Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 11167–11172, September 1999
Biochemistry

SMN mutants of spinal muscular atrophy patients are defective in binding to snRNP proteins

LIVIO PELLIZZONI, BERNARD CHARROUX, AND GIDEON DREYFUSS*

Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6148

Edited by Christine Guthrie, University of California, San Francisco, CA, and approved July 23, 1999 (received for review April 7, 1999)

ABSTRACT **Spinal muscular atrophy (SMA) is a common motor neuron degenerative disease and the leading genetic cause of death of young children. The survival of motor neurons (*SMN*) gene, the SMA disease gene, is homozygously deleted or mutated in more than 98% of SMA patients. The SMN protein interacts with itself, with SMN-interacting protein 1, and with several spliceosomal small nuclear ribonucleoprotein (snRNP) Sm proteins. A complex containing SMN plays a critical role in spliceosomal snRNP assembly and in pre-mRNA splicing. SMN mutants found in SMA patients show reduced self-association and lack the capacity to regenerate the splicing machinery. Here we demonstrate that SMN mutants found in SMA patients are defective in binding to Sm proteins. Moreover, we show that SMN, but not mutants found in SMA patients, can form large oligomers and that SMN oligomerization is required for high-affinity binding to spliceosomal snRNP Sm proteins. These findings directly link the impaired interaction between SMN and Sm proteins to a defect in snRNP metabolism and to SMA.**

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by the degeneration of motor neurons in the spinal cord, resulting in muscular weakness and atrophy. According to the severity and the age of onset of the disease, SMA patients are classified into three types: type I (Werdnig–Hoffmann disease), the most severe lethal form; type II, the intermediate form; and type III, the mildest form (1). The survival of motor neurons (*SMN*) gene has been identified as the disease gene of SMA, and two inverted *SMN* gene copies are present on human chromosome 5 at 5q13 (2–4). Only homozygous deletions or mutations of the SMN telomeric copy (SMN1) result in the SMA phenotype, and the levels of SMN expression driven by the centromeric copy (SMN2) in motor neurons inversely correlate with the severity of the disease (5, 6). The SMN2 gene, which does not provide complete protection from SMA, produces mainly an alternatively spliced form of SMN lacking exon 7 whose ratio, compared with the full length, also correlates with SMA severity (4, 7, 8).

SMN and its associated protein SIP1 (SMN-interacting protein 1) are localized both in the cytoplasm and in the nucleus, where they concentrate in discrete bodies called gems (9, 10). SMN binds to itself, to SIP1, and to some of the spliceosomal small nuclear ribonucleoprotein (snRNP) Sm proteins (9–11). The interaction of SMN with the Sm proteins is likely to be important for the functions of the SMN complex in the assembly of snRNPs in the cytoplasm (12, 13) and in the nuclear regeneration of snRNPs and spliceosomes (14, 15). Consistent with such critical housekeeping functions, SMN is expressed in all tissues of mammalian organisms and the mouse *SMN* gene knock-out displays an embryonic lethal phenotype (16). The evolutionarily highly conserved YG box domain (17), spanning exons 6 and 7, is important for SMN binding to Sm proteins (12) and for SMN self-association (13). A number of SMA patients have been shown to have a deletion of at least exon 7, which also is the main product of SMN2, or single-point mutations within the YG box rather than complete deletions of SMN1 (1). As a result of these mutations, SMN has a reduced ability to self-associate, and this defect correlates with SMA severity (11). The same mutants lack the function of wild-type SMN in regenerating the splicing machinery *in vitro*, a possible functional defect associated with SMA (14). The effect of these SMN mutations on the SMN interaction with Sm proteins is not known. Here we show that SMN mutants found in SMA patients are defective in their interaction with snRNP Sm proteins both *in vitro* and *in vivo*. Moreover, we studied further the possible relationship between SMN self-association, Sm binding, and SMA and found that the functional significance of SMN oligomerization is to generate a high-affinity binding site for the Sm proteins. Only wild-type SMN, but not SMN mutants found in SMA patients, can form large oligomers and bind to Sm proteins. These results establish a direct link between SMN binding to the Sm proteins and SMA.

MATERIALS AND METHODS

Plasmid Construction. DNA fragments corresponding to the ORFs of SMN wild-type and mutant proteins were generated by PCR amplification by using specific primers. All the myc-tagged constructs were generated by cloning the PCR inserts into a modified pcDNA3 vector (18). cDNAs for Sm proteins were a kind gift of R. Luhrmann (19). Untagged SMN and SIP1 cDNAs were from a dihybrid screening described previously (9) and cloned into pSP72 vector. All the constructs were analyzed by DNA sequencing.

Productions of Proteins *in Vitro*. The [^{35}S]methionine-labeled proteins were produced by an *in vitro* coupled transcription–translation reaction (Promega) in the presence of [^{35}S]methionine (Amersham). His$_6$-tagged SMN and SmB fusion proteins, cloned into pET28 vector, were produced and purified as described previously (14). All the glutathione *S*-transferase (GST) fusion proteins were expressed from the GST expression vector pGEX-5X (Pharmacia) in the *Escherichia coli* strain BL21(DE3)pLysS and purified by using glutathione-Sepharose according to the manufacturer's protocol (Pharmacia).

***In Vitro* Protein-Binding Assay.** Purified GST or GST fusion proteins (1–3 μg) bound to 25 μl of glutathione-Sepharose beads were incubated with the *in vitro* translated proteins in 1 ml of binding buffer (50 mM Tris·HCl, pH 7.5/200 mM NaCl/2 mM EDTA/0.1% NP-40/2 μg/ml leupeptin, pepstatin A, and aprotinin). After incubation for 1 hr at 4°C, the resin was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

This paper was submitted directly (Track II) to the *Proceedings* office.
Abbreviations: SMN, survival motor neurons; SMA, spinal muscular atrophy; SIP1, SMN-interacting protein 1; snRNP, small nuclear ribonucleoprotein; GST, glutathione *S*-transferase; wt, wild type.
*To whom reprint requests should be addressed. E-mail: gdreyfuss@hhmi.upenn.edu.

pelleted and washed five times with 1 ml of binding buffer. The bound fraction was eluted by boiling in SDS/PAGE sample buffer and analyzed by SDS/PAGE on a 12.5% polyacrylamide gel, and the signal was enhanced by treatment with Amplify solution (Amersham). In the preincubation experiments, the indicated molar excess of purified recombinant His-tagged SMN proteins were incubated with GST or GST-SMN, previously bound to glutathione-Sepharose beads, for 1 hr at 4°C in 1 ml of binding buffer. Unbound proteins were eliminated by five washes in binding buffer, after which the *in vitro* translated proteins were added and the binding was performed as described above.

Gel-Filtration Chromatography. Purified recombinant His-tagged SMN, SMNY272C or SMNΔEx7 (50 μg), and SmB (25 μg) proteins were incubated, individually or mixed as indicated, for 1 hr on ice in 0.25 ml of a buffer containing 50 mM Hepes, pH 7.9/400 mM KCl/0.5 mM EDTA/2.5 mM DTT. The samples then were applied to a TSK-GEL G3000-SW glass column (08800; Tosohaas, Montgomeryville, PA) equilibrated in the same buffer. One-minute fractions were collected at a 0.25-ml/min flow rate, pooled as indicated, and analyzed by SDS/PAGE and Western blotting with anti-T7 tag mAb (Novagen).

Cell Culture and Immunoprecipitation. 293T cells were cultured in DMEM (GIBCO/BRL) supplemented with 10% FBS (GIBCO/BRL) and transfected by standard calcium phosphate procedure. Thirty-six to 48 hr posttransfection cells were collected and processed by immunoprecipitation. Immunoprecipitations were carried out by using total cell lysates prepared in the presence of 0.5% Triton X-100 as described previously (20). Immunoblotting was performed as described previously (10). The antibodies used for these experiments were as follows: 2E17, mouse monoclonal anti-SIP1 (10); Y12, mouse monoclonal anti-Sm (21); 9E10, mouse monoclonal anti-myc; and mouse monoclonal anti-T7 tag (Novagen).

RESULTS AND DISCUSSION

SMN Mutations of SMA Patients Affect the Direct Interaction of SMN with Itself and with SmB. In an *in vitro* binding assay, purified recombinant His-tagged SMN and SmB proteins bind to a GST-SMN fusion protein but not to GST alone (Fig. 1*a*). This demonstrates that SMN interacts directly with itself and, although with a low affinity, with SmB and rules out a possible bridging effect by other components such as those that may be present in the reticulocyte lysate used in previous reports (11, 12). Next, we examined the effect on these interactions of two well characterized mutations found in SMA patients, the point mutant SMNY272C and the exon 7 deletion mutant (SMNΔEx7). Fig. 1*b* shows that both mutations severely affect not only SMN self-association (11) but also SMN interaction with SmB. In contrast, no effect is observed on the interaction of SMN with SIP1, which involves the amino terminus of SMN (10). GST-SIP1 binds equally efficiently to full-length SMN and to both mutants. GST alone, used as a control, showed no detectable binding (data not shown). Because SMA is a motor neuron disease, we also analyzed the interaction of the neuronal-specific Sm protein, SmN, with SMN wild type, SMNY272C, and SMNΔEx7 and found them to be identical to those of SmB (data not shown). This demonstrates that the SMN self-association and SmB-binding domains share common determinants within the YG box and that both SMN/SMN and SMN/SmB interactions are affected by mutations that cause SMA. A schematic summary of SMN-interacting domains is shown in Fig. 6*a*.

SMN Self-Association Enhances the Interaction with Sm Proteins. To determine whether or not these interactions are mutually exclusive, we preincubated beads containing GST-SMN, or GST as a control, with a molar excess of recombinant His-SMN to form SMN oligomers. Then, after washing away

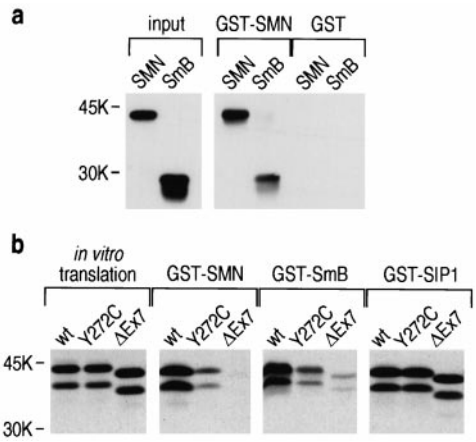

FIG. 1. SMN interacts directly with itself and with SmB, and these interactions are affected by mutations found in SMA patients. (*a*) Binding assay of His-tagged SMN and SmB recombinant proteins (2 μg) with either GST or GST-SMN was performed as described in *Materials and Methods*. Bound SMN and SmB were analyzed by SDS/PAGE and Western blotting with an anti-T7 tag antibody. Ten percent of the input is shown in the first lane. (*b*) *In vitro* translated [^{35}S]methionine-labeled, myc-tagged SMN wild-type and mutant proteins were incubated with the indicated purified GST-fusions (SMN, SmB, or SIP1) as described in *Materials and Methods*. Bound proteins were analyzed by SDS/PAGE and fluorography. The *in vitro* translation area shows 20% of the input. The area corresponding to GST-SmB binding is a 3-fold-longer exposure than the others.

the unbound SMN, *in vitro* translated, [^{35}S]methionine-labeled SMN, SmB, or SIP1 was added and assayed for binding (Fig. 2*a*). SMN binding is reduced only partially by the preincubation with recombinant His-SMN, suggesting that the oligomerization capacity of SMN on the beads has not been saturated. Surprisingly, SmB binding is dramatically enhanced by SMN self-association. SIP1 binding is slightly increased presumably because additional binding sites become available with the bound His-SMN. The specificity of this effect is demonstrated further by the lack of binding to control GST-bearing beads. We asked further whether this effect is seen with other Sm proteins known to bind SMN (10). Fig. 2*b* shows that SMN self-association greatly stimulates its interaction with SmB, SmD1, and SmD3 but not with SmD2 and SmE. SmF and SmG do not bind under any of the conditions we have tested (data not shown). The binding efficiency of Sm proteins to GST-SMN is lower than previously observed (10) because of the more stringent buffer conditions employed in this study. Several lines of evidence argue against the possibility that the increased binding of Sm proteins merely reflects the presence of additional interaction sites on the bound His-SMN: (*i*) even at the highest concentration we tested, the amount of bound His-SMN is roughly equivalent to that of GST-SMN; (*ii*) consistently, SMN contains a binding site for SIP1 independent of that for Sm proteins, and SIP1 binding is only slightly increased by the addition of His-SMN (Fig. 2*a*); and (*iii*) the effect of SMN self-association is not observed with SmD2 and SmE, whose basal binding otherwise is similar to the one of SmB, SmD1, and SmD3 (Fig. 2*b*). These results indicate that SMN self-association and Sm proteins interaction are not mutually exclusive but, on the contrary, that SMN self-association very strongly and specifically increases its affinity for a subset of Sm proteins.

A titration analysis of the stimulating effect of SMN oligomerization on SmB binding is shown in Fig. 3. The amount of His-SMN bound to GST-SMN, after the preincubation step, was determined by Western blotting. The increase in SmB binding correlates with the extent of SMN self-association (Fig. 3*a*). When the bound amount of wild-type His-SMN is equivalent to the amount of GST-SMN on the beads, SmB

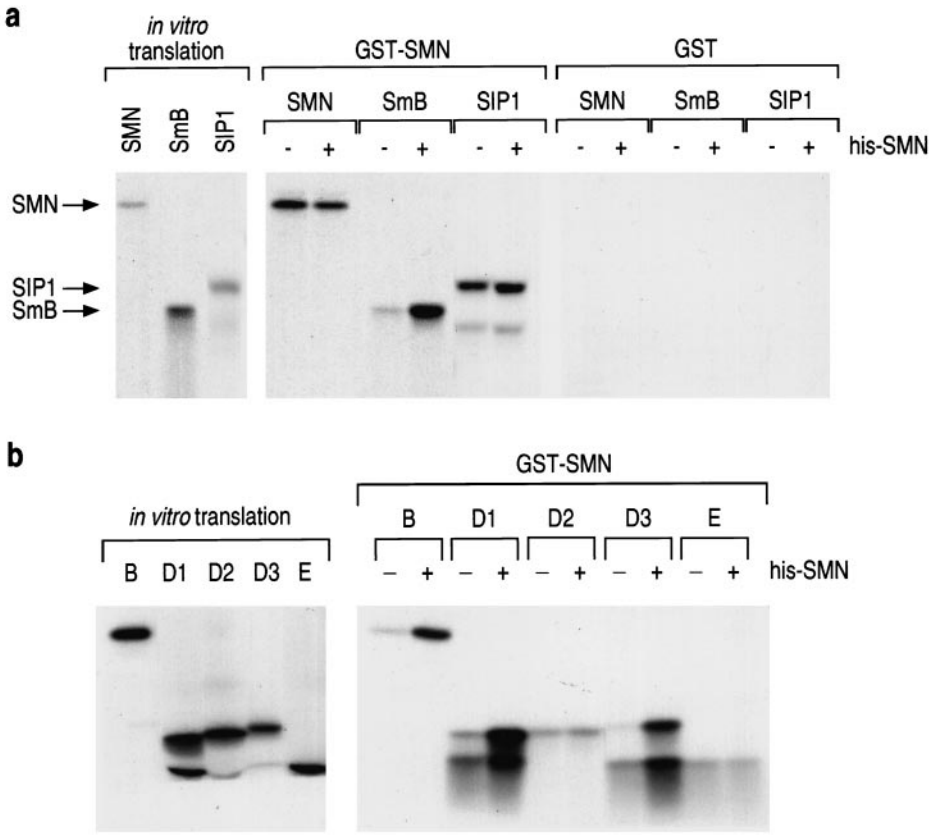

FIG. 2. SMN self-association specifically increases the binding affinity for Sm proteins. (*a*) GST or GST-SMN was preincubated with or without a 4-fold molar excess of His-SMN as described in *Materials and Methods*. After washing away unbound His-SMN, *in vitro* translated [^{35}S]methionine-labeled SMN, SmB, or SIP1 was added and the binding assay was performed as described in *Materials and Methods*. Bound proteins were analyzed by SDS/PAGE and fluorography. The *in vitro* translation area shows 10% of the input. (*b*) GST-SMN was preincubated with or without a 4-fold molar excess of His-SMN as described above. *In vitro* translated [^{35}S]methionine-labeled Sm proteins then were added, and the binding assay was performed as described in *Materials and Methods*. The *in vitro* translation area shows 25% of the input.

binding increases approximately 10-fold (Fig. 3*a*, lane 4×). SmB binding increases about 30-fold, and more than 60% of the input is bound when the amount of His-SMN is 2.5-fold greater than GST-SMN (Fig. 3*a*, lane 8×). It appears that GST-SMN is predominantly in a monomeric form, probably because the GST fusion or the interaction of the GST with the glutathione-Sepharose interferes with the self-association of GST-SMN on the beads. Proportional to their reduced ability to self-associate, a greater molar excess of recombinant SMNY272C and SMNΔEx7 than wild-type SMN (SMNwt) is required to obtain similar levels of association with GST-SMNwt. Although SMNY272C is still able to stimulate SmB binding, SMNΔEx7 does not (Fig. 3*a*). In the case of SMNY272C self-association, the extent of stimulation of SmB

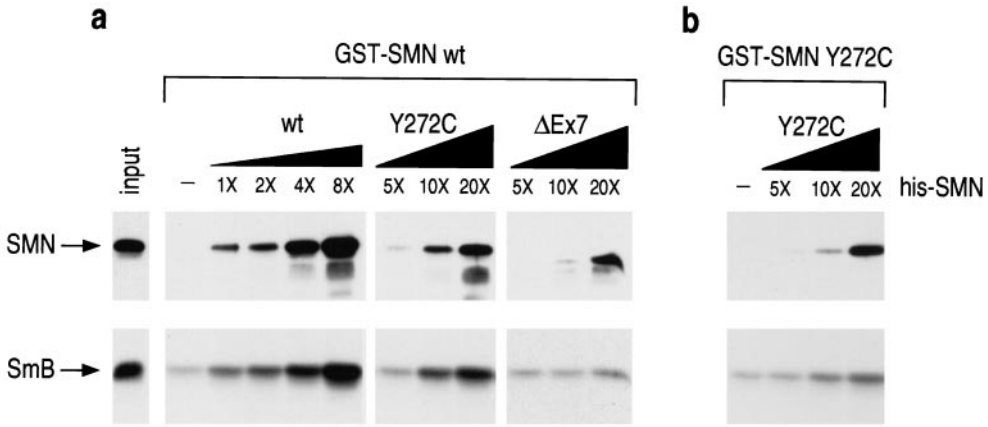

FIG. 3. Titration analysis of the effect of SMN self-association on SmB binding. (*a*) GST-SMN was preincubated with the indicated molar excess of His-tagged SMNwt, SMNY272C, or SMNΔEx7. After washing unbound recombinant proteins, *in vitro* translated [^{35}S]methionine-labeled SmB was added and binding was performed as described in *Materials and Methods*. Each binding was analyzed by Western blotting with anti-T7 tag mAb to show bound His-tagged SMN proteins (*Upper*) and by autoradiography to show bound SmB (*Lower*). (*b*) GST-SMNY272C was preincubated with the indicated molar excess of His-SMNY272C and processed further as described above. The input lane shows an amount equivalent to the 20% of SmB translation and to 1× His-SMNwt recombinant protein used.

binding is very low (Fig. 3*b*). SMNΔEx7 self-association was too inefficient to be analyzed (data not shown). Thus, SMN self-association most likely creates a high-affinity binding site for Sm proteins and SMN mutations found in SMA patients affect the ability of SMN to form the Sm-binding site. Moreover, the binding site formed with the SMN mutants has a lower affinity for Sm proteins than that formed by wild-type SMN.

SMN Oligomerization Is Impaired in Mutants of SMA Patients and Is Required for Binding to Sm Proteins. Although SMN self-associates and is part of a large, macromolecular complex *in vivo*, it could not be distinguished whether this is due to the presence of multiple copies of SMN and/or of additional proteins (9, 10). Moreover, previous *in vitro* experiments showing defective self-association of SMN mutants (11) were performed under solid-state conditions that did not allow a distinction between dimerization and oligomerization. Dimerization and oligomerization likely are different in terms of the interaction surfaces required for a protein to self-associate because oligomerization would involve at least two independent binding sites. Similarly, our present results strongly suggest that SMN oligomerization enhances the interaction with Sm proteins, but no direct evidence that SMN indeed can oligomerize by itself has been provided so far.

We sought to address this question directly by gel-filtration chromatography of purified recombinant His-tagged SMN wild-type and mutant proteins. Fig. 4*a* shows that SMN alone is able to form large oligomers of up to a molecular mass corresponding to approximately 500 kDa. In contrast, SMN Y272C and SMNΔEx7 are impaired severely in their ability to form oligomers. Next, we investigated the predicted requirement of SMN oligomerization for Sm protein interaction. As shown in Fig. 4*b*, SmB associated with SMN large oligomers in the high-molecular-weight fractions. In contrast, no association between SMNΔEx7 and SmB as larger-size complexes could be detected. These results provide direct evidence that SMN is able to oligomerize and is found almost exclusively self-associated in large oligomeric complexes. Furthermore, SMN mutations found in SMA patients disrupt such oligomerization and the interaction with Sm proteins.

Reduced Association of SMN Mutants with snRNPs *in Vivo*. These results predict that *in vivo* mutants such as SMNY272C and SMNΔEx7 would associate with Sm proteins less efficiently than SMN wild type. To test this, 293T cells were transfected with either myc-tagged SMN wild type or SMNY272C or SMNΔEx7. All the transfected proteins were expressed at similar levels as determined by Western blotting by using antibodies against the myc tag (Fig. 5). By coimmunoprecipitation with anti-SIP1 antibodies, comparable levels of wild-type and mutant proteins are found associated with SIP1 as a SMN/SIP1 complex. However, immunoprecipitation using the anti-Sm mAb Y12 shows that the association of SMNY272C and SMNΔEx7 with Sm proteins is reduced markedly compared with the wild-type SMN. A complex containing the SMN mutants and Sm proteins is still detected because the reduced ability of the mutants to form oligomers with the wild-type SMN is partially overcome by the overexpression and by the possible contribution of other SIPs *in vivo* (10).

Fig. 6 presents a summary model of several interactions in the SMN complex. SMN appears to be associated with SIP1 most, if not all, of the time (10). The possibility that SIP1

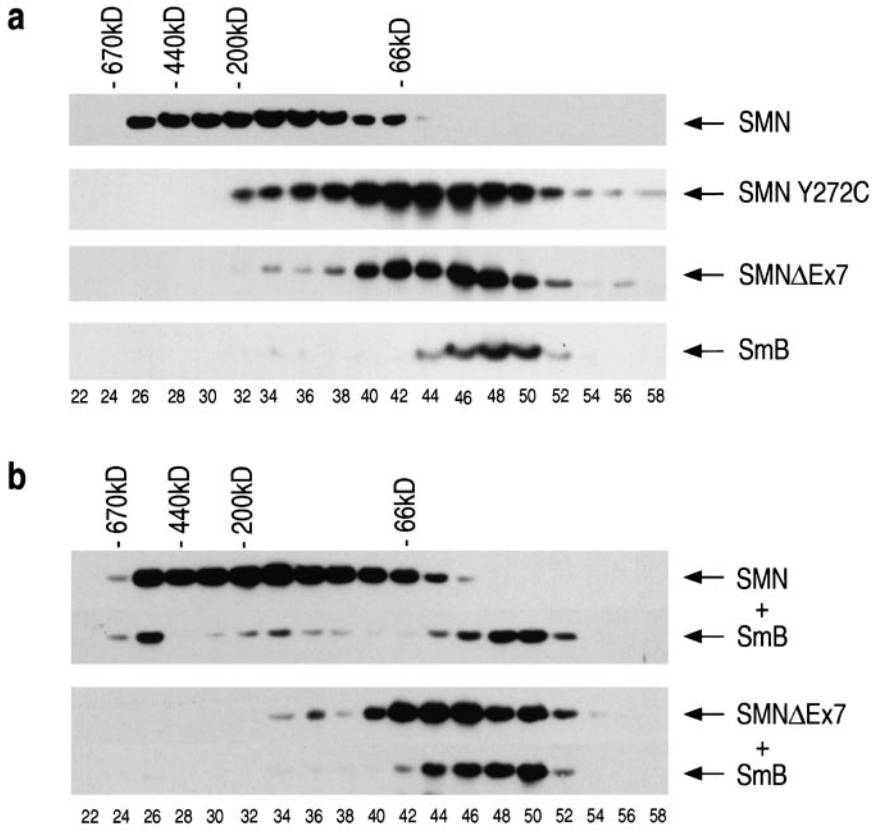

FIG. 4. SMN mutants found in SMA patients are defective in oligomerization and interaction with Sm proteins. (*a*) SMN wild type but not SMN mutants of SMA patients form oligomers. Purified recombinant His-tagged SMN, SMNY272C, SMNΔEx7, and SmB proteins were analyzed individually by HPLC gel filtration as described in *Materials and Methods*. The fractions were analyzed by SDS/PAGE, and the proteins were detected by Western blotting. (*b*) SMN oligomers bind to SmB. The indicated mixtures of purified recombinant His-tagged SMN or SMNΔEx7 with His-tagged SmB were analyzed by HPLC gel filtration. The fractions were analyzed by SDS/PAGE, and the proteins were detected by Western blotting. The indicated positions of the molecular mass markers were determined by independent column chromatographies.

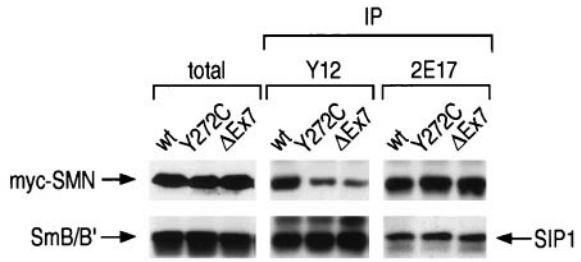

FIG. 5. Reduced association of SMNY272C and SMNΔEx7 with Sm proteins *in vivo*. 293T cells were transiently transfected with the indicated myc-tagged SMN constructs and analyzed by coimmunoprecipitation experiments. Total cell extracts were immunoprecipitated with either anti-Sm (Y12) or anti-SIP1 (2E17) mAbs. Total cell extracts (10% of the input) and the anti-Sm immunoprecipitates were analyzed by Western blotting with anti-myc (9E10) and Y12 antibodies; the anti-SIP1 immunoprecipitates were analyzed by Western blotting with 9E10 and 2E17. A relatively low amount of antibody was used in the immunoprecipitations to improve the detection of SmB/B′ and SIP1 over the light chains of the immunoglobulins, which migrate very closely. The transfected proteins are overexpressed approximately 5- to 10-fold compared with endogenous SMN (data not shown). Only SmB is shown because we are unable to detect the other Sm proteins by Western blotting with Y12 antibody. Note that the transfected SMNΔEx7 migrates close to full-length SMN on a 12.5% polyacrylamide SDS/PAGE.

interaction may have an effect on SMN oligomerization or Sm protein interaction currently is being investigated. Monomeric SMN has only a low affinity for Sm proteins because a high-affinity Sm-binding domain forms only upon SMN oligomerization. For simplicity, we depict SMN here bound to Sm proteins as a dimer; however, the actual stoichiometry of the SMN oligomers is not yet known. Specific protein–protein interactions between the various Sm proteins are required for the ordered assembly of the Sm core (19). We have shown that SMN is able to form large oligomers, and, in such a conformation, it binds with high affinity to a subset of Sm proteins. We propose that the SMN oligomer is the functional core that allows the SMN complex to function in snRNP assembly (12) and spliceosome regeneration (14). Importantly, SMN mutations found in SMA patients directly affect SMN oligomerization and Sm protein binding. Thus, the loss-of-function phenotype of mutant proteins such as SMNY272C and SMNΔEx7 in pre-mRNA splicing (14) is most likely the direct result of an impaired interaction with the Sm proteins. These findings directly link the molecular mechanism of SMA to a deficiency in the interaction of SMN with spliceosomal snRNP Sm proteins. It can be anticipated that a detailed knowledge of the structure of the SMN/SIP1 complex with Sm proteins will lead to further insights into the mechanisms of SMN function and point the way toward possible therapeutic approaches for SMA.

We thank Dr. Joan A. Steitz for the anti-Sm Y12 mAb and Dr. R. Luhrmann for the Sm protein cDNAs. We are grateful to members of our laboratory for stimulating discussions and, in particular, Drs. Wes Friesen, Haruhiko Siomi, and Sara Nakielny for helpful discussions and critical comments on this manuscript. Bernard Charroux was supported by a postdoctoral fellowship from the Association Francaise

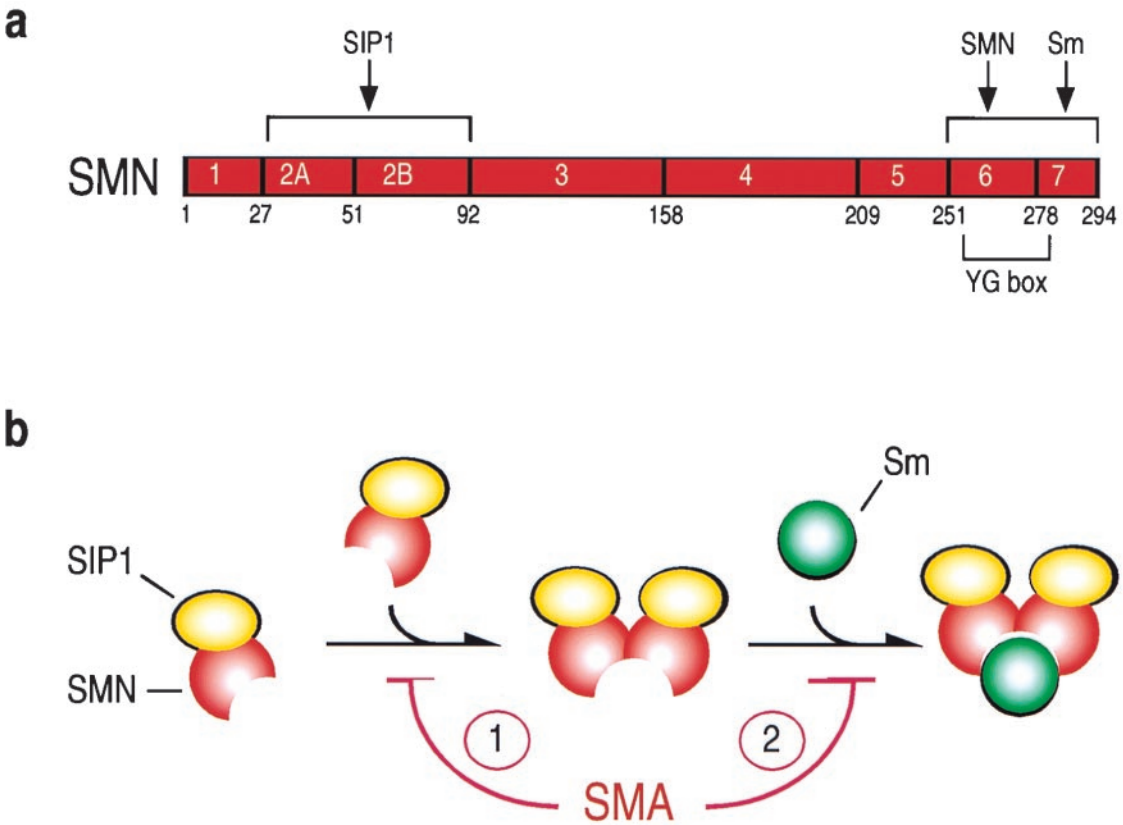

FIG. 6. Schematic model depicting the interaction of SMN/SIP1 with Sm proteins. (*a*) Schematic structure of the SMN protein and its interacting domain. The amino acid numbers and the borders of exons are indicated. SIP1-interacting domain resides at the amino terminus of SMN as determined by competition experiments (10). SMN self-association and SMN/Sm interaction domains overlap with the conserved YG box at the carboxyl-terminus of SMN as determined by deletion, mutation, and competition experiments (refs. 10 and 11 and this paper). (*b*) Monomeric SMN, associated with SIP1, which binds to SMN but not to itself (ref. 10; data not shown), contains a low-affinity binding site for Sm proteins. SMN self-associates, forming at least a SMN/SIP1 tetrameric complex. In this oligomeric conformation a binding site is formed with a much higher affinity for the Sm proteins. SMN mutations found in SMA patients result in a reduced ability of SMN to self-associate (1) and also map within the Sm-binding site itself (2), thus affecting the SMN interaction with Sm proteins.

pour la Recherche contre le Cancer. This work was supported by the Howard Hughes Medical Institute and by a grant from the National Institutes of Health.

1. Burghes, A. H. M. (1997) *Am. J. Hum. Genet.* **61,** 9–15.
2. Brzustowicz, L. M., Lehner, T., Castilla, L. H., Penchaszadeh, G. K., Wilhelmsen, K. C., Daniels, R., Davies, K. E., Leppert, M., Ziter, F., Wood, D., *et al.* (1990) *Nature* (*London*) **344,** 540–541.
3. Melki, J., Abdelhak, S., Sheth, P., Bachelot, M. F., Burlet, P., Marcadet, A., Aicardi, J., Barois, A., Carriere, J. P., Fardeau, M., *et al.* (1990) *Nature* (*London*) **344,** 767–768.
4. Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., *et al*. (1995) *Cell* **89,** 155–165.
5. Lefebvre, S., Burlet, P., Liu, Q., Bertrandy, S., Clermont, O., Munnich, A., Dreyfuss, G. & Melki, J. (1997) *Nat. Genet.* **16,** 265–269.
6. Coovert, D., Le, T. T., McAndrew, P. E., Strasswimmer, J., Crawford, T. O., Mendell, J. R., Coulson, S. E., Androphy, E. J., Prior, T. W. & Burghes, A. H. M. (1997) *Hum. Mol. Gen.* **6,** 1205–1214.
7. Gennarelli, M., Lucarelli, M., Capon, F., Pizzuti, A., Merlini, L., Angelini, C., Novelli, G. & Dallapiccola, B. (1995) *Biochem. Biophys. Res. Commun.* **213,** 342–348.
8. Gavrilev, D. K., Shi, X., Das, K., Gilliam, T. C. & Wang, C. H. (1998) *Nat. Genet.* **20,** 230–231.
9. Liu, Q. & Dreyfuss, G. (1996) *EMBO J.* **15,** 3555–3565.
10. Liu, Q., Fischer, U., Wang, F. & Dreyfuss, G. (1997) *Cell* **90,** 1013–1021.
11. Lorson, C. L., Strasswimmer, J., Yao, J. M., Baleja, J. D., Hahnen, E., Wirth, B., Le, T., Burghes, A. H. M. & Androphy, E. J. (1998) *Nat. Genet.* **19,** 63–66.
12. Fischer, U., Liu, Q. & Dreyfuss, G. (1997) *Cell* **90,** 1023–1029.
13. Mattaj, I. W. (1998) *Curr. Biol.* **8,** 93–95.
14. Pellizzoni, L., Kataoka, N., Charroux, B. & Dreyfuss, G. (1998) *Cell* **95,** 615–624.
15. Dietz, H. (1998) *Nat. Genet.* **20,** 321–322.
16. Schrank, B., Gotz, R., Gunnersen, J. M., Ure, J. M., Toyka, K. V., Smith, A. G. & Sendtner, M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 9920–9925.
17. Talbot, K., Ponting, C. P., Theodosiou, A. M., Rodrigues, N. R., Surtees, R., Mountford, R. & Davies, K. (1997) *Hum. Mol. Genet.* **6,** 497–500.
18. Siomi, H. & Dreyfuss, G. (1995) *J. Cell Biol.* **129,** 551–560.
19. Raker, V. A., Plessel, G. & Luhrmann, R. (1996) *EMBO J.* **15,** 2256–2269.
20. Pinol-Roma, S., Choi, Y. D., Matunis, M. & Dreyfuss, G. (1988) *Genes Dev.* **2,** 215–227.
21. Lerner, E. A., Lerner, M. R., Hardin, J. A., Janeway, C. A. & Steitz, J. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2737–2741.