Gemin8 Is Required for the Architecture and Function of the Survival Motor Neuron Complex*1

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The biogenesis of spliceosomal small nuclear ribonucleoproteins (snRNPs) in higher eukaryotes requires the functions of several cellular proteins and includes nuclear as well as cytoplasmic phases. In the cytoplasm, a macromolecular complex containing the survival motor neuron (SMN) protein, Gemin2–8 and Unrip mediates the ATP-dependent assembly of Sm proteins and snRNAs into snRNPs. To carry out snRNP assembly, the SMN complex binds directly to both Sm proteins and snRNAs; however, the contribution of the individual components of the SMN complex to its composition, interactions, and function is poorly characterized. Here, we have investigated the functional role of Gemin8 using novel monoclonal antibodies against components of the SMN complex and RNA interference experiments. We show that Gemin6, Gemin7, and Unrip form a stable cytoplasmic complex whose association with SMN requires Gemin8. Gemin8 binds directly to SMN and mediates its interaction with the Gemin6/Gemin7 heterodimer. Importantly, loss of Gemin6, Gemin7, and Unrip interaction with SMN as a result of Gemin8 knockdown affects snRNP assembly by impairing the SMN complex association with Sm proteins but not with snRNAs. These results reveal the essential role of Gemin8 for the proper structural organization of the SMN complex and the involvement of the heteromeric subunit containing Gemin6, Gemin7, Gemin8, and Unrip in the recruitment of Sm proteins to the snRNP assembly pathway.

Pre-mRNA splicing is a key step in the pathway of eukaryotic gene expression. The accurate excision of introns and joining of exons of pre-mRNAs is carried out by the spliceosome, a dynamic macromolecular assembly of hundreds of proteins and a few RNAs (1) whose essential constituents are the small nuclear ribonucleoprotein (snRNP)2 particles. Despite steady-state localization in the nucleus, where they function in pre-mRNA splicing, the biogenesis pathway of snRNPs involves an ordered series of events that take place in both the cytoplasm and the nucleus of higher eukaryotes (2). Spliceosomal snRNPs contain an snRNA molecule, a set of common proteins known as Sm proteins and specific proteins unique for each snRNP. The hallmark of snRNPs is a ring structure of seven Sm proteins around a single-stranded region of the snRNAs (3) called the Sm-core and Sm-site, respectively. A large macromolecular complex containing the survival motor neuron (SMN) protein and at least eight additional integral components, which are tightly associated by a network of protein-protein interactions and include Gemin2–8 and Unrip proteins (4–12), takes center stage in the process of Sm core formation (13, 14).

Precursor snRNAs are transcribed in the nucleus by RNA polymerase II and exported to the cytoplasm by the concerted action of the cap-binding complex, PHAX, CRM1, and RanGTP (15). In the cytoplasm, newly translated Sm proteins associate with pICln and the PRMT5 complex and, following symmetrical dimethylation of arginine residues of a subset of Sm proteins by the PRMT5 complex, are transferred to the SMN complex (16–18). The pool of cytoplasmic SMN complexes bound to the seven Sm proteins represents the functional unit competent for snRNP assembly in cells. These SMN complexes bind directly to specific domains of the snRNAs exported from the nucleus and carry out the ATP-dependent assembly of the Sm core (19–21). Cap hypermethylation and trimming of the 3′-end extension of snRNAs follow snRNP assembly and precede import in the nucleus, where snRNPs undergo further maturation steps before functioning in pre-mRNA splicing (2). There is evidence that the SMN complex also plays a direct role in the nuclear import of newly assembled snRNPs and possibly in other steps of snRNP biogenesis (22–25).

In a similar way to the well established role of protein chaperones, the SMN complex is thought to impose efficiency and specificity to the process of Sm core formation on snRNAs and to overcome spontaneous but potentially promiscuous association of Sm proteins with other RNAs (20). This is likely accomplished through the direct and independent interaction of the SMN complex with both Sm proteins and snRNAs (13). Recent studies showed that knockdown of SMN and several Gemin proteins affects snRNP assembly, but in most cases, the molecular defects responsible for reduced SMN complex activity have not been characterized (10, 26–29). Therefore, despite steady progress in the characterization of the SMN complex activity in snRNP biogenesis and the potential implications of its deficiency in the pathophysiology of the human inherited motor neuron disease spinal muscular atrophy (27, 30, 31), the contri-
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bution of the integral components of the SMN complex to its structure, interactions, and function is poorly understood. Here, we have investigated the functional role of Gemin8 within the SMN complex.

EXPERIMENTAL PROCEDURES

DNA Constructs—Plasmids encoding for epitope-tagged SMN, Gemin2, Gemin6, Gemin7, Gemin8, and Unrip have been described previously (9–11). The open reading frame of His$_6$-tagged Gemin8 was cloned into the SalI and NotI sites of pCDF-Duet1 vector (Novagen). All constructs were analyzed by automated DNA sequencing.

Antibodies—For production of monoclonal antibodies, BALB/c female mice were primed with Immunexy adjuvant (Qiagen) and 25 μg of GST-Gemin6/His$_6$-Gemin7, GST-Unrip, or His$_6$-SmB purified recombinant proteins. Following two boosts at two-week intervals, SP2 myeloma cells were fused with mouse splenocytes, and hybridoma supernatants were analyzed onto antigen-coated amino silane-modified slides using an LS400 Scanner (Tecan) and the GenePix Pro version 4.1 software as described previously (32). Selected hybridoma cell lines were further screened by enzyme-linked immunosorbent assay, Western blot, and immunofluorescence analysis of HeLa cells and subcloned by limiting dilution. Other antibodies used were anti-Unrip 3G6 (10), anti-Gemin8 1F8 (10), anti-SMN clone 8 (BD Transduction Laboratories), anti-p300 (Zymed Laboratories Inc.), and purified antibodies in binding buffer (200 mM NaCl, 50 mM Tris-HCl, pH 7.4, 2 mM EDTA) containing 0.1% Nonidet P-40 and EDTA-free protease inhibitor mixture (Roche Applied Science) as previously described (10).

Sucrose Gradient Centrifugation and Immunoprecipitation Experiments—HeLa cell extracts were prepared in RSB-100 buffer (100 mM NaCl, 10 mM Tris-HCl, pH 7.4, 2.5 mM MgCl$_2$) containing 0.1% Nonidet P-40, EDTA-free protease inhibitor mixture (Roche Applied Science), and phosphatase inhibitors (20 mM NaF, 0.2 mM Na$_3$VO$_4$). Centrifugation of HeLa cell extracts on 10–30% sucrose gradients and immunoprecipitation experiments were carried out as previously described (10).

HeLa Cell Fractionation—For preparation of cytoplasmic and nuclear extracts, HeLa cells were washed with cold phosphate-buffered saline and then resuspended gently in cytoplasmic buffer (10 mM KCl, 10 mM Tris-HCl, pH 7.4, 1 mM MgCl$_2$, 1 mM dithiothreitol) containing 0.01% Nonidet P-40, EDTA-free protease inhibitor mixture (Roche Applied Science), and phosphatase inhibitors (10 mM NaF, 0.2 mM Na$_3$VO$_4$). After swelling for 10 min on ice, the cells were passed five times through a 25-gauge needle and centrifuged at 4000 revolutions/min for 1 min at 4 °C to separate the cytoplasm (supernatant) from nuclei (pellet). The supernatant was collected and, after adjusting the salt concentration to RSB-100 containing 0.1% Nonidet P-40, centrifuged again at 10,000 revolutions/min for 15 min at 4 °C to obtain soluble cytoplasmic HeLa extract. The nuclei were washed gently with cytoplasmic buffer and centrifuged again at 4000 revolutions/min for 5 min at 4 °C. The supernatant was then discarded, and the nuclei were resuspended in RSB-100 containing 0.1% Nonidet P-40 and protease and phosphatase inhibitors. Following brief sonication, the extract was centrifuged at 10,000 revolutions/min for 15 min at 4 °C to obtain soluble nuclear HeLa extract.

In Vitro snRNP Assembly and snRNA Binding Experiments—U1, U1ΔSm, SL1, and SL1A3 RNAs were in vitro transcribed in the presence of [α$^32$P]UTP (3000 Ci/mmol) and purified from denaturing polyacrylamide gels according to standard procedures. Bead-bound SMN complexes immunopurified using anti-SMN (7F3) antibodies from HeLa cell extracts or sucrose

rescence microscopy was performed with an Olympus AX70 microscope equipped with a SPOT digital camera (Diagnostic Instruments).

Protein Production and in Vitro Binding Experiments—All recombinant proteins were expressed in Escherichia coli BL21(DE3) (Invitrogen) and purified by affinity chromatography on either nickel-chelated agarose (Pierce) or glutathione-Sepharose (Amersham Biosciences). GST-TEV-Gemin6 and His$_6$-Gemin7 (or GST-TEV-Gemin2 and His$_6$-SMN) proteins were co-expressed and purified on glutathione-Sepharose (9). Gemin6/His$_6$-Gemin7 and Gemin2/His$_6$-SMN protein complexes were eluted from glutathione-Sepharose beads by cleavage with recombinant AcTEV protease (Invitrogen) and further purified by affinity chromatography on nickel-chelated agarose (10). GST or GST-Gemin2/His$_6$-SMN recombinant proteins were also co-expressed with His$_6$-Gemin8 and purified on glutathione-Sepharose. In vitro binding experiments were carried out using 2 μg of GST or GST-tagged proteins bound to glutathione-Sepharose beads and 1 μg of purified recombinant proteins in binding buffer (200 mM NaCl, 50 mM Tris-HCl, pH 7.4, 2 mM EDTA) containing 0.1% Nonidet P-40 and EDTA-free protease inhibitor mixture (Roche Applied Science) as previously described (10).
gradient fractions were analyzed for snRNP assembly or snRNA binding in reconstitution buffer (50 mM KCl, 20 mM Hepes-KOH, pH 7.9, 5 mM MgCl2, 0.2 mM EDTA, 5% glycerol) containing 0.01% Nonidet P-40 as previously described (10, 33).

RESULTS

Identification of a Cytoplasmic Complex Containing Gemin6, Gemin7, and Unrip—To further our understanding of the composition, structure, and function of the SMN complex, we generated novel monoclonal antibodies against Gemin6 (20H8), Gemin7 (5F1), Unrip (2G3), SMN (7F3), SmB (12F5), and plCln (clone 32) or control mouse immunoglobulins (mIgG). 5% of the input (Total) and the immunoprecipitates were analyzed by Western blot.

RESULTS

Identification of a Cytoplasmic Complex Containing Gemin6, Gemin7, and Unrip—To further our understanding of the composition, structure, and function of the SMN complex, we generated novel monoclonal antibodies against Gemin6 (20H8), Gemin7 (5F1), Unrip (2G3), and SmB (12F5) proteins. Western blot analysis shows that each of these antibodies recognizes the corresponding purified recombinant protein as well as a protein of the expected molecular weight in HeLa cell extracts (supplemental Fig. S1). Immunoprecipitation experiments from HeLa cell extract show that antibodies against Gemin6, Unrip, and SmB, similar to SMN antibodies used as a reference, specifically co-immunoprecipitate all the known integral components of the SMN complex (Fig. 1). Strikingly, the anti-

Gemin7 antibody 5F1 co-immunoprecipitates Gemin6, Gemin7, and Unrip but none of the other components of the SMN complex (Fig. 1). Furthermore, immunofluorescence analysis of HeLa cells with the anti-Gemin7 antibody 5F1 shows cytoplasmic and very weak nucleoplasmic staining but no co-localization with SMN in nuclear Gems (Fig. 2, A—C), whereas the anti-Gemin6 antibody 20H8 displays the expected subcellular localization pattern of SMN complex components (Fig. 2, D—F). These results suggest that the Gemin7 epitope recognized by 5F1 is masked in the SMN complex but accessible in a distinct protein complex containing Gemin6, Gemin7, and Unrip.

To address this possibility, we carried out sucrose gradient centrifugation of HeLa cell extracts and immunoprecipitation experiments (Fig. 3). SMN shows the typical heterodisperse distribution in large macromolecular complexes that also contain Gemin6, Gemin7, Gemin8, and Unrip as well as the other components of the SMN complex (Fig. 3, A and B) (10, 11, 34). In contrast, immunoprecipitation with anti-Gemin7 antibodies demonstrates that Gemin6, Gemin7, and Unrip found in slow-sedimenting fractions at the top of the gradient are associated in distinct protein complexes that do not contain SMN or other Gemin proteins (Fig. 3C and data not shown). This finding was further confirmed by immunoprecipitation with anti-Gemin6 and anti-Unrip antibodies, although they also immunoprecipitated SMN complexes (Fig. 3D and data not shown). Importantly, SmB is associated with the entire spectrum of SMN complexes, which are also competent for snRNP assembly (supplemental Fig. S2), but not with the Gemin6-Gemin7-Unrip complex (Fig. 3E).

Next, we sought to investigate the subcellular localization of the Gemin6-Gemin7-Unrip complex. To do so, we carried out immunoprecipitation experiments from cytoplasmic and nuclear HeLa extracts (Fig. 4). The accuracy of cellular fractionation is indicated by proper distribution of poly(A)-binding protein and p300 in the cytoplasmic and nuclear fractions, respectively. Immunoprecipitation with the anti-Gemin7 antibody 5F1 demonstrates that the Gemin6-Gemin7-Unrip protein complex is mostly, if not exclusively, present in the cytoplasm of HeLa cells (Fig. 4). Conversely, as indicated by immunoprecipitation with either anti-SMN or -Gemin6 monoclonal antibodies, Gemin6, Gemin7, and Unrip proteins are associated with SMN in both cytoplasmic and nuclear SMN complexes. Altogether, these results demonstrate that Gemin6, Gemin7, and Unrip form a heteromeric protein complex separate from the SMN complex and present in the cytoplasm of HeLa cells.

Gemin8 Bridges SMN to the Gemin6/Gemin7 Heterodimer—Gemin8 is not found in the Gemin6-Gemin7-Unrip complex identified above, despite its direct interaction with the Gemin6/Gemin7 heterodimer (10). In search for additional Gemin8 interactions, we found that recombinant SMN binds directly and specifically to immobilized GST-Gemin8 but not to GST (Fig. 5A). In the reciprocal experiment, recombinant Gemin8 binds to GST-Gemin2/SMN, although with a lower efficiency compared with GST-Gemin6/Gemin7, but neither to GST nor to GST-Gemin2 alone (Fig. 5B). Therefore, Gemin8 interacts directly with both SMN and the Gemin6/Gemin7 heterodimer. To test whether these interactions occur simultaneously, we
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To carry out snRNP assembly, the SMN complex interacts directly and independently with Sm proteins and snRNAs (13). First, we investigated the potential role of Gemin8 in snRNA binding by using stem loop 1 (SL1) of U1, which binds the SMN complex, and the SL1A3 mutant containing three point mutations as a control (33). Immobilized SMN complexes purified from HeLa cell extracts following Gemin8 knockdown by RNA interference displayed no difference in their ability to interact with SL1 compared with the control (Fig. 8A). These results indicate that Gemin6, Gemin7, Gemin8, and Unrip do not contribute to the snRNA binding activity of the SMN complex. These results are in good agreement with the recent identification of Gemin5 as the snRNA-binding protein of the SMN complex (29), which was reported when our paper was in the final stage of preparation.

Next, we analyzed the effect of Gemin8 knockdown on the association of Sm proteins with the SMN complex. HeLa cells were transfected with control or Gemin8 siRNAs and then pulse-labeled with [35S]methionine before immunoprecipitation of SMN complexes with anti-SMN antibodies. Remarkably, and in addition to that of Gemin6, Gemin7, and Unrip, Gemin8 knockdown affects the association of Sm proteins with the SMN complex (Fig. 8B). Immunoprecipitation with anti-SmB antibodies further highlighted the specific impairment of SmB association with SMN but not with the PRMT5 complex upon Gemin8 knockdown (Fig. 8C). These results indicate that Gemin6, Gemin7, Gemin8, and Unrip are required for the efficient association of Sm proteins with the SMN complex.

**DISCUSSION**

The SMN complex is the large macromolecular machine employed by cells for the assembly of spliceosomal snRNPs (13, 14). Although there is evidence linking impaired snRNP biogenesis to motor neuron degeneration, additional SMN functions have also been implicated in spinal muscular atrophy (35). Detailed knowledge of the SMN complex activities and the function of its integral components are needed to elucidate a fundamental cellular process, such as the biogenesis of the splicing apparatus, and to unravel the pathophysiology of spinal muscular atrophy. Here, we have analyzed the contribution of Gemin8 to the composition, interactions, and function of the SMN complex. We found that Gemin6, Gemin7, and Unrip form a distinct protein complex and that Gemin8 is essential for their efficient association with the SMN complex, which is likely accomplished through the direct and independent interaction of Gemin8 with both SMN and the Gemin6/Gemin7 heterodimer. Importantly, Gemin6, Gemin7, Gemin8, and

**FIGURE 2. Immunofluorescence analysis of HeLa cells with novel monoclonal antibodies against Gemin6 and Gemin7.** A–C, indirect immunofluorescence analysis of HeLa cells double-labeled with anti-Gemin7 (5F1) and anti-SMN (7F3) antibodies. D–F, indirect immunofluorescence analysis of HeLa cells double-labeled with anti-Gemin6 (20H8) and anti-SMN (7F3) antibodies. Combined images and 4',6-diamidino-2-phenylindole (DAPI) staining of nuclei are shown in C and F. Scale bars represent 5 μm.

purified GST or GST-Gemin2/SMN recombinant proteins from E. coli either in the presence or in the absence of co-expressed Gemin8. Gemin8 specifically co-purifies with GST-Gemin2/SMN but not with GST (Fig. 5C). We then analyzed the capacity of Gemin6/Gemin7 heterodimers to bind these protein complexes and found that they interact specifically and efficiently with Gemin2/SMN only in the presence of Gemin8 (Fig. 5C). These results demonstrate that Gemin8 mediates the association of Gemin6/Gemin7 heterodimers with SMN.

**Gemin8 Is Required for SMN Association with Gemin6, Gemin7, and Unrip**—To investigate the role of Gemin8 in the association of Gemin6 and Gemin7 with SMN in vivo, we knocked down Gemin8 expression by RNA interference and analyzed the composition of SMN complexes isolated with anti-SMN antibodies from HeLa cell extracts. Strikingly, Gemin8 knockdown strongly affects the association of Gemin6, Gemin7, and Unrip with SMN, whereas there is little, if any, effect on SMN interaction with Gemin2–5 proteins (Fig. 6). There is also a noticeable reduction of Gemin6 and Gemin7 expression levels upon Gemin8 knockdown, which may be due to partial destabilization as a result of their impaired association with SMN. These results indicate that Gemin8 is required for the efficient association of Gemin6, Gemin7, and Unrip proteins with the SMN complex in vivo.

**Gemin8 Knockdown Affects SMN Association with Sm Proteins**—To investigate the consequence of Gemin8 knockdown on SMN complex activity, we carried out snRNP assembly using SMN complexes isolated with anti-SMN antibodies from HeLa cell extracts following Gemin8 RNA interference. Consistent with our previous results in cell extracts (10), loss of Gemin6, Gemin7, and Unrip association with the SMN complex as a result of Gemin8 knockdown severely impairs Sm core formation (Fig. 7), indicating that these proteins are required for SMN complex activity in snRNP assembly.
Unrip form a heteromeric subunit of the SMN complex required for the efficient association of Sm proteins and snRNP assembly activity. Altogether, these results highlight the essential role of Gemin8 for the architecture of the SMN complex and its function in snRNP assembly.

We have demonstrated that Gemin6, Gemin7, and Unrip are associated in a stable cellular complex separate from the SMN complex (Figs. 1 and 3). Importantly, this heteromeric complex is localized in the cytoplasm of HeLa cells (Figs. 2 and 4). Large pools of Gemin3, Gemin4, and Gemin5 also exist without SMN (11), and Gemin3 and Gemin4 were previously found to associate with Argonaute proteins and micro-RNAs (36). It is therefore becoming increasingly clear that several components of the SMN complex are part of additional multiprotein complexes and likely perform important cellular functions outside the SMN complex. Alterations in the expression and association of Gemin proteins with SMN, as is the case in cells of spinal muscular atrophy patients that have reduced levels of SMN, might therefore influence multiple cellular pathways either directly or indirectly. Although future studies are needed to shed light on these possibilities, it is conceivable that individual components and preformed multiprotein subunits with distinct functional properties are brought together by SMN to carry out specific tasks in the snRNP biogenesis pathway. To do so, SMN interacts directly with a subset of Gemin proteins (13, 14). Here, we uncovered a novel direct interaction between SMN and Gemin8 that, together with Gemin8 binding to the Gemin6/
Gemin7 heterodimer (10), is important for the association of Gemin6, Gemin7, and Unrip with the SMN complex (Figs. 5 and 6). Thus, the SMN complex is a dynamic platform for multiple interactions, and Gemin8 is essential for its structural integrity.

The SMN complex mediates Sm core formation by specifically bringing Sm proteins and snRNAs in close spatial proximity (13). Gemin5 is responsible for the interaction of the SMN complex with snRNAs (29). Direct binding of SMN and several Gemin proteins to distinct subsets of Sm proteins is thought to account for the association of the SMN complex with the seven Sm proteins (4–9, 11, 37). However, these conclusions are mostly based on in vitro binding studies, and it is unknown which of these interactions contribute to the association of Sm proteins and to snRNP assembly in vivo. The interaction between SMN and Sm proteins has been characterized in detail. High-affinity SMN binding to the arginine- and glycine-rich domains at the carboxyl terminus of SmB, SmD1, and SmD3 proteins requires SMN oligomerization and the Tudor domain (37–39). Moreover, symmetrical dimethylation of specific arginines by the PRMT5 complex strongly enhances SMN affinity for the arginine- and glycine-rich domains of Sm proteins (40, 41). Therefore, it is believed that SMN itself plays a crucial role in the recruitment of Sm proteins to the SMN complex. Here, we have shown that Gemin8 knockdown impairs the SMN complex association with Sm proteins and snRNP assembly activity (Figs. 7 and 8). Because Gemin8 does not bind Sm proteins in vitro (10), it is most likely that the reduced interac-

**FIGURE 5.** Gemin8 bridges SMN to the Gemin6/Gemin7 heterodimer. A, recombinant Gemin2/His6-SMN complexes were incubated with GST or GST-Gemin8 immobilized on beads. Input (5%) and bound proteins were analyzed by Western blot. B, recombinant His6-Gemin8 was incubated with GST, GST-Gemin2, GST-Gemin2/His6-SMN, or GST-Gemin6/His6-Gemin7 proteins immobilized on beads. Input (5%) and bound proteins were analyzed by Western blot. C, immobilized GST and GST-Gemin2/His6-SMN, purified from *E. coli* either in the presence (+) or in the absence (−) of co-expressed His6-Gemin8 as indicated, were incubated with recombinant Gemin6/His6-Gemin7 complexes. Input (10%) and bound proteins were analyzed by Western blot.

**FIGURE 6.** Gemin8 knockdown impairs SMN association with Gemin6, Gemin7, and Unrip. Extracts from HeLa cells transfected with siRNAs against Gemin8 or firefly luciferase (Control) were immunoprecipitated with anti-SMN (7F3) antibodies. 5% of the input (Total) and immunoprecipitates (IP) were analyzed by Western blot.
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FIGURE 7. Gemin8 knockdown impairs SMN complex activity in snRNP assembly. Extracts from HeLa cells transfected with siRNAs against Gemin8 or firefly luciferase (Control) were immunoprecipitated (IP) with anti-SMN (7F3) antibodies. Bead-bound SMN complexes were then incubated with radioactive U1 or U1ΔSm under snRNP assembly conditions and analyzed by electrophoresis on native gels and autoradiography. U1 RNP complexes containing the Sm core and U1A proteins are indicated.

FIGURE 8. Gemin8 knockdown affects SMN association with Sm proteins but not with snRNAs. A, extracts from HeLa cells transfected with siRNAs against Gemin8 or firefly luciferase (Control) were immunoprecipitated (IP) with anti-SMN (7F3) antibodies. Bead-bound SMN complexes were then incubated with radioactive SL1 or SL1A3 RNAs under snRNP assembly conditions. Input (5%) and bound RNAs were analyzed by electrophoresis on denaturing gels and autoradiography. B, HeLa cells were labeled with [35S]methionine 60 h post-transfection with siRNAs against Gemin8 or firefly luciferase (Control). Extracts from these cells were analyzed by immunoprecipitation with anti-SMN (7F3) antibodies, SDS-PAGE, and autoradiography. Asterisks mark nonspecific proteins also found in control immunoprecipitates with mouse immunoglobulins (data not shown). C, extracts from HeLa cells transfected with siRNAs against Gemin8 or firefly luciferase (Control) were analyzed by immunoprecipitation with anti-SmB (12F5) antibodies or control mouse immunoglobulins and Western blot.

with Sm proteins is because of the loss of the SMN complex association with Gemin6, Gemin7, and Unrip upon Gemin8 knockdown. Consistent with this possibility, it has been shown that both Gemin6 and Gemin7 have an Sm protein-like structure, which may facilitate the binding and/or organization of Sm proteins with the SMN complex in preparation for snRNP assembly (42). In principle, it is possible that the reduced association of Sm proteins with the SMN complex might reflect a change in the methylation status of Sm proteins upon Gemin8 knockdown. Several lines of evidence argue against this possibility. First, both Gemin8 and the Gemin6-Gemin7-Unrip complex do not associate with pICln or the PRMT5 complex (Fig. 1 and data not shown). Second, decreased Gemin8 expression does not affect Sm protein interaction with the PRMT5 complex (Fig. 8C). Third, no reduction in the methylation of SmB is detected upon Gemin8 knockdown by Western blot analysis with the monoclonal antibody Y12 (10), which recognizes the symmetrically dimethylated arginine- and glycine-rich domains of Sm proteins. One possible view is that the SMN complex relies on both the SmN protein, which interacts with the symmetrically dimethylated arginine- and glycine-rich domains of Sm proteins, and the Gemin6/Gemin7/Gemin8/Unrip heteromeric subunit to efficiently bind Sm proteins in vivo. Surprisingly, however, loss of symmetrical dimethylation of Sm proteins by genetic disruption of PRMT5 complex components in Drosophila does not affect snRNP biogenesis or function (43), indicating that this post-translational modification is dispensable for snRNP assembly in vivo. In conclusion, our findings point to the heteromeric subunit containing Gemin6, Gemin7, Gemin8, and Unrip as a major determinant for the association of Sm proteins with the SMN complex and therefore their recruitment to the snRNP biogenesis pathway.

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