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Why do cells need an assembly machine for RNA–protein complexes?

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Small nuclear ribonucleoproteins (snRNPs) are crucial for pre-mRNA processing to mRNAs. Each snRNP contains a small nuclear RNA (snRNA) and an extremely stable core of seven Sm proteins. The snRNP biogenesis pathway is complex, involving nuclear export of snRNA, Sm-core assembly in the cytoplasm and re-import of the mature snRNP. Although *in vitro* Sm cores assemble readily on uridine-rich RNAs, the assembly in cells is carried out by the survival of motor neurons (SMN) complex. The SMN complex stringently scrutinizes RNAs for specific features that define them as snRNAs and identifies the RNA-binding Sm proteins. We discuss how this surveillance capacity of the SMN complex might ensure assembly of Sm cores only on the correct RNAs and prevent illicit, potentially deleterious assembly of Sm cores on random RNAs.

The small nuclear ribonucleoprotein (snRNP) particles are major components of the spliceosome, which is the pre-mRNA splicing machinery in the eukaryotic nucleus. Each of the major snRNPs contains a small nuclear RNA (snRNA) – U1, U2, U5 or U4/U6 – as well as seven common Sm proteins and a set of proteins that are specific to individual snRNAs [1–7]. The Sm proteins B or B′, D1, D2, D3, E, F and G are common to all spliceosomal snRNPs, except U6 snRNP, and are arranged into an Sm core, which is a seven-membered ring on a uridine-rich sequence (Sm site) [8–12]. The spliceosomal snRNPs are abundant ($>1\times10^6$ U1 snRNP in HeLa cells) and extremely stable. In fact, there is no indication that they turn over. Biogenesis of snRNP in higher eukaryotes involves a complex sequence of discrete steps. The snRNAs, except U6 snRNA, are transcribed by RNA polymerase II and are exported rapidly to the cytoplasm in association with a cap-binding complex and the export factor PHAX [13,14]. The formation of the Sm core, snRNP assembly, occurs in the cytoplasm of vertebrate cells shortly after the nuclear export of nascent snRNAs [15,16]. Proper assembly of the Sm core, 5′-cap hypermethylation and 3′-end processing of the snRNAs are required for the subsequent nuclear import of the snRNPs, which then function in nuclear pre-mRNA splicing [5,17–21].

Early studies using purified total snRNP proteins suggested that the minimal sequence requirement of Sm-core assembly *in vitro* is a region of just 6–10 single-stranded, uridine-rich nucleotides [22]. These studies left unanswered the question of how the Sm proteins distinguish their targets specifically among the myriad of uridine-rich RNA sequences in cells. By contrast, microinjection experiments in *Xenopus* oocytes showed that the Sm site of each snRNA is not functionally interchangeable in Sm-protein binding [23]. These studies suggested that the Sm site, despite being the common binding site for Sm proteins, might cooperate specifically with other elements of snRNAs for snRNP assembly. This implies that, although the assembly of snRNPs shares common Sm proteins and the Sm site, it is not a simple process but, instead, is a strictly regulated and coordinated process involving many factors [23].

Why then, if snRNPs can readily assemble spontaneously *in vitro*, is their biogenesis pathway so complex? Why, in contrast to other small RNAs such as the small nucleolar RNAs, which function and assemble in the nucleus, do snRNAs need to be exported to the cytoplasm and re-imported at a high energy cost? Here, we review recent discoveries regarding a molecular machine that cells employ to assemble snRNPs and suggest possible explanations for these key questions about ribonucleoprotein (RNP) biogenesis in cells.

The SMN complex

Important and unexpected insights into the process of snRNP assembly came from studies on the function of the survival of motor neurons (SMN) protein [24–27]. Reduced levels of SMN, due to a genetic defect, cause degeneration of motor neurons in the spinal cord and result in spinal muscular atrophy (SMA) [28,29]. The SMN protein is expressed in all eukaryotes tested so far, except *Saccharomyces cerevisiae*, and in all cell types of vertebrate organisms. Particularly high levels of SMN are expressed in neuronal cells, including motor neurons of the spinal cord. SMN is found in both the cytoplasm and the nucleus, where it is concentrated in distinct nuclear structures (Gems) that are related to, and often associated with, Cajal bodies [24]. SMN is part of a multiprotein complex that contains Gemins2–7, including the DEAD-box RNA helicase Gemin3 [26,30–34] (Figure 1). The SMN complex is large – sedimenting in sucrose gradients as hetero-disperse particles of 30–70S – and relatively salt resistant (750 mM NaCl) [35]. When SMN complexes are purified at less stringent conditions (e.g. <250 mM NaCl), they contain several snRNP proteins, including the Sm

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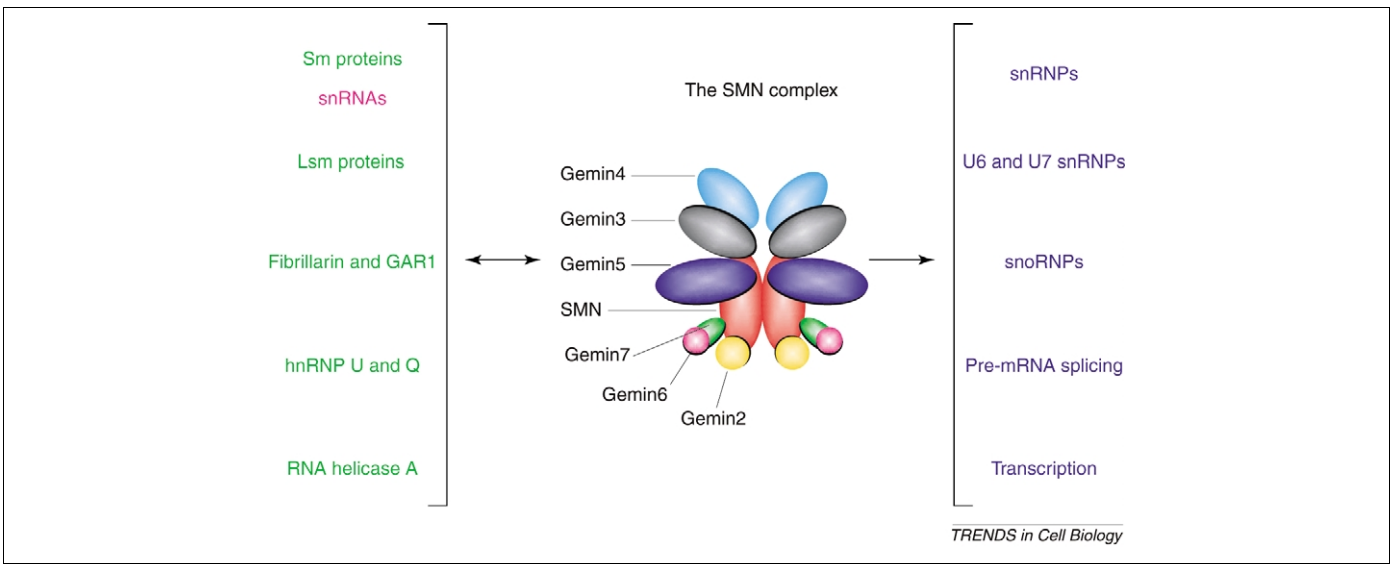

Figure 1. The survival of motor neurons (SMN) complex and its substrates. Gemins2, 3, 5 and 7 bind directly to SMN, whereas Gemins4 and 6 are associated through direct interaction with Gemins3 and 7, respectively. For simplicity, the SMN complex is depicted in dimeric form, although, from its size (30–70S), it is likely to be a much larger oligomeric structure. The SMN complex interacts directly with several protein targets (left-hand side of figure) through arginine- and glycine-rich domains. These protein targets are components of diverse ribonucleoprotein particles (RNPs) that function in a wide range of aspects of RNA metabolism (right-hand side of figure) [26,37,39,47–51,58–61]. In addition to interacting with RNA-binding proteins, the SMN complex interacts with several RNA targets [37,54]. Abbreviations: hnRNP, heterogeneous nuclear RNP; Lsm protein, Sm-like protein; snoRNP, small nucleolar RNP; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein.

proteins [36,37]. Several of the components of the SMN complex, including SMN itself, interact with the Sm proteins [26,30–34,38–40]. Arginine- and glycine-rich (RG) domains found in the Sm proteins B, D1 and D3 are required for the interaction of these proteins with SMN. This interaction is enhanced greatly by a post-translational, symmetric dimethylarginine, modification that occurs at specific arginines in the RG domains of the Sm proteins: a reaction carried out by the 20S methylosome that contains an arginine methyltransferase called JBP1 or PRMT5 [41–46] (Figure 2). The co-immunoprecipitation of Sm proteins with the SMN complex, and the close subcellular association of SMN-containing Gems with snRNP-rich Cajal (coiled) bodies first suggested a possible role for the SMN complex in snRNP biogenesis and metabolism [24] – a role subsequently demonstrated by direct-binding experiments [26,30–34,38–40] (Figures 2,3).

The SMN-dependent assembly of Sm cores

Experiments in *Xenopus* oocytes and mammalian somatic cells revealed an essential role for the SMN complex in spliceosomal snRNP assembly [25,31,38,47]. Further evidence that the SMN complex is needed for assembly of both Sm-site-containing snRNPs and the mixed Sm- and Lsm (Sm-like)-containing core found in U7 snRNP was provided using cell extracts and purified components [36,48–50]. The SMN-dependent assembly of Sm cores in

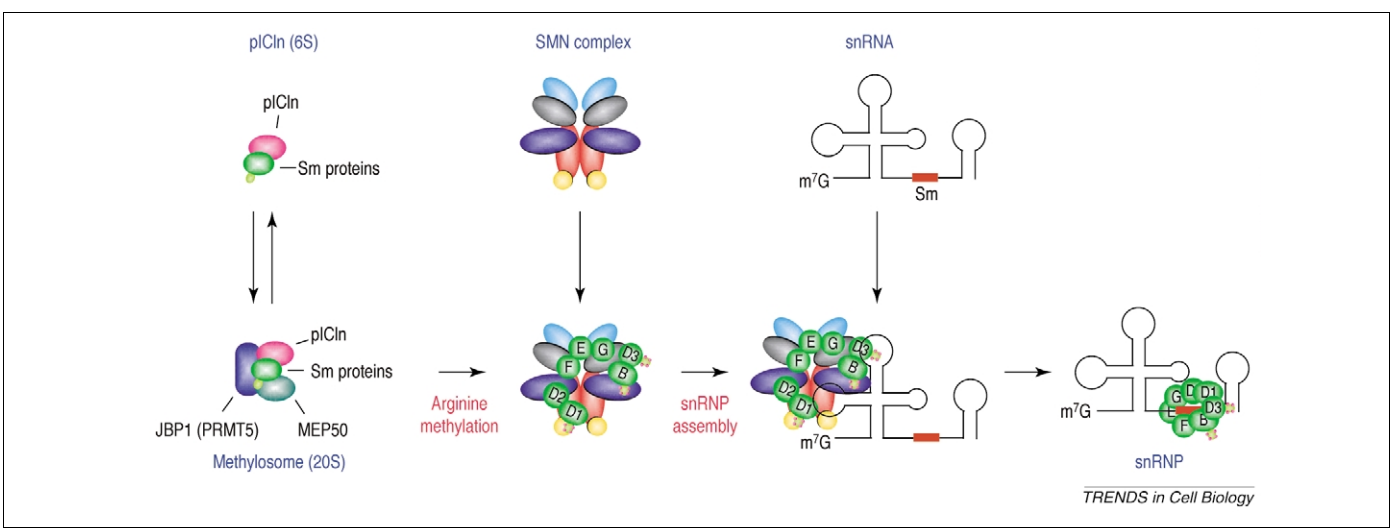

Figure 2. The survival of motor neurons (SMN) complex binds to Sm proteins and small nuclear RNAs (snRNAs) in the cytoplasm. The binding of the SMN complex to the snRNAs depends on the presence of specific, high-affinity (nanomolar) binding domains in the snRNAs. The SMN complex binds the Sm proteins through unique arginine- and glycine-rich (RG) domains found in three of these – SmB, SmD1 and SmD3 – and through additional interactions of the Gemins with Sm domains. The association with RG domains is strongly enhanced by the post-translational symmetric dimethylation of specific arginines in these domains, a process that is carried out by the methylosome (JBP1 or PRMT5) complex. Several Sm proteins also form a 6S complex with pICln. Through these specific interactions, the SMN complex brings together the Sm proteins and snRNAs, and facilitates the assembly of small nuclear ribonucleoproteins (snRNPs). Abbreviations: MEP50, methylosome protein 50; m^7G, m^7GpppG.

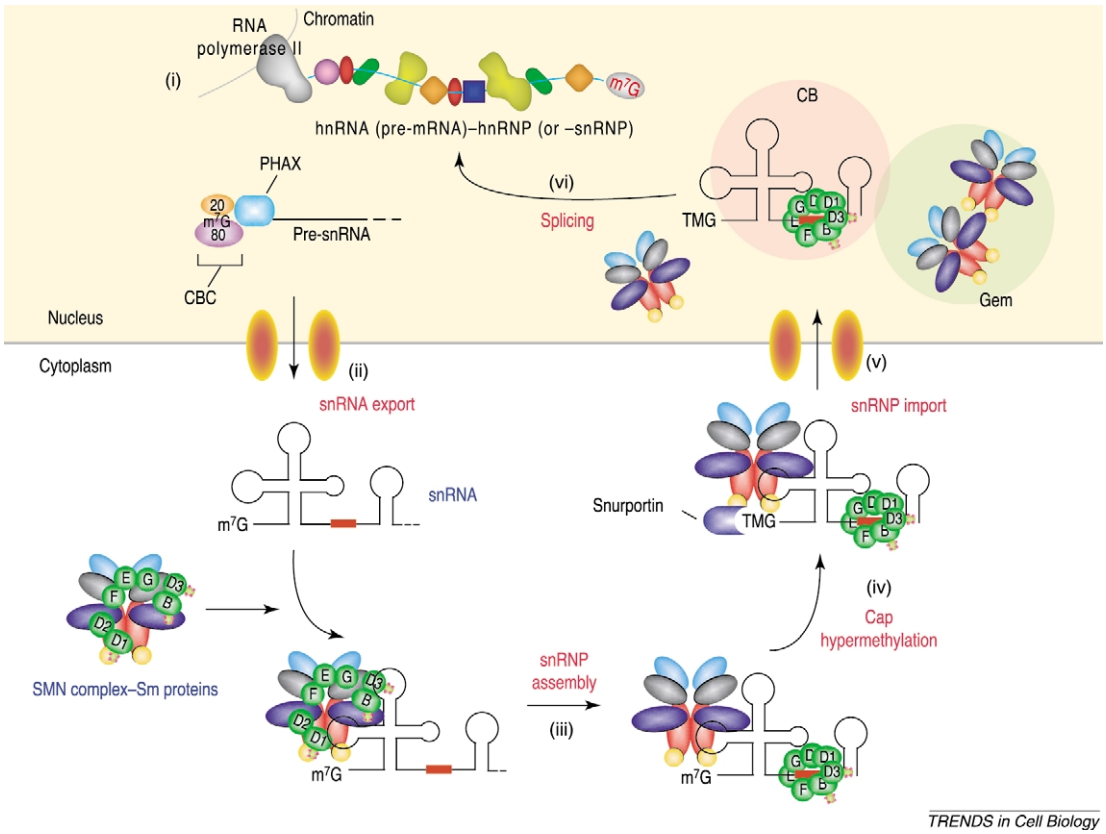

Figure 3. The role of the survival of motor neurons (SMN) complex in the assembly of spliceosomal small nuclear RNAs (snRNAs). **(i)** The major Sm-site-containing snRNAs – U1, U2, U4 and U5 – and the minor spliceosomal snRNAs are transcribed in the nucleus by RNA polymerase II. The primary transcripts contain a monomethylated m^7GpppG (m^7G) cap structure at the 5′-end and are **(ii)** rapidly exported to the cytoplasm with the 20-kDa and 80-kDa nuclear cap-binding-complex (CBC) proteins and the export adaptor PHAX. **(iii)** The formation of the Sm core is required for the subsequent **(iv)** hypermethylation of the m^7G cap of these snRNAs to convert it to a 2,2,7-trimethyl guanosine (m_3G or TMG) and for 3′-end maturation [18,62]. A properly assembled Sm core and the TMG cap structure are prerequisites for small nuclear ribonucleoprotein (snRNP) import into the nucleus **(v)**. In the nucleus, the newly imported snRNPs are initially concentrated in Cajal bodies (CBs), from which they transit to pre-mRNAs for splicing **(vi)**. The SMN complexes in the nucleus are found throughout the nucleoplasm but are particularly concentrated in Gems, the 'twins' of the snRNP-rich CBs.

cell extracts requires ATP hydrolysis [36,48,49]. Depletion of the SMN complex from cell extracts prevents the cell extract from assembling Sm cores, even though only a small fraction of the Sm proteins are removed with the SMN complex. Therefore, only Sm proteins bound to SMN complexes are active in snRNP assembly – the rest, most of which are associated with the 20S methylosome and the 6S complex that contains the Sm-binding protein pICln complex, must be sequestered in an inactive form [30,51] (Figure 2).

To facilitate snRNP assembly, the SMN complex must bring together the Sm proteins and an Sm-site-containing snRNA (Figure 3). *In vitro* experiments have shown that there is a strict requirement for ordered binding of the Sm proteins and the snRNAs to the SMN complex. For assembly to occur, the SMN complex must bind to the Sm proteins before binding to the snRNAs [36] (Figures 2,3). An RNA-binding activity for SMN was first indicated in ribo-homopolymer-binding experiments [52,53]. Because binding to snRNAs must occur after the SMN complex has been bound with at least some of the Sm proteins, it seemed possible that the Sm proteins might bridge the binding of the snRNAs to the SMN complex. However, SMN complexes washed with high-salt solution, so that all detectable Sm or other snRNP proteins are removed, still bind to snRNAs efficiently [33,36,37,54]. Direct-binding experiments with U1 snRNA demonstrated that the deletion of the Sm site does not reduce the binding of U1 snRNA to the SMN complex, which indicates that sequences in U1 snRNA other than the Sm site are necessary and sufficient for binding to the SMN complex [37]. Therefore, the SMN complex has an intrinsic capacity, independent of snRNP proteins, to bind to snRNAs and bring them into the complex for assembly with the Sm proteins. Subsequent experiments have identified the 5′-most stem–loop 1 (SL1) of U1 snRNA as the high-affinity binding sequence for the SMN complex, as well as for the assembly of U1 snRNP [37].

Spliceosomal snRNAs contain high-affinity binding domains for the SMN complex

There are other Sm-site-containing spliceosomal snRNAs that do not contain the U1 SL1 sequence, and yet SMN mediates their assembly with Sm proteins [36]. The SMN complex binds directly to all Sm-site-containing major spliceosomal snRNAs, and delineation of the sequence elements of the snRNAs responsible for SMN-complex binding has revealed that, unlike for U1 snRNA, the SMN complex binds to U2, U4 and U5 snRNAs through domains near their 3′-ends. All of these snRNAs contain at least one

well-defined stem–loop structure and include the Sm site [54] (Figure 4). Although there is no extensive nucleotide sequence similarity or obvious consensus RNA sequence among these SMN-complex-binding domains, the SMN complex binds to all of them with remarkable affinity. Mapping and deletion analysis cannot separate the Sm-site sequence from the minimal recognition domain for SMN-complex binding. Further mutagenesis and more-detailed binding experiments are needed to determine whether it is the specific sequence of the Sm sites that is crucial for binding to the SMN complex or whether the Sm sites are important for the overall structure and presentation of the adjacent stem loop.

Nevertheless, these findings suggest that the interaction between the SMN complex and snRNA occurs through specific recognition of stem–loop structure(s) in an orientation-dependent and/or sequence-specific interaction. Binding competition experiments indicate that the affinities of the various SMN-complex-binding domains for the SMN complex are not the same, although they all appear to be in the low nanomolar range, with order of affinities U4 ~ U1 > U5 > U2. The binding experiments also suggest that there are at least two high-affinity RNA-binding sites on the SMN complex [54]. Further studies are necessary to understand the interaction of the SMN complex with RNAs.

The SMN complex determines the specificity of snRNP assembly

The SMN complex binds directly and sequence-specifically to SL1 of U1 snRNA. Mutations in the SL1 sequence that impair this binding also impair the assembly of U1 snRNP *in vitro* and in *Xenopus* oocytes [37]. The sequence-specific binding of the SMN complex to a specific snRNA is crucial for selection of the snRNA as a target to ensure Sm-core assembly only on targeted RNA, thus preventing

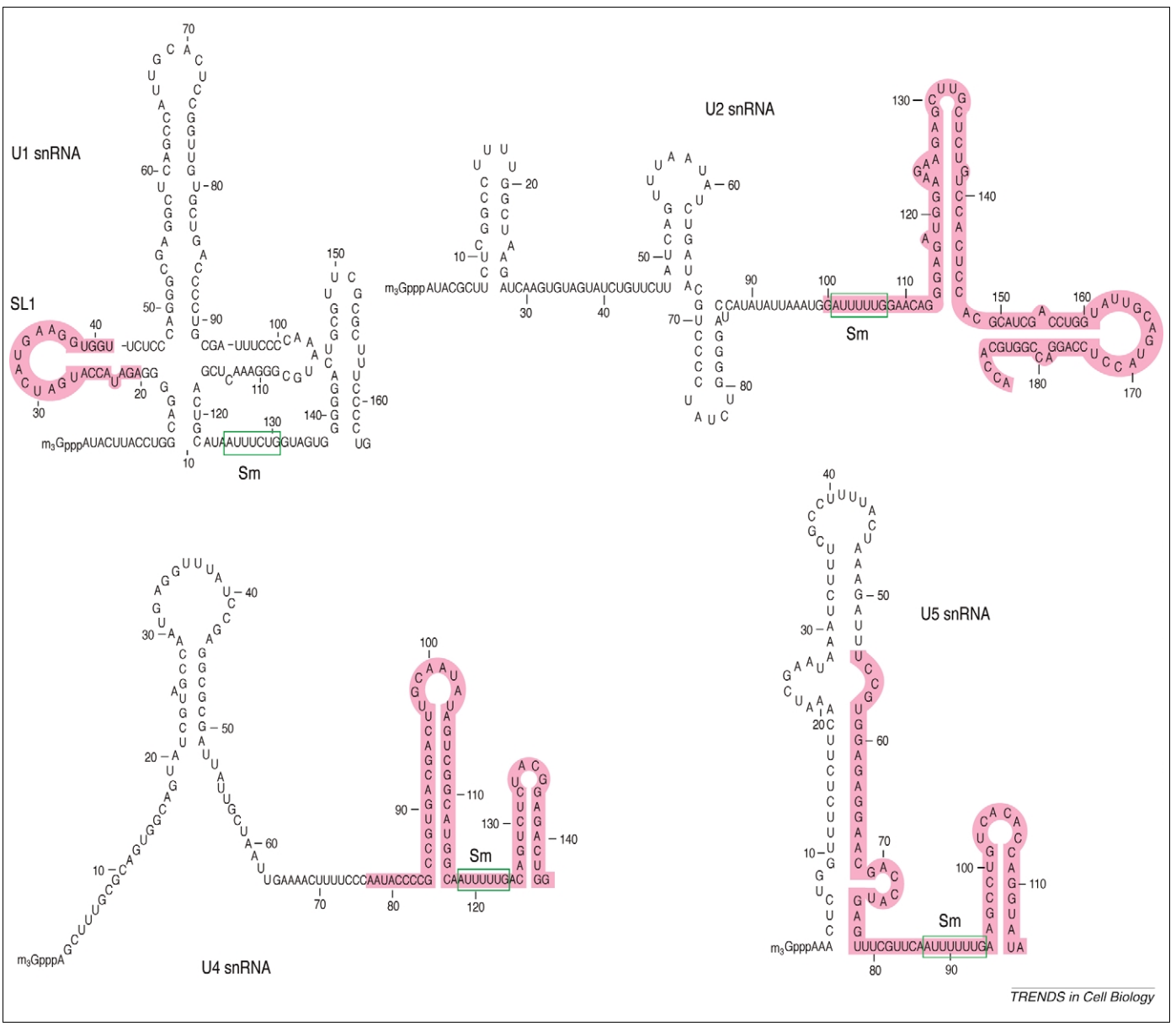

Figure 4. Specific domains in small nuclear RNAs (snRNAs) mediate their binding to the survival of motor neurons (SMN) complex. These domains are contained within the sequences indicated by pink boxes. Abbreviations: m_3G, 2,2,7-trimethyl guanosine; SL1, stem–loop 1.

promiscuous and deleterious binding of Sm proteins to other RNAs [36]. Although an Sm site and a high-affinity binding site for the SMN complex are necessary for Sm-core assembly, it is not sufficient for an RNA simply to contain these elements. For example, the relative position of SL1 and the Sm site in U1 snRNA is crucial for SMN-dependent snRNP assembly. By contrast, purified total Sm proteins alone assemble Sm cores on these snRNAs [54]. Thus, the SMN complex scrutinizes the RNA to ensure that these elements, by both their sequence and their spatial arrangement, define an authentic snRNA on which Sm-core assembly can occur. This suggests that the SMN complex not only provides the platform for binding both Sm proteins and RNAs (and brings these components into close spatial proximity for assembly) but also confers strict specificity to the assembly pathway. In this way, it functions as an 'assemblyosome' to ensure that Sm-core assembly occurs only on the correct RNA targets.

In physiological systems, there is a need to control the Sm proteins and prevent their illicit assembly on RNAs other than the intended snRNA targets. Adventitious binding of Sm proteins to RNAs would be deleterious to cells because it would interfere with the functions of the RNAs. To prevent this, Sm proteins are probably captured by the methylosome and the 6S pICln complex as soon as they are translated (Figure 2). After methylation, the Sm proteins are transferred to the SMN complex so that there is no free pool of Sm proteins available for assembly outside of this complex. The SMN complex provides stringent control over selection of RNA targets by binding directly to specific sequences found in snRNAs [37,54]. This ensures the delivery of Sm proteins for assembly only on the appropriate RNAs, and the assembly can then occur on the adjacent Sm site, at least in part, through the intrinsic capacity of the Sm proteins for self-association. In addition to this vital role, the SMN complex probably enhances the efficiency of Sm-core assembly by reducing the dimensionality of diffusion of the newly synthesized Sm proteins and the snRNAs in the complex microenvironment of the cell. Furthermore, a recent study reported a direct interaction between SMN and trimethylguanosine synthase 1, suggesting that SMN also functions to recruit the cap hypermethylase [55]. This indicates that the SMN complex associates with snRNPs during the entire cytoplasmic phase and coordinates the correct assembly and maturation of snRNPs [56,57] (Figure 3).

The SMN complex in the metabolism and assembly of diverse RNPs

Various studies have suggested that the SMN complex also plays a role in the assembly and metabolism of other types of RNPs, including small nucleolar RNPs (snoRNPs), heterogeneous nuclear RNPs, the complexes that carry out transcription and pre-mRNA splicing and, possibly, micro RNPs [26,37,39,47–51,58–61]. It is likely that, given the numerous RNA-binding proteins with which the SMN complex interacts, there are several RNA targets for it in cells, and that it is also involved in the assembly of other classes of RNPs. For example, SMN interacts with fibrillarin, a component of box C/D snoRNPs, and GAR1, a component of box H/ACA snoRNPs. The interaction of these proteins with SMN is, like that of the Sm proteins, mediated by specific RG domains contained within them [58,60]. By analogy with the role of the SMN complex in the assembly of snRNPs, it is reasonable to expect that the SMN complex is also crucial in snoRNP biogenesis.

Sedimentation in sucrose gradients reveals heterogeneity of SMN complexes because it contains particles of 30–70S. This suggests the existence of SMN complexes that are compositionally similar, although not identical. All of these complexes probably contain the SMN-complex core components, as well as various SMN-complex-interacting substrates (Figure 1). The possibility of diversity among SMN complexes is consistent with the recent finding of two non-identical SMN complexes [50]. One contains the seven Sm proteins and is, thus, charged for spliceosomal snRNP assembly. The other contains some Sm proteins but, because Sm D1 and D2 have been replaced with Lsm 10 and 11, it is charged for assembly of the mixed Sm–Lsm core found in U7 snRNP, which participates in the processing of histone mRNA 3′ ends.

Concluding remarks

Because RNA-binding proteins interact readily with their cognate RNAs, and because specific RNP structures such as Sm cores can form even on minimal RNAs, there does not seem to be a reason for cells to need a specialized molecular machine to govern their associations. Perhaps the key to understanding the essential function of the SMN complex in these assembly processes is the fact that such RNP-assembly reactions can occur so readily. In the case of the spliceosomal snRNPs, the Sm proteins form heptameric rings of extraordinary stability on their own, with minimal RNA sequence specificity. This proclivity to form such structures poses a great danger to cells because of the possibility that Sm cores could form on many RNAs, not only the intended ones. It is reasonable to envision that, to prevent the Sm proteins from exercising their promiscuity, they are prevented from roaming freely in the cell, and that the RNAs with which they are designed to assemble are selected carefully and escorted to them. To accomplish this, the Sm proteins, as nascent polypeptide chains, are captured by the methylosome and/or the 6S pICln complex. The subsequent interaction between the Sm proteins and the snRNAs is orchestrated by the SMN complex.

We suggest that the assembly of Sm cores on snRNAs in the cytoplasm, rather than the nucleus, serves to prevent the Sm proteins from entering the nucleus on their own. Specific signals in the proteins (in the form of methylated RG domains) and in the snRNAs ensure high-affinity binding to the SMN complex. Therefore, a key function of the SMN complex is to define what a proper snRNA is – to guarantee the specificity of interaction of Sm proteins and to restrict it exclusively to intended RNAs. We believe that the general principle is that, if the resulting RNP is relatively stable, it is essential to prevent the proteins from forming the complex in the wrong place, because illicit assembly would result in occlusion and loss of function of the RNAs. The remarkable specificity and high affinity of the SMN complex for several different RNAs that do not have any obvious sequence similarity raises the question

of how it binds to RNA – what is the common motif or structure in the RNAs, and which protein(s) of the SMN complex binds to RNA? It will also be important to know how many (sn)RNAs the SMN complex can handle simultaneously. Investigation of the function and structure of the SMN complex promises to yield important information about a remarkable RNA–protein assemblyosome, as well as provide insights into the molecular mechanism of SMA, a devastating neurodegenerative disease.

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References

1 Luhrmann, R. (1990) Functions of U-snRNPs. *Mol. Biol. Rep.* 14, 183–192
2 Luhrmann, R. *et al.* (1990) Structure of spliceosomal snRNPs and their role in pre-mRNA splicing. *Biochim. Biophys. Acta* 1087, 265–292
3 Mattaj, I.W. *et al.* (1993) Nucleocytoplasmic transport and snRNP assembly. *Mol. Biol. Rep.* 18, 79–83
4 Will, C.L. and Luhrmann, R. (2001) Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.* 13, 290–301
5 Fischer, U. and Luhrmann, R. (1990) An essential signaling role for the m_3G cap in the transport of U1 snRNP to the nucleus. *Science* 249, 786–790
6 Zieve, G.W. and Sauterer, R.A. (1990) Cell biology of the snRNP particles. *Crit. Rev. Biochem. Mol. Biol.* 25, 1–46
7 Andersen, J. and Zieve, G.W. (1991) Assembly and intracellular transport of snRNP particles. *Bioessays* 13, 57–64
8 Branlant, C. *et al.* (1982) U2 RNA shares a structural domain with U1, U4, and U5 RNAs. *EMBO J.* 1, 1259–1265
9 Raker, V.A. *et al.* (1996) The snRNP core assembly pathway: identification of stable core protein heteromeric complexes and an snRNP subcore particle *in vitro*. *EMBO J.* 15, 2256–2269
10 Kambach, C. *et al.* (1999) Crystal structures of two Sm protein complexes and their implications for the assembly of the spliceosomal snRNPs. *Cell* 96, 375–387
11 Stark, H. *et al.* (2001) Arrangement of RNA and proteins in the spliceosomal U1 small nuclear ribonucleoprotein particle. *Nature* 409, 539–542
12 Urlaub, H. *et al.* (2001) Sm protein–Sm site RNA interactions within the inner ring of the spliceosomal snRNP core structure. *EMBO J.* 20, 187–196
13 Ohno, M. *et al.* (2000) PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. *Cell* 101, 187–198
14 Segref, A. *et al.* (2001) The evolutionarily conserved region of the U snRNA export mediator PHAX is a novel RNA-binding domain that is essential for U snRNA export. *RNA* 7, 351–360
15 Sauterer, R.A. *et al.* (1988) Cytoplasmic assembly of snRNP particles from stored proteins and newly transcribed snRNAs in L929 mouse fibroblasts. *Exp. Cell Res.* 176, 344–359
16 Zieve, G.W. *et al.* (1988) Newly synthesized small nuclear RNAs appear transiently in the cytoplasm. *J. Mol. Biol.* 199, 259–267
17 Bringmann, P. *et al.* (1983) Purification of snRNPs U1, U2, U4, U5 and U6 with 2,2,7-trimethylguanosine-specific antibody and definition of their constituent proteins reacting with anti-Sm and anti-(U1)RNP antisera. *EMBO J.* 2, 1129–1135
18 Mattaj, I.W. (1986) Cap trimethylation of U snRNA is cytoplasmic and dependent on U snRNP protein binding. *Cell* 46, 905–911
19 Hamm, J. *et al.* (1990) The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal. *Cell* 62, 569–577
20 Fischer, U. *et al.* (1991) Diversity in the signals required for nuclear accumulation of U snRNPs and variety in the pathways of nuclear transport. *J. Cell Biol.* 113, 705–714
21 Fischer, U. *et al.* (1993) Nucleo-cytoplasmic transport of U snRNPs: definition of a nuclear location signal in the Sm core domain that binds a transport receptor independently of the m_3G cap. *EMBO J.* 12, 573–583
22 Raker, V.A. *et al.* (1999) Spliceosomal U snRNP core assembly: Sm proteins assemble onto an Sm site RNA nonanucleotide in a specific and thermodynamically stable manner. *Mol. Cell. Biol.* 19, 6554–6565
23 Jarmolowski, A. and Mattaj, I.W. (1993) The determinants for Sm protein binding to *Xenopus* U1 and U5 snRNAs are complex and non-identical. *EMBO J.* 12, 223–232
24 Liu, Q. and Dreyfuss, G. (1996) A novel nuclear structure containing the survival of motor neurons protein. *EMBO J.* 15, 3555–3565
25 Fischer, U. *et al.* (1997) The SMN–SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* 90, 1023–1029
26 Liu, Q. *et al.* (1997) The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell* 90, 1013–1021
27 Mattaj, I.W. (1998) Ribonucleoprotein assembly: clues from spinal muscular atrophy. *Curr. Biol.* 8, R93–R95
28 Lefebvre, S. *et al.* (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80, 155–165
29 Melki, J. (1997) Spinal muscular atrophy. *Curr. Opin. Neurol.* 10, 381–385
30 Charroux, B. *et al.* (1999) Gemin3: a novel DEAD box protein that interacts with SMN, the spinal muscular atrophy gene product, and is a component of gems. *J. Cell Biol.* 147, 1181–1194
31 Charroux, B. *et al.* (2000) Gemin4: a novel component of the SMN complex that is found in both gems and nucleoli. *J. Cell Biol.* 148, 1177–1186
32 Gubitz, A.K. *et al.* (2002) Gemin5, a novel WD repeat protein component of the SMN complex that binds Sm proteins. *J. Biol. Chem.* 277, 5631–5636
33 Pellizzoni, L. *et al.* (2002) Purification of native survival of motor neurons complexes and identification of Gemin6 as a novel component. *J. Biol. Chem.* 277, 7540–7545
34 Baccon, J. *et al.* (2002) Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. *J. Biol. Chem.* 277, 31957–31962
35 Paushkin, S. *et al.* (2002) The SMN complex, an assemblyosome of ribonucleoproteins. *Curr. Opin. Cell Biol.* 14, 305–312
36 Pellizzoni, L. *et al.* (2002) Essential role for the SMN complex in the specificity of snRNP assembly. *Science* 298, 1775–1779
37 Yong, J. *et al.* (2002) Sequence-specific interaction of U1 snRNA with the SMN complex. *EMBO J.* 21, 1188–1196
38 Buhler, D. *et al.* (1999) Essential role for the tudor domain of SMN in spliceosomal U snRNP assembly: implications for spinal muscular atrophy. *Hum. Mol. Genet.* 8, 2351–2357
39 Friesen, W.J. and Dreyfuss, G. (2000) Specific sequences of the Sm and Sm-like (Lsm) proteins mediate their interaction with the spinal muscular atrophy disease gene product (SMN). *J. Biol. Chem.* 275, 26370–26375
40 Meister, G. *et al.* (2000) Characterization of a nuclear 20S complex containing the survival of motor neurons (SMN) protein and a specific subset of spliceosomal Sm proteins. *Hum. Mol. Genet.* 9, 1977–1986
41 Brahms, H. *et al.* (2000) The C-terminal RG dipeptide repeats of the spliceosomal Sm proteins D1 and D3 contain symmetrical dimethylarginines, which form a major B-cell epitope for anti-Sm autoantibodies. *J. Biol. Chem.* 275, 17122–17129
42 Brahms, H. *et al.* (2001) Symmetrical dimethylation of arginine residues in spliceosomal Sm protein B/B′ and the Sm-like protein Lsm4, and their interaction with the SMN protein. *RNA* 7, 1531–1542
43 Friesen, W.J. *et al.* (2001) SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. *Mol. Cell* 7, 1111–1117
44 Friesen, W.J. *et al.* (2001) The methylosome, a 20S complex containing JBP1 and pICln, produces dimethylarginine-modified Sm proteins. *Mol. Cell. Biol.* 21, 8289–8300
45 Friesen, W.J. *et al.* (2002) A novel WD repeat protein component of the methylosome binds Sm proteins. *J. Biol. Chem.* 277, 8243–8247
46 Meister, G. *et al.* (2001) Methylation of Sm proteins by a complex containing PRMT5 and the putative U snRNP assembly factor pICln. *Curr. Biol.* 11, 1990–1994
47 Pellizzoni, L. *et al.* (1998) A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell* 95, 615–624

48 Meister, G. *et al.* (2001) A multiprotein complex mediates the ATP-dependent assembly of spliceosomal U snRNPs. *Nat. Cell Biol.* 3, 945–949
49 Meister, G. and Fischer, U. (2002) Assisted RNP assembly: SMN and PRMT5 complexes cooperate in the formation of spliceosomal UsnRNPs. *EMBO J.* 21, 5853–5863
50 Pillai, R.S. *et al.* (2003) Unique Sm core structure of U7 snRNPs: assembly by a specialized SMN complex and the role of a new component, Lsm11, in histone RNA processing. *Genes Dev.* 17, 2321–2333
51 Pu, W.T. *et al.* (1999) pICln inhibits snRNP biogenesis by binding core spliceosomal proteins. *Mol. Cell. Biol.* 19, 4113–4120
52 Liu, Q. *et al.* (1996) Molecular characterization of the protein products of the fragile X syndrome gene and the survival of motor neurons gene. *Cold Spring Harbor Symp. Quant. Biol.* 61, 689–697
53 Lorson, C.L. and Androphy, E.J. (1998) The domain encoded by exon 2 of the survival motor neuron protein mediates nucleic acid binding. *Hum. Mol. Genet.* 7, 1269–1275
54 Yong, J. *et al.* (2004) snRNAs contain specific SMN-binding domains that are essential for snRNP assembly. *Mol. Cell. Biol.* 24, 2747–2756
55 Mouaikel, J. *et al.* (2003) Interaction between the small-nuclear-RNA cap hypermethylase and the spinal muscular atrophy protein, survival of motor neuron. *EMBO Rep.* 4, 616–622
56 Massenet, S. *et al.* (2002) The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. *Mol. Cell. Biol.* 22, 6533–6541
57 Narayanan, U. *et al.* (2002) SMN, the spinal muscular atrophy protein, forms a pre-import snRNP complex with snurportin1 and importin β. *Hum. Mol. Genet.* 11, 1785–1795
58 Jones, K.W. *et al.* (2001) Direct interaction of the spinal muscular atrophy disease protein SMN with the small nucleolar RNA-associated protein fibrillarin. *J. Biol. Chem.* 276, 38645–38651
59 Mourelatos, Z. *et al.* (2001) SMN interacts with a novel family of hnRNP and spliceosomal proteins. *EMBO J.* 20, 5443–5452
60 Pellizzoni, L. *et al.* (2001) The survival of motor neurons (SMN) protein interacts with the snoRNP proteins fibrillarin and GAR1. *Curr. Biol.* 11, 1079–1088
61 Mourelatos, Z. *et al.* (2002) miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. *Genes Dev.* 16, 720–728
62 Plessel, G. *et al.* (1994) m_3G cap hypermethylation of U1 small nuclear ribonucleoprotein (snRNP) *in vitro*: evidence that the U1 small nuclear RNA-(guanosine-N2)-methyltransferase is a non-snRNP cytoplasmic protein that requires a binding site on the Sm core domain. *Mol. Cell. Biol.* 14, 4160–4172