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Minireview

Deciphering the assembly pathway of Sm-class U snRNPs

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Abstract The assembly of the Sm-class of uridine-rich small nuclear ribonucleoproteins (U snRNPs), albeit spontaneous in vitro, has recently been shown to be dependent on the aid of a large number of assisting factors in vivo. These factors are organized in two interacting units termed survival motor neuron (SMN)- and protein arginine methyltransferase 5 (PRMT5)-complexes, respectively. While the PRMT5-complex acts early in the assembly pathway by activating common proteins of U snRNPs, the SMN-complex functions to join proteins and RNA in a highly ordered, apparently regulated manner. Here, we summarize recent progress in the understanding of this process and discuss the influence exerted by the aforementioned *trans-acting* factors.

Keywords: U snRNP biogenesis; SMN-complex; PRMT5-complex; Sm proteins; Splicing; U snRNA

1. Assembly of RNA–protein complexes in the cellular environment

The ability to reconstitute macromolecular complexes from purified single components in vitro has fostered the concept of self-assembly, one of the central principles of molecular biology. From a theoretical, "in vitro" point of view, the formation of macromolecular complexes depends on diffusion-driven, random, and reversible encounters of the cognate subunits [1,2]. The stability of the resulting entities is determined by the ratio of the respective association and dissociation rate constants. While these considerations hold true for idealized, aqueous solutions, the situation in vivo is markedly different. In cells, the local concentration of individual components of macromolecular complexes and other proteins is usually relatively low, yet as a whole they occupy a significant fraction of the total volume. Hence, the possibility for unspecific interactions arises, which hinder the assembly pathway. Individual molecules therefore need to be directed to the site of complex assembly, in order to increase their local concentration and protect them against unfavorable interactions. Furthermore, a separation of both the site of assembly and the site of function should help to prevent assembly intermediates from adversely affecting the function of fully assembled macromolecular complexes. Taking these considerations into account, it is not surprising that cells have evolved strategies to ensure the faithful generation of macromolecular assemblies [1]. Among others, three features seem to predominate: (1) the segregation of biosynthesis of individual components and their assembly into higher-order structures into different subcellular compartments; (2) the evolution of molecular chaperones, which promote formation of intermediates, shielding these intermediates from adverse, premature interactions with substrate molecules of the finally assembled macromolecular complexes; and (3) *trans-acting* factors, working as scaffolds to coordinate several processes of the assembly reaction. A number of biological processes follow, at least in part, these principles and well known examples are the assembly of proteasomes and nucleosomes [3,4].

In this review, we summarize recent experimental advances in the understanding of the in vivo biogenesis pathway of macromolecular RNA–protein complexes found in the spliceosome uridine-rich small nuclear ribonucleoproteins (U snRNPs). We speculate that many aspects of this pathway have evolved as a consequence of the theoretical considerations outlined above.

2. Biogenesis of snRNPs

The spliceosome is a macromolecular machine comprising several RNP subunits that catalyzes the removal of intervening (non-coding) sequences from pre-mRNA (pre-messenger RNA). U snRNPs of the major spliceosome (responsible for splicing the majority of pre-mRNA introns) consist of either one (U1, U2, U5) or two (U4/U6) small nuclear RNAs (snRNAs), and a large number of proteins [5,6]. As individual classes of U snRNPs perform distinct functions in the spliceosome, it is not surprising that each is characterized by a specific set of proteins. However, all U snRNPs also contain a set of seven common, so-called Sm proteins B/B′, D1, D2, D3, E, F and G (B′ is a splicing variant of B). These evolutionarily related proteins form a heptameric ring around a conserved sequence

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Abbreviations: snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein; SMN, survival motor neuron; PRMT5, protein arginine methyltransferase 5; WD45, WD repeat domain 45; pICln, chloride conductance regulatory protein; CBC, cap-binding complex; PHAX, phosphorylated adaptor for RNA export; CRM1, chromosome region maintenance 1; RanGTP, Ras-related nuclear protein bound to GTP; NPC, nuclear pore complex; Tgs1, trimethylguanosine synthetase1; NLS, nuclear localization signal; SPN1, snurportin-1; Lsm proteins, like Sm protein

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motif ($PuAU_{4-6}GPu$) on the snRNA termed the "Sm-site" [7], which is a structural hallmark of these particles [8,9]. The biochemical composition of the minor spliceosome, specialized for processing of a small group of introns (so-called ATAC introns), requires a different set of U snRNPs (termed U11, U12, U4atac and U6atac; U5 appears to be identical in both the major and the minor spliceosome) [10]. However, the general architecture and hence the biogenesis of the U snRNPs of the major and minor spliceosome is believed to be very similar. Thus, although we will concentrate on the description of biogenesis of the components of the major spliceosome, the same principles will most likely also apply for the minor snRNPs.

A large number of studies performed mainly in *Xenopus laevis* oocytes but also in somatic cells have contributed to the understanding of the transport pathways enabling the biogenesis of spliceosomal U snRNPs [6]. Due to these studies the biogenesis of U snRNPs could be divided into several steps, some of which may actually be coupled. Initially, the RNA polymerase II (pol II) transcribes precursor snRNAs (pre-snRNAs) of U1, U2, U4 and U5 in the nucleus that are co-transcriptionally m^7G-capped (Fig. 1, step 1). Transcriptional termination was recently shown to be dependent on a megadalton complex termed Integrator [11]. In the mechanistic model, the Integrator complex is assumed to interact with RNA polymerase II at the promoter of the snRNA genes. Remaining attached to RNA polymerase during transcription, the Integrator complex endonucleolytically cleaves the pre-snRNA upon interaction with the 3′-box; a *cis-acting* element of 13–16 nucleotides that is required for efficient pre-snRNA formation. The m^7G-cap of the pre-snRNA is specifically recognized by the cap-binding complex (CBC), which itself associates with phosphorylated adaptor for RNA export (PHAX), chromosome region maintenance 1 (CRM1) and Ras-related nuclear protein bound to GTP (RanGTP) to form the nuclear export complex [12]. After transport through the nuclear pore complex (NPC) (step 2), GTP hydrolysis of Ran and dephosphorylation of PHAX cause the transport factors to dissociate from the snRNA [13,14] (step 3). U6 RNA (and also U6atac) differ from other snRNAs in that they are transcribed by RNA polymerase III, acquire a γ-monomethyl cap and appear not to leave the nucleus post-transcriptionally [15,16].

In the cytoplasm, the survival motor neuron complex (SMN-complex) facilitates the transfer of seven Sm proteins onto the snRNA's "Sm-site", which results in the formation of the Sm core domain (step 4; see next paragraph for a detailed description). Recent evidence suggests that the U snRNP remains bound to the SMN-complex even after assembly is completed [17]. The Sm core domain and the SMN-complex then cooperate to recruit the cap-hypermethylase trimethylguanosine synthetase1 (Tgs1) [18]. This leads to the conversion of the m^7G-cap of the snRNA to its hypermethylated form, the 2,2,7-trimethylguanosine-cap (m_3G, also termed TMG) (step 5). At an as yet to be defined step in the cytoplasm, the mature 3′-end of U snRNAs is generated by unknown (exo)ribonuclease(s) in a process referred to as 3′-trimming [19,20].

The assembled and processed particle is then imported into the nucleus by means of a bipartite nuclear localization signal (NLS) on the U snRNP and at least two specific transport factors. One part of the NLS is the m_3G-cap that is recognized by the protein snurportin-1 (SPN1) [21]. This interaction alone is not sufficient for import of U snRNPs but requires assistance of importin β [22]. It is believed that importin β has a docking site on the Sm core-bound SMN-complex [23] and hence may constitute the second part of the bipartite NLS, which has long-since been suspected to lie on the Sm core domain [24]. Once both transport factors have bound to their respective signals, nuclear import can be effected (step 6). Within the nucleus, the import complex dissociates and the transport factors are recycled to the cytoplasm (step 7). The U snRNPs, presumably still attached to the SMN-complex, transiently accumulate in subnuclear domains termed Cajal bodies (step 8). Within these domains, small Cajal body RNAs (scaRNAs) introduce site-specific pseudouridylation (Ψ) and 2′-*O*-methylation (m) in the snRNAs [25,26] and thereby complete processing of U snRNAs (step 9). For most snRNP-specific proteins it remains unknown whether they join the particle already in the cytoplasm, as is the case for Sm proteins, or only after re-import into the nucleus. Ultimately, the mature spliceosomal snRNPs accumulate in interchromatin regions in structures referred to as splicing speckles (step 10). The SMN-complex, which is dissociated from the U snRNP at a yet to be identified stage in the nucleus, is then believed to return into the cytoplasm (step 11), where it can re-enter the biogenesis cycle (step 12).

3. *Trans-acting* factors mediate the assembly of spliceosomal U snRNPs

When isolated Sm proteins are incubated with U snRNA under appropriate conditions in vitro, efficient Sm core formation can be observed. This process takes place in an apparently ordered and defined manner. Prior to RNA-binding, Sm proteins form heterooligomeric complexes composed of D1–D2, B/B′–D3, and E–F–G [27,28]. RNP-binding occurs in two steps, first, the so-called subcore particle is formed by interaction of D1–D2 and E–F–G, which is then transformed by the addition of B/B′–D3 into the core particle [28]. These observations have led to the conclusion that all structural information required for the formation of this core-RNP is encoded within these components (i.e. RNA and proteins). But the fact that these structures form in vitro does not necessarily prove that it is also the case in vivo, in particular if the deliberations of the first paragraph are taken into account. Indeed, a body of recent evidence indicates that formation of the Sm core domain of U snRNPs requires ATP and assisting factors, the number of which exceeds that of proteins actually assembled onto the U snRNA [29,30].

The first factor implicated in the biogenesis of U snRNPs was the survival motor neuron (SMN) protein. This factor, whose reduced expression elicits the neuromuscular disease spinal muscular atrophy (SMA), was found to transiently interact with U snRNAs in the cytoplasm but was not an integral part of mature U snRNPs [31]. As detailed biochemical studies further revealed, SMN is a constituent of a macromolecular complex consisting of at least eight key subunits (termed Gemins2–8 and unrip; Fig. 2) [29,30,32–34]. In addition to these integral components, spliceosomal Sm proteins and U snRNAs can be found transiently associated with the SMN-complex. Initial insights into the function of the SMN-complex in U snRNP biogenesis were gained by the biochemical reconstitution of the in vivo assembly reaction from isolated components [29]. These studies have revealed that Sm proteins must first bind to

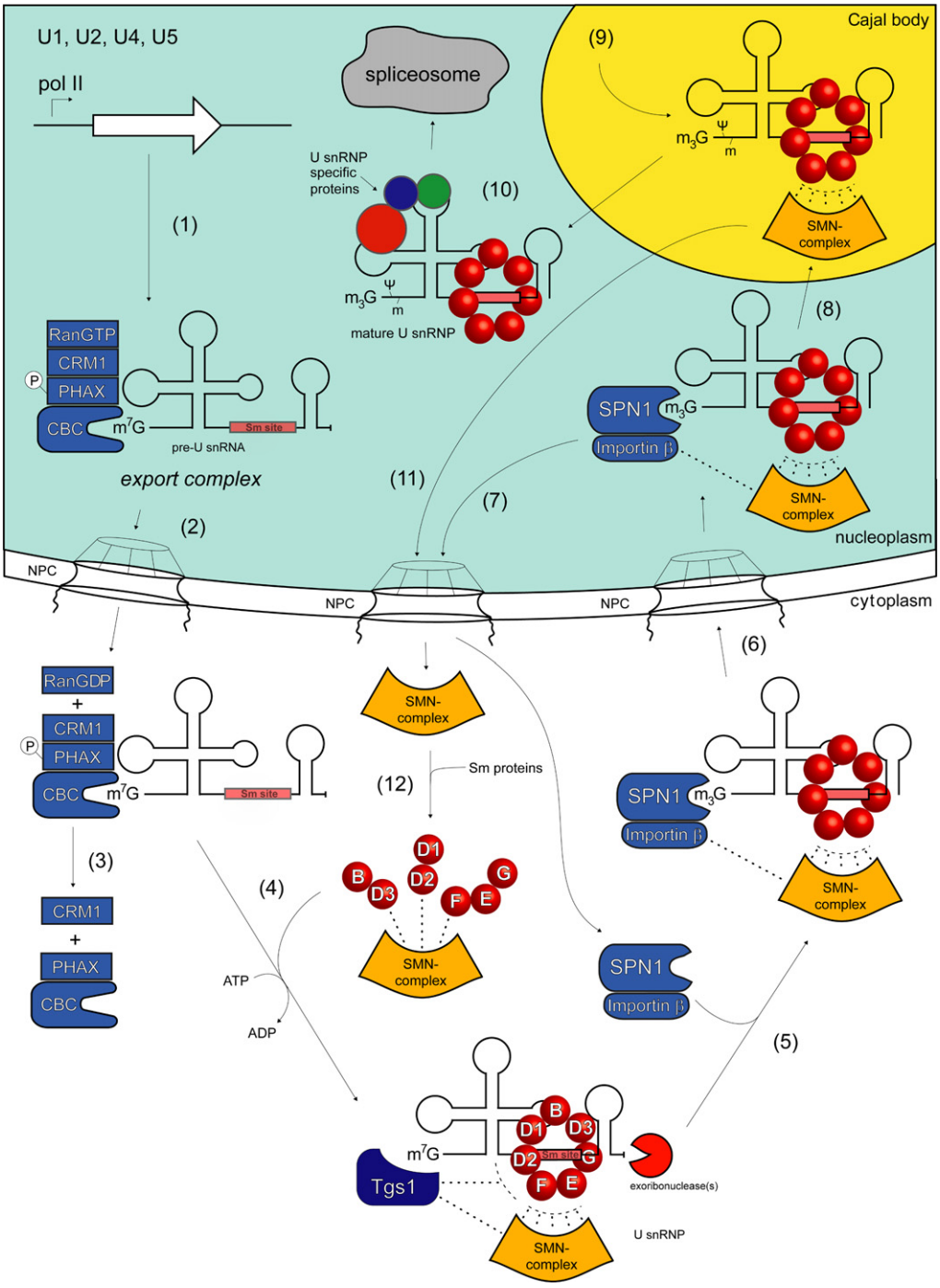

Fig. 1. Biogenesis pathway of spliceosomal U snRNPs. Pre-U snRNA is transcribed by RNA polymerase II (pol II) and m^7G-capped in the nucleus (step 1). After the export complex, consisting of pre-U snRNA, CBC, PHAX, CRM1 and RanGTP, has formed, it is actively transported into the cytoplasm via the NPC (step 2). There, export factors and pre-U snRNA dissociate from each other (step 3) and Sm proteins provided by the SMN-complex are assembled onto the "Sm-site" of pre-U snRNA (step 4). Following recruitment by the SMN-complex and Sm core domain, the hypermethylase Tgs1 modifies the m^7G-cap to m_3G (step 5), before the import factors SPN1 and importin β mediate translocation into the nucleus (step 6). There, both factors dissociate and are recycled into the cytoplasm (step 7), and U snRNPs associated with the SMN-complex enrich in Cajal bodies (step 8). After scaRNA guided pseudouridylation (Ψ) and 2′-*O*-methylation (m; step 9), the mature U snRNP is directed to the spliceosome, (step 10), whereas the SMN-complex is believed to be exported into the cytoplasm (step 11), where it can re-enter the biogenesis cycle (step 12).

the SMN-complex before they can be transferred onto U snRNA. Thus, unlike in vitro, Sm proteins cannot be directly delivered onto U snRNA within the context of a living cell and hence the assembly reaction does not strictly follow a "self-assembly route" (see Fig. 1, step 4).

Although the SMN-complex loaded with all Sm proteins is alone necessary and sufficient for U snRNP assembly, this process is influenced by another complex, whose name-giving constituent is the methyltransferase protein arginine methyltransferase 5 (PRMT5) [35–37]. This enzyme, in conjunction

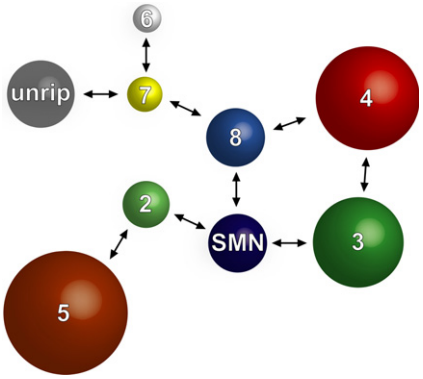

Fig. 2. Interaction map of the human SMN-complex. Schematic of protein interactions within the human SMN-complex as described in [34]. The SMN protein together with Gemin8 and Gemin7 form a core scaffold of the SMN-complex by which the remaining components are recruited. SMN is directly connected to Gemin2, which itself is associated with Gemin5. Furthermore, both Gemin6 and unrip are recruited by Gemin7, whereas Gemin3 and Gemin4 are cooperatively bound by SMN and Gemin8, respectively.

with its interaction partners WD repeat domain 45 (WD45), also termed Mep50, and chloride conductance regulatory protein (pICln), catalyzes the formation of symmetrical dimethylarginines within the C-terminal tails of the Sm proteins B/B′, D1 and D3. Since these modifications increase the affinity of the Sm proteins for the SMN protein in vitro, it is assumed that one function of the PRMT5-complex is the stimulation of the assembly process [35,38]. Recently, evidence for yet another arginine methyltransferase protein arginine methyltransferase 7 involved in Sm protein activation has been reported in HeLa cells which acts in a non-redundant fashion on Sm protein modification [39]. Interestingly, however, genetic inactivation of PRMT5 in *Drosophila melanogaster* has no recognizable influence on U snRNP biogenesis [40]. Therefore, the question arises whether the symmetric dimethylation of Sm proteins is an absolute prerequisite or whether it is only an accessory function in snRNP biogenesis. SMN- and PRMT5-complexes directly interact with each other in a higher-order structure [41]. The cooperating SMN- and PRMT5-complexes can be hence envisaged as the functional unit that promotes and regulates the assembly of spliceosomal U snRNPs.

4. A model for the assisted assembly of spliceosomal U snRNPs

Based on reported data from several laboratories, a model for the assisted U snRNP assembly process can be proposed [42,43]. Sm proteins, synthesized in the cytoplasm, are initially sequestered by the PRMT5-complex (Fig. 3). PICln is likely to play an important role in complex formation as it directly binds to Sm proteins and PRMT5 [35–37]. These initial steps commit Sm proteins to the SMN-mediated assembly pathway. Once recruited onto the PRMT5-complex, Sm proteins B/B′, D1 and D3 are symmetrically dimethylated on arginine residues and may hence become "activated" for subsequent steps (Fig. 3, step 1). We speculate that the PRMT5-complex (or parts thereof) facilitate(s) additional events in the assembly pathway, such as organization of specific Sm protein sub-complexes. In this context, it is noteworthy that each Sm protein occupies a specific spatial position within the Sm core domain of the assembled U snRNP [8]. PRMT5- and SMN-complexes then join to form the SMN-/PRMT5-complex, in which the complete set of Sm proteins is first transferred onto the SMN-complex (step 2), and then passed onto U snRNA (step 3). Ultimately, the assembled U snRNP is transferred along with the SMN-complex to the nucleus, where the U snRNP is further processed and targeted to its site of function. The SMN-complex is then exported into the cytoplasm to engage in another assisted U snRNP biogenesis cycle (step 4, see also Fig. 1, step 12).

5. Open questions and future directions

Some questions need to be addressed to understand the mechanism of this unique assembly system. One of them is how the flow of Sm proteins through the assembly machinery onto U snRNA is facilitated and regulated. We favor a model, in which Sm proteins are pre-assembled on the PRMT5-complex to form the heterooligomers B/B′–D3, D1–D2 and E–F–G, as a prerequisite for the transfer onto the SMN-complex. This model implies that Sm proteins on the PRMT5-complex do not have the propensity of binding to U snRNA, a situation that obviously changes upon transition to the SMN-complex associated state. What could be the switch to turn it from an assembly incompetent into an assembly active state? The answer may lie within the architecture of the components that make up the Sm core domain of U snRNPs, with the RNA being tightly surrounded by seven Sm proteins. Two obvious scenarios could explain formation of such a structure: In one scenario, the Sm protein ring is formed on the SMN-complex and subsequently the RNA threaded through the central hole. Considering the spatial organization of the U snRNP, this mechanism appears to be rather unlikely. A more probable scenario may be a clamp-loading like mechanism. In this process, the Sm proteins are kept on the SMN-complex in an "open ring" configuration. Upon binding of the U snRNA, the SMN-complex may undergo structural rearrangements leading to the closure of the Sm protein ring around the "Sm-site". Therefore, such an "open ring" conformation should be induced by the topology of the SMN-complex. In preceding steps of the assembly line, however, it should be disallowed. This model implies a conformational switch of the SMN-complex and a step in which the RNA is identified and bound onto the open Sm ring. Gemin3 and Gemin5 may play crucial roles in these postulated events. The Gemin3 protein belongs to the DEAD-box family of RNA helicases and may explain why assembly is dependent on the hydrolysis of ATP. Gemin5, in contrast, has been shown to specifically recognize "Sm-site" containing RNAs and may hence guide the U snRNA to the "Sm-site" [44]. Further studies are required to clarify, whether this scenario holds true, or another yet to be discovered mechanism, accounts for the formation of the Sm core domain.

As outlined above, U snRNPs (like other RNPs) can assemble spontaneously in vitro. Therefore, one may ask why *trans-acting* assembly factors are required in vivo. Life without this system is impossible as illustrated by the fact that inactivation of SMN, Gemin2 and pICln is lethal in several organisms,

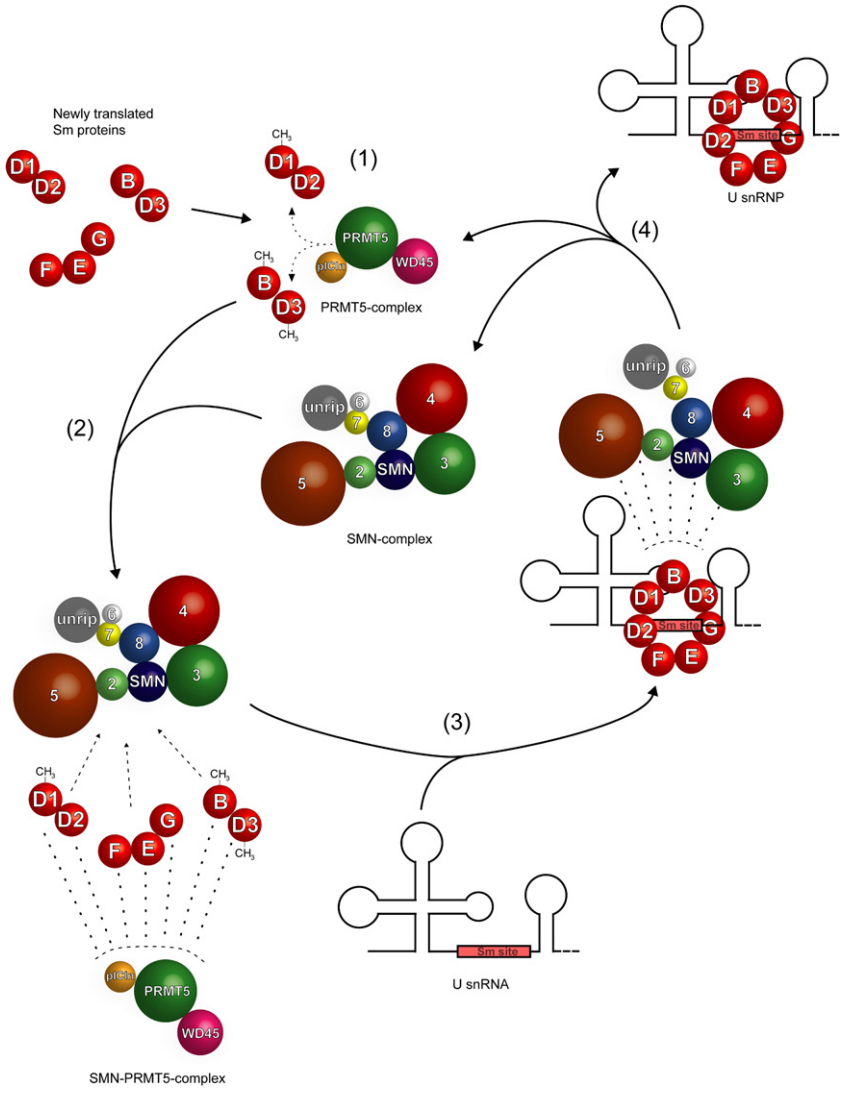

Fig. 3. Model of assisted assembly of U snRNPs. Sm proteins are initially translated in the cytoplasm and sequestered by the PRMT5-complex, consisting of the Type II methyltransferase PRMT5, WD45 (also termed Mep50) and pICln, which promotes symmetric dimethylation of arginines on Sm proteins B/B′, D1 and D3 (step 1). Next, the SMN-complex interacts with the PRMT5-complex to form an SMN-PRMT5-complex in which the Sm proteins are transferred onto the SMN-complex (step 2). These Sm proteins are assembled onto the "Sm-site" of U snRNAs to form U snRNPs (step 3). Finally, the U snRNP, the SMN-complex and PRMT5-complex dissociate and the latter two engage in a new round of U snRNP biogenesis (step 4).

including mice (see [32] for a review). This excludes the possibility that spontaneous assembly is a default pathway in vivo, which is redundant with assisted RNP formation. We speculate that one function of the SMN–PRMT5-system is to serve as a chaperone system that prevents mis-assembly of Sm proteins to non-target RNA and Sm protein aggregation. Indeed, first experimental evidence for this activity has been provided recently [30].

Spliceosomal U snRNPs are certainly the most abundant class of particles, assembly of which is mediated by the SMN-PRMT5-system. But are there also other classes of particles depending on this system? This is true for U snRNPs of the minor spliceosome (i.e. U11, U12, U4atac), which contain an Sm core domain indistinguishable from their counterparts of the major spliceosome. Thus, we are confident to postulate a common assembly for most, probably all, particles with "canonical" Sm core domains. However, a large number of RNPs contain core structures composed, at least in part, of different components. One such case is the U7 snRNP, a particle involved in 3′-end processing of histone mRNA. The core domain of this particle consists of the canonical Sm proteins B, D3, E, F and G, whereas D1 and D2 are replaced by the "like Sm proteins" 10 and 11 (termed Lsm10 and 11) [45]. Interestingly, assembly of the Lsm/Sm core of the U7 snRNP has been shown to be dependent on the SMN-complex charged with the U7-specific set of core proteins [46]. Thus, also a particle with a mixed Lsm/Sm core depends on this assembly machinery.

A group of related like Sm proteins (Lsm proteins) has been identified recently (termed Lsm1–8) which can form heptameric ring-like structures very similar in shape and size to the "canonical" Sm core domain [47]. Depending on their composition, they mediate either RNA degradation (Lsm1–7) [48,49], or function as core components of the spliceosomal snRNAs U6 and U6atac (Lsm2–8) [50–52]. Interestingly, these

Lsm-exclusive rings can form in the absence of RNA and hence behave in this respect markedly different than Sm (and Lsm10/11) proteins [50]. If the SMN-complex acts exclusively as a clamp loader onto the respective cognate RNAs, as outlined above, assembly of the Lsm rings may occur independently of this system. However, SMN (as a single protein) has been shown to bind to Lsm proteins in vitro, providing the possibility that at least some proteins of the SMN-complex also play a role in the biogenesis of Lsm rings [53].

Finally, some nuclear and nucleolar RNAs such as box C/D small nucleolar RNA, box H/ACA and telomerase RNA have been shown to associate with distinct subsets of Sm proteins or other classes of proteins, which are able to interact with SMN [54–58]. We regard the development of in vitro assembly assays, which recapitulate the in vivo situation, an obligate prerequisite to address the question whether the SMN–PRMT5-system is indeed a master assembler for a large variety of different RNPs or whether this system is restricted to a smaller class harboring only specific sets of Sm and Lsm proteins.

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