Synemin May Function to Directly Link Muscle Cell Intermediate Filaments to Both Myofibrillar Z-lines and Costameres

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Synemin is a large intermediate filament (IF) protein that has been identified in all types of muscle cells in association with desmin- and/or vimentin-containing IFs. Our previous studies (Bellin, R. M., Sernett, S. W., Becker, B., Ip, W., Huiatt, T. W., and Robson, R. M. (1999) J. Biol. Chem. 274, 29493–29499) demonstrated that synemin forms heteropolymeric IFs with major IF proteins and contains a binding site for the myofibrillar Z-line protein α-actinin. By utilizing blot overlay assays, we show herein that synemin also interacts with the costameric protein vinculin. Furthermore, extensive assays utilizing the Gal4 yeast two-hybrid system demonstrate interactions of synemin with desmin and vimentin and additionally define more precisely the protein subdomains involved in the synemin/α-actinin and synemin/vinculin interactions. The C-terminal ~300-amino acid region of synemin binds to the N-terminal head and central rod domains of α-actinin and the ~150-amino acid C-terminal tail of vinculin. Overall, these interactions indicate that synemin may anchor IFs to myofilibrilar Z-lines via interactions with α-actinin and to costameres at the sarcolemma via interactions with vinculin and/or α-actinin. These linkages would enable the IFs to directly link all cellular myofibrils and to anchor the peripheral layer of myofibrils to the costameres.

Intermediate filaments (IFs), along with microfilaments and microtubules, comprise the three cytoskeletal filament classes found in animal cells (1–4). The IFs are composed of cell type-specific proteins that are assembled into very long ~10-nm-diameter filaments (4–9). The superfamily of IF proteins currently contains ~60 members (10), and novel members continue to be identified (10–12). Because of the diversity among members of the protein superfamily, it is possible that IFs within different cell types may be formed that have diverse functions (4). Additionally, because the majority of IFs found in living cells are heteropolymeric in nature (i.e. composed of more that one IF protein member), even more diverse functionality of an individual IF is possible (4).

The IFs have long been considered to play an important role as mechanical integrators of cellular space (2, 13, 14). The actual mechanism by which this cytoskeletal integration occurs within cells, however, remains poorly understood. For IFs to connect cell components, some form or type of attachment of IFs to other cytoskeletal components is necessary. These attachments could be indirect, via a cross-linking protein such as plectin that binds to several cytoskeletal proteins (10, 15), or direct, by linking IF proteins themselves to protein components within other cellular structures (12).

Synemin was initially described as an IF-associated protein because it colocalized and copurified with the IF proteins desmin and vimentin (16–18). However, we recently demonstrated that synemin is, in fact, an IF protein because the sequence contains the ~310-amino acid rod domain characteristic of IF proteins (12, 19). Furthermore, we demonstrated that synemin (1,604 residues, 182,187 Da) is a novel member of the IF protein superfamily in that it contains an unusually large C-terminal tail domain (1,290 residues). Synemin has been localized with desmin- and/or vimentin-containing IFs in all types of developing chick (20) and adult avian and porcine muscle cells (21), chick lens cells (22, 23), human SW13 adrenal cortex adenocarcinoma cells (12), and, most recently, rat radial glial cells (24). Because to date synemin has always been found in the presence of at least one major IF protein, and because it fails to form IFs by itself in vitro (21) and when transfected into cells that lack other IF proteins (12), we believe it is very likely that synemin only functions within cells as a component of heteropolymeric IFs.

We have shown previously by blot overlay assays that synemin interacts with both desmin and α-actinin (12). We now show, by blot overlay assays, a direct interaction between synemin and the tail domain of vinculin. Furthermore, we demonstrate these interactions with α-actinin and vinculin and further define the specific binding sites for these interactions by using the Gal4 yeast two-hybrid system. These specific interactions further support a role for synemin, as a component of heteropolymeric IFs, in directly attaching these IFs to other cytoskeletal structures via direct protein/protein interactions involving synemin.

Our major interest is to define how the cytoskeletal elements are organized and attached to each other within muscle cells. We present a schematic showing how synemin in striated muscle cells may serve as a cytoskeletal cross-linking component by enabling synemin-containing heteropolymeric IFs to (a) directly bind to myofilibrillar Z-lines and thereby link all cellular myofibrils via synemin/α-actinin interactions and (b) help in anchoring the peripheral layer of cellular myofibrils to the...
two-hybrid interactions were done by using the FluorAce described by Meng and Ip (31). Quantitative determinations of all fluorescence-based detection method was developed similar to that recently mined optimal amount of 400 ng of plasmid DNA was used for each lane 4, V8 protease-digested vinculin) that were overlaid with buffer only (A), purified intact synemin (B), and bacteriologically expressed synemin tail domain (C) before washing and detection with synemin polyclonal antibody. D and E depict blots resulting from transfer of SDS-polyacrylamide gel electrophoresis gels (lane 1, whole gizzard homogenate; lane 2, purified synemin; lane 3, purified vinculin; lane 4, V8 protease-digested vinculin) that were overlaid with buffer only (D) and synemin rod domain (E) before washing and detection with synemin polyclonal antibody. H and T denote the approximate migration positions of the V8 protease-produced vinculin head and tail domains, respectively.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**—Synemin (12, 21) and vinculin (25) were purified from avian smooth muscle as described previously. Proteolytic fragments of vinculin were prepared by treatment with V8 protease (26). The precise cleavage site(s) in vinculin was determined by protein microsequencing from polyvinylidene difluoride membrane in the Iowa State University Protein Facility. The specific rod and tail domains of synemin were expressed in bacteria and purified (12).

**Blot Overlay Assays**—Blots of samples of purified synemin, desmin, vinculin, and proteolytically digested vinculin, plus a sample of avian smooth muscle homogenate, were probed, by using blot overlay procedures (12), with synemin, expressed synemin rod, and expressed synemin tail domains. Binding was detected by use of polyclonal antibody 2856, which has been characterized previously and shown to recognize intact synemin, and both the rod and tail domains of synemin (12).

**Preparation and Transformation of Yeast Two-hybrid Constructs**—Constructs used for desmin (27) and vimentin (28) have been described. Constructs encode the entire synemin molecule, as well as specific domains of synemin, α-actinin, and vinculin, were generated by polymerase chain reaction and cloned into both the pPC86 and pPC97 yeast vectors originally described by Chevray and Nathans (29). The pPC86 and pPC97 vector pair have been used in other two-hybrid studies on IF proteins (27, 28, 30). The PCY2 strain of yeast (29) was cotransformed with the Alkali-Cation Yeast Transformation kit (Bio101) by using the manufacturer’s protocol. An empirically determined optimal amount of 400 ng of plasmid DNA was used for each construct.

**Fluorescence-based Semiquantitative Two-hybrid Assays**—A fluorescence-based detection method was developed similar to that recently described by Meng and Ip (31). Quantitative determinations of all two-hybrid interactions were done by using the FluorAce β-galactosidase Reporter Assay Kit (Bio-Rad) modified for use with yeast cells as follows. Yeast cultures were started with 2 ml of double drop-out Leu-Trp media and incubated in a rotary incubator at 180 rpm, 30 °C for 72 h, followed by 3 h of protein induction in galactose-containing Leu-Trp media at 180 rpm, 30 °C. Yeast lysates were prepared by freezing the yeast pellet in liquid nitrogen for 5 min and resuspending it in 100 µl of Lysate Buffer (1 mM MgCl2, 0.1% NaN3, and 10 mM sodium phosphate, pH 7.0). An empirically determined amount of yeast lysate (20 µl) was mixed with 50 µl of the kit’s 1× Reaction Buffer, which included β-methylumbelliferyl β-D-galactoside and 1% mercaptoethanol, directly in the wells of a black microtiter plate kept on ice. The plate was incubated for 3 h at 37 °C, followed by the addition of 150 µl of the kit’s 1× Stop Buffer. The plate was then read with a TiterTek Fluoroskan fluorescent microplate reader with excitation at 355 nm and emission at 460 nm. The specificity of interactions was confirmed by performing vector-swap experiments for each protein pair. Both the original vector pair and the vector-swap pair were independently tested twice, with the numbers for each determination resulting from reaction wells set up in triplicate and averaged. The quantitative data reported in the figures were calculated by using an Amax measurement of each individual culture to normalize the respective fluorescence reading. The resulting data were then used to calculate relative interaction affinities by comparing each reading with the value determined for the synemin rod/desmin interaction that was included with each set of assays.

**RESULTS**

**Blot Overlay Assays**—Analyses utilizing purified synemin and vinculin, as well as bacteriologically expressed rod and tail domains of synemin, and the proteolytic products of purified vinculin resulting from V8 protease treatment were conducted to identify interactions of specific protein domains. Vinculin was selected as a potential interaction partner for synemin because it is a key cytoskeletal protein potentially involved in signal transduction pathways (32, 33) and because it is a component of the costameres (34), one of the attachment sites for striated muscle cell IFs (35). V8 protease digestion of vinculin resulted in the production of variable amounts of two low molecular mass bands (~24 kDa and ~22 kDa in Fig. 1). Protein microsequencing of these fragments revealed newly generated N termini having the sequences LAPPKPPLPE and GEVPPPRPPP. These sequences agree precisely with residue numbers 858–867 and 868–877, respectively, in the avian vinculin sequence (GenBank™ accession number Y00312), therefore showing that they both are tail fragments of vinculin. As shown in the Control buffer overlay blots lacking any probe protein in the overlay solution (Fig. 1, A and D), the synemin polyclonal antibody 2856 labeled synemin present in the gizzard homogenate (Fig. 1, A and D, lanes 1), purified synemin (Fig. 1A, lane 2), and expressed synemin rod domain (Fig. 1D, lane 2) but did not label purified vinculin (Fig. 1A, A and D, lanes 3), the small amount of intact purified vinculin remaining after V8 protease treatment (Fig. 1A, A and D, lanes 4), or any other proteins in the gizzard homogenate (Fig. 1, A and D, lanes 1). As is shown in Fig. 1B, probing blots of vinculin and V8-digested vinculin with purified intact synemin in the overlay revealed an interaction of synemin with purified intact vinculin (lane 3), the very small amount of intact vinculin remaining in the V8 protease digest of vinculin (lane 4), and the small tail domain fragment(s) of vinculin produced by the V8 protease digestion (lane 4), but not with the large head domain fragment of vinculin that migrated with an approximate size corresponding to 90 kDa. Additionally, an interaction between the probe...
Synemin May Help Anchor Myofibrils to the Sarcolemma

SN Rod
SN Tail
SN Tail I
SN Tail II
SN Tail III
SN Tail IV
SN Tail Iab
SN Tail Ibb
Desmin
Vimentin
α-Actinin
α-Act Head
α-Act Rod
α-Act Tail
Vinc Head I
Vinc Head II
Vinc Tail

Fig. 2. Constructs used in two-hybrid studies. SN, avian synemin (GenBank™ accession number U28143); Desmin, murine desmin (GenBank™ accession number L22550); Vimentin, murine vimentin (GenBank™ accession number M24849); α-Actinin-α-Act, avian α-actinin (GenBank™ accession number J03486); Vinc, avian vinculin (GenBank™ accession number Y00312). Numbers at the right of each construct refer to the amino acid residues in the construct, with the N terminus of the intact protein corresponding to residue number 1.

Results from two-hybrid assays of the interaction between synemin and desmin (Fig. 4) were very similar to those observed between synemin and desmin (Fig. 4), as may be expected because of the high sequence homology between the type III IF proteins vimentin and desmin (37), which are the major IF proteins of developing and mature striated muscle cells, respectively (12, 16, 20). The rod domain of synemin reacts most strongly with vimentin (Fig. 5, column 1), when compared with the vector control (Fig. 5, column 9). The results shown in Fig. 5 (columns 2-8) demonstrate that none of the tail domain constructs of synemin exhibit interactions with desmin in these assays.

The results of the two-hybrid assays of the interactions between synemin and vimentin (Fig. 5) were also evident in the gizzard homogenate lane (Fig. 1B, lane 1) was seen, as we reported previously (12). Probing blots (Fig. 1) with expressed synemin tail (Fig. 1C) and rod domains (Fig. 1E) resulted in binding patterns similar to that seen when using intact synemin, although only weak binding to the vinculin tail domain is seen (Fig. 1, C and E, lanes 4). It is evident that synemin binds specifically to the vinculin tail domain because neither synemin (Fig. 1B) nor synemin domains (Fig. 1, C and E) bound to any of the other proteins (e.g. filamin, myosin heavy chains, and actin) present in the gizzard homogenate (Fig. 1B, C, and E, lanes 1). The lack of binding to α-actinin in the gizzard homogenate (Fig. 1, B, C, and E, lanes 1) was also evident in the original blot overlay experiments depicting the synemin/α-actinin interaction (12). In those studies, it appeared that the relatively large amount of desmin in the gizzard homogenate, in comparison to the amount of α-actinin, seemed to “bind up” the available synemin overlay protein, thereby obscuring the identification of the interaction between synemin and α-actinin. The synemin/α-actinin interaction was evident, however, with heavier loads of α-actinin (12).

Two-hybrid Analysis—Identification and analysis of interactions by the Gal4 two-hybrid system included sufficient controls to demonstrate that seemingly positive interactions are not due to false positives of the assay system. This is especially of concern when testing helical proteins, such as an IF protein rod domain, because a charged helix attached to the DNA-binding portion of Gal4 (as is present in the pPC97 vector) has been shown, in some cases, to activate β-galactosidase reporter gene expression (36). The constructs used herein are shown in Fig. 2. As demonstrated in Fig. 3, when each of the constructs encoding a domain of synemin (see Fig. 2) is cotransfected with a negative control consisting of an empty two-hybrid vector (e.g. synemin rod insert in pPC86, but no insert in pPC97), the resulting values from the fluorescence assays were quite low, which indicates that none of the synemin constructs significantly activates transcription by itself.

Results from two-hybrid assays testing synemin/desmin interactions are shown in Fig. 4. The rod domain of synemin interacts strongly with desmin (Fig. 4, column 1), as compared with the vector control (Fig. 4, column 9). The strong interaction between synemin and desmin was expected because other studies have shown that the rod domains of IFs are the primary interaction domains between IF protein pairs (reviewed in Ref. 4). Results shown in Fig. 4 (columns 2-8) demonstrate that none of the tail domain constructs of synemin exhibit interactions with desmin in these assays.

The results of two-hybrid analysis of interactions between synemin and α-actinin are shown in Fig. 6. Analysis of these interactions must take into account that the full-length α-actinin pPC97 construct activated transcription by itself to a moderate degree (Fig. 6, column 9). When compared with the vector controls (Fig. 6, column 9), the interaction of synemin with α-actinin is specific for the tail domain of synemin (Fig. 6, column 2). Analysis of the constructs of the synemin tail domain split into four nonoverlapping parts indicates that SN tail Ibb has the strongest interaction (Fig. 6, column 8), thus mapping the interaction domain to the last quarter of the C-terminal tail of synemin. Additionally, testing this tail domain construct with domains of α-actinin demonstrates interaction of the SN tail Ibb with both the N-terminal head domain of α-actinin (Fig. 6, column 10), which contains the actin-binding site, and the rod domain of α-actinin (Fig. 6, column 12), which contains four spectrin-like repeats (38, 39). Each of these interactions is much stronger than the respective vector control results (cf. columns 10 and 11 and columns 12 and 13 of Fig. 6).

Two-hybrid assays of the remaining synemin constructs with the three α-actinin constructs revealed no other strong interactions.2

The results of the two-hybrid assays of the interaction between synemin and vinculin are shown in Fig. 7. Because the head and tail domains within a single vinculin molecule are known to interact in a fashion that blocks some protein interaction sites in solution (26, 40), interactions with vinculin were tested on subdomains of vinculin, rather than on the whole molecule. In agreement with the blot overlay assays shown earlier (Fig. 1), the synemin rod (Fig. 7, column 7) and synemin tail domains (Fig. 7, columns 8, 10, and 12) interact strongly with the vinculin tail domain in comparison with the vector control (Fig. 7, column 13). Within subdomains of the synemin tail domain, the strongest interaction with the vinculin tail was found with the SN tail Ibb construct (Fig. 7, column 12). Thus, the end of the tail domain and the rod domain (Fig. 7, column 7) of synemin show affinity for the vinculin tail domain. Two-hybrid assays of the remaining tail domain constructs of synemin with the vinculin constructs revealed no other strong interactions.2

DISCUSSION

Identification of the interaction between synemin and vinculin is an important advance in understanding linkages within the cytoskeleton. Although it has long been recognized that IFs attach or insert into and are thus somehow linked to protein-rich cytoskeletal/membrane attachment sites, the only well

2 R. M. Bellin and R. M. Robson, unpublished observations.
established mechanism for cytoskeletal attachment of IFs involves members of the plakin family (30, 41–43). Vinculin is a component of both cell-cell and cell-matrix type adhesion plaques (44). The cell-matrix adhesion plaques include the membrane-associated dense bodies in smooth muscle, the neuromuscular and myotendenious junctions in skeletal muscle, and the costameres of striated muscle cells (34, 45). The costameres, which are located proximal to the sarcolema at sites adjacent to the myofibrillar Z-lines of the peripheral layer of myofibrils are, in fact, often defined by the presence of vinculin (46–48). Thus, the interaction between synemin and vinculin may serve as a mechanism for IF attachment to the costameric regions. Vinculin, in turn, can be anchored to the cell membrane in an interaction with the integrin-binding proteins talin and α-actinin (44, 49–51), which are also present in costameres (52, 53). As a result, the synemin/vinculin/talin and/or synemin/vinculin/α-actinin interaction(s) may also serve to link IFs to the cell membrane.

The results of the Gal4 yeast two-hybrid assays provide direct confirmation of the interactions involving synemin that were originally identified by blot overlay methods (Ref. 12 and the studies herein). Although blot overlay methods have the advantage of utilizing proteins prepared from their normal tissue source (i.e. synemin, desmin, α-actinin, and vinculin purified from muscle tissue), the Gal4 yeast two-hybrid assay has the advantage of testing protein interactions within the context of a living cell. Thus, the combination of approaches and results reported herein and previously (12) provide complementary evidence, obtained by at least two different methods, of the interactions of synemin with desmin, α-actinin, and vinculin.

Although the usual and most basic application of the yeast two-hybrid system is to determine whether two proteins have affinity for each other (54), use of the yeast two-hybrid system in conjunction with a quantitative detection method, as was used herein, also provides a relative measurement of the strength of the protein-protein interaction (31). The interactions between IF proteins were the strongest of those we tested. To provide a useful "ruler" of relative protein interaction strength, all values reported in this article are relative to the value of the interaction measured between the synemin rod domain and desmin (column 9) as described under "Experimental Procedures."
Synemin rod. Although it might be expected that all IF protein interactions would be of similar strength because of the relatively high sequence homology and conservation in overall length of the cytoplasmic IF rod domains, the strengths of the self-dimerization interactions of desmin and of vimentin were significantly greater than all of those that included the synemin rod domain. It is notable that, based on the strength of interactions measured, the interactions between synemin and actinin are unlikely to result from coiled-coil interactions (5, 7, 8). The major force driving this protein interaction is thought to be due to interactions of a hydrophobic stripe of apolar residues along each of the α-helical rod domains of two polypeptides (6, 57) as well as to charge-charge interactions that further stabilize the rod domain interactions (58, 59). By comparison, the interactions of synemin with actinin and with vinculin are unlikely to result from coiled-coil interactions because the tail domain of synemin lacks any strongly helical domains needed for a coiled-coil interaction (12).

Results of the two-hybrid studies define more precisely the protein domains involved in the interactions we reported recently between synemin and actinin (12). The actinin molecule is composed of an N-terminal head domain containing an actin-binding site, a central rod domain composed of four spectrin-link repeats, and a C-terminal tail domain containing two EF hand homology regions (38, 39, 60). Assays utilizing several constructs of domains of both the synemin tail and within both the head and rod either actinin or vinculin are considerably weaker. Considering the nature of these protein interactions, however, this finding is not unexpected. The IF proteins have been shown repeatedly to interact with each other by strong coiled-coil interactions between their ~310-amino acid-long rod domains (5, 7, 8). The major force driving this protein interaction is thought to be due to interactions of a hydrophobic stripe of apolar residues along each of the α-helical rod domains of two polypeptides (6, 57) as well as to charge-charge interactions that further stabilize the rod domain interactions (58, 59). By comparison, the interactions of synemin with actinin and with vinculin are unlikely to result from coiled-coil interactions because the tail domain of synemin lacks any strongly helical domains needed for a coiled-coil interaction (12).

Results of the two-hybrid studies define more precisely the protein domains involved in the interactions we reported recently between synemin and actinin (12). The actinin molecule is composed of an N-terminal head domain containing an actin-binding site, a central rod domain composed of four spectrin-link repeats, and a C-terminal tail domain containing two EF hand homology regions (38, 39, 60). Assays utilizing several constructs of domains of both the synemin tail and actinin revealed the smallest interacting domains between the two proteins to reside within the C-terminal ~300 amino acid residues of the synemin tail and within both the head and rod.
domains of α-actinin (Fig. 6). The binding site for α-actinin identified within synemin seems logical because the end of the synemin tail may be the domain extended farthest from the surface of the 10-nm core of the IF and would therefore be more likely to reach α-actinin molecules at the myofibrillar Z-line.

The blot overlay results demonstrate an interaction between synemin and vinculin. The two-hybrid assays provide further important information regarding the specific protein domains involved in the synemin/vinculin interaction. Use of the three separate constructs, two of which each code for approximately half of the large vinculin head domain and one that codes for the small C-terminal vinculin tail domain, reveals that only the tail domain of vinculin has affinity for synemin (Fig. 7). This small ~150-amino acid domain is a very interesting part of the vinculin molecule (61). The tail domain of vinculin contains two actin-binding sites, and their affinity for actin is regulated by binding of the tail domain to a site on the large head domain of vinculin, an interaction that, in turn, has been proposed by some investigators to be controlled by the binding of specific phospholipids (32, 33, 40, 62). Additionally, it was recently reported that the vinculin tail domain may act as a dimerization domain for vinculin, with actin filaments inducing the dimerization by stabilizing interactions between the tail domains of two vinculin molecules (63). Whether some of these dynamic activities of the vinculin tail also affect the binding to synemin is unknown. Whether vinculin head-tail association also regulates synemin binding was not directly addressed in the blot overlay assays shown (see Fig. 1) because full-length vinculin exists in an "open" conformation when blotted to a membrane (64). In preliminary in vitro studies, however, we have found that purified full-length vinculin does not bind when it is overlaid onto synemin (i.e. the vinculin would be in the closed conformation), in contrast to the positive interaction obtained when bacterially expressed vinculin tail is overlaid

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**Fig. 7.** Two-hybrid assays of interactions involving synemin and vinculin. All fluorescence values were calculated relative to the value (defined as 100) obtained for the interaction of synemin rod domain with desmin as described under "Experimental Procedures." Note that interactions are positive for both the rod (column 7) and tail domains (columns 8, 10, and 12) of synemin with the small vinculin tail domain. For details on graph format and labeling, see the Fig. 3 legend.

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**Fig. 8.** Summary of synemin protein interactions and a schematic showing a proposed role for synemin in striated muscle cells. A, the protein interactions involving the synemin molecule as determined by the yeast two-hybrid system are depicted. Numbers refer to the amino acid residues of the avian synemin transcript (see Fig. 2). B, the diagram shows the known location of heteropolymeric IFs at the Z-lines of striated muscle cells. Also depicted are attachment points of IFs to structures containing proteins that we have found to interact with synemin. Our hypothesis is that heteropolymeric IFs are firmly anchored to (a) the myofibrillar Z-lines by interactions between synemin and α-actinin, an integral Z-line protein (this interaction would enable heteropolymeric IFs to link all adjacent myofibrils together at their Z-lines), and (b) the costameres by interactions of synemin with vinculin and/or α-actinin (this interaction would enable heteropolymeric IFs to firmly anchor the peripheral layer of myofibrils to the costameres at the cell membrane). The schematic in B was adapted from Ref. 13.
onto synemin. It is intriguing that the tail domain of vinculin has affinity for both the rod domain and the C-terminal ~300 amino acids of the very long tail domain of synemin. Perhaps these two discrete vinculin-binding sites within synemin bind to separate vinculin molecules in the costamere, which would foster formation of a matrix/network between the IFs and vinculin and thereby result in strong anchoring of the IFs to these sites.

The binding sites for synemin on both α-actinin and vinculin exist within protein domains that also contain actin-binding sites. Whereas these findings may indicate that α-actinin and vinculin act as “tight borders” between synemin-containing heteropolymeric IFs and actin filaments, it is also possible that these IFs compete with actin filaments for binding to these proteins. The latter option raises the possibility that signal transduction pathways that affect actin filament dynamics could also alter binding to synemin-containing IFs. Conversely, it is possible that dynamic changes in the IF proteins, such as those induced by covalent modifications including phosphorylation (65) and ADP ribosylation (66), may also impact the actin cytoskeleton. Synemin itself has been found to be an excellent substrate for both phosphorylation (17) and ADP ribosylation (12) reactions.

Although the interactions identified for synemin with α-actinin and vinculin demonstrate a possible mechanism(s) of direct linkage between IFs and other cytoskeletal structures, some recent studies have implicated the protein plectin as an indirect mechanism for anchoring muscle cell IFs to other cytoskeletal structures (67–69). Plectin is a member of the plakin family of proteins that also includes the protein desmo-

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