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The SMN Complex Is Associated with snRNPs throughout Their Cytoplasmic Assembly Pathway

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The common neurodegenerative disease spinal muscular atrophy is caused by reduced levels of the survival of motor neurons (SMN) protein. SMN associates with several proteins (Gemin2 to Gemin6) to form a large complex which is found both in the cytoplasm and in the nucleus. The SMN complex functions in the assembly and metabolism of several RNPs, including spliceosomal snRNPs. The snRNP core assembly takes place in the cytoplasm from Sm proteins and newly exported snRNAs. Here, we identify three distinct cytoplasmic SMN complexes, each representing a defined intermediate in the snRNP biogenesis pathway. We show that the SMN complex associates with newly exported snRNAs containing the nonphosphorylated form of the snRNA export factor PHAX. The second SMN complex identified contains assembled Sm cores and m_3G-capped snRNAs. Finally, the SMN complex is associated with a preimport complex containing m_3G-capped snRNP cores bound to the snRNP nuclear import mediator snurportin1. Thus, the SMN complex is associated with snRNPs during the entire process of their biogenesis in the cytoplasm and may have multiple functions throughout this process.

The small nuclear ribonucleoprotein particles (snRNPs) consist of snRNAs (U1, U2, U4/U6, or U5), several specific proteins that are unique to each snRNA, and a set of seven common Sm proteins (B/B′, D1, D2, D3, E, F, and G). The biogenesis of the snRNPs is a complex process that involves both the nucleus and the cytoplasm (for a recent review, see reference 59). The snRNAs, with the exception of U6, are transcribed by RNA polymerase II as precursors that contain additional nucleotides at the 3′ end and a monomethylated m^7GpppG (m^7G) cap structure at the 5′ end. This cap structure is recognized by the nuclear cap binding complex (CBC), a heterodimeric complex composed of two subunits, CBP20 and CBP80, both of which are required for binding to the m^7G cap structure (30, 31, 35, 36). The adaptor protein PHAX binds both CBC and snRNAs and mediates their interaction with the nuclear export receptor CRM1/Exportin1 (Xpo1) (49, 57). CRM1, together with RanGTP, exports the newly transcribed snRNAs to the cytoplasm (9, 13, 29). In vitro, phosphorylation of PHAX is required for the formation of the snRNA export complex but is not necessary for the formation of the precomplex containing snRNAs, CBC, and PHAX but not RanGTP and CRM1 (49). Following export to the cytoplasm, GTP hydrolysis of Ran and dephosphorylation of PHAX lead to disassembly of the snRNA export complex (49). Each snRNA then associates with the Sm proteins, which form a seven-membered ring (snRNP core particle) around the Sm site (34, 59). A properly assembled Sm core is required for cap hypermethylation and 3′-end maturation (40, 48, 56). Both a properly assembled Sm core and an m_3G cap structure are required for snRNP import into the nucleus (11, 12, 24, 25, 33, 41). The m_3G cap structure is specifically bound by snurportin1, which then interacts with the nuclear import receptor importin-β and, together with an unidentified import receptor that recognizes the Sm core, mediates the import of the assembled snRNP (12, 28, 50).

The neuromuscular disease spinal muscular atrophy (SMA) is characterized by degeneration of motor neurons of the spinal cord leading to muscular weakness and atrophy (reviewed in reference 45). Over 98% of SMA patients have mutations or deletions of the survival of motor neurons 1 (*SMN1*) gene, and decreased levels of the SMN protein correlate with the phenotypic severity of SMA (7, 19, 27, 32, 45, 46). The SMN protein is expressed in all tissues of metazoan organisms. SMN is associated with several proteins, including Gemin2 (formerly SIP1) (39), the DEAD box RNA helicase Gemin3 (3, 4), Gemin4 (5, 42), a WD repeat protein, Gemin5 (23, 43), and Gemin6 (52) to form large complexes. The SMN complex is found both in the nucleus and in the cytoplasm and appears to be involved in the assembly, restructuring and metabolism of several RNPs, including snRNPs, snoRNPs, and transcriptosomes (2, 10, 39, 53, 55).

Previous experiments have shown that the SMN complex functions in the cytoplasmic assembly of snRNP core particles. Microinjections of anti-SMN complex antibodies in *Xenopus* oocytes inhibit or stimulate snRNP core particle formation (2, 10), and expression of a dominant-negative mutant of SMN in mammalian cells sequesters Sm proteins and snRNAs in cytoplasmic accumulations (55). Moreover, the SMN complex is required for the assembly of U1 snRNP cores in *Xenopus laevis* egg extracts (43). SMN binds preferentially and directly to the symmetrical dimethylarginine-modified RG-rich domains of SmD1, SmD3, and SmB (15, 16). This modification is carried out by the methylosome, a complex containing the methyl-

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transferase JBP1 (PRMT5), and it likely serves to direct the Sm proteins to the SMN complex (17, 18, 44). Several SMN mutants found in SMA patients are defective in Sm protein binding, suggesting that a defect in these interactions may play a role in the pathogenesis of SMA (2, 53).

To determine more precisely the role of the SMN complex in snRNP core assembly, we asked at what step the SMN complex interacts with snRNAs and whether the SMN complex is released from the snRNP after Sm core assembly. We show that the SMN complex binds newly exported snRNAs in an RNA-dependent manner and remains associated with the snRNPs during Sm core assembly, m_3G cap formation, and snurportin1 binding to the m_3G cap structure. These findings indicate that the SMN complex is directly associated with snRNPs during the various steps of their biogenesis in the cytoplasm.

MATERIALS AND METHODS

DNA constructs and antibodies. Plasmids expressing myc-tagged SMN and SMNΔN27 were described previously (55). The DNA fragment corresponding to the open reading frame of snurportin1 was generated by PCR amplification using specific primers. For transient expression in HeLa cells, the insert was cloned downstream of the cytomegalovirus promoter into a modified pcDNA3 vector (InVitrogen) containing the Flag tag sequence (17).

The antibodies used in these experiments were as follows: anti-SMN (2B1) (38), anti-Gemin2 (2E17) (39), anti-Gemin3 (11G9) (4), anti-Gemin4 (22C10) (5), anti-Gemin5 (10G11) (23), anti-Gemin6 (6H5) (60), anti-importin-β (31H4) (A. Perkinson and G. Dreyfuss, unpublished data), anti-Sm proteins (Y12) (37), anti-2,2,7 trimethylguanosine (ab1; Oncogene Research) (R1131) (11), anti-myc (9E10), anti-poly(A)-binding protein (10E10) (21), anti-Flag (Sigma), anti-PHAX (49), anti-Ran (Perkinson and Dreyfuss, unpublished), anti-CRM1 (Transduction Laboratories), and nonimmune antibody SP2/0 (6).

Cell culture and transfection. 293 cells or HeLa cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. Cells growing on 100-mm-diameter culture dishes (about 40% confluent) were transfected with 5 μg of DNA using the CalPhos Mammalian Transfection kit (Clontech Laboratories) according to the manufacturer's recommendations. For immunofluorescence staining, HeLa cells plated on glass coverslips were transfected with 4 μg of myc-SMNΔN27-expressing vector. Following overnight incubation with DNA, the cells were washed, and fresh medium was added. The transfected cells were fixed and processed by immunofluorescence staining after 48 h of incubation.

Immunofluorescence microscopy. Immunofluorescence staining was carried out as previously described (55). Laser confocal fluorescence microscopy was performed with a Leica (Bensheim, Germany) TCS 4D confocal microscope. Images from each channel were recorded separately and then merged.

Immunoprecipitation experiments. Cytoplasmic extracts were prepared as previously described (58) and incubated with specific antibodies bound to GammaBind G Sepharose (Amersham) for 2 h at 4°C in RSB100 (10 mM Tris-HCl [pH 7.5], 100 mM NaCl, 2.5 mM $MgCl_2$) containing 0.01% NP-40. The beads were extensively washed with RSB200 (10 mM Tris-HCl [pH 7.5], 200 mM NaCl, 2.5 mM $MgCl_2$) containing 0.05% NP-40, and the immunoprecipitated proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting as previously described (39). For RNA analysis, bound RNAs were isolated as previously described (10), 3′-end labeled with [5′-^{32}P]pCp (3,000 Ci/mmol; Amersham) and T4 RNA ligase according to the method of England and Uhlenbeck (8), and separated on a denaturing 10% polyacrylamide gel containing 7 M urea. The bands were visualized by autoradiography.

RESULTS

The SMN complex is associated with newly exported PHAX-containing snRNAs. To investigate a possible interaction between the SMN complex and the snRNA export complex in vivo, HeLa cell cytoplasmic extracts were prepared and immunoprecipitations were carried out using either anti-SMN (2B1), anti-PHAX, or nonimmune (control) antibody (Fig. 1). The quality of the cellular fractionation was monitored by the presence in cytoplasmic and nuclear fractions of the nuclear-restricted hnRNP C protein (compare Fig. 1B and C). The immunoprecipitated proteins were then analyzed by SDS-PAGE and Western blotting. The snRNA export factor PHAX is the earliest detectable marker of newly exported snRNAs in the cytoplasm (49). Phosphorylated PHAX is associated with the snRNAs in the nucleus, and following snRNA export, PHAX is dephosphorylated (49). The anti-SMN antibody specifically coimmunoprecipitated the nonphosphorylated form of PHAX from the cytoplasm (Fig. 1A). In contrast, the poly(A)-binding protein (PABP), as a control, was not coimmunoprecipitated with the anti-SMN antibody. The interaction between the SMN complex and PHAX in the cytoplasm is RNA dependent, because PHAX was not coimmunoprecipitated with SMN after RNase treatment. No dephosphorylation of PHAX occurred in the extract during the course of the immunoprecipitation experiment (data not shown). In a reciprocal experiment (Fig. 1B and C), all the known components of the SMN complex, namely, SMN, Gemin2, Gemin3, Gemin4, Gemin5, and Gemin6, were coimmunoprecipitated from the cytoplasm in an RNA-dependent manner using the anti-PHAX antibody. No interaction between PHAX and SMN was detected in the nuclear fraction (Fig. 1C). These findings indicate that the SMN complex is associated with snRNAs (10, 60) and nonphosphorylated PHAX in the cytoplasm but not in the nucleus. Even though PHAX can bind directly to snRNAs in vitro, PHAX interaction with the snRNA export complex requires its binding to both CBC and snRNAs (57). Therefore, snRNAs and CBC bound to the m^7G cap structure likely mediate the association between nonphosphorylated PHAX and the SMN complex. Unfortunately, we were not able to probe for the components of the CBC complex due to the limited affinities of available anti-CBC antibodies. SmB also coimmunoprecipitated with PHAX in an RNA-dependent manner (Fig. 1B). Finally, anti-PHAX antibody did not coimmunoprecipitate Ran and CRM1, likely because RanGTP hydrolysis occurs immediately after snRNA export (Fig. 1B). Therefore, the SMN complex associates with Sm proteins and the postexport complex that contains nonphosphorylated PHAX, CBC, and snRNAs.

The SMN complex is associated with m_3G cap-containing snRNPs in the cytoplasm. Subsequent to snRNP core assembly, the m^7G cap structure of the snRNAs is converted to an m_3G cap structure, and the snRNAs undergo 3′-end trimming. Both events are dependent on proper snRNP core assembly (see the introduction). The snRNP core assembly likely takes place in association with the SMN complex. To determine if the SMN complex is still associated with the snRNPs after Sm core assembly, HeLa cell cytoplasmic extracts were prepared and immunoprecipitated with anti-m_3G cap (TMG) or nonimmune (SP2/0) antibody (Fig. 2). SnRNP cores were efficiently coimmunoprecipitated with anti-m_3G cap antibody, as indicated by the presence of SmB and snRNAs (Fig. 2 and data not shown). All the components of the SMN complex were also coimmunoprecipitated with the m_3G-capped snRNP core. In contrast, PHAX, as well as PABP as a control, were not coimmunoprecipitated with the anti-m_3G cap antibody. Thus, the SMN complex does not dissociate following Sm core assembly and remains bound to snRNPs after cap hypermethylation whereas PHAX is removed before hypermethylation. The dis-

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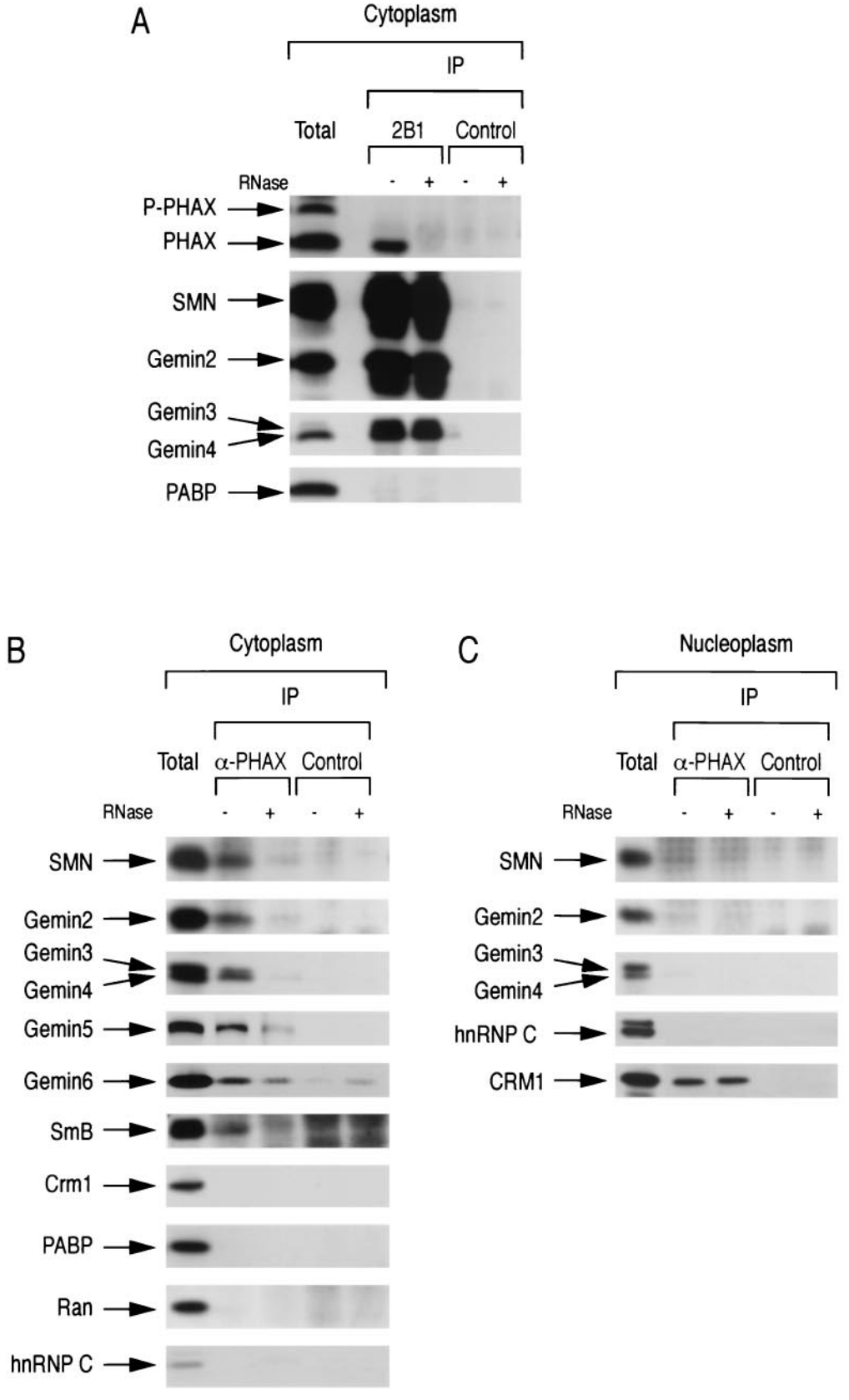


FIG. 1. Association of the SMN complex with PHAX in vivo. Immunoprecipitation (IP) experiments were carried out on HeLa cell cytoplasmic extracts (Cytoplasm) or on HeLa cell nucleoplasmic extracts (Nucleoplasm) using anti-SMN (2B1) or nonimmune (Control) antibody (A) and anti-PHAX (α-PHAX) or nonimmune (Control) antibody (B and C) in the presence (+) or absence (−) of RNase. The immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting with antibodies to the indicated proteins; 2% of the inputs are shown (Total). P-PHAX, the phosphorylated form of PHAX. PABP, poly(A)-binding protein.

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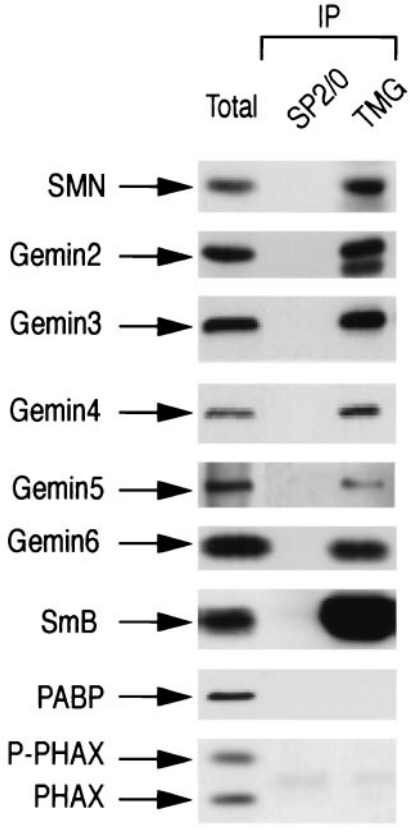


FIG. 2. Association of the SMN complex with m_3G-capped snRNAs in the cytoplasm. Immunoprecipitation (IP) experiments were carried out on HeLa cell cytoplasmic extracts using anti-m_3G cap structure (TMG) or nonimmune (SP2/0) antibody. The immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting with antibodies to the indicated proteins; 2% of the inputs are shown (Total). P-PHAX, the phosphorylated form of PHAX. PABP, poly(A)-binding protein.

sociation of PHAX and CBC might be expected to expose the m^7G cap structure, allowing the cap methyltransferase access to it. The association between the SMN complex and cytoplasmic m_3G-capped snRNPs strongly suggests that snRNP core assembly, as well as the formation of the m_3G cap structure, occur in the presence of the SMN complex.

The SMN complex is associated with snurportin1-containing preimport snRNPs. The next step in the pathway of snRNP biogenesis is the binding of snurportin1 to the newly formed m_3G cap structure, which in turn recruits importin-β and, together with an unidentified import receptor that recognizes the Sm core, mediates snRNP import into the nucleus (28, 50). To test whether there is an interaction between the SMN complex and snRNPs bound to snurportin1, cytoplasmic extracts were prepared from 293T cells transiently expressing Flag-tagged snurportin1 (Flag-snurportin1) or the Flag tag alone (Mock) and immunoprecipitated with anti-Flag antibodies. The immunoprecipitated proteins were then analyzed by Western blotting (Fig. 3A), and the immunoprecipitated RNAs were analyzed by denaturing polyacrylamide electrophoresis after 3′-end labeling (Fig. 3B). SmB and the snRNAs (U1, U2, U4, and U5) were specifically coimmunoprecipitated with snurportin1. We note that snurportin1 also associates with U11 snRNA, suggesting that the minor and major snRNAs likely share the same import pathway.

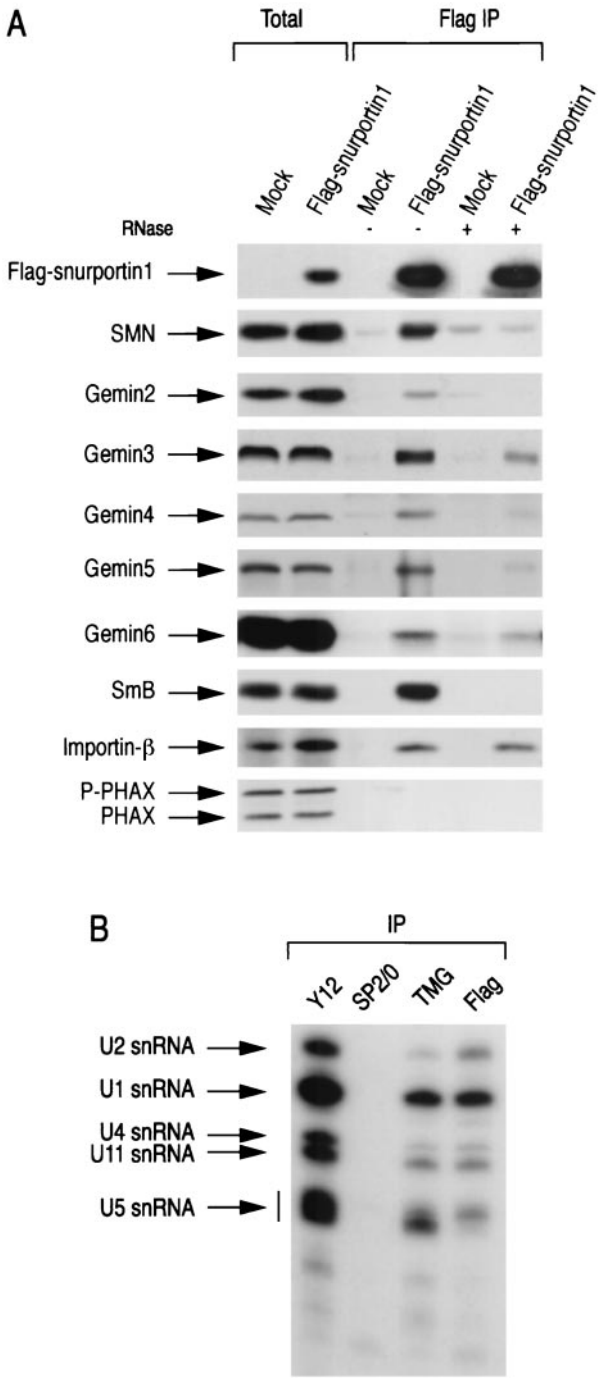


FIG. 3. Association of the SMN complex with snurportin1 in vivo. Immunoprecipitation (IP) experiments were carried out on cytoplasmic extracts prepared from 293 cells transiently expressing Flag-snurportin1 (Flag-snurportin1) or Flag-pcDNA3 alone (Mock). (A) Immunoprecipitation experiments were carried out using anti-Flag antibody in the presence (+) or absence (−) of RNase. The immunoprecipitated proteins were analyzed by SDS-PAGE and Western blotting with antibodies to the indicated proteins; 2% of the inputs are shown (Total). P-PHAX, the phosphorylated form of PHAX. (B) Immnunoprecipitation experiments were carried out using anti-Sm proteins (Y12), anti-m_3G cap structure (TMG), anti-Flag (Flag), and nonimmune (SP2/0) antibodies. Immunoprecipitated RNAs were extracted from the beads, 3′-end labeled, analyzed by denaturing polyacrylamide gel electrophoresis, and visualized by autoradiography. The immunoprecipitated RNAs are indicated by arrows (20).

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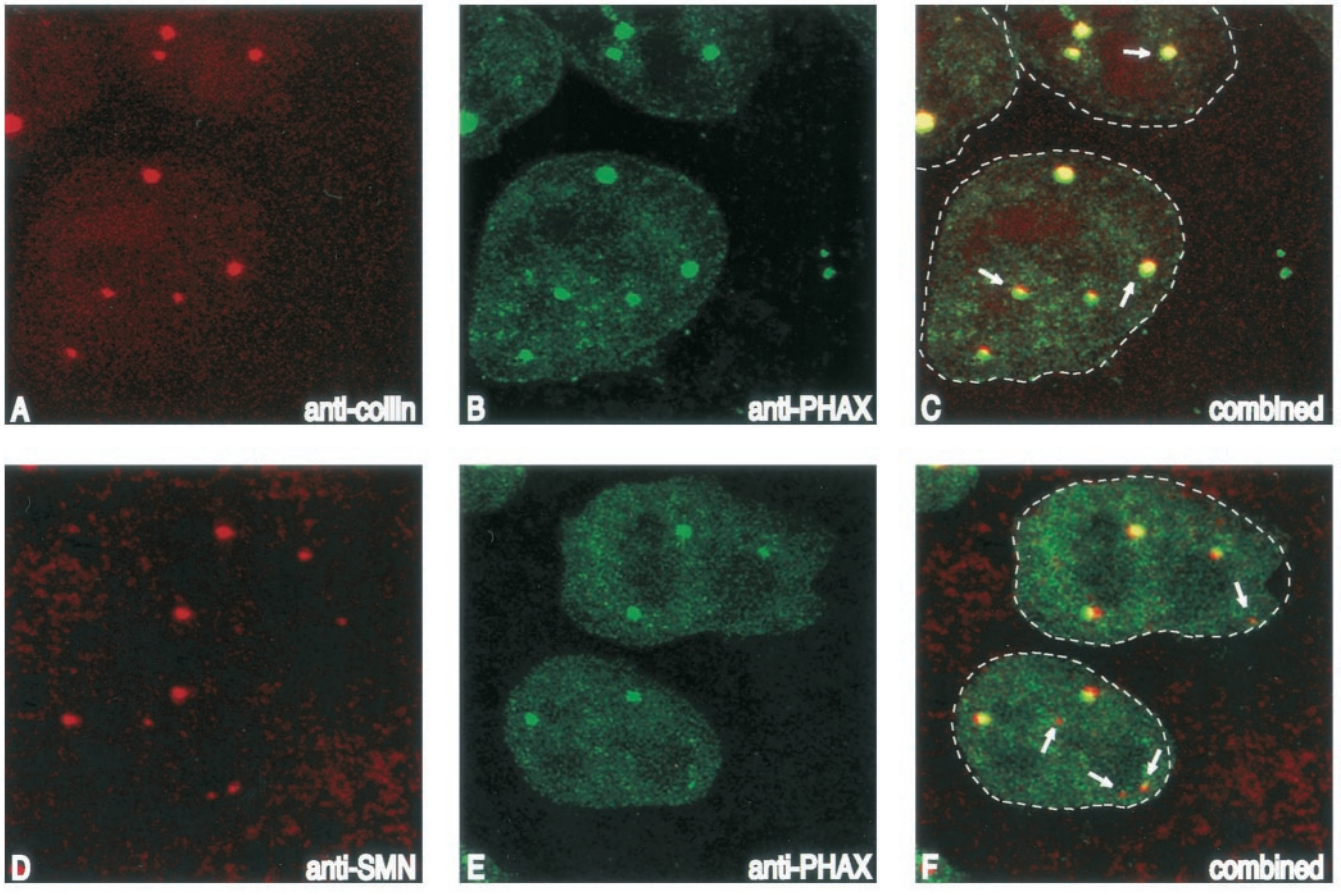


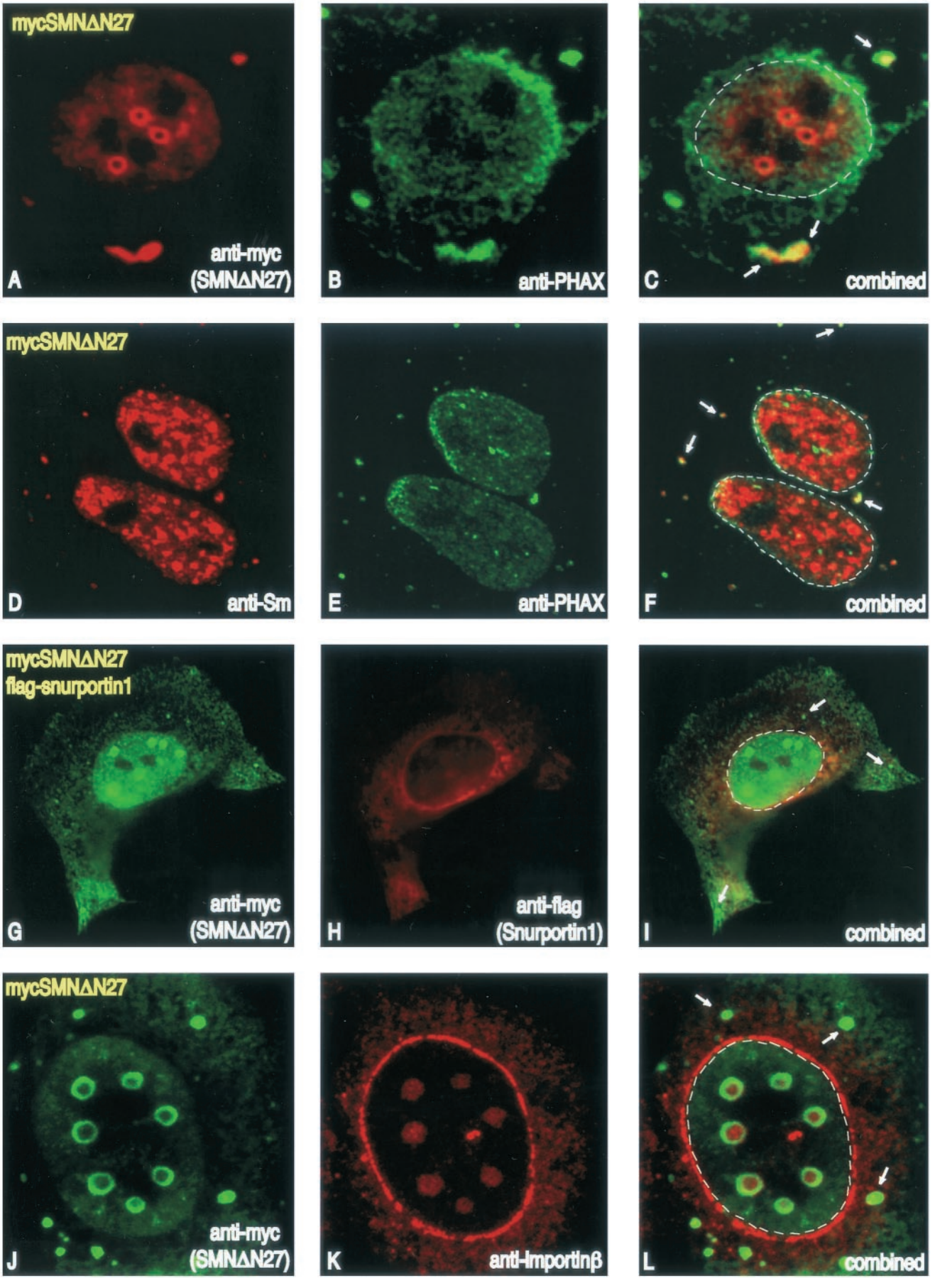
FIG. 4. In the nucleus, endogenous PHAX accumulates in Cajal bodies. A double-label confocal immunofluorescence experiment using anti-coilin (A), anti-PHAX (B and E), and anti-SMN (D) antibodies on HeLa cells is shown. The respective combined images are shown in panels C and F. Colocalization results in a yellow signal. The dashed lines demarcate the nuclei. The Cajal bodies (C) and the gems (F) are indicated by arrows.

snurportin1 was associated with importin-β, as well as with the SMN complex. When the cytoplasmic extracts were treated with RNase during immunoprecipitations, SmB and the SMN complex components were not coimmunoprecipitated with Flag-snurportin1, indicating that snurportin1 and the SMN complex are associated with the same RNPs but do not bind each other. As expected, the interaction between snurportin1 and importin-β was stable under these conditions. Thus, the SMN complex is associated with the last preimport snRNP intermediate, which contains snurportin1.

Expression of the SMN dominant-negative mutant, SMNΔN27, causes reorganization of PHAX in the cytoplasm. PHAX localizes in the nucleoplasm and is highly concentrated in nuclear domains (57). Frey and Matera (14) showed that green fluorescent protein-PHAX accumulates in the Cajal (coiled) bodies. We analyzed the localization of PHAX in HeLa cells (strain PV), where gems and Cajal bodies are often observed as separate structures (38). As shown by costaining with anti-coilin and anti-SMN antibodies, endogenous PHAX accumulates in Cajal bodies (Fig. 4A to C) but not in gems (Fig. 4D to F). The presence of PHAX in the Cajal bodies is consistent with the possibility that these nuclear bodies are the site of early events of snRNP biogenesis (14).

The SMN dominant-negative mutant, SMNΔN27, inhibits splicing in vitro (55). In vivo, the expression of SMNΔN27 causes a profound rearrangement of snRNPs, snoRNPs, and components of the RNA polymerase II transcription and processing machinery and inhibits transcription in the nucleus (51, 54, 55). SMNΔN27 also leads to the cytoplasmic accumulation of endogenous SMN complexes, Sm proteins, and m^7G-capped snRNAs in discrete accumulations, indicating that this mutant blocks the snRNP assembly pathway prior to m_3G cap formation (55). We investigated whether the expression of SMNΔN27 has an effect on the subcellular localization of PHAX in HeLa cells. As shown in Fig. 5A to F, PHAX specifically accumulates with both SMNΔN27 and Sm proteins in the cytoplasmic but not in the nuclear accumulations. In cells transfected with wild-type SMN, no reorganization of PHAX or Sm proteins was observed (reference 55 and data not shown). The accumulation of PHAX in these cytoplasmic accumulations is consistent with the association between the SMN complex and the newly exported PHAX-containing snRNAs detected by coimmunoprecipitation experiments (Fig. 1). In cells transfected with SMNΔN27, gems and Cajal bodies are completely merged in large nuclear accumulations (55). Consistent with the presence of PHAX in Cajal bodies (Fig. 4B), low levels of PHAX colocalize with SMNΔN27 in the nuclei of SMNΔN27-tranfected cells. However, nucleoplasmic PHAX does not accumulate in the enlarged Cajal bodies or gem accumulations.

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Flag-snurportin1 mostly localizes in the cytoplasm (data not shown). In cells cotransfected with myc-SMNΔN27 and Flag-snurportin1, Flag-snurportin1 does not accumulate with the mutant SMNΔN27 in either the cytoplasmic or the nuclear accumulations (Fig. 5G to I) and shows the same localization observed in cells transfected with wild-type SMN or transfected only with Flag-snurportin1 (data not shown). Similarly, importin-β does not accumulate in the cytoplasmic accumulations (Fig. 5J to L). We note that some importin-β localizes within the SMNΔN27 nuclear accumulations. The significance of this observation is not known. The absence of snurportin1 and importin-β in the cytoplasmic accumulations is consistent with the suggestion that SMNΔN27 blocks the snRNP assembly pathway prior to cap hypermethylation.

DISCUSSION

Over the past several years, it has become evident that the SMN complex functions in several aspects of mRNA biogenesis, such as splicing and the assembly of various RNPs in cells. Our experiments reveal not only that the SMN complex plays a role in proper Sm core assembly but also that it is associated with the snRNPs throughout their biogenesis in the cytoplasm. We found that the SMN complex is associated with three distinct snRNP complexes in the cytoplasm, each representing a well-defined intermediate in the pathway of snRNP assembly that spans the entire cytoplasmic phase. Figure 6 presents a model depicting these different complexes and summarizing the current view of the role of the SMN complex in this pathway.

To accomplish its function in snRNP core assembly, the SMN complex must bring together the Sm proteins and the snRNAs. The SMN complex contains all seven Sm proteins, and SMN binds directly to a subset of Sm proteins including SmB, SmD1, and SmD3 (2, 4, 5, 15, 39, 53). Methylation of specific arginine residues in the RG-rich domains of SmB, SmD1, and SmD3 by the methylosome dramatically increases their affinity for SMN and thus directs them to the SMN complex (Fig. 6) (1, 16–18, 44). The SMN complex also has the capacity to directly interact with the U1 snRNA (60). We detected an RNA-dependent association between the nonphosphorylated form of PHAX and the Sm-containing SMN complex in the cytoplasm but not in the nucleus. This indicates that the SMN complex binds the newly exported snRNAs (Fig. 6), and the direct interaction of SMN complexes with U1 snRNA likely facilitates this association (60). The interaction between PHAX and the Sm-containing SMN complex occurs after hydrolysis of RanGTP and dephosphorylation of PHAX. These two events are believed to lead to the dissociation of Ran and CRM1 from the snRNAs and therefore to the disassembly of the snRNA export complex immediately after snRNA export into the cytoplasm (49). Both in vivo and in vitro, nonphosphorylated PHAX, CBC, and snRNAs form a stable complex (this work and reference 49). It is not yet known how PHAX and CBC are removed from the snRNAs, but the interaction between CBC and the importin-α/β heterodimer can release capped RNAs from CBC (22). Our results do not address whether the PHAX/CBC complex is released before or after Sm core assembly. The dominant-negative SMNΔN27 mutant causes accumulation of the SMN complex, Sm proteins, and m^7G cap snRNAs in the cytoplasm (55). This suggested that SMNΔN27 blocks the snRNP pathway in the cytoplasm at a step preceding cap hypermethylation (55). The observation that PHAX is also accumulated in the same cytoplasmic accumulations suggests that SMNΔN27 interferes with proper Sm core assembly and blocks the release of the PHAX/CBC complex from the m^7G cap structure. An interesting possibility is that the PHAX/CBC complex may protect the m^7G cap structure until the Sm core is correctly assembled. Therefore, the formation of the snRNP core within the SMN complex may be necessary for the release of the PHAX/CBC complex from the m^7G cap structure, making it accessible for hypermethylation.

The association of the SMN complex with cytoplasmic m_3G-capped snRNAs indicates that the SMN complex is not released from the snRNP after Sm core assembly and is present during and after cap hypermethylation (Fig. 6). The snRNA-(guanosine-N_2)-methyltransferase responsible for the snRNA m_3G cap formation in yeast has recently been identified (47). It has been shown that the methyltransferase binds the Sm core directly through its interaction with SmB (47, 56). Our results raise the possibility that the SMN complex may help recruit the methyltransferase or play some role in its activity.

The third distinct snRNP-associated SMN complex detected in the cytoplasm contains snurportin1 (Fig. 6). The RNA-dependent coimmunoprecipitation of the SMN complex and snurportin1 indicates that the SMN complex remains bound to the snRNPs prior to their import. This observation raises the possibility that the SMN complex may be imported into the nucleus together with the newly assembled snRNPs. Indeed, the SMN complex is also found in the nucleus (38). It is conceivable that the SMN complex may play a direct role in snRNP import because at least one factor that interacts with the Sm core and plays a role in snRNP import remains unidentified (12, 59). If this hypothesis is correct, coilin may dissociate the SMN/snRNP complex in the Cajal bodies as previously suggested (26). Alternatively, the SMN complex may be released from the snRNPs immediately, prior to or coincident with their import via the nuclear pore complex. The binding of importin-β to snurportin1 may be the trigger to

FIG. 5. Expression of SMNΔN27 causes accumulation of PHAX, but not snurportin1 and importin-β in the cytoplasm. (A and B) Double-label confocal immunofluorescence experiment using anti-myc (A) and anti-PHAX (B) antibodies on HeLa cells transiently expressing myc-SMNΔN27. (D and E) Double-label confocal immunofluorescence experiment using anti-Sm protein (D) and anti-PHAX (E) antibodies on HeLa cells transiently expressing myc-SMNΔN27. (G and H) Double-label confocal immunofluorescence experiment using anti-myc (G) and anti-Flag (H) antibodies on HeLa cells transiently expressing myc-SMNΔN27 and Flag-snurportin1. (J and K) Double-label confocal immunofluorescence experiment using anti-myc (J) and anti-importin-β (K) antibodies on HeLa cells transiently expressing myc-SMNΔN27. The respective combined images are shown in panels C, F, I, and L. Colocalization results in a yellow signal. The dashed lines demarcate the nuclei. The cytoplasmic accumulations are indicated by arrows. The different sizes of the nuclear and cytoplasmic accumulations observed in panels A to C and panels D to F are due to different incubation times of the cells after transfection.

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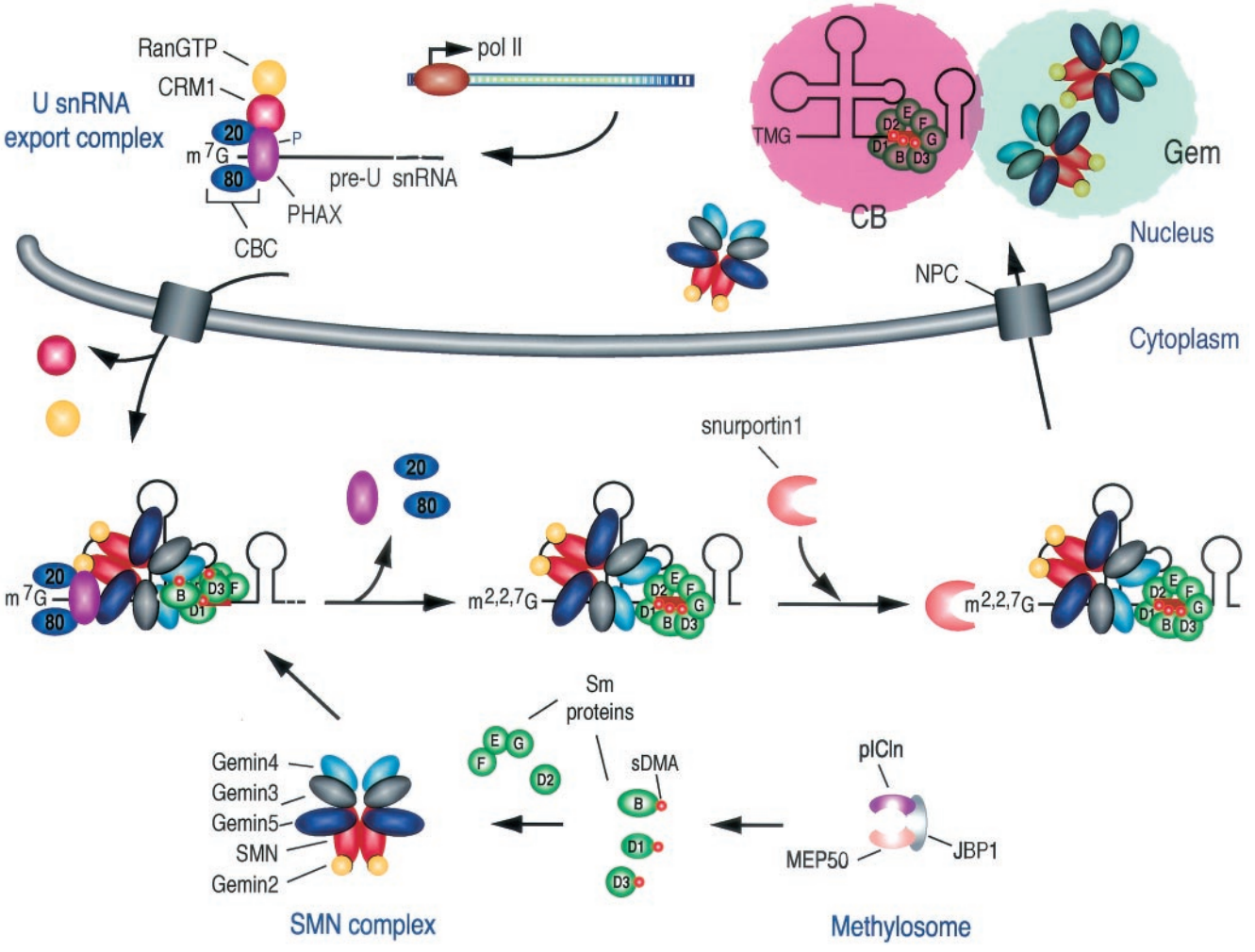


FIG. 6. Schematic model of the role of SMN in snRNP core biogenesis in the cytoplasm. The cytoplasmic chaperone function of the SMN complex in snRNP core formation is discussed in the text. The methylosome consists of the MEP50, pICln, and JBP1 (PRMT5) proteins (16, 17, 44). sDMA, symmetrical dimethylarginine; CB, cajal body.

release the SMN complex from the snRNP core, and the SMN complex may be imported into the nucleus via a distinct pathway.

In conclusion, we showed that the SMN complex binds newly exported snRNAs and is associated with snRNPs during m_3G cap formation and with an snRNP preimport complex containing snurportin1. Therefore, the SMN complex not only plays a role in snRNP core assembly but is an integral component of, and likely serves as a chaperone during, the entire snRNP core biogenesis process in the cytoplasm.

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