Proper Perinuclear Localization of the TRIM-like Protein Myospryn Requires Its Binding Partner Desmin

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Desmin, the muscle-specific intermediate filament protein, surrounds the Z disks and links the entire contractile apparatus to the sarcolemmal cytoskeleton, cytoplasmic organelles, and the nucleus. In an attempt to explore the molecular mechanisms of these associations, we performed a yeast two-hybrid screening of a cardiac cDNA library. We showed that the desmin amino-terminal domain (N-(1–103)) binds to a 413-kDa TRIM-like protein, myospryn, originally identified as the muscle-specific partner of dysbindin, a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). Binding of desmin with myospryn was confirmed with glutathione S-transferase pulldown assays and coimmunoprecipitation experiments. Western blot analysis revealed that the complex immunoprecipitated by desmin antibodies, in addition to myospryn, contained the BLOC-1 components dysbindin and palladin. Deletion analysis revealed that only the (N-(1–103)) fragment of desmin binds to myospryn carboxyl terminus and that this association takes place through the 24-amino acid-long carboxyl-terminal end of the SPRY domain of myospryn. Using an antibody against the COOH terminus of myospryn, we demonstrated that myospryn colocalizes with desmin at the periphery of the nucleus, in close proximity to the endoplasmic reticulum, of mouse neonatal cardiomyocytes. In adult heart muscle, the two proteins colocalize, predominantly at intercalated disks and costameres. We also showed that myospryn colocalizes with lysosomes. Using desmin null hearts, we determined that desmin is required for both the proper perinuclear localization of myospryn, as well as the proper positioning of lysosomes, thus suggesting a potential role of desmin intermediate filaments in lysosomes and lysosome-related organelle biogenesis and/or positioning.

The intermediate filaments (IFs) form one of the three cytoskeletal networks found in higher eukaryotes. They display a tissue-specific and developmentally regulated pattern of expression. IFs were initially characterized as "integrators of cellular space" (1), because of their relative stable organization. Desmin, the muscle-specific member of the intermediate filament protein family, is one of the earliest known myogenic markers, both in heart and in somites (2–4). Desmin is expressed in satellite stem cells (5) and replicating myoblasts (1), and its inhibition interferes with myoblast fusion and proper expression of myogenic transcription regulators (6, 7). In mature striated muscle, desmin IFs form a three-dimensional scaffold that seems to extend across the entire diameter of the myofibril. Desmin IFs surround the Z disks, extend from one Z disk to the other, and potentially associate with different cellular organelles. Finally, desmin IFs extend from the Z disk to the plasma membrane at the level of costameres and intercalated disks in the case of cardiac muscle, and project from the Z disks of the perinuclear myofibrils to the nuclear membrane. The molecular mechanism responsible for the majority of these associations remains unknown. (For review see Ref. 8).

Ablation of desmin expression in mice by gene targeting (9, 10) and identification of numerous mutations in the human desmin gene (for review see Refs. 8 and 11) demonstrated that this protein is crucial for the maintenance of healthy muscle. Defects because of the lack of desmin are more severe in the heart, where transient cardiomyocyte hypertrophy and chamber dilation characterized by extensive myocyte death lead to heart failure (9, 10, 12). Because the earliest observed defects were found in mitochondrial positioning, structure, and function (13, 14), it has been suggested that these might be the target organelles in desmin null pathogenesis. Accordingly, analysis of the mitochondrial proteome from wild type and desmin null heart demonstrates several changes in proteins involved in energy metabolism and apoptosis (15). These data, so far, strongly suggest potential defects either in proper protein targeting to mitochondria and other cellular organelles, without excluding destabilization of specific regions or complexes on them. Amelioration of mitochondrial abnormalities by overexpressing the anti-apoptotic protein Bcl2, located at the mitochondrial contact sites and known to regulate the voltage-dependent anion channel function, supports the above hypothesis (16). Moreover, new evidence from studies in other cell types...
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also support the involvement of IFs in similar functions, including organelle positioning and protein targeting to the proper intracellular compartments (17, 18).

In an attempt to identify new desmin-associated proteins, we performed a yeast two-hybrid screening of a cardiac cDNA library. Here, we describe a novel association of desmin with myospryn, a member of the tripartite motif (TRIM) superfamily of proteins, initially identified as a dysbindin-associated protein (19) and as a Mef-2 target gene (20). Dysbindin is a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1), which is involved in protein trafficking and organelle biogenesis. We provide evidence that the interaction between desmin and myospryn is important for the proper perinuclear localization of the latter and that it potentially facilitates lysosome biogenesis and/or positioning.

EXPERIMENTAL PROCEDURES

The procedures for the care and treatment of animals were according to institutional guidelines, which follow the guidelines of Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) and the recommendations of Federation of European Laboratory Animal Science Association (FELASA). Mice lacking desmin were generated as described previously (10) and were of the 129Sv genetic background.

Yeast Two-hybrid Library Screening—The Matchmaker GAL-4 two-hybrid system was used as described by the manufacturer (Clontech). A cDNA fragment encoding the NH2 terminus of mouse desmin (nucleotides 80–410 bp) was inserted downstream of the GAL4 DNA binding domain in the pGBK7 bait vector. A yeast two-hybrid cDNA library (Clontech, catalog number 638865) derived from human heart muscle was screened for interacting proteins as described by the distributor. For interaction, positive clones were selected in selection medium (SD/-Trp/-Leu/-Ade/-His + X-α-galactosidase plates).

Generation and Purification of GST Fusion Proteins—The myospryn fragment was inserted into pGEX-5x-1 at EcoRI/ Xhol sites (Amersham Biosciences) to generate GST fusion proteins. The recombinant polypeptides were expressed into BL21 bacteria by induction with 0.1 mM isopropyl 1-thio-β-D-galactopyranoside for 4 h. The bacteria were harvested by low speed centrifugation, resuspended in lysis buffer 1: 20 mM Tris, pH 8.5, 5 mM EDTA, 5 mM DTT, 0.2% (v/v) Triton X-100 with protease inhibitors (protease inhibitor mixture (Sigma P8340) plus 2 mM phenylmethylsulfonyl fluoride). Lysozyme was added to 1 mg/ml and incubated for 1 h at 4 °C. The suspension was centrifuged at 11,000 rpm for 20 min. The inclusion bodies which contained the GST-myospryn fusion proteins were dissolved in lysis buffer 2 containing 6 M urea, 20 mM Tris, pH 8.5, 5 mM EDTA, 5 mM DTT, 5% (v/v) glycerol and dialyzed against buffer 3: 100 mM NaCl, 20 mM Tris, pH 8.5, 0.5% (v/v) Tween 20, 1 mM EDTA, 1 mM DTT, 5% (v/v) glycerol.

Preparation of Desmin-enriched Cardiac Extract—Cardiac tissue was homogenized in PBS, 0.6 M KCl, 1% (v/v) Triton X-100 with protease inhibitors, and the homogenate was centrifuged for 10 min at 3000 × g. The pellet was resuspended in gel filtration buffer (PBS, 6 M urea, 1 mM EDTA, 2 mM DTT with protease inhibitors) and was further purified with gel filtration column chromatography using the Shodex KW-804 column (Thomson Instruments, Clear Brook, VA) connected to AKTA-P900 purifier (Amersham Biosciences). Fractions enriched in desmin were chosen by SDS-PAGE and Western blot analysis (data not shown).

Preparation of Heart Homogenates—Cardiac tissue from wild type and desmin null mice was homogenized in extraction buffer containing 10 mM Tris, pH 8.5, 0.01% (v/v) SDS, 20 mM NaCl, 0.01% (v/v) Nonidet P-40, 0.01% (v/v) DOC, 5 mM EDTA, 2 mM DTT, and protease inhibitors. The homogenate was centrifuged for 10 min at 3000 × g, and the supernatant was used.

GST Pulldown Assay—Equal amounts of recombinant GST and GST-myospryn, or GST-desmin were bound to glutathione–Sepharose and mixed with either 0.1 mg of desmin-enriched cardiac extract or 1.5 mg of total heart homogenates at room temperature for 2 h. Bound proteins after washing in the cold washing buffer (10 mM Tris, pH 8.5, 0.01% (v/v) Nonidet P-40, 0.01% (v/v) DOC, 5 mM EDTA, 2 mM DTT, 0.05% (v/v) SDS) were eluted by heating for 5 min at 90 °C in SDS-PAGE sample buffer (50 mM Tris, pH 6.8, 50 mM DTT, 2% (v/v) SDS, 0.2% (v/v) bromophenol blue, 10% (v/v) glycerol). The soluble fractions were analyzed by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and probed with antibodies to desmin and myospryn, respectively.

In Vitro Transcription/Translation and Co-IP—Proteins encoded by the various constructs, hemagglutinin (HA), HA-myospryn in pGADT7 and c-Myc, c-Myc-full-length desmin in pGBKT7, were synthesized and labeled with [35S]methionine using the Tnt QuickCoupled transcription/translation system (catalog number L4610, Promega, Madison, WI). 30 µl from each protein lysate was mixed and incubated for 2 h to allow the complexes to be formed. 5 µl of chimeric protein A/G (Pierce, catalog number 53132) with 10 µl of antibody anti-HA or anti-c-Myc (Clontech) were used for each reaction to capture the protein complexes. The mixture was centrifuged at 1,000 × g for 1 min, and the pellet was washed four times with 1× PBS, 0.5% (v/v) Nonidet P-40, 0.25% (v/v) DOC and then resuspended in SDS-PAGE sample buffer. The samples were heated at 90 °C for 5–10 min, separated by electrophoresis on 10–15% gradient acrylamide gel, and proteins were visualized by autoradiography.

Coimmunoprecipitations Experiments with Cardiac Extract—Heart muscle was dissected from a normal adult 129Sv mouse, flash-frozen in liquid nitrogen, and homogenized in 500 µl of solubilization buffer (150 mM NaCl, 1% (v/v) Nonidet P-40, 0.05% (v/v) SDS, 50 mM Tris, pH 7.4) containing protease inhibitors (Sigma). Samples were incubated on ice for 30 min and centrifuged at 13,000 rpm at 4 °C for 45 min. The following procedures were performed at 4 °C unless stated otherwise. 1.5 mg of soluble protein in a volume of 0.5 ml of washing buffer (10 mM Tris, pH 8.0, 0.5% (v/v) Nonidet P-40, 0.5% (v/v) DOC, 5 mM EDTA, 2 mM DTT) was pre-cleared with 50 µl of chimeric protein A/G (Pierce, catalog number 53132) for 3 h. Anti-desmin antibodies (15 µl of D3 from Hybridoma Bank plus 15 µl of H-76 (in case of dysbindin and pallidin experiment) or D3 plus Y-20 (in case of myospryn experiment) from Santa Cruz Biotechnology) were applied on 50 µl of protein A/G for 3 h.
The pre-cleared cardiac extract was combined with protein A/G carrying the desmin antibodies for 16–18 h. The beads were washed five times with washing buffer by centrifugation at 1000 rpm for 2 min, eluted in 60 μl of elution buffer (75 mM Tris, pH 6.8, 3.8% (v/v) SDS, 4 M urea, 5% mercaptoethanol, 20% glycerol) and analyzed by Western blotting for the detection of dysbindin (1:200, Novus, CO, catalog number NB100-40808), pallidin (1:200, Novus, catalog number NB100-1234), and myospryn (1:1000, rabbit anti-peptide antibody). A sample lacking primary antibody acted as negative control.

**Primary Culture of Cardiomyocytes from Neonatal Mice**—Hearts were harvested from 3-day old neonatal mice, and after removal of the atria, the ventricles were subjected to trypsin (Invitrogen, catalog number 840-7250IL) digestion, in a final concentration of 100 μg/ml, for 16–18 h at 4 °C. Digestion with 150 units collagenase (Invitrogen, catalog number 17101015) was followed and the mixture of cells was plated on collagen-coated Petri dishes in a concentration of 100,000 cells/cm².

For LysoTracker-positive organelles visualization, cells were incubated with LysoTracker dye (Molecular Probes, Leiden, Netherlands) at a final concentration of 100 nM for 30 min. After extensive washes, cells were visualized with a Leica confocal laser scanning microscope (see under “Immunofluorescence Labeling of Cardiomyocytes from Neonatal Mice and Cryosections from Adult Heart and Skeletal Muscle”).

**Anti-myospryn Antibody Production**—A rabbit polyclonal antibody was raised against a synthetic 15-aa peptide (EGVHPAFALEKPGKC) near the COOH terminus of myospryn, coupled to preactivated keyhole limpet hemocyanin (antiserum was generated by Sigma Genosys). Its specificity was confirmed using the 15-peptide as competitor in Western blots and in immunocytochemistry studies (see supplemental data; Fig. 1).

**Immunofluorescence Labeling of Cardiomyocytes from Neonatal Mice and Cryosections from Adult Heart and Skeletal Muscle**—Cells were fixed in cold acetone (100%) for 3 min in 20 °C and then were permeabilized with 0.02% (v/v) saponin and were blocked with 5% bovine serum albumin in PBS for 1 h at room temperature. For cryosections, methanol:ethanol (70:30%) was used for 20 min at −20 °C, and the fixed sections were subjected in two 20-min washes, one with 0.1% (v/v) saponin and the second with 0.5% (v/v) Triton X-100. Primary antibodies including anti-myospryn (1:400, rabbit polyclonal), anti-desmin (1:20, mouse monoclonal, D3, Hybridoma Bank), anti-α-actinin (1:1000 mouse monoclonal; Sigma, clone EA-53 catalog number A7811), anti-cathepsin D (1:50 goat polyclonal; Santa Cruz Biotechnology, sc-6487), anti-KDEL (1:100, mouse monoclonal; StressGen Bioreagents, catalog number SPA-827), TGN38 (1:100, mouse monoclonal; BD Biosciences, catalog number 610898), and anti-PKA RII subunit (1:50, goat polyclonal; Upstate, catalog number 09-411) were incubated with the cells for 3 h at room temperature.
Samples were stained with Alexa Fluor 488, 594, or 568 (Molecular Probes, Leiden, Netherlands) for room temperature. Cells as well as sections were extensively washed with PBS/Tween 20 (0.01%), mounted with fluorescent mounting medium from DAKO (Carpinteria, CA), and analyzed with a Leica confocal laser scanning microscope (TCS SP5, DMI6000, inverted, with the acquisition software LAS-AF, at 23–24 °C) equipped with /H11003 63 NA 1.4 objective.

RESULTS

The Amino-terminal Domain of Desmin Binds to the SPRY Domain of Myospryn—In an effort to explore the role of the terminal domains of the desmin molecule and to identify potential binding partners of desmin in heart, we used the amino-terminal domain, aa 1–103, as a “bait” to screen a cDNA library from human cardiac muscle. Approximately 1/106 transformants were screened, of which 170 “prey” clones met the stringent criteria for positive interactions. From the sequencing analysis of these clones we identified myospryn (GenBank accession number NM_153610; fragment 12,078–12,884 bp) as a potential binding partner of desmin.

Confirmation of the desmin-myospryn interaction was achieved by both reciprocal GST pulldown assays and co-IP experiments. Recombinant GST-myospryn and GST-desmin were prepared and used for binding studies to endogenous desmin and myospryn, respectively. Gel filtration chromatography was applied to enrich for desmin the cardiac extracts (data not shown), used in the pulldown assay with GST-myospryn. When the desmin-enriched cellular extract was incubated with GST-myospryn bound to a glutathione matrix, desmin was efficiently and specifically absorbed, as detected by Western blot analysis using anti-desmin antibodies (Fig. 1I, A2, 2nd lane). The inability of GST alone to absorb desmin confirmed this interaction. In the reverse experiment, we produced recombinant GST-desmin, which was incubated with cardiac homogenates from wild type mice. After binding to glutathione matrices, recombinant GST-desmin absorbed myospryn from cardiac muscle preparation, as shown by SDS-PAGE and immunoblotting with anti-myospryn antibodies (Fig. 1I, B2, 2nd lane). In this experiment, a very small portion of myospryn was absorbed nonspecifically by GST.

We also performed in vitro co-immunoprecipitation experiments with differently tagged proteins, HA-myospryn and c-Myc-desmin. Proteins were expressed and 35S-labeled with the TNT QuickCoupled transcription/translation system. After incubation with protein A/G-Sepharose, each 35S-labeled translated protein was immunoprecipitated with either anti-c-Myc or anti-HA antibody and then analyzed by SDS-PAGE (Fig. 1II). Myospryn can be coimmunoprecipitated using anti-c-Myc, the desmin tag antibody. Anti-c-Myc can specifically precipitate desmin, whereas desmin alone cannot be precipitated using anti-HA, the antibody of myospryn tag. These results demonstrated that desmin interacts directly with myospryn.

To determine the precise corresponding region of desmin and myospryn molecules, necessary and sufficient for their interaction, we extended the yeast two-hybrid analysis by generating deletions constructs. For desmin, bait constructs encoding aa 1–61, 58–103, 1–33, 107–470, 408–470, and the entire molecule were coexpressed in yeast with the prey vector encoding the myospryn region corresponding to aa 4003–4069 (Fig. 2A). Only when the desmin region aa 58–103 was included in the used bait constructs, colony growth and -galactosidase gene activation occurred. In addition, we showed that this region by itself was sufficient for myospryn binding. The data demonstrate that the binding to the 4003–4069-aa region of myospryn occurs only with the amino-terminal domain of the
Desmin molecule, because the bait construct encoding the entire desmin molecule lacking the amino-terminal domain did not allow the growth of yeast. Furthermore, it was shown that the 58–103-aa region of desmin is both necessary and sufficient for binding to the myospryn carboxyl terminus (Fig. 2A).

Similarly, we expressed two fragments of the originally isolated myospryn clone in the prey vector and assayed their ability to interact with the amino terminus of desmin (Fig. 2B). From the two fragments, aa 4003–4054 and 4045–4069, only the latter gave a positive response, suggesting that the desmin amino terminus binds to aa 4045–4069 of the myospryn molecule, which corresponds to the last 24 aa of its carboxyl terminus. This region includes a part of the SPRY (SPIa/ryanodine recep-

dominantly at intercalated disks where it colocalizes with desmin, as the yellow color of the merged image reveals (Fig. 4, A1 and A2). Myospryn is also localized at the Z-line connection level of the sarcolemma (Fig. 4B1), as described previously for skeletal muscle (19, 20). However, myospryn is not easily detected at the internal sarcomeric Z-lines, as the double labeling with anti-myospryn and anti-α-actinin reveals (Fig. 4B2). The colocalization of desmin with myospryn at the costamere Z-line level was also confirmed in skeletal muscle (Fig. 5). In longitudinal cryosections of adult mouse skeletal muscle, nuclei were easily detected, and thus it was possible to confirm the intense colocalization of myospryn with desmin at the periphery of the nucleus as well (Fig. 5).

Myospryn Colocalizes with Desmin in Vivo—To determine the subcellular colocalization of myospryn with desmin, we generated an antibody against a 15-aa long peptide corresponding to the carboxyl terminus of myospryn. The peptide chosen for this purpose was unique to myospryn, and the specificity of the generated antibodies was confirmed by competition with the peptide in Western blot and immunocytochemistry studies (see supplemental Fig. 1). We initially examined the colocalization of these two proteins in primary cultures of mouse neonatal cardiomyocytes using confocal microscopy. As shown in Fig. 3A, myospryn is found predominantly at the periphery of the nucleus. As expected, in addition to the perinuclear localization, desmin also forms a continuous network throughout the cell (Fig. 3B). As shown in the merged image (Fig. 3C), the two proteins colocalize at the periphery of the nucleus. We also performed immunocytochemistry on longitudinal cryosections of adult mouse hearts where desmin exhibits the known Z-line pattern, in addition to the strong localization at intercalated disks. Myospryn is found predominantly at intercalated disks (Fig. 2B), corresponding to the carboxyl terminus of the TRIM-like motif as shown in Fig. 2B. These data demonstrate that the 24 carboxyl-terminal amino acids of the SPRY domain of myospryn are necessary and sufficient for its association with desmin, but it does not exclude the possibility that another region of myospryn might bind to desmin, as well.

FIGURE 4. Colocalization of myospryn and desmin in cardiac muscle sections. Immunofluorescence staining and confocal microscopy for myospryn and desmin in cardiac muscle cryosections reveal that myospryn colocalizes with desmin predominantly at the intercalated disks (A1 and A2) as well as at the costameres of the sarcolemma (B1), and it is not easily detectable at the internal sarcomeric Zlines (B2). No labeling was observed when nonimmune (myospryn serum) was used (A3). A1, A2, B1, B2, confocal image projections of 16 z-stacks through the cardiac tissue section of 0.2 μm intervals.

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Desmin Is Necessary for the Proper Localization of Myospryn around the Nucleus—To address the role that desmin plays in myospryn behavior, we used the desmin knock-out mouse model. We examined the localization of myospryn in cardiomyocytes from neonatal wild type and desmin null mice by immunofluorescence staining for myospryn. As shown in Fig. 6, in the absence of desmin (B), myospryn loses its sharp localization pattern around the nucleus and seems to diffuse throughout the cytoplasm, in contrast to wild type cardiomyocytes (A). Similar studies in adult heart cryosections revealed no significant change in the localization of myospryn at intercalated disks between wild type (C) and desmin null (D) myocardium. Note that the different intercalated disk shapes reflect the previously observed changes in its architecture in desmin null hearts.

**FIGURE 5.** Colocalization of myospryn and desmin in skeletal muscle sections. As in Fig. 4, confocal microscopy reveals that in adult skeletal muscle myospryn colocalizes with desmin at the costamere Z-line level and at the periphery of the nucleus (enlargement).

**FIGURE 6.** Loss of myospryn perinuclear localization in desmin-null cardiomyocytes. Immunofluorescence labeling for myospryn in wild type (+/+) (A) and desmin null (−/−) (B) neonatal cardiomyocytes reveals that, in the absence of desmin, myospryn loses its distinct presence at the periphery of the nucleus and is more abundant in the cytoplasm in a diffused pattern. In adult mouse heart cryosections (C and D), there is no significant quantitative change in the localization of myospryn at intercalated disks (IDs) between wild type (C) and desmin null (D) myocardium. Note that the different intercalated disk shapes reflect the previously observed changes in its architecture in desmin null hearts.
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Desmin Is Essential for Proper Lysosome Behavior—As described above, immunofluorescence labeling with anti-cathepsin antibodies revealed extensive mislocalization of cathepsin D-positive organelles in desmin null cardiomyocytes (Fig. 8, right panels −/−, and Fig. 9D). To further confirm the effect of desmin deficiency and myospryn redistribution on lysosomal positioning, we used the lysosomal marker LysoTracker. We showed that in the absence of desmin, the LysoTracker-positive organelles seem bigger, and instead of being distributed throughout the cardiomyocyte, as in wild type (see Fig. 9A), they aggregate and are localized to the juxtanuclear region (Fig. 9B). These data suggest that desmin, and possibly myospryn, play an important role in the proper localization and/or biogenesis of lysosomes.

FIGURE 7. Myospryn resides in close proximity to the endoplasmic reticulum. Double immunofluorescence for myospryn and markers of the cell sorting machinery (KDEL for endoplasmic reticulum and TGN38 for trans-Golgi), reveals that it colocalizes mainly with the KDEL receptor (A), whereas there is a little colocalization with TGN38 (B).

Myospryn Colocalizes with Lysosomal Markers—Myospryn has been found to interact with dysbindin, a component of the BLOC-1 complex. However, its link to lysosomes has not yet been established. In an attempt to determine a potential connection of myospryn with the endo/lysosomal machinery, we performed double immunofluorescence labeling of mouse neonatal cardiomyocytes using anti-myospryn and an antibody against cathepsin D, a known lysosomal marker. As shown in Fig. 8, myospryn colocalizes extensively with cathepsin D in lysosomes of mouse neonatal cardiomyocytes. To determine the importance of desmin in the association of myospryn with lysosomes, a similar study was performed with cardiomyocytes from desmin null mice. As shown in Fig. 8 (−/−), in the absence of desmin the association of myospryn with the lysosomes was partially-retained. However, extensive mislocalization of the lysosomes was found in the absence of desmin (Fig. 8).

Myospryn Colocalizes with the Endoplasmic Reticulum—We sought to determine more precisely the perinuclear compartment at which myospryn localizes in primary cardiomyocytes from wild type mice. We used markers for the main parts of the cell sorting machinery and, specifically, KDEL receptor for endoplasmic reticulum and TGN38 for trans-Golgi. Indirect immunofluorescence labeling for myospryn and either KDEL receptor or TGN38 revealed that myospryn resides closer to the endoplasmic reticulum, as revealed by its colocalization with KDEL (Fig. 7). We have also used an antibody against the RII subunit of PKA, which resides in the endoplasmic/sarcoplasmic reticulum, and we have shown that the two proteins colocalize (see supplemental data Fig. 3). This finding is in agreement with the work of Reynolds et al. (21), where it was shown that myospryn is an AKAP protein and that it colocalizes with the RII subunit of PKA.

Myospryn Colocalizes with Lysosomal Markers—Myospryn has been found to interact with dysbindin, a component of the BLOC-1 complex. However, its link to lysosomes has not yet been established. In an attempt to determine a potential connection of myospryn with the endo/lysosomal machinery, we performed double immunofluorescence labeling of mouse neonatal cardiomyocytes using anti-myospryn and an antibody against cathepsin D, a known lysosomal marker. As shown in Fig. 8, myospryn colocalizes extensively with cathepsin D in lysosomes of mouse neonatal cardiomyocytes. To determine the importance of desmin in the association of myospryn with lysosomes, a similar study was performed with cardiomyocytes from desmin null mice. As shown in Fig. 8 (−/−), in the absence of desmin the association of myospryn with the lysosomes was partially-retained. However, extensive mislocalization of the lysosomes was found in the absence of desmin (Fig. 8).

DISCUSSION

The biological significance of the muscle-specific IF protein desmin has been linked so far to proper plasma membrane and membranous organelle behavior and function (for review see Refs. 13, 14, and 22). Desmin is important in fusion events (6, 7, 23), in proper costamere protein organization (22), nuclear morphology (24) and localization (25), as well as in proper mitochondrial positioning, morphology, structure, and function (13, 14).

Although the association of desmin with mitochondria behavior and function has been extensively demonstrated, a potential role of desmin in other membranous organelle behavior and function has not been explored. Here, we describe the association of desmin with myospryn, a TRIM-like protein, initially identified as an associated partner to the BLOC-1 protein dysbindin (19). Dysbindin has been shown recently to be involved in protein trafficking and organelle biogenesis, as it is mutated in patients with the bleeding and pigmentation disorder Hermansky-Pudlak syndrome type 7 and in sandy (sdy/sdy)
mouse, a murine model of the disease (26). These disorders are characterized by defects in the biogenesis of lysosome-related organelles, including melanocytes and platelet-dense granules (27, 28). Dysbindin comprises a subunit of a soluble 200-kDa protein complex, BLOC-1, and binds directly to two other subunits of this complex, pallidin and muted. The above proteins are encoded by genes mutated in two additional models of Hermansky-Pudlak syndrome (26, 29).

It has been proposed that myospryn might be a part of a BLOC-1-like complex in muscle (19), and that it might also serve as a cell type-specific effector for the ubiquitously expressed Hermansky-Pudlak syndrome protein dysbindin (30). Data presented in Fig. 10 support this proposal and further demonstrate that there is a physical link between desmin and BLOC-1, because desmin can immunoprecipitate the BLOC-1 subunits, dysbindin and pallidin, most possibly through myospryn. Myospryn may serve as a link between desmin and lysosomes. Indeed, we demonstrated that myospryn colocalizes with lysosomes and desmin is required for both the proper perinuclear localization of myospryn and the proper positioning of lysosomes, thus suggesting for the first time a potential role of desmin IFs in lysosomes and lysosome-related organelle biogenesis and/or positioning. A direct role of IFs with lysosome positioning and/or membrane trafficking has been reported recently with AP-3 and vimentin (17). In addition, several older reports had previously implied an interaction of IFs with membrane-bound organelles. A direct interaction of vimentin with the Golgi complex, through a Golgi-specific protein, has been suggested (31, 32). Moreover, formation of the autophagosome intermediate organelle, which converges with lysosomes during autophagic process, requires intact cytokeratin and vimentin IFs (33, 34). Finally, maturation of glycosphingolipids has been found to be impaired in vimentin-deficient fibroblasts, most possibly because of a defect in a step that requires endosome-Golgi recycling (35, 36). Thus, it has been suggested that intra-organelle lipid sorting might be linked to IFs.

The observed redistribution of lysosomes in cardiomyocytes from desmin null myocardium suggests that there is a failure to control the endo/lysosomal compartments in the cytosol of desmin null cardiomyocytes. This may be, at least in part, due to the redistribution of myospryn from the periphery of the nucleus to the diffused form throughout the cytoplasm and could suggest that the proper BLOC-1 formation might require proper myospryn-desmin association. This hypothesis remains to be proven. The recently reported interaction of vimentin with AP-3 (37) is believed to regulate the subcellular localization of AP-3 and AP-3-dependent traffic in endocytic organelles, as
well as lysosome positioning. It should be emphasized that the abnormal juxtanuclear localization of lysosomes, observed in desmin-deficient cardiomyocytes, is very similar to that observed in the absence of vimentin in fibroblasts (37). The observed mislocalization might just reflect a defect of vesicle formation, either because of compromised vesicle budding, fusion, or recruitment of adaptor proteins to sites of vesicle formation. On the other hand, IFs could serve as an anchor for lysosomes or as a bridge between vesicle and motor proteins that could move them. Considering the most recent report where it was shown that BLOC-1 is present in AP-3-derived vesicles and that both can regulate the targeting of AP-3 cargo proteins (38), we consider that myospryn together with desmin might regulate the BLOC-1/AP-3 coexistence in the same vesicle and consequently proper cellular trafficking.

Myospryn has been characterized as a TRIM-like protein (19). Members of this protein family are known to identify novel specific cell compartments (39). The role of myospryn in muscle cells and how this correlates with its presently identified compartmentalization around the nucleus and at the costameres and intercalated disks requires further investigation. Indirect immunofluorescence for myospryn and markers for compartments of sorting machinery (KDEL receptor and TGN38; Fig. 7) revealed that myospryn resides mainly close to membrane structures of the endoplasmic reticulum and potentially the endoplasmic reticulum-Golgi intermediate compartment. This observation further supports the potential involvement of myospryn in the process of protein transport through endoplasmic reticulum and Golgi compartment, as well as in the process of membrane trafficking in the secretory pathway. The observed localization of BLOC-1 complex to the endosomes, which facilitates through its interaction with AP-3 (40) the protein sorting from endosomes to lysosomes, further supports the potential role of myospryn in the above processes as a tissue-specific component in the sorting machinery.

While this manuscript was in preparation, it was reported that myospryn is an anchoring protein for PKA, and it was demonstrated that it colocalizes with the regulatory subunit RII\(\alpha\) (41). The latter is localized to the endoplasmic reticulum and the neighboring perinuclear compartment, where it forms a complex with the ryanodine receptor. Indeed, we have confirmed these data both with RII\(\alpha\) antibodies (supplemental Fig. 3), as well as with KDEL receptor antibodies (Fig. 7) and shown that myospryn resides close to the membranes of the endoplasmic reticulum in isolated cardiomyocytes. PKA has already been identified as an enzyme phosphorylating type III intermediate filaments and more precisely desmin (42, 43). Because myospryn might be also phosphorylated by PKA, as in vitro studies suggest (41), it is plausible to speculate that the association of desmin with myospryn may be involved in a feedback mechanism for the local regulation of PKA action. Interestingly, the amino-terminal domain of desmin that interacts with...
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the carboxyl terminus of myospryn, which in turns interacts with RIIα subunit, is important not only for the intermediate filament assembly but also for cardiogenesis (44). As we have demonstrated, desmin is necessary for the proper perinuclear localization of myospryn at the membranes of endoplasmic reticulum where, at least in skeletal muscle, myospryn interacts with the subunit RIIα of PKA. Thus, it can be suggested that desmin may regulate the proper targeting of myospryn with the RIIα subunit at the membranes of the endoplasmic/sarcoplasmic reticulum so these molecules can exert their function. A more direct link of IFs to AKAPs was recently established with synemin (45). It is important to note that AKAP proteins have been found to serve also as guanidine exchange factors in protein trafficking (46, 47). Myospryn might indeed be an AKAP protein that could be involved in such processes.

Although desmin is required for the appropriate localization of myospryn around the nucleus, it does not seem to be absolutely necessary for its localization at intercalated disks and costameres, although these structures seem to be altered in the desmin null myocardium (48, 49). These data suggest that the association of myospryn with these compartments is facilitated by other proteins. For costameres, potential candidates include dysbindin and α-actinin, which have been found to associate with myospryn (20). For intercalated disks there is no known candidate yet.

The yeast two-hybrid experiments revealed that the interaction of myospryn with desmin takes place through amino acids 58–103 of the desmin amino-terminal domain. This region shows no similarity with other members of the IF protein family (glial fibrillary acidic protein, vimentin, and synemin), suggesting that the tissue specificity of the interaction is consistent with the functional diversity of the IF terminal domains (head and tail). As far as the myospryn molecule is concerned, the deletion analysis with the yeast two-hybrid assay reveals that the interaction involves amino acids 4045–4069. This region is part of the SPRY domain of the myospryn TRIM motif. In addition to TRIM proteins, the SPRY domain can be identified in at least 10 other protein families (for a review see Ref. 50). These proteins cover a wide range of functions, including regulation of cytokine signaling (suppressor of cytokine signaling, SOCS), RNA metabolism (DDX and heterogeneous nuclear ribonucleoprotein), intracellular calcium release (ryanodine receptor receptors), immunity to retroviruses (TRIM5α), and developmental processes (HERC1 and Ash2L). Several of the above processes are of utmost importance for the functional and structural integrity of muscle, and it is tempting to speculate that the interaction between desmin and myospryn might facilitate some of them.

In summary, this work presents evidence of a novel association of desmin with the TRIM protein myospryn. This is the first report showing an interaction between IFs and a TRIM protein. We described the interaction, and we validated it with in vitro and in vivo assays. Moreover, we showed that desmin is essential for proper perinuclear localization of myospryn. This might be linked to a dual role of myospryn both as AKAP protein as well as a BLOC-1 interacting protein, thus shedding light on new potential regulatory roles for desmin intermediate filaments in muscle.

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