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In Vitro and *in Cellulo* Evidences for Association of the Survival of Motor Neuron Complex with the Fragile X Mental Retardation Protein*[S]

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Spinal muscular atrophy (SMA) is caused by reduced levels of the survival of motor neuron (SMN) protein. Although the SMN complex is essential for assembly of spliceosomal U small nuclear RNPs, it is still not understood why reduced levels of the SMN protein specifically cause motor neuron degeneration. SMN was recently proposed to have specific functions in mRNA transport and translation regulation in neuronal processes. The defective protein in Fragile X mental retardation syndrome (FMRP) also plays a role in transport of mRNPs and in their translation. Therefore, we examined possible relationships of SMN with FMRP. We observed granules containing both transiently expressed red fluorescent protein(RFP)-tagged SMN and green fluorescent protein(GFP)-tagged FMRP in cell bodies and processes of rat primary neurons of hypothalamus in culture. By immunoprecipitation experiments, we detected an association of FMRP with the SMN complex in human neuroblastoma SH-SY5Y cells and in murine motor neuron MN-1 cells. Then, by *in vitro* experiments, we demonstrated that the SMN protein is essential for this association. We showed that the COOH-terminal region of FMRP, as well as the conserved YG box and the region encoded by exon 7 of SMN, are required for the interaction. Our findings suggest a link between the SMN complex and FMRP in neuronal cells.

The neuromuscular disease spinal muscular atrophy (SMA)[3] is characterized by degeneration of motor neurons of the spinal cord leading to muscular weakness and atrophy (reviewed in Refs. 1 and 2). The SMN (survival of motor neuron) protein is encoded by two genes, a centromeric *SMN1* gene and a telomeric *SMN2* gene. Over 98% of SMA patients have mutations in or deletions of the *SMN1* gene, and decreased levels of the SMN protein correlate with the phenotypic severity of SMA (3–5). The *SMN2* gene mostly produces a functionally defective form of the protein that lacks the domain encoded by exon 7 at the carboxyl terminus (6, 7). The SMN protein is ubiquitously expressed in all eukaryotes tested so far, except *Saccharomyces cerevisiae*. Its function is essential in all studied organisms (for review, see Ref. 8). SMN is associated with the proteins Gemin2 to Gemin8 in HeLa cells, to form a large stable complex called the SMN complex (9–19). This complex localizes to both the cytoplasm and the nucleus where it accumulates in nuclear bodies called Gems (20). Unrip is also a component of the SMN complex, except in Gems where this protein could not be detected (21, 22).

The SMN complex is thought to function in multiple cellular pathways related to RNA metabolism. More precisely, the SMN complex may play a central role in the assembly and metabolism of various RNPs in cells (reviewed in Refs. 23 and 24). The best characterized function of the SMN complex is the assembly of spliceosomal U snRNPs (for reviews, see Refs. 25 and 26). In the cytoplasm, each U snRNA except U6 associates with a set of Sm proteins (B/B′, D1, D2, D3, E, F, and G), which forms a seven-membered ring (Sm core) around the Sm site (27). The SMN complex interacts directly with both the Sm proteins and the U snRNAs and mediates the ATP-dependent assembly of the Sm core (14, 26, 28–35). The SMN complex likely plays additional roles in U snRNP biogenesis, *i.e.* cap hypermethylation of U snRNAs and nuclear import of U snRNPs (36–39). The SMN complex is also necessary for the formation of Sm and Lsm cores on small noncoding RNAs other than U snRNAs (33, 34, 40).

It is still unclear why motor neurons are specifically affected in SMA. Defect of a motor neuron-specific function of the SMN protein may be responsible for motor neuron degeneration in SMA (41, 42, and for reviews, see Refs. 2, 24, 43, and 44). In light

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[3] The abbreviations used are: SMN, survival of motor neuron; SMA, spinal muscular atrophy; FMRP, Fragile X mental retardation protein; G3BP, ras-GAP SH3 domain-binding protein; GST, glutathione *S*-transferase; snRNP, small nuclear ribonucleoprotein; hnRNP, heterogeneous nuclear ribonucleoprotein; PBS, phosphate-buffered saline; IP, immunoprecipitation; RFP, red fluorescent protein; GFP, green fluorescent protein.

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of this hypothesis, the SMN protein was found to be present in dendrites, axons, and growth cones, suggesting a function of the SMN protein in specific neuronal mechanisms, distinct from U snRNP assembly (45–53). More precisely, the SMN protein localizes in granules that are actively transported into neuronal processes and growth cones, and which show frequent colocalization with ribosomal RNAs (51). Only a subset of these SMN granules also contains the Gemin proteins, indicating the presence of diverse SMN-containing multiprotein complexes in axons and dendrites (47, 52, 53). Local protein synthesis in axons and dendrites is crucial for axonal growth, pathfinding, and regeneration, as well as synapse formation and plasticity (reviewed in Refs. 54–58). It was shown that SMN-deficient motor neurons have reduced axon outgrowth and pathfinding, as well as synapse dysfunction (2, 41, 50, 59). Moreover, the SMN protein was shown to interact with the RNA-binding protein hnRNP R (49, 60), and this interaction may be necessary for hnRNP R association with the 3′ untranslated region of β-actin mRNA and for efficient transport of this mRNA to growth cones (49, 50). Taken together, these observations point out a possible function of the SMN protein, and perhaps of the SMN complex, in the assembly, translation regulation, and/or transport of localized mRNP complexes (reviewed in Refs. 2, 43, and 44).

One way to get additional insights into SMN function in transport and translation of mRNAs in dendrites and axons is to identify SMN protein partners that are known to be involved in these neuronal mechanisms. The Fragile X mental retardation protein (FMRP), which is defective in the Fragile X mental retardation syndrome, has been shown to be a regulator of translation (Refs. 61–64, for a review, see Ref. 65). This RNA-binding protein shuttles between the nucleus and cytoplasm, suggesting that it may also be involved in RNA trafficking (66–68). Despite this shuttling property, FMRP is predominantly localized in the cytoplasm, and is associated in an RNA-dependent manner with polyribosomes. In neurons, FMRP has been proposed to play a role in dendritic transport of some specific mRNAs and to regulate local protein synthesis in synapses in response to synaptic stimulation signals (for reviews, see Refs. 65 and 69). In particular, exaggerated activation of protein synthesis by metabotropic glutamate receptors could be responsible for long term depression defects observed in the hippocampal neurons lacking FMRP (reviewed in Ref. 70). FMRP is also localized to growth cones of developing axons, and is proposed to be important for growth cone morphology and motility and for synapse formation (71, 72).

In the present study, we provide experimental evidences for a physical association of the SMN complex with FMRP in neuronal cell extracts. The SMN and FMRP protein domains required for this association are identified by site-directed mutagenesis and *in vitro* assays. The biological significance of these data is supported by analysis of the respective localizations of the SMN and FMRP proteins in rat primary neurons of hypothalamus in culture.

EXPERIMENTAL PROCEDURES

DNA Constructs and Antibodies—Plasmids expressing FLAG-tagged FMRP (pTL1 iso7 Flag-FMRP), His-tagged FMRP, His-tagged FMRP I304N, and glutathione *S*-transferase (GST)-tagged FMRP were described previously (73, 74). Deletion into the pTL1 iso7 Flag-FMRP vector was generated by PCR using appropriate oligonucleotides to produce pTL1 iso7 Flag-FMRPΔ470–485 vector, expressing FLAG-tagged FMRPΔ470–485 (deleted of amino acids 470 to 485), and iso7 Flag-FMRPΔ1–134 vector, expressing FLAG-tagged FMRPΔ1–134 (FMRPΔNDF) (deleted of the amino-terminal 134 amino acids). DNA fragments coding for FMRPΔ527–632 (FMRPΔRGG) (deleted of the carboxyl-terminal 106 amino acids) and FMRPΔ470–632 (deleted of the carboxyl-terminal 162 amino acids) were subcloned downstream of the cytomegalovirus promoter into a modified pcDNA3 vector (Invitrogen) containing an in-frame FLAG tag sequence at the amino terminus (FLAG-pcDNA3) (75). The GFP-FMRP and SMN-RFP constructs were generated with Gateway technology (Invitrogen).

The plasmids expressing FLAG-tagged SMN WT (pcDNA3-Flag-SMN), GST-tagged SMN WT, SMNΔYG (deleted of the carboxyl-terminal 26 amino acids), SMNΔEx7 (deleted of amino acids encoded by exon 7), SMNΔTudor (deleted of amino acids encoded by exon 3), and SMNΔEx2B (deleted of amino acids encoded by exon 2B) were kind gifts from Gideon Dreyfuss (9, 20, 76, 77). These constructs produce two bands, likely because translation starts at both the ATG tag and the native SMN ATG. The DNA fragment coding for SMNΔN27 (deleted of the amino-terminal 27 amino acids) was generated by PCR amplification using specific primers from the pcDNA3-Flag-SMN plasmid. The insert was cloned downstream of the cytomegalovirus promoter into the FLAG-pcDNA3 vector (75).

The constructs for production of FLAG-tagged Gemin2, myc-tagged Gemin3, myc-tagged Gemin4, FLAG-tagged Gemin5, untagged Gemin8, His-tagged Unrip, and FLAG-tagged SmB were described previously and are kind gifts from G. Dreyfuss and L. Pellizzoni (10, 12, 15, 18, 21, 29, 78). Plasmid expressing FLAG-tagged Snurportin1 was described previously (Flag-pcDNA3-Snurportin1) (36). For production of GST-tagged Snurportin1 in bacteria, a DNA fragment corresponding to the open reading frame of Snurportin1 was generated by PCR amplification using specific primers, and cloned into the pGEX-6P-2 plasmid (Amersham Biosciences). DNA fragments corresponding to the open reading frame of Gemin6 and Gemin7 were generated by PCR amplification using specific primers and genomic cDNA, and cloned downstream of the cytomegalovirus promoter into the FLAG-pcDNA3 vector. For the generation of the stable cell line that expresses FLAG-tagged Gemin2, a DNA fragment coding for FLAG-tagged Gemin2 was generated by PCR amplification using specific primers from pcDNA3-Flag-Gemin2 plasmid and cloned into the pTRE2 plasmid downstream of a promoter containing the tetracycline-responsive element (BD Biosciences). All the constructs were analyzed by automated sequencing.

The antibodies used in these experiments were as follows: anti-SMN (2B1) (20), anti-Gemin2 (2E17) (9), anti-Gemin3 (12H12) (10), anti-Gemin4 (17D10) (12), anti-Gemin5 (10G11) (15), anti-Gemin6 (20H8) (79), anti-Gemin8 (1F8) (18), anti-Unrip (3G6) (18), anti-hnRNP A1 (4B10) (80), anti-hnRNP

C1/C2 (4F4) (81), and anti-FMRP (1C3) used for Western blotting (82) and (7G1) used for immunoprecipitation (83), anti-Sm proteins (Y12) (84), anti-FLAG (Sigma), anti-β tubulin (SAP.4G5) (Sigma), and anti-G3BP monoclonal antibody (1F1) (Rhône-Poulenc Rorer).

Cell Culture and Transfection—SH-SY5Y and MN1 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 10 units/ml penicillin/streptomycin, and 2.9 mg/ml glutamine (Invitrogen). Cells growing on 100-mm culture dishes (about 60% confluent) were transfected with 8 μg of DNA using the JetPEI Transfection Kit (PolyPlus Transfection) according to the manufacturer's recommendation. Following overnight incubation with DNA, cells were washed and fresh medium added. Transfected cells were processed for immunoprecipitation 48 h after transfection. The transfection efficiency of SH-SY5Y cells was in average equal to 10% as indicated by the ratio of GFP-FMRP positive cells to total cells.

For protein localization experiments, primary cultures were prepared by mechanoenzymatic dissociation of fetal (day 7) Sprague-Dawley rat hypothalami, as previously described (85). Then, primary neurons were transfected using the calcium phosphate method according to Xia *et al.* (86). Briefly, cells were placed in a transfection medium containing Dulbecco's modified Eagle's medium, DMKY (1×) (0.5% phenol red, 1 M Hepes, 1 M $MgCl_2$), and received precipitate that was formed for 30 min, then cells were incubated for 45 min with 2 μg of each plasmid. After transfection, the medium was removed and replaced by 1× HBes (274 mM NaCl, 10 mM KCl, 1.4 mM Na_2HPO_4, 15 mM D-glucose, 42 mM Hepes, pH 7) for 1 min. Cells were put back in their filtered medium for 24 h. These cells were transfected with plasmids expressing GFP-FMRP or RFP-SMN. The percentage of transfected cells was determined directly using a microscope and is on average equal to 5%. To induce stress granule formation, the cells were treated with 0.5 mM arsenite for 1 h.

Generation of a Stable Cell Line That Expresses FLAG-tagged Gemin2—The generation of a stable cell line that expresses FLAG-tagged Gemin2 was done according to the manufacturer's recommendation (BD Biosciences) and according to Ref. 16, except that the Tet-Off system was used instead of the Tet-On system. Briefly, HeLa Tet-Off cells that constitutively express the tetracycline transactivator were cotransfected with the pTRE2 plasmid encoding FLAG-Gemin2 under the control of a promoter containing the tetracycline-responsive element, and the pTK-Hyg plasmid (BD Biosciences) carrying the hygromycin resistance gene. Stable clones were obtained by double selection in the presence of G418 (100 μg/ml) and hygromycin (200 μg/ml). Individual clones were isolated and analyzed by Western blotting for the expression of FLAG-Gemin2. To avoid possible side effects of Gemin2 overexpression, a stable cell line that expresses low levels of FLAG-Gemin2 was selected for SMN complex purification.

Affinity Purification of SMN Complex—Native SMN complexes were affinity purified as described by (16). In summary, total cell extracts from parental HeLa Tet-Off cells and from the HeLa Tet-Off stable cell line expressing FLAG-Gemin2 were prepared by resuspending cell pellets from five 100-mm culture dishes in RSB 100 buffer (10 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 100 mM NaCl) containing 0.1% Igepal and protease inhibitors (Roche). Cells were sonicated briefly. Following centrifugation at 10,000 × *g* for 15 min, supernatants were passed through a 0.2-μm filter and added to anti-FLAG M2 beads (Sigma) for 2 h at 4 °C. Supernatants were discarded, and beads were extensively washed with RSB 100 containing 0.02% Igepal. Then, three high salt washes were performed with 10 bead volumes of RSB 500 buffer (10 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 500 mM NaCl) containing 0.02% Igepal for 15 min at 4 °C. Following three washes with RSB 100 containing 0.02% Igepal, native SMN complexes were eluted from the beads by competitive displacement of FLAG-Gemin2 with 0.5 mg/ml of 3X-FLAG peptide (Sigma) in RSB 100 buffer containing 0.02% Igepal for 1 h at 4 °C. The composition of native SMN complexes were analyzed by SDS-PAGE on 12% polyacrylamide gels and silver staining or Western blotting.

Recombinant Protein Production and in Vitro Binding Experiments—Recombinant GST-FMRP, His-FMRP, and His-FMRP I304N were produced in a baculovirus system. The other GST-tagged recombinant proteins were expressed in *Escherichia coli* BL21(DE3)pLysS cells (Promega) and purified by affinity chromatography on glutathione-Sepharose beads (Amersham Biosciences). *In vitro* translated proteins were produced in the presence of [^{35}S]methionine (GE Healthcare) using a rabbit reticulocyte lysate and a coupled transcription-translation system (Promega). Arginine methylation of the proteins was inhibited by preincubating the lysate with different amounts of *S*-adenosylhomocysteine (250 and 500 μM) (Sigma) at room temperature for 10 min prior to the addition of the DNA template. For *in vitro* binding experiments, 4 μg of GST or GST-tagged proteins bound to glutathione-Sepharose beads were incubated with *in vitro* translated [^{35}S]methionine-labeled proteins in RSB 200 buffer (10 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 200 mM NaCl) containing 0.05% Igepal for 2 h at 4 °C, or with native purified SMN complex in RSB 100 buffer containing 0.02% Igepal for 2 h at 4 °C. RNase A (0.2 mg/ml) was added to the translation product and the mixture was incubated for 15 min at 30 °C prior to the binding experiment. Following five washes with the same buffer, bound proteins were eluted by boiling in SDS-PAGE sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 3.7 M β-mercaptoethanol), and analyzed by SDS-PAGE on 12% polyacrylamide gels followed by autoradiography or Western blotting.

Immunoprecipitation Experiments—For total cell extract preparation, SH-SY5Y cells were resuspended in RSB 100 buffer containing 0.01% Igepal and protease inhibitors (Roche), briefly sonicated three times on ice, and centrifuged 15 min at 10,000 × *g* at 4 °C. Total cell extracts were incubated with anti-FLAG beads (Sigma) for 2 h at 4 °C in RSB 100 buffer containing 0.01% Igepal. The beads were extensively washed with RSB 200 buffer containing 0.05% Igepal, and the immunoprecipitated proteins were eluted by boiling in SDS-PAGE sample buffer and analyzed by SDS-PAGE and Western blotting. For immunoprecipitation using anti-FMRP 7G1 antibody, MN-1 cells were resuspended in RSB 250 buffer (10 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 250 mM NaCl) containing 0.01% Igepal and protease inhibitors. Total extracts were prepared as indicated above

and incubated with anti-FMRP 7G1 and anti-β-tubulin antibodies immobilized on G-Sepharose beads for 2 h at 4 °C in RSB 250 buffer containing 0.01% Igepal. The beads were extensively washed with RSB 250 buffer containing 0.05% Igepal, and the immunoprecipitated proteins were analyzed as described above.

Immunofluorescence Microscopy—After 24 h transfection, cells were fixed in 4% paraformaldehyde/PBS for 20 min. After three washes with PBS, cells were permeabilized in 0.2% Triton X-100 for 20 min, then rinsed three times with 1× PBS. Blocking was performed for 1 h in PBS containing 1% bovine serum albumin and 5% goat normal serum. Cells were incubated overnight at 4 °C in a humid chamber with primary antibody in blocking buffer. After rinsing in PBS, they were incubated for 2 h at room temperature in a humid chamber with anti-goat IgG conjugated with Alexa Fluor 488 (Molecular Probes, Leiden, The Netherlands). The secondary antibodies were diluted in the same buffer as the primary antibodies. After three washes with 1× PBS, sections were mounted in Vectashield containing 4′,6-diamidino-2-phenylindole.

Image Acquisition and Processing—Images were acquired on a DMRA microscope equipped for epifluorescence, and with a 100 PlanApo objective and a 1.6 eyepiece. Digital images were recorded with a 12-bit C4795-NR CCD camera (Hamamatsu). Both the camera and the microscope were controlled by the software Metamorph (Universal Imaging). When necessary, maximal likelihood estimation deconvolution was performed with the software Huygens (Bitplane, Zurich, Switzerland), on stacks of 11 images taken with a Z-step of 0.5 μm. Maximal image projections of the resulting stacks were then converted to 8-bit images and colorized with Photoshop. For colocalization analysis, we counted all the spots and reported the percentage of SMN in FMRP foci. This analysis was done on a total of 4 cells with an average of 60 spots.

RESULTS

The SMN Complex Is Associated with FMRP in Human Neuroblastoma SH-SY5Y and Murine Motor Neuron MN1 Cells in Culture—To test whether the SMN complex can associate with FMRP in cells, total extracts were prepared from human neuroblastoma SH-SY5Y cells transiently expressing FLAG-tagged FMRP (FLAG-FMRP), or FLAG-tag alone (mock) as a negative control. The extracts were immunoprecipitated with anti-FLAG antibodies. Immunoprecipitated proteins (IP) and aliquots of the respective extracts (Total) were analyzed by SDS-PAGE and Western blotting using specific antibodies. All the tested components of the SMN complex, namely SMN, Gemin2, Gemin3, Gemin4, Gemin5, Gemin6, Gemin8, and Unrip were coimmunoprecipitated with FLAG-FMRP (Fig. 1*A*, *lane 3*). In contrast, the abundant RNA-binding proteins hnRNP C1/C2 were not coimmunoprecipitated, which indicated that the immunoprecipitation was specific (Fig. 1*A*, *lane 3*). Because we did not have an antibody against Gemin7, this protein was not probed in our assays. However, because Gemin6 and Gemin7 form an heterodimer and Gemin7 mediates the interaction of Gemin6 and Unrip with the SMN complex (17, 19, 87), Gemin7 was likely present in the immunoprecipitate. None of the SMN complex components were detected

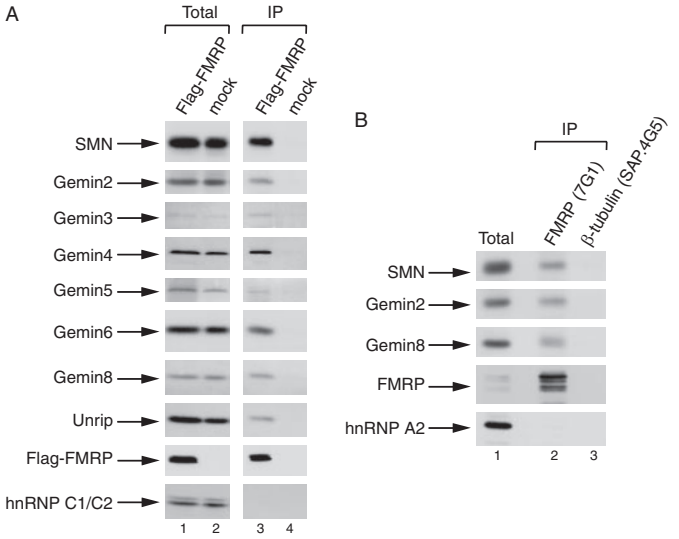


FIGURE 1. **The SMN complex is associated with FMRP in human and murine neuronal cell lines in culture.** *A*, immunoprecipitation experiments using anti-FLAG antibody (*IP*, *lanes 3* and *4*) were carried out on total extracts prepared from human neuroblastoma SH-SY5Y cells that were transiently transfected with a DNA construct expressing FLAG-tagged FMRP (*Flag-FMRP*, *lanes 1* and *3*) or the empty vector (*mock*, *lanes 2* and *4*). The immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting using antibodies directed against the indicated proteins. 2.5% of the extracts used in each experiment are shown (*Total*, *lanes 1* and *2*). *B,* immunoprecipitation experiments using anti-FMRP 7G1 antibody (*IP*, *lane 2*) and anti-β tubulin SAP.4G5 antibody (*IP*, *lane 3*) were carried out on total extracts prepared from murine motor neuron MN-1 cells. The 7G1 antibody recognizes the various FMRP isoforms. The immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting using antibodies directed against the indicated proteins. 2.5% of the extract used in the experiments is shown (*Total*, *lane 1*).

in the control immunoprecipitate from mock-transfected extracts (Fig. 1*A*, *lane 4*). Our experiment showed that transiently expressed FLAG-FMRP associates with the entire SMN complex. Because it is difficult to define the stoichiometry of each component of the SMN complex in the immunoprecipitate by Western blotting, it is also possible that FLAG-FMRP associates with some of the SMN complex components taken individually.

To verify whether endogeneous FMRP also associates with the SMN complex, total extracts from murine motor neuron MN-1 cells (88) were immunoprecipitated with the anti-murine FMRP 7G1 antibody, which was shown to efficiently immunoprecipitate the various endogeneous FMRP isoforms (83). Anti-β tubulin antibody was used as a negative control. Immunoprecipitated proteins (IP) and aliquot of the respective extracts (Total) were analyzed by SDS-PAGE and Western blotting using specific antibodies (Fig. 1*B*). Only a few of the available antibodies against the human SMN complex components cross-react with the murine proteins. However, the experiment showed that the analyzed components of the SMN complex, *i.e.* SMN, Gemin2, and Gemin8, were coimmunoprecipitated with endogeneous FMRP (Fig. 1*B*, *lane 2*). In contrast, the hnRNP A2 protein was not coimmunoprecipitated. Neither SMN, nor Gemin2 or Gemin8 were detected in the control immunoprecipitate using anti-β-tubulin antibody (Fig. 1*B*, *lane 3*).

SMN and FMRP Proteins Partially Colocalize in Cell Bodies and Processes of Rat Primary Neurons in Culture—Previous studies on cultured neurons have demonstrated the presence of

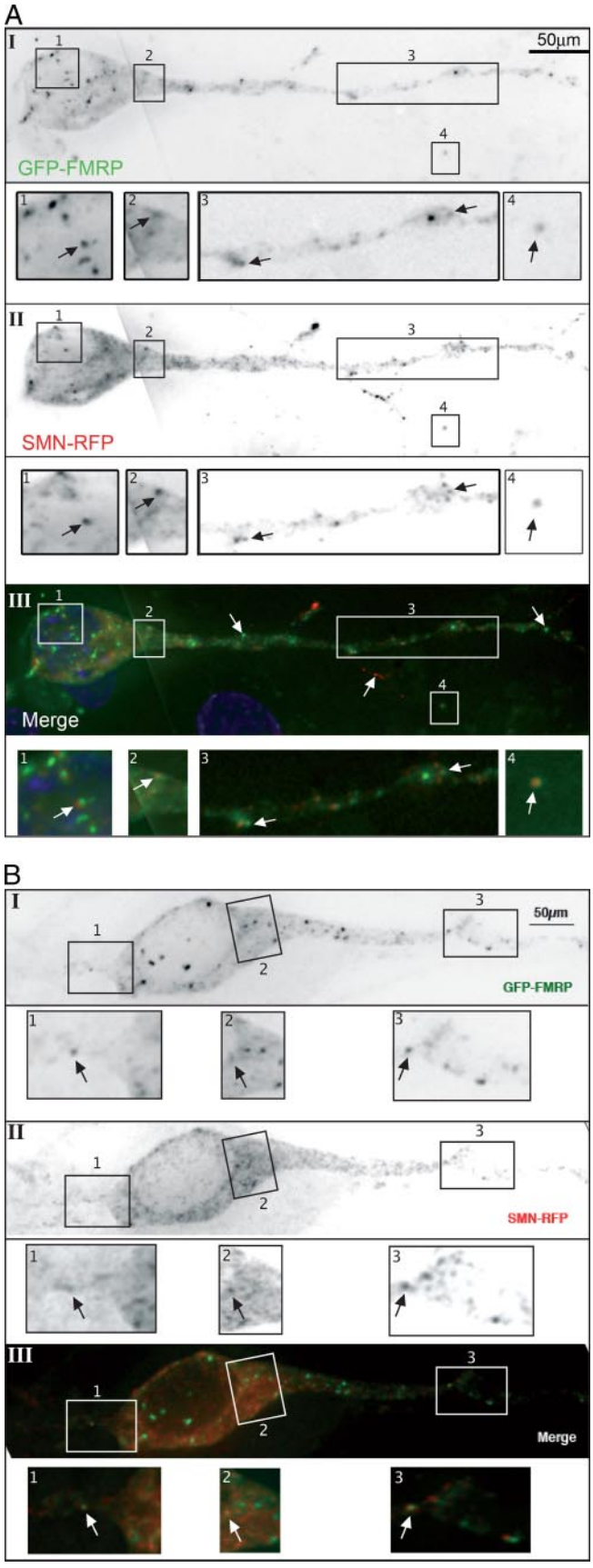


FIGURE 2. **A fraction of neuronal SMN granules contains FMRP protein.** *A* and *B*, rat primary neurons of hypothalamus were co-transfected with plasmids expressing GFP-FMRP and SMN-RFP fusion proteins. Localization of GFP-FMRP is shown in *I* and localization of SMN-RFP is shown in *II*. The combined images are shown in *III*. *Blue* signals demarcate nuclei visualized by 4′,6-diamidino-2-phenylindole staining in *A*. Eleven images were taken with a *Z*-step of 0.5 μm and colocalization analysis was done on these separate images. The figures represented here are a stack of the 11 images. Magnified views show examples of granules containing both SMN and FMRP (in *A*, *arrows in the enlarged insets 2–4*, as examples, in *B*, *arrows in the enlarged insets 1–3*, as examples), SMN-containing granules juxtaposed to FMRP-containing granules (in *A*, see *arrow in the enlarged insets 1*, as example), and SMN-containing granules independent from FMRP-containing granules (in *A*, see *arrows in the upper figure* of *Panel III*, as examples). *Scale bar*, 50 μm.

SMN-containing granules in processes and growth cones (47, 51–53). FMRP was also observed within trafficking granules in neuronal processes (89, 90). To gain further insights into the relationship between the SMN complex and FMRP in cells, we looked for possible colocalization of SMN and FMRP in rat primary neurons of hypothalamus in culture. To this end, primary neurons were transiently transfected to express GFP-tagged FMRP and RFP-tagged SMN (Fig. 2, *A* and *B*). We observed some granules containing either FMRP or SMN that were independent one from the other (for examples, Fig. 2*A*, *arrows* in the *upper part* of *panel III*). In addition, in both cell bodies and processes, some of the SMN-containing granules also contained FMRP (26.6 ± 6% and 45.7 ± 2%, respectively) (Fig. 2*A*, *arrows* in the enlarged *insets 2–4*, as examples, Fig. 2*B*, *arrows* in the *enlarged insets 1–3*, as examples). The indicated percentages of colocalization were average values of percentages established by analysis of four transfected primary neurons, as described in Costes *et al.* (91). Moreover, some SMN-containing granules were juxtaposed to FMRP-containing granules (Fig. 2*A*, *arrow*, in the *enlarged insets 1*, as example). Thus, our data indicated the presence of granules containing either one of the two FMRP and SMN proteins, or both of them.

Massive overexpression of SMN or FMRP was previously found to induce the accumulation of stress granules (63, 92). Therefore, we had to verify that the low expression of SMN-RFP and GFP-FMRP in our experiments did not induce stress granule formation (supplementary data Fig. 1). The ras-GAP SH3 domain-binding protein (G3BP), which is diffusely distributed throughout the cytoplasm (93, 94), is known to be recruited to cytoplasmic stress granules under stress conditions (95). We used this protein as a marker of stress granules. Rat primary neurons were exposed 1 h to arsenite treatment (concentration of 0.5 mM). As revealed by immunolocalization of G3BP, the arsenite treatment induced the formation of stress granules in cell bodies of cells transiently expressing or not SMN-RFP. Interestingly, no stress granules were detected in neurites after arsenite treatment. Moreover, in contrast to what was previously observed in HeLa cells and in neuronal SKN-MC cultured cells (92), SMN-RFP was still evenly distributed throughout the cell body of rat primary neurons after arsenite treatment, and did not accumulate in stress granules in our experimental conditions. The diffuse distribution of G3BP observed throughout the cytoplasm in cells that were transiently expressing SMN-RFP and were not treated by arsenite demonstrated the absence of stress granule formation in these conditions (supplementary data Fig. 1). A similar observation was made after transient expression of GFP-FMRP (data not shown). Therefore, we excluded the possibility that the SMN-

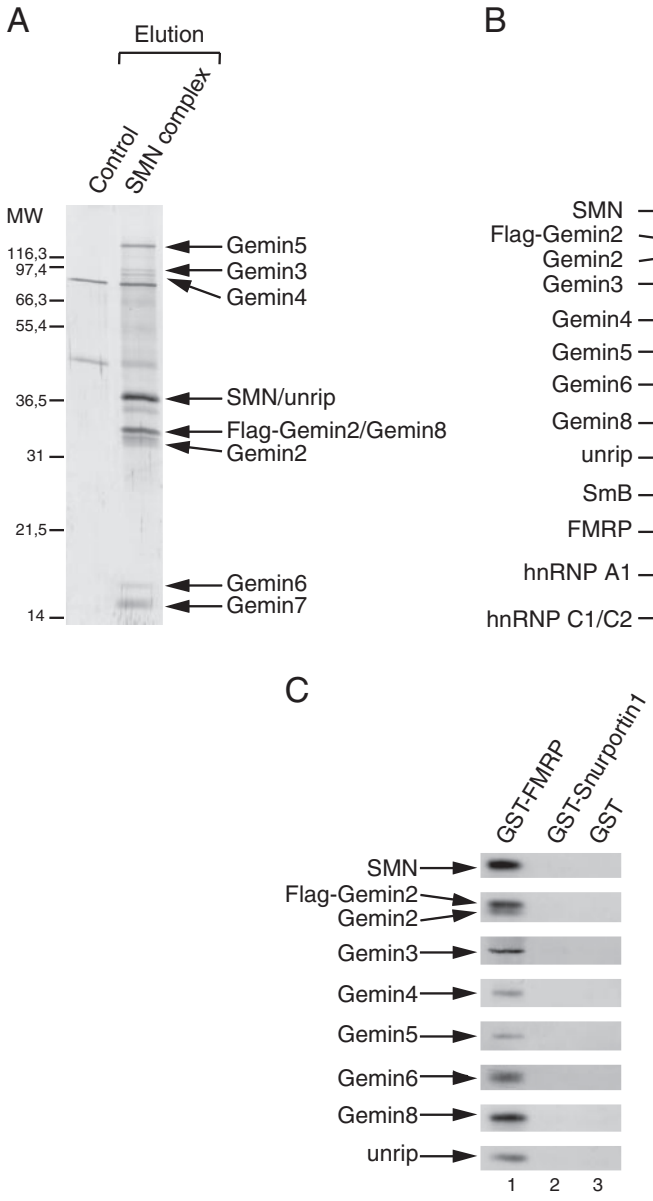


FIGURE 3. **Purified native SMN complex associates with FMRP *in vitro*.** *A*, native SMN complexes (SMN complex) were purified from total extracts prepared from HeLa Tet-Off cells that stably expressed FLAG-tagged Gemin2 by immunoprecipitation with anti-FLAG antibodies. After elution with an excess of FLAG peptides, the protein composition of the complexes was analyzed by SDS-PAGE followed by silver staining (*Elution*). The nonspecific proteins purified from HeLa Tet-off cells that do not express FLAG-Gemin2 are shown as a control (*Control*). The protein pattern was compared with those previously observed in Refs. 16, 18, and 21 and the proteins were assigned to known proteins of the SMN complex according to their molecular weights (*MW*). The identity of the proteins was confirmed by Western blotting analysis (see *B*). *B*, the purified native SMN complex (SMN complex) shown in *A* was analyzed by SDS-PAGE and Western blotting with antibodies directed against the indicated proteins (*Elution*, *lane 4*). A control experiment was performed with HeLa Tet-Off cells that do not express FLAG-Gemin2 (*Elution*, *lane 3*). *Lanes 1* and *2* correspond to 8% of the cellular extracts used in each assay. *C*, purified native SMN complexes shown in *A* and *B* were incubated with recombinant GST-FMRP immobilized on beads (*lane 1*), GST-Snurportin1 (*lane 2*), or GST alone (*lane 3*). The bound proteins were analyzed by SDS-PAGE and Western blotting with antibodies directed against the indicated proteins. *Lane 4* of *B* was loaded with 25% of the amount of SMN complex used in each binding reaction.

RFP and GFP-FMRP containing granules detected in the transfected cells were stress granules.

FMRP Associates in Vitro with Purified SMN Complexes—We next examined whether FMRP can associate *in vitro* with purified SMN complexes, which were prepared using a stably transfected cell line expressing a FLAG-Gemin2 construct (Fig. 3). The stringent purification conditions used in our assays (500 mM NaCl) were previously shown to produce complexes containing all the known components of the SMN complex, but no detectable amount of Sm proteins or other proteins known to interact with the SMN complex, as well as no detectable amount of RNAs (16, 18) (see "Experimental Procedures"). We incubated cell extracts from the FLAG-Gemin2 stable cell line (SMN complex) and the parental HeLa cell line used as a control (Control) with anti-FLAG antibodies. The proteins bound on the beads were eluted by competition with an excess of FLAG peptide (Elution) and analyzed by SDS-PAGE and silver staining (Fig. 3*A*) or Western blotting using appropriate antibodies (Fig. 3*B*, *lanes 3* and *4*). As previously observed (16, 18), the purified complexes contained all the components of the SMN complex (Fig. 3, *A* and *B*), but no detectable amounts of SmB (Fig. 3*B*, *lane 4*). The absence of the abundant RNA-binding proteins hnRNP A1 and hnRNP C1/C2 in the immunoprecipitate confirmed the specificity of the purification (Fig. 3*B*, *lane 4*). Importantly, FMRP was not detected in these complexes by Western blotting (Fig. 3*B*, *lane 4*). Then, the purified SMN complexes were incubated with a recombinant GST-FMRP protein fusion, immobilized on glutathione-Sepharose beads (Fig. 3*C*). GST-Snurportin1 or GST alone were used as negative controls. The bound proteins were separated by SDS-PAGE and analyzed by Western blotting using specific antibodies. As shown in Fig. 3*C*, purified SMN complexes were retained on GST-FMRP (*lane 1*), but not on GST-Snurportin1 or GST alone (*lanes 2* and *3*). We concluded that FMRP can associate *in vitro* with the SMN complex.

Among the Components of the SMN Complex, Only SMN Associates in Vitro with FMRP—Next, we determined which component(s) of the SMN complex can associate with FMRP *in vitro*. For this assay, a GST-FMRP fusion protein, or the GST protein alone as a negative control, was immobilized on glutathione-Sepharose beads. The beads were then incubated with one of the following [^{35}S]methionine-labeled proteins: SMN, Gemin2 to Gemin8, or Unrip, which

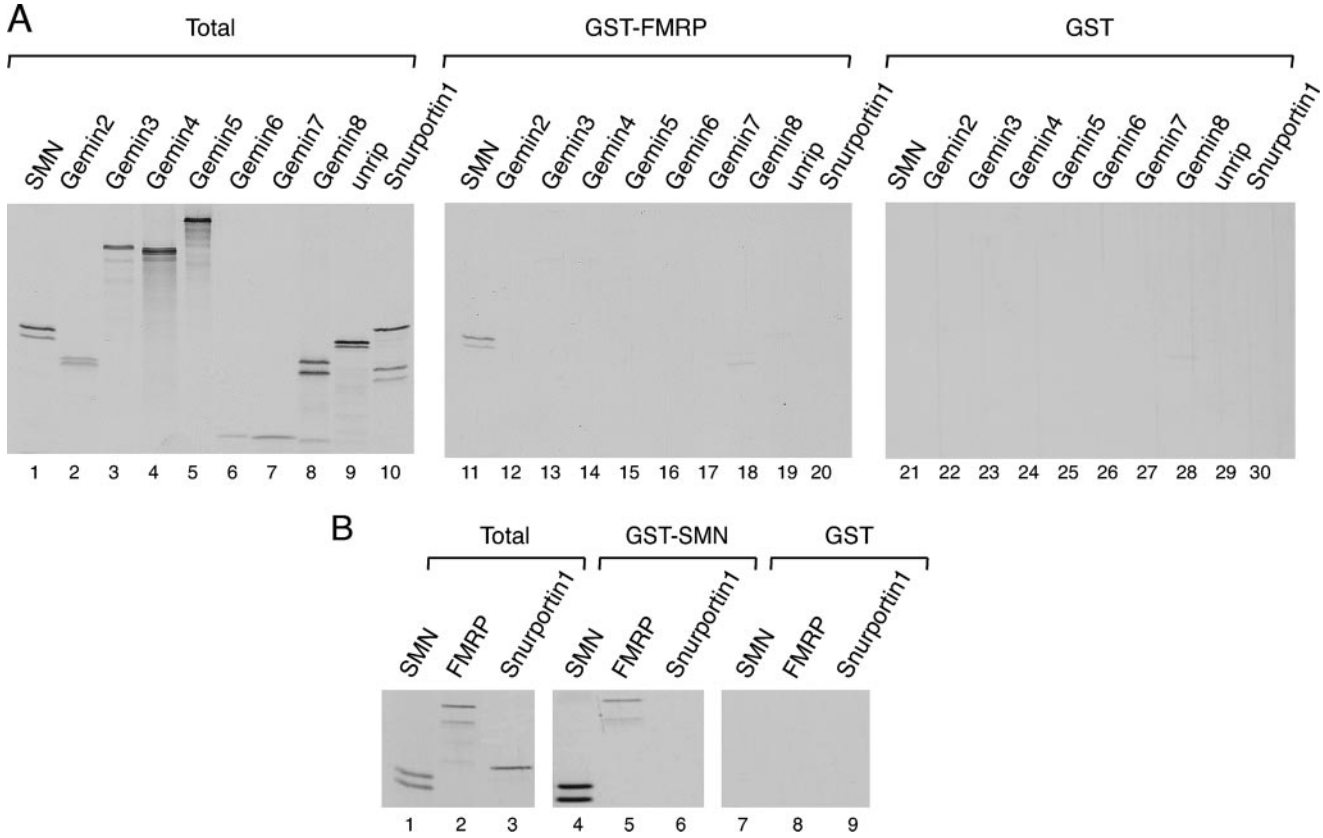


FIGURE 4. **The SMN protein, but none of the other components of the SMN complex, interacts with FMRP *in vitro*.** *A*, *in vitro*-translated [^{35}S]methionine-labeled SMN, Gemin2, Gemin3, Gemin4, Gemin5, Gemin6, Gemin7, Gemin8, Unrip, as well as Snurportin1, which was used as a negative control, were incubated with recombinant GST-FMRP immobilized on beads (*lanes 11–20*). Recombinant GST immobilized on beads was used as a negative control (*lanes 21–30*). After extensive washes, the bound proteins were analyzed by SDS-PAGE and autoradiography. The *Total* lanes show 10% of each labeled protein used in binding reactions (*lanes 1–10*). *B*, *in vitro* translated [^{35}S]methionine-labeled FMRP, as well as SMN and Snurportin1, which were used, respectively, as positive and negative controls, were incubated with recombinant GST-SMN immobilized on beads (*lanes 4–6*). Recombinant GST immobilized on beads was used as a negative control (*lanes 7–9*). Following extensive washes, the bound proteins were analyzed by SDS-PAGE and autoradiography. The *Total* lanes show 10% of each labeled protein used in binding reactions (*lanes 1–3*).

were produced by *in vitro* transcription-translation using a rabbit reticulocyte lysate (Fig. 4). To exclude RNA-mediated interactions, the *in vitro* translated products were treated with RNase A. As a control, a similar experiment was performed with Snurportin1, which is known to associate *in cellulo* with the SMN complex through an interaction with spliceosomal U snRNAs (36). As shown in Fig. 4*A*, in contrast to the other components of the SMN complex or Snurportin1 (*lanes 12–20*), SMN bound to GST-FMRP (*lane 11*). However, one cannot exclude the existence of a faint association of FMRP with components of the SMN complex other than SMN, which could not be detected in our experimental conditions. None of the tested proteins showed binding to GST (*lanes 21–30*), except Gemin8, which bound slightly and unspecifically to both GST-FMRP and GST (*lanes 18* and *28*). The association between SMN and FMRP was also observed when a [^{35}S]methionine-labeled SMN protein was produced by *in vitro* transcription-translation in *E. coli* extract (data not shown). In a reverse experiment, a recombinant GST-SMN protein was immobilized on glutathione-Sepharose beads and incubated with [^{35}S]methionine-labeled SMN, FMRP, or Snurportin1 produced by *in vitro* transcription-translation in a rabbit reticulocyte lysate. The labeled SMN protein was used as a positive control because SMN self-associates (96, 97). As shown in Fig. 4*B*, in contrast to Snurportin1, both FMRP and SMN associated with GST-SMN. None of these proteins showed binding to GST alone. These experiments indicated that FMRP associates *in vitro* with SMN and that this association is not mediated by RNAs.

A COOH-terminal Region of FMRP Is Necessary for Interaction with SMN—SMN interacts directly with the arginine- and glycine-rich domains of several proteins including the Sm proteins, fibrillarin, GAR1, coilin, and hnRNP Q/R (reviewed in Ref. 32). The COOH-terminal domain of FMRP also contains an arginine- and glycine-rich domain called the RGG box (98, 99) and several RG and GR repeats are present downstream from the RGG box (RG/GR repeats, amino acids 470 to 485) (Fig. 5*A*). Moreover, several domains of FMRP are already known to mediate interactions with protein partners, *i.e.* the region encoded by exon 7 is necessary for FXR1P, FXR2P, CYFIP1, and CYFIP2 binding, as well as for FMRP dimerization (100, 101), the region encoded by exons 4 and 5 within the NDF region (NH_2-terminal domain of FMRP) (amino acids 66–134) is necessary for NUFIP and 82-FIP binding (102, 103), the region spanning amino acids 490–526 is necessary for MSP-58 binding (104), and the FMRP COOH-terminal region (amino acids 419 to 632) is sufficient for Ran-BPM binding (105).

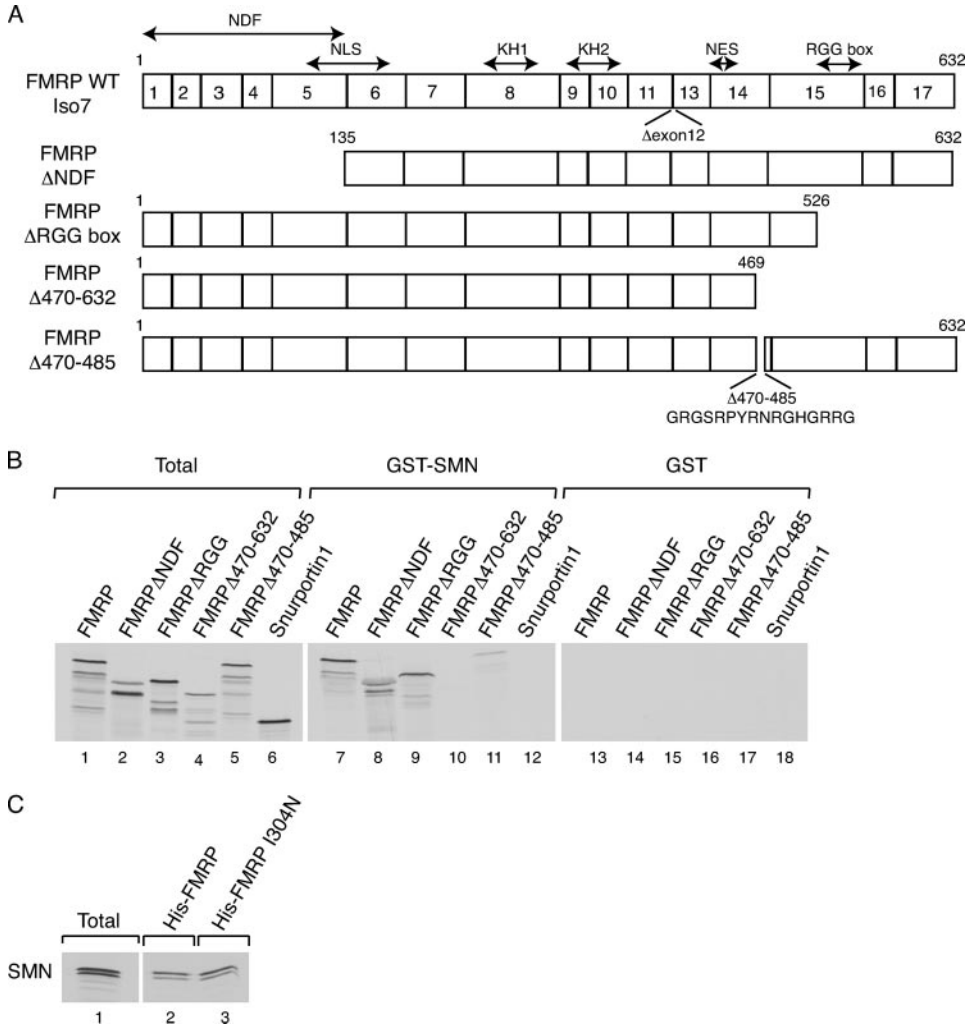


FIGURE 5. **A small COOH-terminal region of FMRP is necessary for interaction with SMN.** *A*, schematic representation of FMRP wild-type and deletion mutants used in binding assays. Regions denoted *1–17* correspond to the regions encoded by exons 1–17. Wild-type FMRP Iso7 has been used. It lacks the region encoded by exon 12 and is the most frequent FMRP isoform (150). *NDF* (NH_2-terminal domain of FMRP), *NLS* (nuclear localization signal), *NES* (nuclear export signal), *KH1*, *KH2*, and the *RGG* box are indicated. The sequence spanning amino acids 470–485 is shown. *B*, *in vitro* translated [^{35}S]methionine-labeled wild-type FMRP or the indicated deletion mutants were incubated either with recombinant GST-SMN immobilized on beads (*lanes 7–12*), or with GST alone used as a negative control (*lanes 13–18*). Following extensive washes, the bound proteins were analyzed by SDS-PAGE and autoradiography. The *Total* lanes show 10% of the labeled proteins used in each binding reaction (*lanes 1–6*). *C*, *in vitro* translated [^{35}S]methionine-labeled wild-type SMN was incubated with either recombinant His-FMRP (*lane 2*) or His-FMRP I304N mutant (*lane 3*) immobilized on beads. Following extensive washes, the bound proteins were analyzed by SDS-PAGE and autoradiography. The *Total* lanes show 10% of labeled SMN protein used in each binding reaction (*lane 1*).

To determine which of the FMRP domain(s) is/are important for binding to SMN, a series of truncated versions of the FMRP protein were produced by *in vitro* translation in the presence of [^{35}S]methionine (Fig. 5*A*). The wild type and mutated FMRP were incubated with an immobilized GST-SMN fusion protein. The GST protein alone was used as a negative control. As shown in Fig. 5*B*, deletion of the COOH-terminal 106 amino acids of FMRP (amino acids 527–632) (FMRPΔRGG, *lane 9*), including the RGG box, as well as deletion of the NH_2-terminal domain (FMRPΔNDF, amino acids 1–134, *lane 8*) did not impair the association with SMN. In contrast, deletion of amino acids 470–632 (FMRPΔ470–632, *lane 10*) abolished the interaction and deletion of the RG/GR repeats (FMRPΔ470–485, *lane 11*) reduced it significantly. These results indicated that the FMRP region spanning amino acids 470–526 is necessary for binding to SMN and that the RG/GR repeat domain of FMRP (amino acids 470–485) is important but not essential for the association.

The point mutation I304N within the FMRP KH2 domain is known to lead to a severe mental retardation phenotype (106). FMRP protein carrying this mutation does not interact with RNAs harboring a "kissing complex" and does not associate with polyribosomes (107, 108). Therefore, we compared the capability of immobilized recombinant FMRP and FMRP I304N proteins to associate with *in vitro* translated [^{35}S]methionine-labeled SMN protein. We observed that the I304N mutation did not impair association with SMN *in vitro* (Fig. 5*C*, compare *lane 3* with *lane 2*).

The symmetric dimethylarginines present within the RG-rich domain of several SMN-interacting proteins, *i.e.* SmB, SmD1, SmD3, Lsm4, and coilin, strongly enhance their binding to SMN (109–112). As FMRP contains monomethylated and asymmetrically dimethylated arginines in its RGG box and perhaps in other regions (113, 114), we compared the binding of SMN to methylated and unmethylated forms of FMRP and showed that FMRP methylation is not essential for efficient association of FMRP with SMN (data not shown).

Association of SMN with FMRP Requires the Conserved SMN YG Box and Is Defective for SMNΔEx7—Three regions of SMN are particularly conserved throughout evolution, *i.e.* the region encoded by exon 2A, the Tudor domain, and the YG box (Fig. 6*A*). These three regions have been implicated in SMN oligomerization and SMN binding to several proteins (reviewed in Refs. 8 and 32). Most of the mutations found in the *SMN1* gene of SMA patients are clustered in these three domains (115). To define the SMN domain(s) that is required for binding to FMRP, we tested the association of *in vitro* translated SMN deletion mutants with an immobilized GST-FMRP fusion protein or with GST alone. As shown in Fig. 6, SMNΔN27, lacking the NH_2-terminal 27 amino acids, bound to FMRP as efficiently as wild-type SMN (*lane 9*). The same result was obtained for SMNΔEx2B deleted of the amino acids encoded by exon 2B (Fig. 6, *lane 10*) and SMNΔTudor deleted of the amino acids encoded by exon 3 (amino acids 92–144)

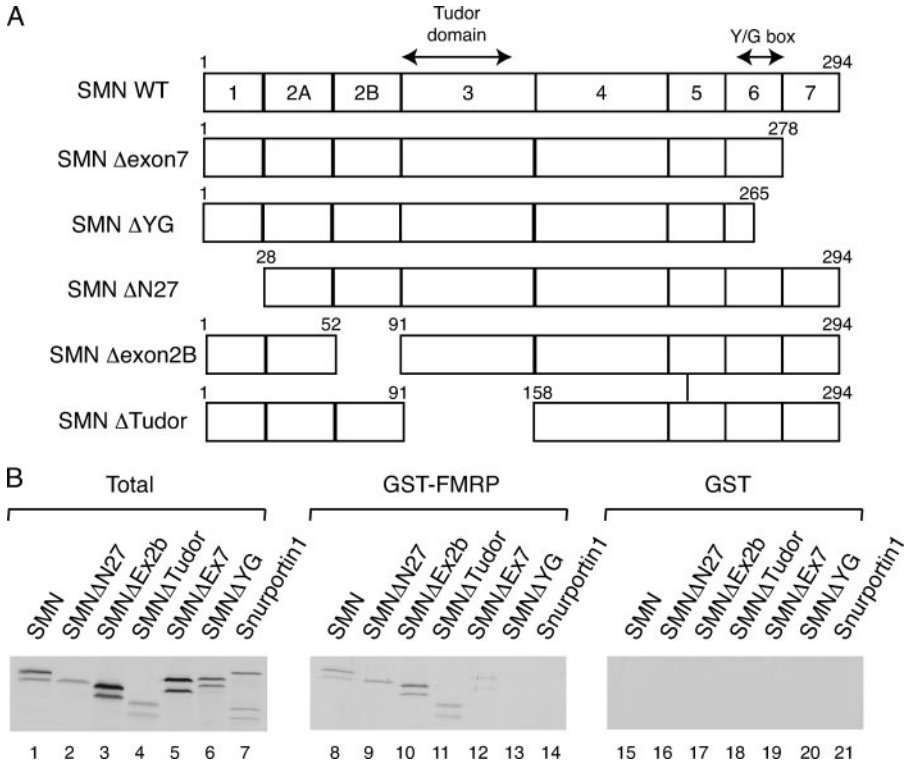


FIGURE 6. **The association of SMN with FMRP requires the COOH-terminal domain of SMN.** *A*, schematic representation of SMN wild-type and deletion mutants used in binding assays. Regions denoted *1–7* correspond to the regions encoded by exons 1–7. The Tudor domain and the YG box are indicated. *B*, *in vitro* translated [^{35}S]methionine-labeled wild-type SMN or the indicated mutants were incubated with recombinant GST-FMRP immobilized on beads (*lanes 8–14*), or with GST alone used as a negative control (*lanes 15–21*). Following extensive washes, the bound proteins were analyzed by SDS-PAGE and autoradiography. The *Total* lanes show 10% of labeled proteins used in each binding reaction (*lanes 1–7*).

(Fig. 6, *lane 11*). In contrast, deletion of the COOH-terminal 26 amino acids of SMN (SMNΔYG), which encompasses the YG box, completely abolished the SMN interaction with FMRP (Fig. 6, *lane 13*). Moreover, we showed that SMN binding to FMRP is strongly reduced by deletion of the region encoded by exon 7 (SMNΔExon7, Fig. 6, *lane 12*). None of the tested proteins showed binding to GST (Fig. 6, *lanes 15–21*). These results demonstrated that the highly conserved YG box is necessary for SMN association with FMRP. Moreover, SMNΔEx7, the main product of the *SMN2* gene, is severely impaired in FMRP association.

DISCUSSION

Here, we show that the SMN and FMRP proteins, which both play a very important role in dendrite and axon morphogenesis and activity, interact *in vitro* and in extracts from the human neuroblastoma SH-SY5Y cell line and from the murine motor neuron MN1 cell line, and partially colocalize in cell bodies and processes of rat primary neurons of hypothalamus in culture. As discussed below, these results open the question of possible functional links between these two proteins in neuronal mRNA transport and translation regulation, and/or in the plasticity of actin cytoskeleton in growth cones and synapses.

SMN and FMRP May Interact Together in Neuronal mRNP Granules—The highly polarized neuronal cells use specific localization and translation of mRNAs to respond rapidly to a local requirement of specific proteins and to regulate gene expression independently in different parts of the cell. Translation of localized mRNAs in growth cones is crucial for axon guidance and synapse formation, and the local translation in dendrites contributes to synaptic plasticity (54–56, 58). The mRNAs are transported along neuronal processes in an untranslatable form within RNP granules (for reviews, see Refs. 116 and 117). Although FMRP is predominantly found in the cell body of neuronal cells, a small fraction is associated with trafficking mRNP granules in neuronal processes and is localized at growth cones and synapses (71, 72, 89, 90, 118, 119). As the knockout of the *FMR1* gene encoding FMRP induces subtle changes both in location and abundance of several neuronal specific mRNAs (120), FMRP was proposed to play a role for specific mRNA transport in neurons. Based on its ability to limit translation efficiency *in vitro*, FMRP was also proposed to be involved in silencing of some of the mRNAs that are transported in mRNP granules (for reviews, see Refs. 65 and 121). Previous studies have revealed a punctuate and granular distribution of SMN in the cytoplasm of neuronal cells. More precisely, its localization extends throughout processes and into growth cones (47, 51–53). SMN granules in neurites show frequent colocalization with ribosomal RNAs and lack spliceosomal Sm proteins (51, 53). The present data reveal the presence of granules containing either one or both of the SMN and FMRP proteins within cell bodies and processes of rat primary neurons of hypothalamus in culture. Therefore, a subset of these two proteins may be transported together in some of the granules. Based on our observation of an ability of FMRP to associate with SMN, we can imagine a physical association of the two proteins within the granules. It will be interesting to know whether colocalization of the two proteins in granules depends upon the domains required for their interaction. According to previous data, some of the SMN-containing granules also contain Gemin proteins (47, 52, 53). Therefore, our observation of the ability of FMRP to interact with the purified SMN complex and the *in vitro* translated SMN protein alone opens the question of an interaction of FMRP with SMN alone and/or the SMN complex in the neuronal granules.

FMRP was already shown to be part of large mRNP complexes containing several proteins that interact directly or indirectly with FMRP (for reviews, see Refs. 65 and 69). However, none of the proteins identified up to now corresponds to SMN. The absence of previous detection of the SMN protein may be due to the fact that not all of the FMRP-containing granules

contain SMN. Interestingly, beside FMRP, several proteins known to associate directly or indirectly with SMN were found to be present in RNA transport granules purified from neuronal and non-neuronal cells, *i.e.* hnRNP Q, hnRNP R, RNA helicase A, and nucleolin (49, 60, 122–128). It will be important to analyze whether these proteins are present in the FMRP/SMN-containing granules. Altogether, our observations suggest the existence of different classes of mRNP granules in neuronal processes or a dynamic composition of these granules.

Association of SMN and FMRP in mRNP Granules May Take Place in the Nucleus or Cytoplasm—Although FMRP is predominantly cytoplasmic, it may already interact with mRNAs in the nucleus and play an active role in the export of specific mRNAs in the cytoplasm. This hypothesis has been recently strengthened by the discovery of an interaction between FMRP and NXF2, an mRNA nuclear export factor (129). In neurons, once the FMRP-containing mRNP complexes reach the cytoplasm, they are directed to a so-called "triage center," which in turn directs them to be translated or to be transported to neurites as translationally silent mRNP granules (for review, see Ref. 65). FMRP associates with specific neuronal mRNAs either directly or indirectly through interaction with non-coding RNAs (64, 108, 130–135). As it has already been proposed for other FMRP-interacting proteins (65, 105), the SMN complex or the SMN protein alone may modulate the affinity of FMRP for different classes of mRNAs by inducing structural changes of the FMRP conformation.

Interestingly, Unrip, one of the components of the SMN complex, is also able to associate *in vitro* and *in vivo* with the mRNA nuclear export NXF family proteins (at least NXF1/TAP, NXF2, and NXF7), as well as with the brain-specific microtubule-associated protein MAP1B (136). The MAP1 family of proteins are supposed to be molecular adaptors linking specific proteins to microtubules (for review, see Ref. 137). It is therefore possible that the SMN complex via Unrip could play a role in coupling nuclear export of mRNP complexes mediated by NXF proteins with subsequent trafficking both in non-neuronal and neuronal cells (136). In addition, SMN together with its protein partner hnRNP R, were found to interact with β-actin mRNA and are both essential for transport of this mRNA to growth cones of motor neurons (49, 50). Furthermore, the SMN complex is known to be involved in U snRNP assembly and is expected to play a role in the assembly of other RNP complexes (for reviews, see Refs. 23–26). An interesting possibility is that the SMN complex plays a role in the assembly of some specific mRNPs, for instance, some FMRP-containing mRNPs.

Based on all these considerations and on the data presented in this work, we can imagine several scenario: (i) the SMN complex may be involved in assembly of FMRP-containing mRNPs in the nucleus and, either the entire complex, or the SMN protein alone, may remain bound to some of these mRNPs throughout their transport. By interaction with the NXF protein family, FMRP and the SMN complex may contribute together to the mRNP export from the nucleus, (ii) some of the mRNP complexes may be associated with only FMRP in the nucleus, FMRP interaction with NXF2 may facilitate nuclear export and then some of the exported mRNPs may bind to the SMN complex or the SMN protein in the cytoplasm, and (iii) vice versa, some mRNP complexes may be associated with the SMN complex in the nucleus, Unrip may favor export through its interaction with NXF proteins and FMRP may associate in the cytoplasm. A more thorough analysis of the granule content in the various cellular compartments will be required to choose among these various possibilities.

SMN and FMRP May Have Complementary Activities on Actin Filament Organization in Neurons—Whereas a large number of mRNAs have been identified *in vitro* and *in vivo* as potential FMRP targets (Refs. 83, 120, 131, and 132; for reviews, see Refs. 138 and 139), only an involvement of SMN in the transport and regulation of the translation of β-actin mRNA in axons have been described (49, 50). This regulation by SMN may be mediated by its interaction with hnRNP R that interact with β-actin mRNA *in vitro* (50). It was shown that hnRNP R overexpression enhances β-actin mRNA localization in axons and, conversely, reduced localization of β-actin mRNA and protein was detected in axonal growth cones of motor neurons cultured from the SMA transgenic mouse model (50). In addition to its role in β-actin production at growth cones, SMN was proposed to have a direct role in the regulation of actin polymerization through its interaction with profilin IIa (52, 140). Very interestingly, it was recently shown that dFMRP, the *Drosophila* homologue of FMRP, can bind the profilin mRNA and negatively regulate its translation (141). Moreover, FMRP was also proposed to play an important role in remodeling the actin cytoskeleton by its interaction with CYFIP1, a partner of the Rho GTPase Rac1 (101, 142, 143). The Rho GTPase pathway regulates actin dynamics in response to extracellular stimuli and is crucial in neurons for neurite outgrowth as well as for synapse development (for review, see Ref. 144). In addition, FMRP inhibits translation of the mRNA of the phosphatase 2A catalytic subunit (145), which is also a crucial effector of the Rac1 signaling pathway. Therefore, it is possible that the physical link that we discovered between SMN and FMRP plays a role in the synchronization of their functions on actin dynamic in dendrites and axons.

SMN and FMRP Interact Through Their COOH-terminal Domains—We showed that the COOH-terminal region of FMRP from positions 470 to 526 is essential for the association with SMN. Within this region, the RG/GR repeats (positions 470–485) seem to be important. However, in contrast to the Sm proteins or coilin (109–112), methylation of FMRP is not essential for the interaction. A similar observation was already made for SMN association with GAR1 and fibrillarin (146). Interestingly, an interaction of SMN with the COOH-terminal domain of FMRP is not expected to alter FMRP interaction with RNAs mediated by the KH domains, and with the FXR1P, FXR2P, CYFIP1, and CYFIP2 proteins. Indeed, these interactions require the region encoded by exon 7 of FMRP (100, 101). In addition, FMRP in association with SMN can probably self-associate because the dimerization domain is located also in the region encoded by exon 7 (100). However, as the region spanning amino acids 490–526 is necessary for MSP-58 binding (104) and the COOH-terminal region (amino acids 419 to 632) is needed for Ran-BPM binding (105), interaction of FMRP with these proteins may be exclusive of the SMN interaction. As the FMRP region necessary for its association with SMN

encompasses the FMRP phosphorylation domain (147–149), the level of FMRP phosphorylation may modulate *in vivo* the interaction with SMN. Importantly, truncated SMN proteins (SMNΔEx7 and SMNΔYG) that are expressed in patients suffering of SMA have no or very low capability to associate with FMRP. In conclusion, although the best known role of the SMN complex is its function in the assembly of U snRNP, recent studies (for reviews, see Refs. 2, 43, and 44) and the present data strongly suggest completely different additional roles of SMN in neuronal cells in localized translation and/or actin metabolism.

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