A Role for Polyproline Motifs in the Spinal Muscular Atrophy Protein SMN

PROFILINS BIND TO AND COLocalize WITH SMN IN NUCLEAR GEMS*

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by the loss of α-motoneurons in the spinal cord followed by atrophy of skeletal muscles. SMA-determining candidate genes, SMN1 and SMN2, have been identified on human chromosome 5q. The corresponding SMN protein is expressed ubiquitously. It is coded by seven exons and contains conspicuous proline-rich motifs in its COOH-terminal third (exons 4, 5, and 6). Such motifs are known to bind to profilins (PFN), small proteins engaged in the control of actin dynamics. We tested whether profilins interact with SMN via its polyproline stretches. Using the yeast two-hybrid system we show that profilins bind to SMN and that this binding depends on its proline-rich motifs. These results were confirmed by communoprecipitation and by in vitro binding studies. Two PFN isoforms, I and II, are known, of which II is characteristic for central nervous system tissue. We show by in situ hybridization that both PFNs are highly expressed in mouse spinal cord and that PFN II is expressed predominantly in neurons. In motoneurons, the primary target of neurodegeneration in SMA, profilins are highly concentrated and colocalize with SMN in the cytoplasm of the cell body and in nuclear gems. Likewise, SMN and PFN I colocalize in gems of HeLa cells. Although SMN interacts with both profilin isoforms, binding of PFN II was stronger than with PFN I in all assays employed. Because the SMN genes are expressed ubiquitously, our findings suggest that the interaction of PFN II with SMN may be involved in neuron-specific effects of SMN mutations.

Spinal muscular atrophies (SMAs)¹ types I, II, and III are autosomal hereditary diseases of graded severity in which loss of motoneurons leads to paralysis and subsequent atrophy of skeletal muscles, and in the most severe Wernding-Hoffmann type I form, to death in early infancy. The corresponding SMA disease genes have been mapped to human chromosome 5q (1). There are two genes in close vicinity, the telomeric SMN1 (or SMN¹) and the centromeric SMN2 (or SMN²; 2). Although they have identical coding sequences for a 294-amino acid SMN polypeptide, pathogenic mutations were found solely in SMN1. Its gene product, the 40-kDa protein “survival motoneuron,” SMN, is expressed ubiquitously, and its concentration is reduced drastically in the spinal cord of SMA patients (3, 4). There is evidence, mostly from a yeast two-hybrid screen and from a Xenopus oocyte model system, that the SMN protein is engaged in the assembly of spliceosomal U snRNPs in the cytoplasm (5–7). Recently, the function of SMN has been demonstrated by a dominant-negative mutant of SMN which inhibits its pre-mRNA splicing by blocking the formation of a mature spliceosome (8).

The severity of SMA has been correlated with a deficient oligomerization of mutated SMN proteins, and it has been hypothesized that the critical level of functional SMN oligomers in normal motoneurons may be controlled by SMN’s binding to a motoneuron-specific factor (9). There are seven coding exons in the human SMN gene, and SMN splice variants lacking exons 5, 7, or both are found. SMN2 expresses predominantly a Δ7 truncated variant that is probably not capable of exerting all biological functions of SMN. This explains why the telomeric SMN1 gene is indispensable for normal survival of motoneurons in humans (4). Pathogenic mutations involve deletions, frameshift and missense mutations in exons 5, 6, and 7 of SMN1 as well as the conversion of SMN1 to SMN2 which drastically reduces exon 7 expression (2, 10–14).

In the mouse, there is only one gene coding for SMN, located on chromosome 13, within the region of conserved synteny with human chromosome 5q13 (15). No alternative splicing of the transcripts has been found in the mouse. The predicted amino acid sequence of mouse SMN is 82% identical to that of the human protein, including a conserved putative nuclear localization signal (15). No murine mutations homologous to human chromosome 5q SMAs have been found (16), and a functional knockout of the gene coding for SMN causes death of early embryos (17) suggesting, in accordance with biochemical evidence (6), that SMN is an essential housekeeping protein (18).

Several ligand binding sites have been identified in SMN protein. Based on a yeast two-hybrid screen using SMN as a bait, SIP-1 (SMN-interacting protein 1) was selected and shown to interact with SMN in vivo and in vitro (6). The SMN-SIP-1 complexes are large (approximately 300 kDa) and contain additional proteins several of which were shown to be U snRNP proteins (6). The proteins within this complex are involved in spliceosomal snRNP biogenesis (7) and in pre-mRNA splicing (8). Two of these proteins contain an Sm

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The abbreviations used are: SMA(s), spinal muscular atrophies; SMN, survival motoneuron; PFN, profilin; sn, small nuclear; SIP, SMN-interacting protein; MBP, maltose-binding protein; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; BSA, bovine serum albumin; poly-Pro, peptides consisting of 50–60 proline residues.

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translated proteins were diluted in PBS containing 1% (w/v) BSA and purified by poly-(L-proline) affinity chromatography as described previously in the coding region of mouse PFN II. The expressed protein was transformed with the expression plasmid pMW172/PFN II, containing the coding region of mouse PFN II. The expressed protein was found to have a lower affinity for polyproline motifs than profilin II (PFN II; 24). PFN I is expressed early in mammalian embryogenesis (25) and is distributed widely in various cells and tissues, whereas PFN II is not expressed in most tissues tested but is highly concentrated in the central nervous system and in cultured neurons (26). Promoted by the conspicuous proline stretches in SMN, we analyzed the possible interaction of this protein with both isoforms of profilin, PFN I and PFN II.

A preliminary report of this work has been given (27).

**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid Assay**—cDNA fragments encoding human SMN, SMNΔ7 (gift of Dr. Judith Melki, Ilikirch), and mouse profilins I and II (gift of Dr. Werner Witke, Monterotondo, Italy) were subcloned into the yeast expression plasmids pGBT9 and pGAD424 in-frame to the GAL4 DNA binding domain and into pGAD424 in-frame to the GAL4 activating domain, respectively. Deletion fragments SMN-N (encoding amino acids 1–188), SMN-C (encoding 188–289), and SMNΔ7-C (encoding 188–278) were inserted into the vectors pGBT9 and pGAD424 using synthetic oligodeoxynucleotides as linkers. For all constructs, the correct reading frames were confirmed by sequencing. The preparation of all media and reagents and all manipulations of the yeast strains were performed according to the manufacturer’s manual (CLONTECH, Heidelberg).

**In Vitro Transcription/Translation and Production of Fusion Proteins**—The coding regions of human SMN and SMNΔ7 and mouse PFN II were cloned into the vector pCDNA3 (Invitrogen, Groningen). The (G)SMN proteins were recognized by indirect immunofluorescence. The antibodies directed against human SMN and against mouse PFN I and II as immunogens) have been used in the course of this study. Several monoclonal antibodies showed isospecificity in immunoblots with SDS-denatured antigens but not on sections. Some antibodies reacted with diffuse PFN but not PFN in gems. A possible explanation for these differences might be epitope masking or modification of the antigen. Several antibodies recognized human and bovine PFN in immunoblots and cultured cells but not mouse PFNs. Problems with mouse monoclonal antibodies on mouse tissue sections, due to endogenous mouse IgG, could in part be overcome by perfusion of the animals.

With the following antibodies were included in this study: monoclonal antibody 2B1 directed against human SMN (IgG; generous gift of Dr. G. Dreyfuss, Ref. 5, and purchased from Transduction Labs, Lexington, KY), henceforth designated mc-a-SMN; a commercial polyclonal antibody that recognizes both PFN isoforms (Alexis Co., San Diego, CA), here designated pc-anti-PFN; monoclonal IgG 2H11, raised against a fusion protein of an α-actinin domain with bovine PFN I, which reacts specifically with PFN I of many mammalian species, but not with rodents (30), designated mc-a-PFN I; and a monoclonal IgG raised against recombinant mouse PFN II, 5C6, here designated mc-a-PFN II. For pc-anti-PFN (Alexis) the specificity was verified on Western blots of extracts from cultured cells and tissues including mouse spinal cord; only a 15–25 kDa band was stained. Monoclonal antibody 2B11 has been described in a previous study (20). Monoclonal antibody 11A8 (20) was tested for its isospecificity by enzyme-linked immunosorbent assay and Western blotting on extracts of E. coli expressing recombinant PFN I and PFN II, respectively, and stained a 15 kDa band only in the presence of PFN II.
Motifs.

Boxes work; SMN, self-association (9).

Vertical lines and numbered above; full-length SMN protein is shown as a bar with exons separated by vertical lines and numbered above; black boxes symbolize proline-rich motifs. Boxes below refer to interaction sites with ligand proteins: SIP-1 (6); SmB, a protein of the U snRNPs complex (6); PFN I and PFN II (this work); SMN, self-association (9). Panel B, SMNΔ7 truncated form (natural splice isoform) and deletion fragments used in the yeast two-hybrid system.

**TABLE I**

Interactions between SMN and its fragments with profilins I and II in the yeast two-hybrid system

|        | PFN I | PFN II | SMN | SMN-N | SMN-C |
|--------|-------|--------|-----|-------|-------|
| SMN    | +*    | +      | +++ | +     | +     |
| SMNΔ7  | −     | −      | −   | −     | −     |
| SMN-N  | +     | +      | ++  | +     | +     |
| SMN-C  | +     | +      | ++  | +     | +     |
| SMNΔ7-C| −     | −      | −   | −     | −     |

* Symbols indicate degree of blue color development indicating β-galactosidase activity. +++, within 1 h; +, weak staining after 3 h; −, no color development within 24 h.

**Fig. 1.** Functional domains in the SMN polypeptide and constructs used for protein-protein interaction studies. Panel A, the full-length SMN protein is shown as a bar with exons separated by vertical lines and numbered above; black boxes symbolize proline-rich motifs. Boxes below refer to interaction sites with ligand proteins: SIP-1 (6); SmB, a protein of the U snRNPs complex (6); PFN I and PFN II (this work); SMN, self-association (9).

**Fig. 2.** SMN-PFN complex formation in HeLa cells as shown by *in situ* cross-linking and subsequent coimmunoprecipitation. HeLa cells were treated with the membrane-permeant cross-linker diethio-bis(succinimidyl propionate) (31, 32). SMN and bound proteins were immunoprecipitated (IP) with mc-a-SMN antibody; mc-a-birch pollen profilin, not reactive with mammalian profilins (33, 34), served as unspecific control. Precipitated proteins were detected by immunoblot (IB) with mc-a-PFN I or mc-a-SMN as indicated. LC, IgG, light chain.

**Fig. 3.** Interaction of SMN with mouse PFN isoforms *in vitro*. Panels A and B, solid phase overlay assays. Panel A, comparison of SMN binding by PFN I and II. 100 pmol of MBP and MBP-SMN fusion protein or 500 pmol of recombinant mouse PFN I and mouse PFN II per spot were immobilized on a membrane and were incubated with 35S-labeled *in vitro* translated proteins in the overlay. Bound protein was detected by autoradiography. Panel B, effect of poly-L-proline on the interaction of PFN II with SMN. 100 pmol of MBP-SMN fusion protein per spot was immobilized and incubated with *in vitro* translated mouse PFN II in the presence of increasing concentrations of poly-L-proline (poly-Pro). Panel C, affinity precipitation of *in vitro* translated SMN and SMNΔ7 with PFN II bound to beads and effects of poly-L-proline. Translation efficiency and purity of [35S]methionine-labeled proteins were checked by PAGE and autoradiography (Total). 120 μg of mouse PFN II was coupled on 10 μl of NHS-HiTrap material. The material was incubated with *in vitro* translated 35S-Met-labeled SMN and SMNΔ7. BSA, negative control. Poly-Pro, a 5-fold excess of poly-L-proline over coupled mouse PFN II. Samples of the supernatants (S) and pellets (P) were separated by SDS-PAGE and blotted. 35S-labeled SMN was detected by autoradiography. Different positions on the gel are the result of interference with electrophoretic mobility by salt in supernatants.

**Immunohistochemistry**—For immunofluorescence, cryosections and cells were fixed with methanol at −20 °C for 6 min. Monoclonal antibodies were used as undiluted tissue culture supernatants. Goat anti-mouse IgG, conjugated with Cy3 (red, Dianova, Hamburg) served as a secondary antibody. For double staining, Fab fragments of Cy2-conjugated (green) goat anti-mouse IgG (Dianova) were used to saturate the anti-

tration of the membrane-permeant cross-linker diethio-bis(succinimidyl propionate) (Pierce, Sankt Augustin, Germany). Excess cross-linker was quenched with 0.2 M glycine. After additional rinsing in PBS, cells were lysed in RIPA buffer (50 mM Tris, pH 7.2, 1% (v/v) Triton X-100, 0.25% deoxycholate, 1 mM EGTA, 150 mM NaCl, 20 mM glycine, 2.5 mM sodium azide, 1 μM pepstatin A, 80 μg/ml Pefabloc SC, 0.46 μg/ml aprotinin; 30 min at 4 °C). Cellular material was then scraped off the dish with a rubber policeman, homogenized by pipetting, and centrifuged at 15,000 × g at 4 °C for 10 min. 2 μl of mc-a-SMN was added to the supernatant, and the samples were incubated for 16 h at 4 °C. Then 50 μl of a 50% slurry of protein G-Sepharose preblotted with 2% (w/v) BSA in RIPA buffer was added to the samples, which were then incubated further under stirring at 4 °C for 1 h. The Sepharose beads were collected by centrifugation and washed twice in RIPA buffer and once with PBS before the samples were boiled in SDS sample buffer containing 20% (v/v) β-mercaptoethanol, to cleave the cross-linker. Finally, samples were analyzed by SDS-PAGE and immunoblotting, using standard procedures. For control precipitations, we used the monoclonal antibody 4A6, which does not react with any vertebrate protein but specifically recognizes an epitope present exclusively in profilins of birch-related plants (33, 34).
SMN before incubation with mc-a-PFN II and Cy3-conjugated goat anti-mouse IgG. Fluorescent images were photographed on a Zeiss Axiophot fluorescence microscope. Color slides were scanned and images processed using Adobe Photoshop (version 5.0).

RESULTS

SMN and Profilins Interact in the Yeast Two-hybrid System—To test for a direct interaction between SMN and PFN we employed the yeast two-hybrid system. Appropriate vectors comprising cDNA fragments encoding mouse PFN I and II and human SMN (Fig. 1A), SMNΔ7, NH2- and COOH-terminal deletion fragments of SMN, and a COOH-terminal deletion fragment of SMNΔ7 were generated (Fig. 1B). SMN-N, encoding amino acids 1–188, and SMN-C, encoding amino acids 188–294, were obtained by cleavage at the NcoI site at base pair 565 within the coding region of the SMN gene. The COOH-terminal deletion fragment contained the proline-rich region with the Pn, P10, F5 motifs. The results of the yeast two-hybrid screens are shown in Table I. SMN protein interacted strongly with itself (5, 9), with PFN II, and less well with PFN I. SMN-SMN interactions were shown to be mediated by the COOH-terminal third of SMN. These findings correspond to the location of the recently determined oligomerization domain of SMN, a 30-amino acid region between residues 249 and 278 (9; Fig. 1A). The same fragment, which contains 54% proline residues between amino acids 190 and 294, was found to be responsible for PFN binding. SMNΔ7 and SMNΔ7-C did not interact with any of the partners (Table I).

Interaction of SMN and Profilin in Mammalian Cells—To corroborate the results obtained in the yeast two-hybrid system we performed coimmunoprecipitations with HeLa cell extracts. Cells were treated with the membrane-permeant cross-linker dithiobis(succinimidyl propionate) before lysis, in order to stabilize protein complexes under the competitive conditions in the cytosol of cells.

SMN and Profilins Interact in Vitro—To characterize further the interaction between SMN and profilin we performed solid phase binding assays on membranes. In vitro translated, radioactively labeled SMN and mouse profilins, a recombinant fusion protein (MBP-SMN) comprising SMN and the MBP of E. coli as a fusion partner, and recombinant mouse profilins were used in this assay. As shown in Fig. 3A, in vitro translated SMN bound to immobilized MBP-SMN but not to MBP alone. Recombinant PFN II bound strongly to immobilized MBP-SMN, whereas the interaction with mouse PFN I was rather weak. In the converse experiment, in vitro translated SMN also bound strongly to immobilized PFN II but weakly to PFN I. The significance of the SMN polyproline stretches for this interaction was tested in competition experiments. The addition of peptides consisting of 50–60 proline residues (poly-Pro) inhibited the complex formation between membrane-adsorbed MBP-SMN and in vitro translated PFN II. As seen in Fig. 3B, a 25-fold molar excess of poly-Pro over SMN virtually abolished the interaction.

Lastly, affinity precipitation with Sepharose-coupled proteins was performed. PFN II covalently coupled to Sepharose beads was incubated with in vitro translated 14C)methionine-labeled SMN. Bound and free SMN were separated by centrifugation and detected by SDS-gel electrophoresis and autoradiography. As shown in Fig. 3C, Sepharose-coupled recombinant PFN II bound SMN, whereas control BSA-Sepharose did not. Again, SMN/PFN II binding was sensitive to the presence of poly-Pro: a 5-fold molar excess over SMN completely abolished the interaction. However, in contrast to the results obtained with the yeast two-hybrid system, in vitro translated SMNΔ7 did bind to Sepharose-coupled PFN II.

The data from the yeast two-hybrid experiments and the in vitro data allow the conclusion that SMN binds PFN II stronger than PFN I. Because mouse PFN II is characteristic for central nervous system tissue (26) we examined the expression of the mRNAs for both profilin isoforms and SMN in the spinal cord.

PFNIs I and II mRNAs Are Highly Expressed in the Spinal Cord—To analyze the expression pattern of PFNs in the spinal cord, the organ affected in SMA, in situ hybridizations were
performed using mouse PFN I- and PFN II-specific riboprobes (Fig. 4). PFN I mRNA was present in a variety of cell types, including motoneurons and interneurons of the gray as well as in astrocytes of the white matter, whereas PFN II mRNA expression was restricted to motoneurons in the anterior horn and some interneurons. Hence, motoneurons appeared especially rich in the mRNA of both PFN isoforms.

Colocalization of PFNs and SMN—On the protein level, the distribution of profilins and SMN was analyzed by immunohistochemistry (Fig. 5). HeLa cells were stained for PFN and SMN as examples of non-neuronal cells, using a monoclonal antibody specific for PFN I (mc-a-PFN I, Fig. 5, A–C). A similar distribution, diffuse in the cytoplasm and concentrated in nuclear gems, was observed for PFN I and SMN. Whereas this distribution is well known for SMN (5), the localization of PFN in gems, i.e., nuclear dots that come in pairs or in quadruplets, is a novel finding. Not all cells contained gems in their nuclei, but all gems inspected were positive for both proteins, SMN and PFN I. We further analyzed mouse spinal cord because the spinal cord is the central nervous system region affected in SMA patients. Spinal cord sections were stained with a variety of antiprofilin antibodies, in single as well as in double-labeled experiments, in combination with anti-SMN. Examples are shown in Fig. 5, D–I. High levels of PFN were seen in large

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**Fig. 5.** Immunocytochemical localization by double-staining of PFNs (Cy3, red) and SMN (Cy2, green). Panels A–C, cultured HeLa cells; panels D–I, frozen sections from the ventral region of mouse spinal cord. Panels A, D, and G, profilin staining with antibodies mc-a-PFN I (panel A, 2H11; Ref. 30), mc-a-PFN II (panel D, 5C6), and pc-a-PFN (panel G, Alexis). Panels B, E, and H, SMN staining with mc-a-SMN (2B1; Ref. 5). Panels C, F, and I, double exposures, to show colocalization (yellow). In all cases, there is a diffuse staining of the cytoplasm for profilin and SMN. In the non-neuronal HeLa cells (panels A–C), a nuclear gem in a quadruplet configuration is indicated by arrowheads. In panels D–F, a gem in a twin (“gemini”) configuration in the nucleus of a motoneuron is indicated by arrowheads. In panels G–I, gems are indicated by arrowheads in quadruplet (upper left) and in twin (lower right) configurations. Dark area to the lower left is white matter (see Fig. 4). In all cases, gems are stained for both profilin and SMN (yellow in panels C, F, and I). Bars in panel A (valid for panels A–C) and panel G (valid for panels D–I), 10 μm.
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anterior horn neurons using both monoclonal (Fig. 5, D–F) and polyclonal anti-PFNs (Fig. 5, G–I).

In the many of these neurons, profilins were seen to colocalize with SMN in gems, and, like SMN, they were also distributed diffusely in the cytoplasm of the cell bodies (Fig. 5).

Again, wherever gems were seen on spinal cord sections, they were labeled for both SMN and PFN. Similar observations were made (data not shown) on a motoneuron-derived mouse cell line, NSC-19 (35).

**DISCUSSION**

In this study we show that SMN and profilins are capable of forming complexes under a variety of experimental conditions, including communoprecipitation, the yeast two-hybrid system, and in vitro assays. We demonstrate that this interaction is mediated by the polyproline stretch in SMN and the corresponding polyproline binding site in profilins. Our data expand the previously known functional domain structure of SMN by a profilin-binding site, which comprises the polyproline stretches in the COOH-terminal half of SMN. Within the region of the SMN sequence delineated by amino acid residues 244–248 (the COOH-terminal Pro5 motif), there is an overlap between the domains responsible for complex formation with the spliceosomal protein SmB (amino acids 240–267), the oligomerization domain in exon 6, and the profilin interaction domain. This suggests that profilin binding may regulate the interactions with these proteins.

Although the SMNΔ7 deletion fragment still contains all identified binding motifs in the COOH-terminal region of the molecule, i.e., for SmB, SMN, and profilin binding, we found that this fragment failed to interact with itself as well as with PFN II in the yeast two-hybrid system. The fact that SMNΔ7-C gave the same negative result might emphasize the importance of exon 7 for these interactions. However, we cannot exclude misfolding or degradation of this short fragment. Liu and Dreyfuss (5), however, mention an interaction between SMN and two SMN cDNA clones that lack exon 7 in their two-hybrid experiments. Because this notion is not documented by experimental data, it is difficult to explain why these results are at variance with ours. In contrast to our finding in the yeast two-hybrid system, SMNΔ7 did bind to PFN II in solution, as seen by affinity precipitation with radioactively labeled proteins and a sensitive detection system. The discrepancy between the data obtained with the yeast two-hybrid system and those from in vitro binding studies may reflect relevant but gradual differences in the ability of SMN and SMNΔ7 to bind profilin in the competitive environment of the cytoplasm. Alternatively, it could be caused by a diminished translation efficiency, aberrant folding, or increased sensitivity to degradation of SMNΔ7 compared with SMN, which in itself might also be relevant for the etiology of spinal muscular atrophy.

Although a variety of proline-rich ligands for the corresponding binding site in profilins has been identified (23), not all proteins with polyproline stretches interact with profilins. For example, the proline cluster containing protein CAP (36) does not bind to profilins in vitro, whereas its isolated polyproline peptide does so (20, 37). Moreover, the difference in affinity for proline-rich ligands between profilin I and II, as reported for VASP (20), has also been seen with SMN. These observations support the notion that the interaction between SMN and PFNs is not an unspecific consequence of the presence of polyproline stretches in SMN.

Our cytochemical studies indicate that large neurons are particularly rich in profilins. As we have shown both on the mRNA and on the protein level, PFN II is expressed predominantly in motoneurons that are the primary targets for neurodegeneration in SMA patients, whereas PFN I is expressed in many different cell types. In all cells and tissues examined, profilins were found diffusely distributed in the cytoplasm and diffusely or as dot-like structures or gems in the nucleus. The same distribution was observed for the SMN protein in HeLa cells, in mouse spinal cord (this work and Ref. 38), in neuroblastoma (38), in a motoneuron-like mouse cell line (this work), and in spinal cord tissue biopsies from human fetuses (3). A role of SMN in gem formation is suggested by the correlation of gem number in explanted fibroblasts with the severity of SMA symptoms in patients (4). Furthermore, SIP-1, a small protein that is involved in snRNP biogenesis, interacts with SMN and is also localized in gems, suggesting that SMN, SIP-1, and profilins are components of the same complex. In this context, it is noteworthy that coprecipitation of an unidentifed 15-kDa polypeptide from HeLa cell lysates with either SMN or SIP-1 antibodies has been reported previously (6). We suspect this polypeptide to be profilin.

The identification of profilins as components of a physiologically important nuclear structure defines these proteins as members of a growing list of microfilament-associated proteins located in the cytoplasmic and in the nuclear compartment, with discrete functions in both locations. Such proteins comprise β-catenin (39) plakoglobin (40), vinculin (41), and actin (42). Except for β-catenin (43), the precise role of these proteins in the nucleus has yet to be elucidated.

It has been shown that SMN is essential for mRNA splicing (8). However, the etiology of the chromosome 5q spinal muscular atrophy, in particular its neuron-specific primary pathology, is not yet understood. The correlation between self-association of SMN protein (3, 8) and the severity of the disease strongly suggests that oligomerization of SMN is necessary for SMN’s function, at least in motoneurons. Oligomerization and cytoplasmic/nuclear transport of SMN, in turn, might be modulated by profilins, especially by neuronal PFN II.

Thus our results are suggestive for the understanding of how a defect in the ubiquitous protein SMN would cause neurodegeneration while leaving other cell types unaffected (the wasting of skeletal muscle is a secondary consequence of neurodegeneration). Following this argument, PFN II might be considered as a possible target of mutations causing SMA. Genetic knockout or other manipulations of the profilin II gene of mice should provide further insights into the role of PFN II in neuronal survival.

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