceptibility factor (13, 14). Several groups have now found genetic linkage of schizophrenia to polymorphisms in the gene encoding dysbindin in different populations (15). Although no mutations in the coding region of the dysbindin gene have been found, cis-acting factors have a significant influence on the levels of the dysbindin expression in different individuals (16).

Although dysbindin has now been implicated in a number of different diseases, little is known about its function. To determine the role of dysbindin in skeletal muscle, we undertook a yeast two-hybrid screen to identify potential dysbindin binding partners. In this study, we describe the cloning and characterization of myospryn, a 413-kDa tripartite motif (TRIM)-related protein expressed in skeletal and cardiac muscle. Transcripts orthologous to myospryn show altered regulation in DMD and stretched, hypertrophic muscle.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screens—PCR products encoding amino acids 1–189 and amino acids 1–88 of dysbindin (Table I) were subcloned into the EcoRI site of pHyblexA (Invitrogen). Dysbindin-(1–189) was used as a targeted mutation in the gene encoding the dystrophin-glycoprotein complex (DGC)1 that interact with α- and β-dystrobrevin (1). The dystrobrevins are components of the dystrophin-glycoprotein complex (DGC)1 that bind directly to the dystrophin and syntrophin families of proteins (2, 3). Mutations in several of the components of the DGC cause different forms of muscle disease, including Duchenne muscular dystrophy (DMD) caused by the lack of dystrophin, and limb girdle muscular dystrophies, caused by mutations in the genes encoding the sarcoglycans (4). Mice carrying a targeted mutation in the gene encoding the α-dystrobrevins also have muscle disease (5). It has been proposed that α-dystrobrevin may be involved in intracellular signaling, because α-dystrobrevin-deficient mice develop a mild muscular dystrophy without disrupting the assembly of the DGC or the integrity of the sarcolemma, as is commonly seen in the other diseases involving DGC components (5, 6). One possible explanation of this phenotype is that an unknown protein linked to α-dystrobrevin or disruption of an unidentified signaling cascade may cause muscular disease in these mice.

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Myospryn Is a Novel Binding Partner for Dysbindin in Muscle

Molecular Biology—The 3′ myospryn cDNA sequence was obtained by sequencing the cDNA clones identified in the yeast two-hybrid screen. The remainder of the sequence was obtained by sequencing a full-length clone retrieved from a mouse skeletal muscle cDNA library (a kind gift from Prof. Kay E. Davies) as described previously (18). Protein sequence alignments were made using the PSI-BLAST program (19), and protein domains were determined using the SMART program (20, 21). Mouse multiple tissue northern blots were purchased from Origene and hybridized with the full-length cDNA fragment using Rapid-hyb buffer as per the manufacturer’s instructions (Amer sham Biosciences). The hybridized blots were processed as described previously (18). Western blotting was performed as described previously (1). The myospryn expression construct MD7:pEGFP was produced by cloning the SalI fragment of MD7 into the XhoI site of pCMVtag2C. DNA from each bait and prey pair was ligated into the EcoRI and XhoI sites of pET-28a (Novagen) and pET-28a (Novagen) respectively (18). Western blotting was performed as described previously (1). Protein Pull-down Assay and Immunoprecipitation—PCR products of myospryn and the corresponding RYR1 antisense oligonucleotide were amplified using the following primers: BBC domain, BBCF (5′-CACAAAGACAATCTGCTAACACAGCAC-3′); BBCR (5′-CTCTCTAATGGAGACGTAGCTATCCA-3′); PhyLex (5′-GGTCTCCTTTGATTCTTAACTGAC-3′); PhyLexR (5′-GGGAGAAGGCTTCCGGGTCAGCAGC-3′); SPRY (5′-GTCAAGGCTCAGTTTCTTCTCT-3′). The PCR products were ligated into pQE30-UA (Qiagen). This construct was transformed into E. coli BL21 (DE3) (Stratagene) to produce fusion proteins. Thioredoxin-tagged proteins were purified under denaturing conditions on Talon resin columns (Clontech). Proteins were washed while on the column containing decreasing concentrations of urea (8, 6, 4, and 2 M) to refold the protein prior to elution with sonication buffer containing 100 mM sodium phosphate, pH 8 containing 2 M urea. The RYR1 monoclonal anti-Myc antibody was purchased from Covance. The RyR binding domain 2166 have been described elsewhere (1, 22, 23). The 9E10 monoclonal anti-Myc antibody was purchased from Sigma. The anti-myospryn antibody des122 was produced from a PCR product spanning amino acids 2791

Table 1

| Bait       | Prey       | Activity (A405/mg protein) |
|------------|------------|-----------------------------|
| Dysbindin (1–189) | Myospryn MD9 | 80.2 ± 0.2                   |
| Dysbindin (1–88) | Myospryn MD9 | 0.6 ± 0.3                    |
| PhyLexA | Myospryn MD9 | 0.1 ± 0.1                     |
| Dysbindin (1–189) | PYesTrp2 | 0.7 ± 0.3                       |
| Dysbindin (1–88) | PYesTrp2 | 0.3 ± 0.1                       |
| β-Dystrobrevin | α-Syntrophin | 11.3 ± 5.3                     |

Fig. 1. Yeast two-hybrid clones and primary sequence. A, the distribution of a representative selection of myospryn clones isolated from a mouse muscle cDNA library relative to the 3′-end of the myospryn gene. The locations of the sequences encoding predicted protein domains (Fig. 2) and the minimal dysbindin-binding region determined by the yeast two-hybrid screen. The remainder of the sequence was obtained by sequencing the cDNA clones identified in the yeast two-hybrid screen. The anti-dystrophin antibody des122 was produced from a PCR product spanning amino acids 2791–2913. The single line leading to the poly(A) tail represents the 3′-untranslated region. B, the primary amino acid sequence of mouse myospryn. The italicized text shows the position of a low complexity domain with sequence consensus sNHRLREHAE (upper-case, 80% identity).
FIG. 2. Identification and annotation of myospryn domains and homologues. A, comparison of the protein domains found in the C terminus of myospryn with FSD1/MIR1 (30–31) and midline1/TRIM18 (40). The asterisk indicates a representative member of the tripartite motif protein family. The shaded shapes represent shared domains between the three proteins. B, multiple sequence alignment of the primary sequences of the myospryn C terminus (amino acids 3215–3739) aligned against midline1 (mid1), FSD1, and spring1 (32). The protein domains are illustrated with a line above the sequence. Identical residues are decorated in black, with similar residues shaded gray. C, sequence alignment between myospryn (amino acids 1748–1868) and the orthologous human protein, genethonin-3.

Myospryn Is a Novel Binding Partner for Dysbindin in Muscle
Myospryn Is a Novel Binding Partner for Dysbindin in Muscle 10453

imidazole. GST-tagged dysbindin was produced by PCR amplification of the coding region using the primers m1SalF (5'-CTGTCGACGGG-GACGGCTGCTGAG-3') and m1SalR (5'-CTGTCGACAAATGTC-CTGAGTTGAGTCACA-3'). This product was ligated into pGemT (Promega) and excised using Sall. The Sall fragment was then cloned in frame into Sall cut pGEX-4T3 (Amersham Biosciences). The m10-pGEX-4T3 recombinant plasmid was transformed into E. coli XL1 Blue, and a fusion protein was produced as per the manufacturer’s recommendations. The GST-dysbindin fusion protein was purified using a glutathione-Sepharose 4B column as per the manufacturer’s instructions (Amersham Biosciences). 50 μg of thioredoxin-tagged domains and GST-dysbindin were mixed at room temperature for 3 h in a total volume of 1 ml of binding buffer (Tris-buffered saline; 10 mM Tris, pH 7.4, and 150 mM NaCl) containing 0.5% Tween 20, 20 mM imidazole, and 1× protease inhibitors (Sigma). 50 μl of nickel-nitriiltriacetic acid beads (Qiagen) pre-equilibrated in binding buffer were added to the protein mixture and incubated with shaking for 1 h. The beads were captured magnetically and washed 3× in binding buffer. The bound proteins were eluted into 25 μl of binding buffer mixed with 25 μl of 2× SDS-PAGE sample buffer and separated on two 10% PAGE gels. One gel was stained with Coomassie Blue R-250, whereas the other gel was Western blotted as described earlier (22).

Mouse quadriceps muscle (3g) was homogenized in 15 ml of 0.25× RIPA buffer (37.5 mM NaCl, 12.5 mM Tris-HCl, pH 8, 0.25% Triton X-100, 0.125% sodium deoxycholate, and 250 μM EGTa) containing protease inhibitor mixture (Sigma). After incubation on ice for 30 min, the homogenate was clarified by centrifugation (141,000 × g for 45 min in a Beckman SW41 rotor). Proteins were immunoprecipitated using 4 μg of des122 or PA31111A antibody and analyzed as described previously (1).

Muscle Immunocytochemistry and Subcellular Fractionation—8-μm transverse sections of rat quadriceps muscle were fixed in −20 °C acetone for 30 s and allowed to air dry for 5 min. The fixed sections were then stained as described previously (24). Muscle microsomes were prepared from rabbit muscle as described by Chu et al. (25). Briefly, a New Zealand White rabbit was sacrificed by cervical dislocation. 60 g of quadriceps muscle was minced in a meat grinder, resuspended in 250 ml of homogenization buffer (5 mM imidazole, pH 7.4, 0.3 M sucrose, 100 μM ZnSO4, and 1× EDTA-free protease inhibitor mixture from Roche Applied Science), and homogenized in a Waring blender for 1 min at full speed. This homogenate was centrifuged at 11,000 × g for 20 min. The supernatant was decanted, and the pellet was resuspended in 250 ml of homogenization buffer and re-homogenized. The homogenate was then centrifuged at 11,000 × g for 20 min. The supernatant was filtered through six layers of sterilized cheesecloth and then centrifuged at 110,000 × g for 60 min. The microsomal pellet was resuspended in 10 ml of homogenization buffer and stored at −70 °C. All procedures were carried out at 4 °C to minimize protein degradation, and samples were taken at each stage. Protein concentrations were determined using the BCA assay (Pierce), and 20 μg of each was analyzed by PAGE gels or Western blotting.

RESULTS

Dysbindin Binds to a Novel Protein in Yeast Two-hybrid Experiments—To identify potential dysbindin-interacting proteins in skeletal muscle, a yeast two-hybrid screen was performed on a mouse muscle cDNA library using amino acids 1–189 of dysbindin as the bait. Screening a total of 1.8 × 106 independent clones produced 87 interacting clones, 44 of which were overlapping clones derived from the same novel gene (Fig. 1A). We have named this new gene myospryn. The interaction between myospryn and dysbindin was confirmed in vitro using a liquid phase β-galactosidase assay on a variety of bait and prey plasmids that were co-transformed into Saccharomyces cerevisiae L40 (Table I). This assay also revealed that the binding site for myospryn is located within the coiled-coil region of dysbindin. The interaction between myospryn and dysbindin occurs only when the coiled-coil domain of dysbindin is present but does not occur in its absence. Reporter gene activation is not seen when myospryn is co-transformed with the empty bait plasmid pHyflexA or when the N-terminal constructs of dysbindin are co-transformed with the empty prey plasmid, pYesTrp2. A control experiment utilizing the well-characterized β-dystrofibrin-a-syntrophin C terminus interaction (22, 26) was performed and, as expected, reporter gene expression was activated.

Cloning and Characterization of Myospryn—The complete myospryn cDNA was isolated and found to encode a protein of 3,739 amino acids (Fig. 1B) with a predicted molecular mass of 413.1 kDa and a pI of 4.71. The C terminus of myospryn contains the BBC, FN3, and SPRY domains in a configuration that is reminiscent of members of the TRIM protein family (27) (Fig. 2A). It is particularly similar to the proteins midline-1 (28, 29), FSD1/MIR1 (30, 31), and Spring (32) (Fig. 2, A and B). A region with apparent homology to a BBox domain is present prior to the BBC domain (Fig. 3A). However, a true BBox domain is characterized by the presence of four pairs of Zn2+-coordinating cysteine and histidine residues. The correspond-
ing myospryn domain only contains two pairs of these conserved residues, so we have named this domain BBox\textsuperscript{1}. Also of note in the primary sequence of myospryn is a 12-amino acid imperfect repeat (consensus sequence pIVhREEEHAPE; uppercase, 80\% identity) that occurs nineteen times between amino acids 476–715 (Fig. 1B). A BLAST search performed on the non-redundant protein data base using the myospryn primary amino acid sequence showed that genethonin-3 (AAD55265), a transcript that is down-regulated in DMD (33), and stretch-responsive protein 553 (sr553) (CAC084950), a transcript that is up-regulated in a model of muscle hypertrophy (34), are orthologous to the C-terminal encoding region of the myospryn gene (Fig. 2C).

**Myospryn Is Expressed in Heart and Skeletal Muscle**—To determine the tissue distribution of myospryn, a polyclonal rabbit antibody (des122) was raised. The specificity of this antibody was confirmed by Western blotting protein extracts from COS-7 cells transiently transfected with the plasmids indicated. MD9 encodes the myospryn C terminus (amino acids 2729–3739; Fig. 1A). Co-immunoprecipitated proteins were detected with anti-myospryn (top), anti-Myc (middle), and anti-β-dystrobrevin (bottom) antibodies. ± represents the presence of precipitating anti-dystrobrevin antibody (IP). The original protein extracts are shown to indicate the presence of protein in the transfected cells. dabn, dystrobrevin; β-db, β-dystrobrevin. B, in vivo association of myospryn and dystrobrevin. Proteins extracted from mouse skeletal muscle were immunoprecipitated (IP) using either anti-myospryn or anti-dystrobrevin antibodies. Immunoprecipitated proteins were detected using des122 or biotinylated PA3111A. Dystrobrevin is specifically immunoprecipitated with PA3111A (+), which also co-immunoprecipitates myospryn. A control immunoprecipitation performed in the absence of the primary antibody (−) is included to show specificity. The anti-myospryn antibody des122 specifically immunoprecipitates its cognate antigen but does not show the same level of enrichment compared with PA3111A. The presence of myospryn in the muscle lysate is shown to facilitate a direct comparison.

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![Fig. 4. Co-immunoprecipitation of myospryn and dysbindin. A, in vitro association of myospryn and dysbindin. Protein extracts were prepared from COS-7 cells transfected with the plasmids indicated. MD9 encodes the myospryn C terminus (amino acids 2729–3739; Fig. 1A). Co-immunoprecipitated proteins were detected with anti-myospryn (top), anti-Myc (middle), and anti-β-dystrobrevin (bottom) antibodies. ± represents the presence of precipitating anti-dystrobrevin antibody (IP). The original protein extracts are shown to indicate the presence of protein in the transfected cells. dabn, dystrobrevin; β-db, β-dystrobrevin. B, in vivo association of myospryn and dysbindin. Proteins extracted from mouse skeletal muscle were immunoprecipitated (IP) using either anti-myospryn or anti-dystrobrevin antibodies. Immunoprecipitated proteins were detected using des122 or biotinylated PA3111A. Dystrobrevin is specifically immunoprecipitated with PA3111A (+), which also co-immunoprecipitates myospryn. A control immunoprecipitation performed in the absence of the primary antibody (−) is included to show specificity. The anti-myospryn antibody des122 specifically immunoprecipitates its cognate antigen but does not show the same level of enrichment compared with PA3111A. The presence of myospryn in the muscle lysate is shown to facilitate a direct comparison.](http://www.jbc.org/)[Fig. 4. Co-immunoprecipitation of myospryn and dysbindin. A, in vitro association of myospryn and dysbindin. Protein extracts were prepared from COS-7 cells transfected with the plasmids indicated. MD9 encodes the myospryn C terminus (amino acids 2729–3739; Fig. 1A). Co-immunoprecipitated proteins were detected with anti-myospryn (top), anti-Myc (middle), and anti-β-dystrobrevin (bottom) antibodies. ± represents the presence of precipitating anti-dystrobrevin antibody (IP). The original protein extracts are shown to indicate the presence of protein in the transfected cells. dabn, dystrobrevin; β-db, β-dystrobrevin. B, in vivo association of myospryn and dysbindin. Proteins extracted from mouse skeletal muscle were immunoprecipitated (IP) using either anti-myospryn or anti-dystrobrevin antibodies. Immunoprecipitated proteins were detected using des122 or biotinylated PA3111A. Dystrobrevin is specifically immunoprecipitated with PA3111A (+), which also co-immunoprecipitates myospryn. A control immunoprecipitation performed in the absence of the primary antibody (−) is included to show specificity. The anti-myospryn antibody des122 specifically immunoprecipitates its cognate antigen but does not show the same level of enrichment compared with PA3111A. The presence of myospryn in the muscle lysate is shown to facilitate a direct comparison.](http://www.jbc.org/)
precipitated myospryn strongly (Fig. 4A, top panel). When dysbindin is visualized using the rabbit polyclonal PA3111A, a rabbit IgG heavy chain band obscures the dysbindin band. For this reason, immunoprecipitation of dysbindin was confirmed using the monoclonal anti-Myc antibody, 9E10 (Fig. 4A, middle panel). In a control experiment, the known dysbindin-interacting protein, H9252-dystrobrevin (1), is only co-immunoprecipitated when co-expressed with Myc-dysbindin (Fig. 4A, bottom panel).

Similarly, in vivo immunoprecipitation experiments performed on mouse muscle lysate using anti-dysbindin antibodies demonstrated that dysbindin is able to robustly co-immunoprecipitate myospryn (Fig. 4B). Myospryn immunoprecipitation using anti-myospryn antibodies was visualized with des122 as a positive control. The respective proteins are only immunoprecipitated in the presence of the precipitating antibody and do not immunoprecipitate with the antibody-conjugated beads alone (Fig. 4B). Dysbindin is specifically immunoprecipitated with its cognate antibody and can be detected in the presence of rabbit IgG with biotinylated PA3111 and horseradish peroxidase-streptavidin (Fig. 4B).

**Dysbindin Binds within the C Terminus of Myospryn**—Sequence alignment of the yeast two-hybrid cDNA clones revealed a region encompassing the BBox domain and the N terminus of the BBC domain (amino acids 3151–3283) that was required for the interaction of myospryn and dysbindin (Fig. 1A). To delineate the dysbindin-binding site of myospryn, recombinant proteins containing these domains were produced as thioredoxin fusion proteins in E. coli (Fig. 5B). Pull-down experiments using GST-tagged dysbindin (GST-dysbindin) showed that the optimal interaction with dysbindin occurs when both the BBC and the BBox domains are present in the fusion protein. A small amount of binding does occur when only the BBC domain is present in the fusion protein, presumably through a coiled-coil motif interaction; however the presence of the BBox domain improves this interaction. Thioredoxin and the BBox alone do not display any apparent dysbindin binding.
capability (Fig. 5C). These data further confirm an *in vitro* association between myospryn and dysbindin.

**Co-localization of Myospryn with Dysbindin in Skeletal Muscle**—Myospryn is found at the sarcolemma of most muscle fibers in the rat quadriceps. Diffuse myospryn immunoreactivity is also associated with intracellular structures and the sarcoplasm (Fig. 6, upper panel). Double immunofluorescence shows that myospryn co-localizes precisely with dysbindin at the sarcolemma and also in the sarcoplasm (Fig. 6, center and lower panels). Myospryn was not detected in large blood vessels or endomysial capillaries (Fig. 6, upper panel). The biochemical distribution of myospryn in muscle was determined by Western blotting different fractions prepared from rabbit skeletal muscle with a panel of antibodies. Myospryn is found in the high speed supernatant (S2) prepared from the P1 fraction after the myofibrillar proteins and nuclei had been spun out (Fig. 7). Myospryn is also found in the microsomal fraction, which contains the sarcolemma, sarcoplasmic reticulum (SR), and other intracellular membranes. Similarly, dysbindin is found in the S2, S3, and microsome fractions, showing that both proteins co-fractionate (Fig. 7). In control experiments the distribution of α-dystrobrevin-1 (sarcolemma) and dystrophin (sarcolemma), and the ryanodine receptor (of the SR) was determined. Dys-}



**FIG. 6. Co-localization of myospryn with dysbindin.** As shown in the merged image, myospryn (labeled with des122 directly conjugated with Cy3.5) co-localizes precisely with dysbindin (labeled with an Alexa 488-conjugated anti rabbit antibody) at the sarcolemma and sarcoplasm of most muscle fibers. Scale bar is 50 μm.

**FIG. 7. Subcellular distribution of myospryn.** Samples from each step of the microsome preparation were probed with antibodies raised against myospryn, dysbindin (detected with biotinylated PA3111A), α-dystrobrevin-1 (sarcolemma and costameres, detected with αICT-FP), dystrophin (sarcolemma and costameres), and the ryanodine receptor (of the SR). A Coomassie Blue-stained PAGE gel of the same samples is also shown. The majority of the myospryn and dysbindin remains in the soluble (S3) fraction after ultracentrifugation as opposed to the membrane-associated proteins, dystrophin and the ryanodine receptor, that are enriched in the P1 and microsomal fraction. Note that myospryn is also found in the microsomal fraction. The major components of myofibril, myosin, and actin can clearly be seen in P1. To, total muscle extract; S1, supernatant from initial homogenization; P1, low speed pellet; S2, low speed supernatant; S3, high speed supernatant; Mic, microsome.
Fig. 8. **Self-association of myospryn.** A, location of yeast two-hybrid clones interacting with the C-terminal encoding region of myospryn. The clone names and the location of the first amino acid are shown. The shortest clone, C3, shows that there is no overlap between the dysbindin-binding site and the self-association site. B, self-association was confirmed in transfected cells. Myc-MD7, encoding the C terminus of myospryn (Fig. 1A), is only co-immunoprecipitated in the presence of MD9 and with the des122 antibody (+) and not when the antibody is omitted (−). Control experiments show that MD9 is immunoprecipitated with des122, whereas Myc-MD7 is not. Immunoprecipitation only occurs in the presence of the precipitating antibody demonstrating specificity and efficacy.

(Fig. 8B, upper panel). Myc-MD7 is not immunoprecipitated with des122 and does not co-immunoprecipitate with MD9 if the primary antibody is omitted. Thus, myospryn, in common with the midline proteins, self-associates. By contrast, no evidence was found for myospryn targeting to microtubules (data not shown).

**DISCUSSION**

This study presents the cloning and characterization of myospryn, a novel 413-kDa protein that interacts with dysbindin. We have named this protein myospryn due to its restricted expression in skeletal muscle and heart in contrast to its ubiquitous expression in yeast two-hybrid screening (36). The altered distribution of dysbindin in dystrophin-deficient muscle and brain also supports our hypothesis that dysbindin may play a role in DMD, possibly linking the DGC to an unidentified protein complex (1, 3, 7). We have now identified myospryn as a tissue-specific ligand for dysbindin in muscle. Although our data does not preclude a signaling role for dysbindin, it seems that a role in intracellular trafficking or protein complex assembly is more likely. Dysbindin is a component of the soluble BLOC-1 complex that has been characterized in bovine liver and HeLa cells (8, 12, 35). Although this is a soluble complex, dysbindin, in common with many TRIM proteins, does self-associate (41, 42). This self-association has been implicated as a possible regulatory feature, and it is interesting to speculate whether dysbindin may participate in this process due to its binding within the region containing features similar to those of the TRIM protein family. Furthermore, the homologues midline-1 and -2 heterodimerize, suggesting that there may be a myospryn homologue that could be an additional binding partner (41).

In a study analyzing gene expression changes in DMD, Tkatchenko et al. showed that the myospryn orthologue gene, sr553, is up-regulated in X-linked Opitz syndrome, a developmental defect in ventral midline formation (36). Myospryn is however, not a true TRIM protein because it lacks the requisite RING finger domain. This RING domain has been implicated in degradation of proteins via the ubiquitin pathway (29). Furthermore, many TRIM proteins, including midline-1 and -2, are associated with the microtubular cytoskeleton (39, 40). Although no evidence for an association between myospryn and microtubules was found (data not shown), myospryn, in common with many TRIM proteins, does self-associate (41, 42). This self-association has been implicated as a possible regulatory feature, and it is interesting to speculate whether dysbindin may participate in this process due to its binding within the region containing features similar to those of the TRIM protein family. Furthermore, the homologues midline-1 and -2 heterodimerize, suggesting that there may be a myospryn homologue that could be an additional binding partner (41).

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