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REVIEW

SMN - A chaperone for nuclear RNP social occasions?

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ABSTRACT

Survival Motor Neuron (SMN) protein localizes to both the nucleus and the cytoplasm. Cytoplasmic SMN is diffusely localized in large oligomeric complexes with core member proteins, called Gemins. Biochemical and cell biological studies have demonstrated that the SMN complex is required for the cytoplasmic assembly and nuclear transport of Sm-class ribonucleoproteins (RNPs). Nuclear SMN accumulates with spliceosomal small nuclear (sn)RNPs in Cajal bodies, sub-domains involved in multiple facets of snRNP maturation. Thus, the SMN complex forms stable associations with both nuclear and cytoplasmic snRNPs, and plays a critical role in their biogenesis. In this review, we focus on potential functions of the nuclear SMN complex, with particular emphasis on its role within the Cajal body.

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Background

Cajal bodies and their components

Cajal bodies were first discovered over one hundred years ago by Santiago Ramon y Cajal.[1,2] He adapted Golgi's silver staining technique to identify a variety of structures within mammalian nuclei, one of which he termed the nucleolar "accessory body." These structures were 'rediscovered' over the ensuing decades as they were studied in increasing detail using more advanced microscopy techniques.[3] However, it was not until the 1990s that a molecular characterization of Cajal bodies began.[4-7] Since that time, the number of known cellular components that are enriched in the Cajal body has been growing.[3,8-10] These components include factors important for the maturation and function of Sm-class small nuclear ribonucleoproteins (snRNPs), core particles of the spliceosome. The colocalization of Cajal bodies with relevant small nuclear RNA (snRNA) gene clusters, RNAs that guide post-transcriptional modification of snRNPs, and snRNP specific proteins provide important clues regarding the functions of Cajal bodies.[11-14] Additional studies in mouse embryonic fibroblasts revealed that a primary protein constituent of these structures, called coilin, was responsible for recruitment of another important component, the survival motor neuron (SMN) protein.[15,16]

SMN and spinal muscular atrophy (SMA)

Reduced levels of SMN protein cause SMA,[17] whereas complete loss of *Smn* expression is embryonic lethal.[18] SMA is a common neuromuscular disorder, recognized as the most prevalent genetic cause of early childhood mortality.[19] Clinically, SMA patients show progressive muscle weakness of proximal muscle groups, ultimately leading to paralysis; death is typically caused by progressive and restrictive respiratory failure. Patients with the most severe (and most common) form of SMA become symptomatic in the first 6 months of life and rarely live past 2 y.[20-24]

SMA typically results from homozygous deletion of *survival motor neuron 1* (*SMN1*) gene; however, a small fraction of SMA patients have lost one copy of *SMN1* and the remaining copy contains a point mutation.[25] Decreased levels of the SMN protein correlate with the phenotypic severity of SMA. Since the onset of symptoms and their severity can vary, SMA has been historically classified into 3 subtypes. More recently, clinicians have recognized that SMA is better characterized as a continuous spectrum disorder, ranging from severe (prenatal onset) to nearly asymptomatic.[20] While the genetic etiology of the disease is well-established, the molecular role of SMN in the disease is largely unknown and is the topic of many reviews.[25-34]

SMN and cytoplasmic snRNP assembly

The best-characterized function for the SMN protein, which is expressed in all tissues of metazoan organisms, is in the cytoplasmic assembly of Sm-class snRNPs, core particles of the spliceosome.[34-37] The Sm-class snRNPs consist of uridine-rich snRNAs (e.g. U1, U2, U4, U5), several specific proteins that are unique to each snRNA, and a set of 7 common Sm proteins (B/B', D1, D2, D3, E, F, and G). Sm-class snRNAs are transcribed by RNA polymerase II as precursors that contain additional nucleotides at the 3′ end and a monomethylated m7GpppG

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(m7G) cap structure at the 5′ end. The pre-snRNA transcripts are then exported from the nucleus by a distinct set of factors that includes the cap-binding complex (CBP80 and CBP20), the snRNA-specific export adaptor phosphorylated adaptor RNA export (PHAX), and arsenite resistance 2 (ARS2).[38] These proteins form a link between the 5′ cap and the export receptor chromosome region maintenance 1 (CRM1/Exportin1), which interacts with nuclear pore proteins to promote export.[39] Various reviews focus on this function of SMN.[34,36,37]

Once the pre-snRNAs translocate to the cytoplasm, the snRNA nuclear export complex dissociates.[40,41] The SMN complex, which includes SMN and several tightly associated proteins, collectively called Gemins binds newly exported snRNAs.[42-46] The SMN protein complex regulates the entire cytoplasmic phase of the snRNP cycle including Sm core assembly, trimethylguanosine (TMG) cap formation, and snurportin1 binding to the TMG cap structure.

The SMN complex combines newly exported snRNAs with a set of Sm proteins to form a 7-membered ring around a binding site that is present within each of the Sm-class snRNAs.[47,48] The Sm proteins are delivered to the SMN complex via the activity of the PRMT5 complex, which methylates C-terminal arginine residues within SmB, SmD1, and SmD3[42,49] and then chaperones delivery of partially assembled Sm subcomplexes.[50,51] Gemin5, a component of the SMN complex, is thought to be the factor responsible for recognition of Sm-class snRNAs.[52] Thus, the SMN complex is thought to provide specificity, to avoid assembly of Sm cores onto non-target RNAs,[43,52] and to accelerate formation of the final product from kinetically trapped intermediates.[51] Assembly of the Sm core not only stabilizes the snRNA by protecting it from nucleases, but also is required for the downstream RNA-processing steps.

Following Sm-core assembly, an RNA methyltransferase called trimethylguanosine synthase (Tgs1) is recruited to the m7G cap, whereupon the RNA is hypermethylated to form a 2,2,7-trimethylguanosine (TMG) cap structure.[53] A properly assembled Sm core is required for cap hypermethylation and 3′-end maturation.[54-56] Tgs1 directly interacts with SMN both *in vivo* and *in vitro*.[46] Furthermore, the SMN complex does not immediately dissociate from the RNA after Sm-core assembly, suggesting that SMN may even recruit Tgs1 to the complex. Subsequently, the RNP is imported back into the nucleus by specific snRNP import factors.[57] Once in the nucleus the RNPs undergo additional assembly and maturation steps. Fig. 1A provides a summary of the major events in U snRNP biogenesis.

The SMN complex as a nuclear import adaptor

Nuclear transport of snRNPs involves the use of a bipartite nuclear localization signal (NLS), comprised of the TMG cap and the Sm core.[58-60] In vitro, these NLS pathways are independent, enlisting discrete import adapters[61,62] but sharing a common import receptor, importin β.[63] *In vivo*, the situation likely involves the synergistic use of both pathways, although additional studies are required in order to clarify this issue. In any event, the adaptor for the TMG cap is called snurportin1 (SPN1); this protein binds directly to the TMG cap and significantly improves the kinetics of snRNP import.[61,64,65] At the time, relatively little was known about the factor that mediates the interaction between the Sm core and importin β. Previous studies had indicated that the Sm core adaptor is a cytosolic factor that binds to Sm proteins and importin β.[63] Subsequently, SMN was shown to directly interact with these proteins.[45,66-68] Furthermore, co-fractionation studies with HeLa cytosolic extracts demonstrated that SMN, SPN1 and importin β were present in a novel, pre-import RNP complex.[45,62] Along with several other hints in the literature,[32,44,69] these findings suggested that SMN functions to bridge the gap between the Sm core and the import machinery.

Functional studies of this pre-import RNP complex were carried out using digitonin-permeabilised HeLa cells. The results demonstrated that import of SMN and splicing snRNPs are coupled in somatic cells.[62,65] Two lines of evidence substantiated this conclusion. First, purified SMN complex was required for *in vitro* snRNP import and vice versa. Second, an excess of snRNPs significantly improved SMN import kinetics; the converse also held true. Notably, the SMN complex (but not SMN alone) was sufficient to restore snRNP import defects caused by depletion of SMN from the reconstituted cytosol. Furthermore, mutations in the YG Box self-oligomerization domain of SMN disrupted nuclear import,[62] suggesting the importance of oligomeric SMN in the active import complex. Collectively, these data indicated that additional SMN complex members (e.g., Gemins) are required for cap-independent snRNP import.[62]

SMN import regulation

A model of the putative U snRNP import complex is shown in Fig. 1B. The precise composition of the Gemin proteins and the overall stoichiometry of importin β within this complex is completely unknown. Given the independence of the 2 import pathways in vitro,[61,62,65] the question remains whether the Sm-core and TMG cap import adaptors act synergistically *in vivo*. Moreover, the SMN complex might only play a role in import of newly-assembled snRNPs. In such a scenario, post-mitotic snRNPs would strictly utilize the TMG cap-dependent pathway. Future studies will be needed to clarify this important issue.

Of the various factors that control SMN and U snRNP nuclear import, very little is known. However, a zinc finger protein called ZPR1 was reported to participate in this process.[70] ZPR1 is an essential, highly-conserved protein in eukaryotes.[71,72] The protein was originally identified as a factor that binds to the cytoplasmic tyrosine kinase domains of epidermal growth factor (EGF)-like receptors in the absence of mitogens.[73] Ligand binding and autophosphorylation of the receptor causes ZPR1 to be released from the receptor and subsequently accumulate in the nucleus.[74]

The C-terminal tail of ZPR1 interacts indirectly with SMN, forming cytoplasmic snRNP-containing complexes.[70] Given that ZPR1, SMN and SPN can be detected in a pre-import RNP complex,[45] and that anti-ZPR1 antibodies co-deplete Sm proteins from cytoplasmic lysates,[62] it appears that ZPR1 is involved in snRNP biogenesis. Although ZPR1 forms complexes with U snRNPs, it is not directly required for SMN nuclear transport *in vitro*.[62] However, these complexes are disrupted when there are low levels of SMN, and both ZPR1 and SMN fail to accumulate in nuclear bodies. Moreover, depletion of ZPR1 results in disruption of Cajal bodies and defective nuclear localization of SMN.[75]

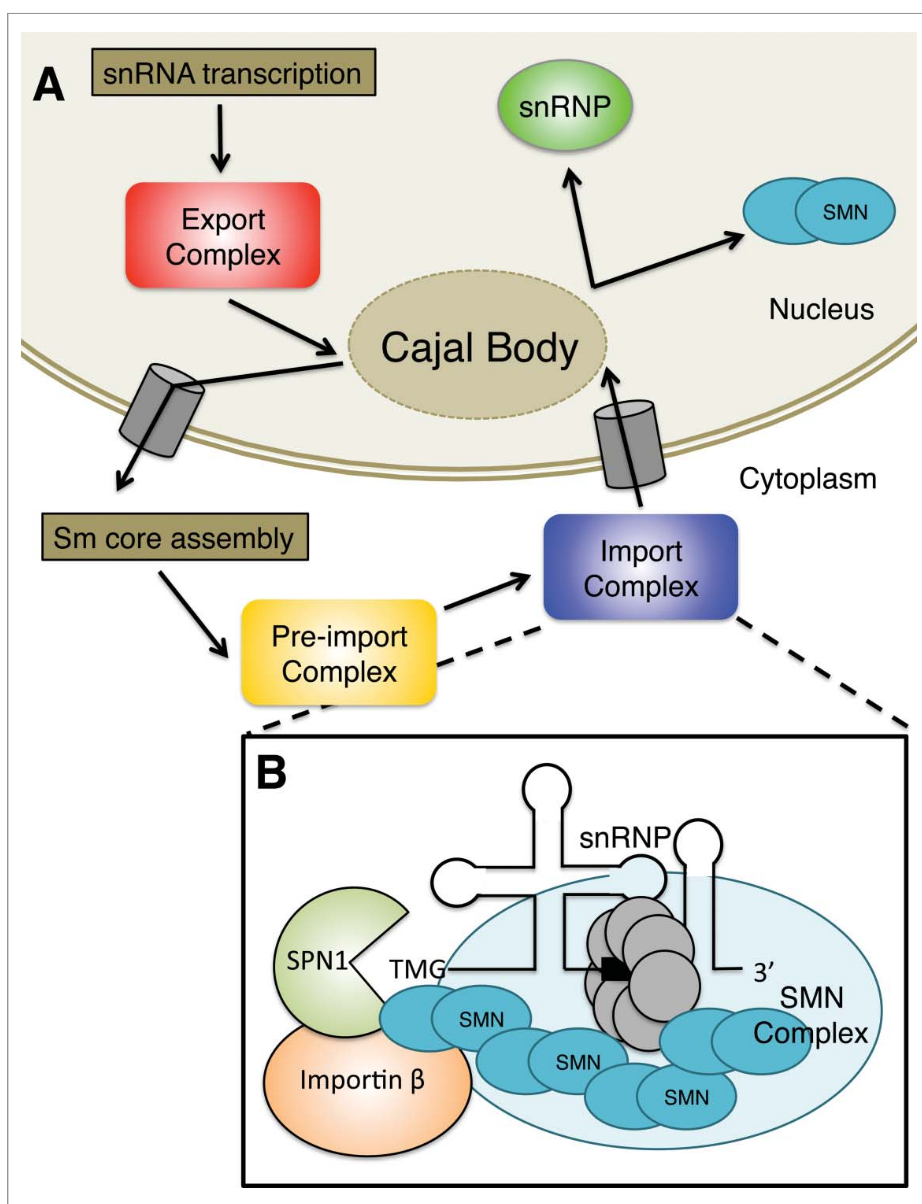

Figure 1. (A) Sm-class snRNAs are transcribed by RNA polymerase II as precursors that contain additional nucleotides at the 3′ end and a monomethylated m7GpppG (m7G) cap structure at the 5′ end. The pre-snRNA transcripts are then exported from the nucleus, often passing through the Cajal body. Once the pre-snRNAs translocate to the cytoplasm, the snRNA nuclear export complex dissociates. The SMN complex combines newly exported snRNAs with a set of Sm in a process known as Sm core assembly. Then, the snRNA is hypermethylated to form a 2,2,7-trimethylguanosine (TMG) cap structure in the pre-import complex. Finally, the RNP is imported back into the nucleus by specific snRNP import factors shown in B. Once in the nucleus, the partially assembled RNPs can transit through Cajal bodies where they undergo additional assembly and maturation steps. (B) The adaptor for the TMG cap is called snurportin1 (SPN1); this protein binds directly to the TMG cap and significantly improves the kinetics of snRNP import. SMN interacts with both SPN1 and Importin β. SMN functions to bridge the gap between the Sm core and the import machinery. These proteins, together with the snRNA and associated snRNP proteins, constitute the pre-import RNP complex.

Overexpression of ZPR1 in SMA patient fibroblasts restores the subnuclear accumulation of SMN and increases overall levels of SMN protein.[76] Because ZPR1 also interacts with EGF-type receptor proteins that regulate cellular proliferation, the most likely scenario places ZPR1 in a signaling cascade upstream of SMN and U snRNP nuclear import.

Nuclear functions of the SMN complex

Following import and localization of newly assembled snRNPs to the Cajal body, coilin may function to disrupt the SMN-snRNP complex and facilitate higher-order snRNP formation.[16,45,77,78] SMN is thought to dissociate from snRNPs soon after their import, as the protein does not co-purify with mature snRNP mono-particles.[79,80] Once released from the SMN complex, the newly assembled snRNP is then free to diffuse throughout the interchromatin space. The fate of the SMN complex following snRNP release in the Cajal body is mostly unknown. In most cell types, the nuclear fraction of the SMN complex localizes primarily within Cajal bodies. However, a small fraction of nuclear SMN protein has been found to co-immunoprecipitate with spliceosomal subcomplexes.[81,82] SMN also accumulates in distinct nuclear substructures called Gemini bodies, or Gems.[83] Cajal bodies contain a plethora of RNAs and their associated proteins, but components of Gems have thus far been limited to constituents of the SMN complex.[9,83] A

U1 snRNP component, U1-70K, also localizes to gems, but its interaction with SMN is thought to be snRNP-independent.[84] Given its recently described role in human U1 snRNP assembly, U1-70K protein might be considered as an auxiliary member of the SMN complex.[85] Notably, a decrease in SMN protein levels (and thus an increase in SMA severity) is correlated with the loss of SMN nuclear foci.[24,86] Thus, the nuclear functions of SMN may well turn out to be important for understanding disease etiology.

Inter- and intra-molecular interactions within the Cajal body

Photobleaching studies in living cells have shown that there are distinct kinetic groups of Cajal body components.[87,88] The proteins with the longest Cajal body residence times are coilin, SMN, Gemin3 and Tgs1. These factors typically reside in Cajal bodies on the order of minutes, whereas other groups of proteins have much shorter residence times.[87] However, despite the direct physical interaction of coilin and SMN, Cajal bodies and Gems are kinetically autonomous compartments.[87] The basis for this kinetic autonomy is likely due to the ability of SMN and coilin to maintain homo-typic interactions (coilin-coilin and SMN-SMN) within the separated nuclear bodies (Fig. 2A). Hetero-typic interactions are therefore likely to regulate overall composition of a given nuclear body.[89] Indeed, fluorescence resonance energy transfer (FRET) by acceptor photobleaching experiments in living nuclei showed that coilin and SMN interact with themselves and with each other inside the Cajal body.[87]

Cajal body homeostasis requires ongoing snRNP biogenesis, as perturbation of SMN or SPN1 function results in disassembly of Cajal bodies and relocalization of coilin to nucleoli.[90] Similarly, depletion of other factors involved in small RNP biogenesis, such as Tgs1, WDR79/WRAP53, PHAX, INTS4 and USPL1 also cause Cajal bodies to disassemble.[91-95] In addition to overall protein levels, post-translational modifications (PTMs) of resident proteins, including SMN and coilin, are almost certainly important for establishing and maintaining inter- and intra-molecular interactions within Cajal bodies.[96-100] Disruption of these PTMs can result in mislocalization of SMN and alterations in Cajal body integrity. These and other factors will need to be addressed by future studies.

Oligomeric properties of SMN complexes

X-ray crystallographic studies of the SMN C-terminal region show that the YG box forms a helical structure whose dimerization is driven by a network of hydrophobic interactions similar to those found in glycine zipper domains of certain transmembrane channel proteins.[101,102] The core of this helical domain contains a highly conserved YxxxYxxxY motif.[101] Notably, phylogenetic analysis reveals that this motif can be extended on both ends to form a longer LxxxLxxxYxxxYxxxYxxxL helix (A. G.M., unpublished observations). Structural analysis of the SMN Tudor domain has also been carried out,[35,103] revealing the existence of an "aromatic cage" of β-sheets that mediates recognition of dimethylarginine residues present on Sm proteins. Future studies will be required to determine how these 2 regions, as well as the α-helical Gemin2 (Gem2) binding domain at the N-terminus[50,104] all fit together in space to carry out SMN's various functions.[105]

The functional importance of the SMN C-terminal domain has been well-documented. More than half of the known SMA patient missense mutations cluster within the YG box.[25,106,107] Furthermore, mutations that completely disrupt SMN's ability to self-oligomerize display severe phenotypes in human SMA patients as well as in animal models.[86,108-113] Despite this strong correlation, the composition and stoichiometry of the various complexes formed by SMN are not well understood. *In vitro*, SMN-Gem2 exists as a stable heterodimer that, for purposes of discussing higher order oligomerization, can be considered as a single structural unit.[66,114] As the concentration increases, this unit exists in an equilibrium mixture containing dimers, tetramers and octamers of SMN-Gem2.[102] Importantly, the SMN-Gem2 complex does not multimerize by forming symmetric bundles.[102] Instead, SMN tetramers are formed by a dimer of dimers (Fig. 2B). In the fission yeast system, dimers and tetramers are the only species observed.[102] Human SMN-Gem2 forms dimers to octamers and possibly even larger complexes (Fig. 2B). Octamers appear to form via self-association of tetramers, although the existence of a hexameric SMN complex cannot be ruled out.[102] *In vivo*, human SMN-Gem2 cosediments with Gemins3–8,[115] however, the relative stoichiometries of these proteins are completely unknown.

Studies in fission yeast suggest that the SMN dimer [i.e. $(\text{SMN-Gem2})_2$] is the basal functional unit. Using in vivo estimates of SMN protein concentration,[116] Van Duyne and colleagues calculated that the concentration of the dimer (~15 nM) lies far, far below the dissociation constant of SMN tetramers (~1 uM).[102] Immunofluorescence studies showed that yeast SMN is diffusely distributed throughout the nucleus and cytoplasm, with no discernible foci.[117] Thus, the SMN dimer is the most abundant species *in vivo*, and is presumed to be functionally active in snRNP biogenesis.[102]

Although changes in the composition and organization of the SMN complex are likely to be regulated by various PTMs, the studies of yeast SMN have important consequences for metazoans, particularly when it comes to the oligomerization status of the protein. For example, the concentration of SMN within nuclear Cajal bodies or cytoplasmic stress granules is significantly greater than that of the surrounding nucleoplasm or cytosol, respectively. Hence, if there are unique functions of SMN that can only be performed by higher-order oligomers, then it is therefore likely that such functions are being carried out in specific cellular locales that contain high concentrations of the SMN complex (Fig. 2C).

SMN and Cajal Body structure

As mentioned above, loss of SMN is detrimental to Cajal body integrity.[90,91,95,100] Cajal bodies consistently associate with specific loci on multiple chromosomes, many of which include snRNA gene arrays, snoRNAs, as well as other small U RNA and histone gene clusters.[11,12,118-120] Following siRNA knockdown of essential Cajal body components, WDR79/WRAP53 or USPL1, these chromosomal regions were no longer clustered, and the expression of many of the associated small U RNA loci were significantly reduced. Thus, disruption of Cajal

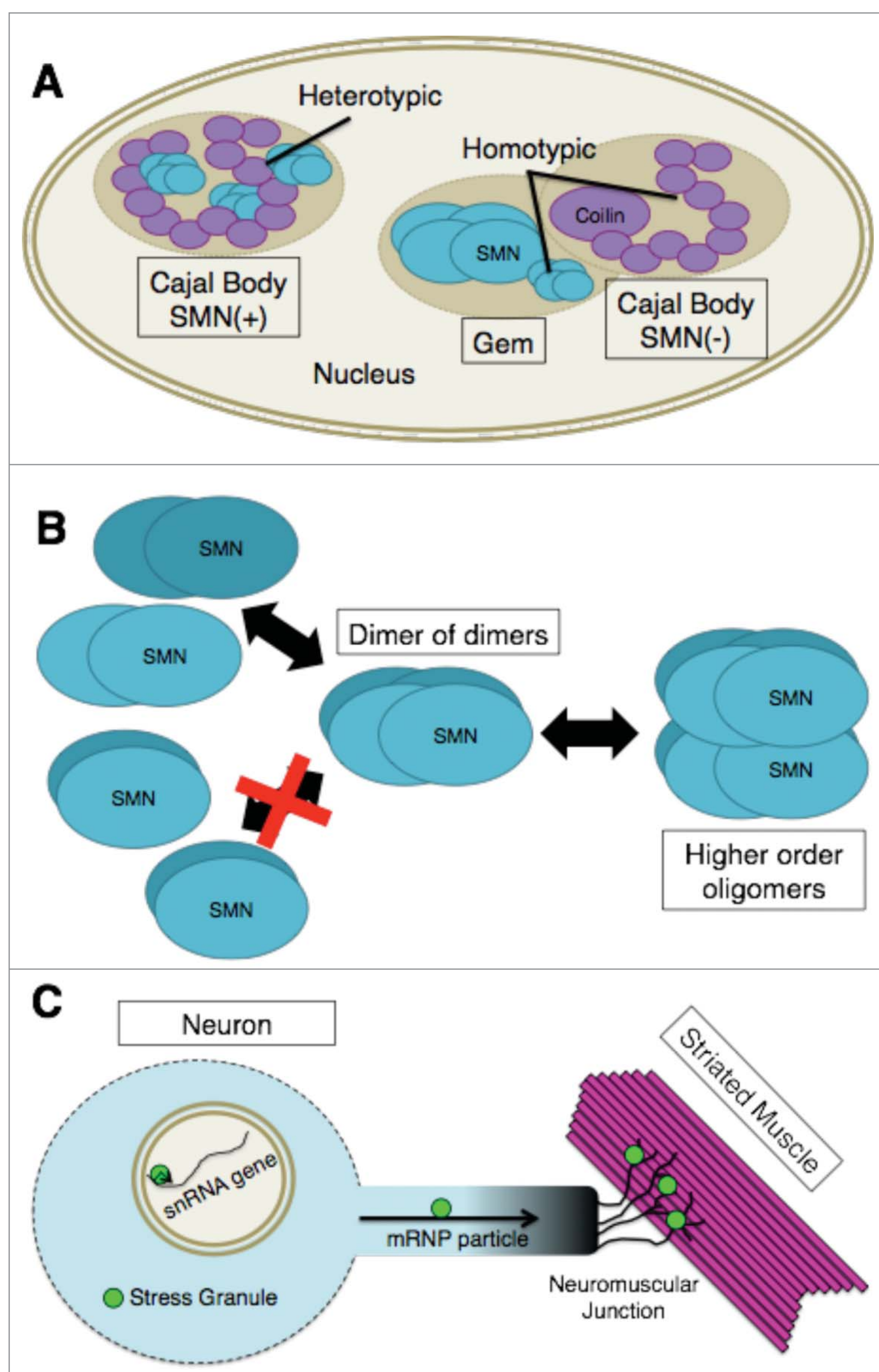

Figure 2. (A) In SMN(+) Cajal bodies, coilin and SMN physically interact. These heterotypic interactions are likely to regulate the overall composition of the nuclear body. Cajal bodies and Gems are kinetically autonomous compartments as illustrated by the Gem and SMN(−) Cajal body. The basis for this kinetic autonomy is likely due to the ability of SMN and coilin to maintain homotypic interactions (coilin-coilin and SMN-SMN) within the separated nuclear bodies. (B) SMN-Gem2 exists as a stable hetero-dimer that, for purposes of discussing higher order oligomerization, has been shown as a single structural unit (labeled "SMN"). At varying concentrations, this complex exists in an equilibrium mixture containing dimers, tetramers (dimer of dimers), and octamers of SMN-Gem2. Human SMN-Gem2 forms dimers to octamers and possibly even larger complexes. Octamers appear to form via self-association of tetramers. (C) The concentration of SMN within certain subcellular compartments (e.g. Cajal bodies, snRNA gene clusters or cytoplasmic stress granules) is significantly greater than that of the surrounding regions of the cell. High concentrations of SMN are also thought to be found in mRNP transport particles along axons as well as at neuromuscular junctions. If there are unique functions of SMN that can only be performed by higher-order oligomers it is likely that such functions are being carried out in specific cellular locales that contain high concentrations of the SMN complex, as illustrated by the bright green circles.

bodies leads to a loss of genome conformation and this may lead to inefficient expression of snRNAs. On the other hand, factors like USPL1 have been shown to participate directly in processing of pre-snRNA transcripts,[94] so it is difficult to distinguish between the function of the protein and that of the nuclear body.

While there is not a direct connection between SMN and genome conformation disruption, SMN is important for

maintaining the structure of the Cajal body. Previously, low levels of SMN have been linked to widespread changes in expression patterns,[121-128] but it is currently an open question as to how these changes might be linked to Cajal bodies. In addition, the mechanism by which these conformational changes in the nucleus affect the rest of the cell and which cellular components and processes are most severely affected has yet to be studied.

SMN and transcriptional regulation

While SMN is definitively important for maintaining the structure of the Cajal body, there is emerging evidence suggesting the involvement of SMN in other processes while in this nuclear body. One potential role for SMN is in RNA Polymerase II (RNAPII) termination. Zhao et al.[129] recently showed that SMN binds to the symmetric dimethylation of arginine-1810 (R1810me2s) of the RNAPII C-terminal domain (CTD). Asymmetric dimethylation of this residue leads to reduced expression of snRNAs and snoRNAs when recognized by TDRD3.[130]

In addition to the interaction of SMN with R1810me2s, SMN interacts with senataxin (SETX) (Fig. 3A). Binding of SMN at the R1810me2s stabilizes interaction of SETX with the CTD. SETX is a RNA/DNA helicase that is responsible for unwinding R-loops around transcription termination sites.[131] This unwinding allows the 5′-to-3′ exonuclease XRN2 to be recruited, thus terminating transcription.[132] Mutation of arginine to alanine at this residue and knockdown of SMN cause accumulation of RNAPII at termination regions of active genes across the genome (Fig. 3B). A CRISPR knockout of SMN led to increased R-loops in termination regions and γ-H2AX, a marker of DNA damage, accumulates at these sites.

R-loops have been shown to both stabilize and destabilize the genome depending on the context.[133,134] The characteristics

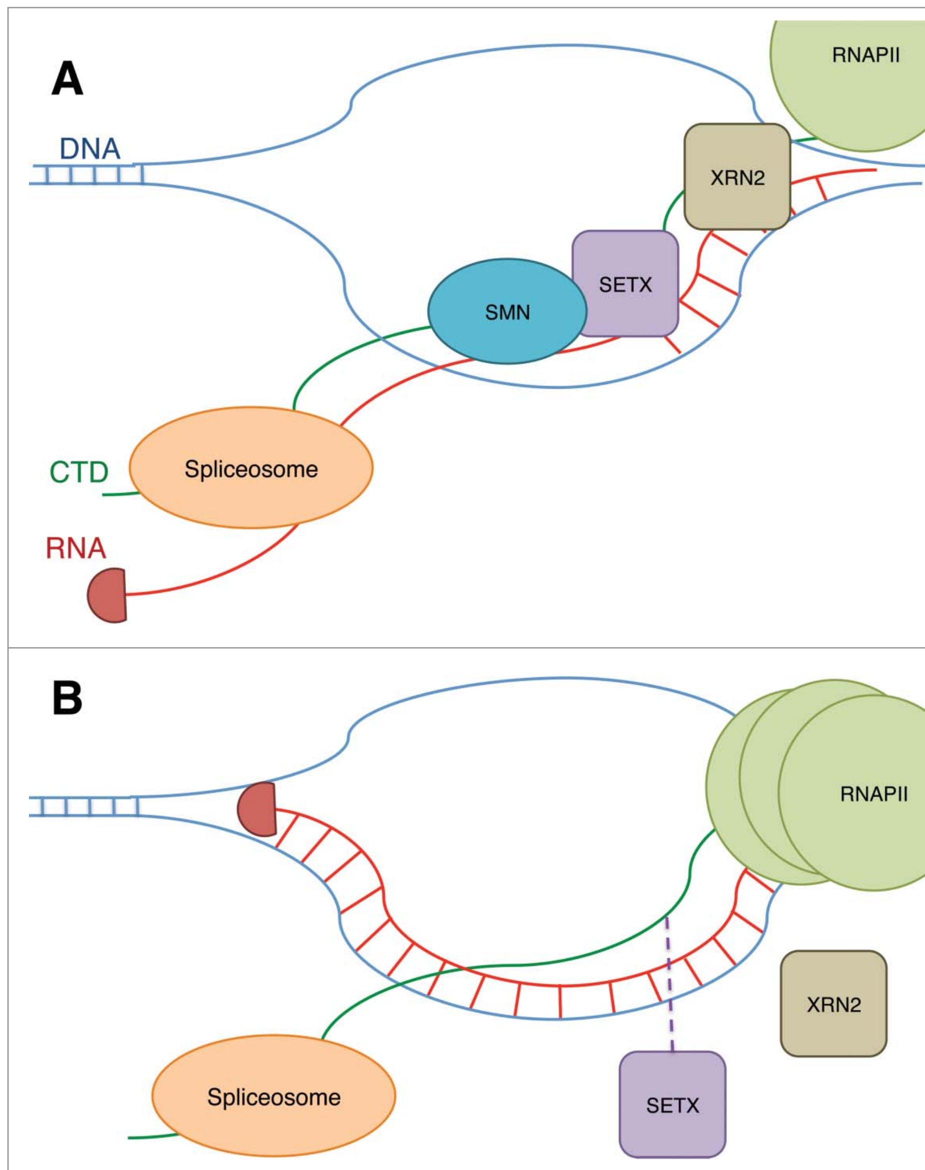

Figure 3. (A) The binding of SMN to R1810me2s on RNAPII CTD is shown to stabilize the binding of an RNA/DNA helicase, SETX, to the CTD. SETX prevents R-loops at termination sites and allows the exonuclease XRN2 to terminate the transcript, and RNAPII is released. The prevention of R-loops by SETX also allows the spliceosome to access the RNA and modify the transcript through splicing. (B) When SMN is reduced, or the R1810 residue is lost, this results in reduced recruitment of SETX and an increase in R-loops and DNA damage. RNAPII also accumulates at the transcription termination site.

and mechanisms that determine whether an R-loop is beneficial or detrimental to the genome are unknown. SMN may contribute to this determination through its interaction with SETX.

If SMN does indeed affect RNAPII termination through its interaction with senataxin, it's possible that its structural and transcriptional functions coincide adjacent to the Cajal body. It has not been shown that this active role of SMN is associated with the Cajal body, but this would provide a parsimonious model for efficient transcript processing of chromosomal regions associated with the Cajal body, including snRNAs gene arrays. This provides a potential mechanism for how these genes are expressed and regulated in a finely tuned manner. It has been shown that snRNAs with extended 3′ regions associate with Cajal bodies[135] and that snRNAs accumulate in the Cajal body before they are exported to the cytoplasm.[136] Conveniently, snRNA export proteins PHAX and CRM1 are also concentrated at Cajal bodies.[13,137] Inhibition of PHAX leads to accumulation of snRNAs in the Cajal body.[136] The return of snRNPs to their initial site of biogenesis is also thought to be a mechanism for feedback regulation of snRNA transcription and gene dosage compensation.[138] It is clear that the Cajal body is used as a hub for snRNAs as they transition from transcription to export, but whether this nuclear body is also directly associated with the active transcriptional regulation of snRNAs remains to be studied.

Conclusions and perspectives

Following its discovery as the product of the SMA-determining gene,[17] the SMN protein was quickly shown to accumulate in nuclear Cajal bodies.[67,139,140] Understanding the function of the SMN complex within the Cajal body has been a much slower process. As discussed in the preceding paragraphs, the nuclear fraction of SMN may well participate in both early and late events in the biogenesis of spliceosomal snRNPs. Particularly appealing is the idea that SMN might also chaperone assembly of other types of RNPs, wherever they may happen to congregate inside the cell. What sets the Cajal body bound fraction of SMN apart from the nucleoplasmic SMN complex? The simple answer is that the apparent protein concentration difference between the 2 subcellular compartments determines the oligomerization potential of nucleoplasmic SMN. Is there a specific molecular function that is performed only by the larger SMN multimers? If so, is a similar function carried out by SMN in stress granules, where the cytoplasmic fraction of SMN is most concentrated?[141-143] Additional studies will be needed to answer these questions.

One possibility is that the larger SMN oligomers might serve as intracellular signaling hubs. Interestingly, SMN depletion is associated with innate immune and stress signaling.[144-146] Moreover, hypomorphic SMN mutations that cause milder forms of SMA in humans and significant viability defects in SMA model flies are neither associated with defects in pre-mRNA splicing nor spliceosomal snRNP biogenesis.[147] Although potential defects in snRNP biogenesis at specific developmental time points are possible, these findings indicate that snRNP-dependent RNA processing changes are unlikely to be primary drivers of SMA pathology. Rather, SMN-dependent activation of innate immune and other types of stress signaling pathways may be more important to neuromuscular pathophysiology than previously envisioned.[147,148,149]

As the storehouse of nuclear SMN protein, the Cajal body likely plays an important role in its nuclear activities. Learning more about mechanisms of SMN nuclear-cytoplasmic trafficking and its various functions within the Cajal body should help elucidate SMN's role in organismal development and SMA etiology. The mysteries surrounding the connection between SMN and Cajal bodies, and the implications of this relationship for human disease are a perfect example of an observation that Cajal himself made in *Advice for a Young Investigator*, saying that, "each problem solved stimulates an infinite number of new questions, and that today's discovery contains the seed of tomorrow's [idea]."

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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