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Properties of Synemin, a Protein Important in Maintaining the Structural Integrity of Muscle Cells

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Summary and Implications
Intermediate filaments (IFs), composed primarily of the protein desmin, link together the myofibrils in skeletal muscle. We have shown that the protein synemin is a novel IF protein and that it interacts with desmin. Together, these proteins are important in maintaining the structural integrity of muscle cells.

Introduction
Skeletal muscle cells are packed with myofibrils, which are responsible for contraction and contain the majority of the protein in muscle/meat. The myofibrils are aligned in register with, and are attached to, all of the neighboring myofibrils. IFs are the cellular structures that organize and link myofibrils together. Desmin is the major protein that makes up the IFs in muscle cells. We have previously shown that another protein, synemin, is located with the desmin IFs. Biochemical and molecular biology methods were used herein to examine the properties and possible function of synemin.

Materials and Methods
Desmin and synemin were purified from avian muscle by conventional protein biochemistry methods. Molecular cloning methods were used to obtain the cDNA and protein sequence of synemin. Synemin rod was prepared by expression in bacteria. Cosedimentation assays, a method for characterizing protein interactions, were used to examine interactions between synemin and desmin.

Results and Discussion

Figure 1. Schematic of the desmin and synemin molecules. Numbers refer to the amino acid residue position in the sequence.

A comparison of the overall structures of desmin, obtained by biochemical methods, and of synemin, obtained by molecular cloning, is shown in Figure 1. The major feature of desmin is the central, ~310 amino acid rod domain, which is responsible for assembly of desmin protein into IFs and is characteristic of all IF proteins. Synemin also contains this rod domain, and is, therefore, also an IF protein. However, synemin is a very large IF protein, compared to desmin, with most of the molecule comprised of a very long tail domain.

Figure 2. Cosedimentation of synemin rod domain with purified desmin.

Pellets (P) and supernatants (S) obtained by centrifuging mixtures of expressed synemin rod and purified desmin were analyzed by gel electrophoresis. Proteins were mixed in non-IF forming conditions, and the buffer conditions then adjusted to induce filament formation before centrifugation. Panel 1 is desmin alone; panel 2 is synemin rod alone; panel 3 is desmin and synemin rod mixed. In each case, soluble bovine serum albumin was added as a control to show that virtually no unbound protein was trapped within the pellet.

Cosedimentation assays were used to show that the rod domain of synemin interacts with desmin. As shown in Figure 2, desmin alone forms IFs that are sedimented in the pellet by centrifugation (Panel 1). Only about half of the synemin rod was sedimented, with the remainder left in the supernatant (Panel 2). In the presence of desmin, virtually all of the synemin rod was found in the pellet with the desmin (Panel 3), demonstrating a strong interaction between the desmin and synemin proteins.

As shown in this study, synemin and desmin are capable of forming heteropolymeric desmin/synemin IFs. Together, these two IF proteins appear responsible for linking together all of the myofibrils. Furthermore, studies in progress suggest that the long tail domain of synemin interacts with proteins in the Z-line region of myofibrils. These results indicate that synemin, therefore, plays a key role in linking the IFs to the myofibrils, and in maintaining the overall structural integrity of muscle cells.

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