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► **To cite this version:**

Séverine Massenet. In vivo assembly of eukaryotic signal recognition particle: A still enigmatic process involving the SMN complex. *Biochimie*, 2019, 164, pp.99-104. 10.1016/j.biochi.2019.04.007 . hal-02279910

HAL Id: hal-02279910

<https://hal.science/hal-02279910>

Submitted on 20 Jul 2022

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***In vivo* assembly of eukaryotic signal recognition particle: a still enigmatic process involving the SMN complex**

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Abstract

The signal recognition particle (SRP) is a universally conserved non-coding ribonucleoprotein complex that is essential for targeting transmembrane and secretory proteins to the endoplasmic reticulum. Its composition and size varied during evolution. In mammals, SRP contains one RNA molecule, 7SL RNA, and six proteins: SRP9, 14, 19, 54, 68 and 72. Despite a very good understanding of the SRP structure and of the SRP assembly *in vitro*, how SRP is assembled *in vivo* remains largely enigmatic. Here we review current knowledge on how the 7SL RNA is assembled with core proteins to form functional RNP particles in cells. SRP biogenesis is believed to take place both in the nucleolus and in the cytoplasm and to rely on the survival of motor neuron complex, whose defect leads to spinal muscular atrophy.

1. RNP biogenesis often requires numerous assembly factors

Molecular assemblies of biomolecules facilitate diverse biological tasks in the cells of all organisms. Many cellular functions are performed by a family of molecular machines made up of RNA-protein complexes, called non-coding ribonucleoprotein (ncRNP) complexes. These ncRNPs include ribosomes and spliceosomes, respectively involved in translation and splicing, and a plethora of other stable ncRNPs involved in multiple essential cellular functions including chromatin modification, transcriptional regulation, ribosome biogenesis, epigenetic and translational regulation. Among these functions, the evolutionary conserved signal recognition particle (SRP) is crucial for protein targeting to the endoplasmic reticulum (ER) in eukaryotes and to the plasma membrane in archaea and bacteria.

Given the fundamental roles played by ncRNPs, it is essential for cells to produce these RNPs in a functional state, and this can be a real challenge. How the complexes are produced and how cells control their quality and their amounts is still far from being understood. Early studies demonstrated that fully active ncRNPs can be reconstituted *in vitro* from purified components. Indeed, despite the highly complex assembly process of the ribosome, active subunits can be reconstituted *in vitro* using purified ribosomal (r-) proteins and ribosomal RNAs (rRNAs) [1,2]. These data imply that the folding determinants are encoded in the RNP components. This is a general rule, since over the years, many laboratories have managed to assemble different ncRNPs from different organisms in spontaneous and sequential reactions, including 1) SRP [3,4,5,6], 2) U rich small nuclear RNPs (UsnRNP), which are components of the spliceosome [7,8], and 3) the archaeal counterpart of the small nucleolar RNPs (snoRNPs) required for post-transcriptional modifications and processing of rRNA precursors [9]. Despite this general tendency for self-assembly of macromolecular complexes *in vitro*, the situation *in vivo* is extremely different and numerous assisting factors can be required for efficient and faithful assembly in cells (discussed in [10]). Ribosome biogenesis is a remarkable example: one human ribosome contains 80 r-proteins and four rRNAs, but more than 200 factors (designated assembly factors) are required for its biogenesis (reviewed in [11,12,13]). Studies of UsnRNP assembly also largely contributed to understanding the importance of assisting factors in cells. Indeed, two macromolecular complexes are indispensable for faithful UsnRNP biogenesis: the methylosome (PRMT5 complex) and the survival of motor neuron (SMN) complex (reviewed in [14]). Studies of the biogenesis of eukaryotic snoRNPs also highlighted how essential the assembly factors are (for a recent review, see [15]).

The assembly factors associate transiently with the individual subunits of the ncRNPs, promoting the efficient and highly specific formation of mature ncRNPs, performing proof-reading function and directing the ncRNPs to their correct location in cells. Assembly factors are absent from the mature complexes, they are often evolutionarily conserved and out-number ncRNP components,

illustrating their importance for the cells. However, even in these most studied cases, how ncRNP assembly is catalyzed and regulated is still not completely understood.

2. Signal Recognition Particle: composition and function in protein secretion

Protein secretion and their correct cellular localization are crucial to maintain cell compartmentalization and homeostasis. In eukaryotes, one third of proteins are translocated into the ER, prior to being transported to their final destinations. There are several strategies to localize proteins to the ER (for a recent review, see [16]); the principal and best characterized one relies on SRP, which is one of the most abundant ncRNPs in cells. It is conserved across all three kingdoms of life, but its composition and size varied during evolution (for reviews, see [17,18,19]). Bacterial SRP is the simplest as it comprises the universal SRP54 protein (Ffh in bacteria) bound to the SRP RNA (4.5S RNA in *Escherichia coli* containing 114 nucleotides). In mammals, SRP contains a larger RNA molecule comprising 300 nucleotides, 7SL RNA, and six proteins: SRP9, 14, 19, 54, 68 and 72 (Figure 1). Mammalian SRP is composed of two distinct functional domains that can be separated by micrococcal nuclease [20]: 1) the Alu domain, which most likely gave rise to the Alu sequence element scattered throughout the human genome (for a review, see [21]), and 2) the S domain. The Alu domain contains the 5' and 3' domains of the 7SL RNA, with the heterodimer composed of two proteins, SRP9 and SRP14 attached to it. The S domain consists of four additional proteins: SRP19, SRP54, and the heterodimer SRP68/SRP72 associated with the central part of the 7SL RNA. In yeast, SRP9/14 heterodimer is replaced by a Srp14p homodimer and a yeast-specific protein, Srp21p that is structurally related to SRP9. Archaeal SRP contains an SRP RNA whose size and secondary structure resemble its mammalian counterpart and homologs of only two mammalian SRP proteins: SRP54 and SRP19.

SRP-mediated targeting is achieved via a series of closely coordinated ordered steps (for reviews, see [19,22,23]). SRP54 recognizes the N-terminal signal sequence (signal-anchor sequence SAS) of the proteins destined for the ER when they are being translated. In fact, SRP rapidly scans all ribosomes by forming transient complexes. These complexes quickly dissociate or, if the nascent chain present in the peptide exit tunnel carries a SAS, they undergo conformational changes to become high affinity complexes [24,25,26,27]. The Alu domain causes a delay in protein synthesis, in a process called elongation arrest. Indeed, the SRP Alu domain localizes in the GTPase center between the 40S and the 60S subunits, therefore competing with the binding of the elongation factors [28,25]. The SRP-ribosome nascent chain complex is then targeted to the ER membrane through a GTP-dependent interaction between SRP54 and the SRP receptor (SR). The signal sequence is released from SRP and inserted into the Sec61 translocon channel. GTP hydrolysis triggers the dissociation of SR and SRP and translation resumes. SRP can start the next targeting round.

SRP is essential for growth in *E. coli*, in the archaea *Haloflex volcanii*, and in the yeast *Yarrowia lipolytica* and *Schizosaccharomyces pombe* [29,30,31,32,33]. In contrast, the yeast

Saccharomyces cerevisiae and the Gram-positive bacterium *Streptococcus mutans* continue to grow, albeit poorly, in the absence of SRP [34,35,36]. These organisms survive 1) by increasing chaperone and protease expression to cope with the accumulation of mislocalized membrane and secretory proteins in the cytosol, and 2) by reducing the expression of ribosomal components to reduce protein synthesis and hence the need for translocation machineries [36,37]. However, a sudden drop in cellular SRP levels is lethal for *S. cerevisiae* [38]. It was recently shown that SRP loss in *S. cerevisiae* leads to the mistargeting of SRP-dependent proteins to mitochondria instead of to ER, triggering mitochondrial dysfunction [39]. In trypanosomes, depletion of SRP proteins resulted in the death of the parasite [40,41] and in mammalian cells, expression of mutated SRP proteins leads to significant growth and translocation defects [42]. Recently, it was observed that mutations in SRP72 cause familial aplasia and myelodysplasia, while mutations in SRP54 lead to congenital neutropenia with Shwachman-diamond-like features [43,44,45]. These data all point to essential aspects of SRP for cell survival and for maintaining the efficiency and specificity of protein targeting, as well as to the need to coordinate SRP and ribosome productions in cells.

3. Signal Recognition Particle biogenesis: stepwise assembly?

In vitro studies and structural analyzes have provided a very good understanding of the assembly of the SRP *in vitro*, as well as of the protein-protein and protein-RNA interactions, and the SRP 3D structure (for reviews, see [46,47,48 49]). The hetero-dimerization of SRP9 and SRP14 is required for their binding to 7SL RNA [50]. SRP68 and SRP72 are also associated with 7SL RNA as a heterodimer [3,51], but each protein has the capability to bind 7SL RNA independently of the other, at least *in vitro* [52,53]. In contrast, both SRP19 and SRP54 bind individually to 7SL RNA. However, SRP54 is not able to associate with 7SL RNA unless SRP19 is bound beforehand 3. The prerequisite of binding of SRP19 is because SRP19 binding clamps 7SL RNA helices 6 and 8 together, creating a binding platform for SRP54 [54,55,56,57]. The formation of this RNA platform is facilitated by the binding of SRP68/72 heterodimer to the 7SL RNA 58.

In contrast, the mechanism of SRP biogenesis *in vivo* is far from being understood. An early study showed that a portion of 7SL RNA could be fractionated with purified nucleoli of rat hepatoma [59]. Transient nucleolar localization of mammalian endogenous 7SL RNA was confirmed by *in situ* hybridization [60], or using fluorescent 7SL RNA microinjected into the nucleus of mammalian cells [61]. Endogenous SRP19, or GFP (green fluorescent protein)-tagged-SRP19, SRP72 and SRP68 transiently expressed in mammalian cells, localized as expected in the cytoplasm but also to some extent within the nucleus and nucleolus, while on the contrary endogenous SRP54 or transiently expressed GFP-SRP54 were only observed in the cytoplasm [60,62]. Concerning the yeast *S. cerevisiae*, Srp68p, Srp72p, Srp14p or Srp21p also displayed a cytoplasmic and nucleolar localization pattern [63,64]. In contrast to mammalian SRP19, its yeast homolog Sec65p has not been observed in

the nucleolus but only in the nucleoplasm [63,64]. Therefore, either Sec65p does not enter the nucleolus or does so only transiently. Taken together, these data led to the hypothesis that SRP assembly takes place at least partially in the nucleolus [61,60]. This property appears to be evolutionarily conserved in eukaryotes, since some SRP components have also been observed in the nucleolus of *X. laevis* and of the parasites *Trypanosoma brucei* and *Plasmodium falciparum* [65,41,66]. This conservation highlights the importance of a nucleolar phase during SRP biogenesis even though the reason is still unknown.

The current model proposed for SRP assembly in eukaryotic cells is based on examination of the location of SRP components in cells and still awaits experimental confirmation. In this model, 7SL RNA is transported into the nucleolus where it associates with five SRP proteins: SRP9, SRP14, SRP19, SRP68 and SRP72, to form an initial pre-SRP (Figure 1). The pre-SRP is then exported to the cytoplasm where it associates with SRP54 to form the mature and functional SRP particle. In *S. cerevisiae*, since Sc65p (the yeast homolog to SRP19) was observed in the nucleoplasm but not in the nucleolus, it may bind the yeast SRP RNA (scR1) in this compartment. *In vitro*, SRP19, SRP54 and 7SL RNA can form a stable but misassembled and unfunctional complex [67,68]. This observation may explain why SRP54 may be sequestered in the cytoplasm and why it may be the last protein to join the particle. However, several data suggest that SRP54 may transit in the nucleus and or the nucleolus and challenge the current hypothesis that SRP54 binding occurs in the cytoplasm of eukaryotic cells. Indeed, although SRP54 is only detectable in the cytoplasm of HeLa cells in WT conditions, it strongly accumulates in the nucleus in the presence of Leptomycin B, a drug that inhibits CRM1 export [69]. Moreover, SRP54 has been detected in the nucleolus of the parasite *P. falciparum* [66].

In yeast, in the absence of either Srp68p, Srp72p, Srp14p or Srp21p (replacing SRP9 in yeast), no SRP complexes was observed and the level of scR1 RNA dropped severely [35]. In contrast, a stable RNA-containing SRP subcomplex was formed in the absence of Sec65p and Srp54p, and the level of scR1 RNA remained unchanged upon depletion of these proteins [35]. These data indicate that Srp68p, Srp72p, Srp14p or Srp21p are essential for SRP RNA stability and that they form a stable core complex with scR1 RNA, to which Sec65p and Srp54p can subsequently bind. The absence of any Srp68p, Srp72p, Srp14p or Srp21p inhibits the export of the pre-SRP in the cytoplasm, while the absence of Sec65p or Srp54p has no effect [64], indicating that the formation of the core complex is a precondition for the export of the pre-particle. Gene silencing of either SRP68 or 72 homologs in *T. brucei* causes massive accumulation of SRP RNA in the nucleolus [41], confirming the importance of these proteins in the formation of a pre-SRP competent for export.

4. Processing and post-transcriptional modification of 7SL RNA

7SL RNA is transcribed by the RNA polymerase III (polIII) and bears a triphosphate 5' end [70]. The 3' extremity of human 7SL RNA has the sequence CUCUUU-OH. The last three terminal uridylic residues are post-transcriptionally removed and an adenylic acid residue is added by the poly(A) polymerase γ [71,72]. The polIII termination factor La binds the 7SL RNA through its 3'-oligo(U) tract and is required for its accurate processing, at least in yeast [73]. The 7SL RNA present in the nucleolus is already 3'-end processed and adenylated, indicating that these two maturation steps are early events in SRP assembly [74]. A RNA mutant that cannot bind SRP9/14 dimer cannot be adenylated *in vitro* [72] suggesting that processing of 7SL RNA occurs in the nucleolus after pre-SRP formation. The post-transcriptional modification pattern of 7SL RNA is not yet known. Early studies suggest that rat 7SL RNA does not contain any pseudouridine (Ψ) residue, and that the U residue at position 10 may be modified, however, the nature of the modification is not known [70]. Human 7SL RNA is thought to contain at least one m⁶A and one m⁵C residues [75,76], while the presence of other types of methylation has not yet been explored. The nucleolus is the site of snoRNP-guided formation of pseudouridines, 2'-O-methylated residues and acetylated cytosines (ac⁴C) within rRNA precursors and U6 snRNA [77,11,78,15]. One interesting hypothesis is that at least some of the modifications carried by the 7SL RNA may be catalyzed by snoRNPs in the nucleolus.

5. Trafficking of SRP during its biogenesis

How are SRP proteins imported into the nucleus and how do they reach the nucleolus? How is the 7SL RNA targeted to the nucleolus? How does the pre-SRP leave the nucleolus and is exported to the cytoplasm? All these processes are still unclear and most of the factors required need to be characterized. In yeast, each SRP protein enters the nucleus independently of the other proteins [63,64]. Srp68p, Srp72p, Srp14p and Srp21p are imported by the importins/karyopherins Pse1p and Kap123p [64]. The import route of Sec65p is less clear but seems to require several import pathways to be efficient [64]. In the human HeLa cell line, *in vitro* nuclear import assays showed that SRP19 is efficiently transported into the nucleus by two members of the importin- β family of transport receptor proteins: importin 8 and transportin [62]. In *X. laevis*, importin- α is required for SRP19 nuclear import [65]. Concerning the localization of 7SL in vertebrates, its Alu domain is indispensable for its nucleolar targeting and Helix 8 in the S-domain is also important [79,61].

In yeast, pre-SRP release from the nucleolus does not require CRM1(Xpo1) but its subsequent export into the cytoplasm does [63,64,69]. Yeast Srp68p contains two NES signals that can serve as adaptors between Xpo1 and pre-SRP [64]. Several studies have also implicated CRM1 in pre-SRP export in vertebrates [80,65], and several SRP proteins have been found to be CRM1 binders in HeLa cells and *X. laevis* [69]. However, a recent study suggested that Exportin-5 rather than CMR1 is involved in this process [81]. Thus, how pre-SRP is exported into the cytoplasm remains to be clarified.

6. Assembly of eukaryotic SRP requires the SMN complex

The SMN complex contains the SMN protein, associated with Gemin2 to 8 and Unrip proteins. This complex is essential for cell survival and is present in all eukaryotes tested so far except in the yeast *S. cerevisiae*. Reduced levels of the SMN protein lead to a severe pathology, spinal muscular atrophy (SMA), which is an autosomal recessive neuromuscular disease characterized by muscle atrophy and paralysis, mainly due to degeneration and loss of the α motor neurons of the spinal cord anterior horn. The development and functioning of other types of neurons may also be impaired, as well as several non-neuronal organs including heart and liver (reviewed in [82]). SMA remains the leading genetic cause of infant mortality and affects 1/8000 children. In more than 98% of patients, the disease is caused by deletions or mutations in the *SMN1* gene [83]. A reduced level of the SMN protein causes the disease, while complete loss of SMN expression results in prenatal death. The SMN complex functions in multiple RNA metabolic pathways, including transcription, splicing, and RNP biogenesis and transport including the essential UsnRNPs; and in neuron-specific functions like neurite and axon outgrowth, growth cone excitability, **protein homeostasis** and the function of the neuromuscular junction (for reviews, see [14,84,85,86]). The previously mentioned pathways are altered when SMN amounts in cells are reduced. The causes of SMA are still not fully understood as it remains unclear how a defect in the ubiquitous SMN protein leads to the tissue- and cell-specific degeneration of the motor neurons of the spinal cord.

The SMN complex has been shown to be required for SRP assembly in eukaryotic cells [87]. The SMN complex associates with SRP in HeLa cells and *X. laevis*, and the 7SL RNA interacts directly with the SMN complex via one of its components, Gemin5. Microinjection of anti-SMN or anti-Gemin2 antibodies into the cytoplasm of *X. laevis* oocytes was shown to strongly interfere with the association of 7SL RNA with SRP54 [87]. The SMN complex might therefore be important for SRP54 binding to pre-SRP. Consistently, SMN deficiency caused a severe reduction in the level of 7SL RNA in the spinal cord of severe SMA mice (but not in other tissues), as well as in the *S. pombe* cells carrying a temperature-degron allele of *SMN* gene, suggesting that a defect in SRP biogenesis may contribute to the severity of the disease [87]. The role of the SMN complex in SRP assembly may not be restricted to SRP54 binding since a fraction of the SMN complex is located in the nucleolus [88,89,90]. Further researches are thus needed to understand the exact molecular function of the SMN complex in SRP biogenesis both in the cytoplasm and in the nucleus.

7. Conclusion

Eukaryotic SRP biogenesis is a stepwise process that takes place at least partially in the nucleolus. Remarkably, apart from the SMN complex and a few other proteins involved in RNA processing and cellular trafficking, no other factor involved in the biogenesis of SRP has been identified to date. How SRP is assembled and how its biogenesis is regulated are far from being

understood. Further studies are needed to 1) understand the precise function of the SMN complex in SRP assembly and to look for other putative SRP assembly factors, 2) elucidate the mechanisms that tightly regulate trafficking of all the SRP components, 3) characterize the intermediate complexes that form during SRP assembly, and 4) identify the pattern of post-transcriptional modifications in 7SL RNA and their role in SRP biogenesis and function.

ACKNOWLEDGEMENTS

Many thanks to Dr. S. Labialle for comments on the manuscript. S. Massenet is supported by the French *Centre National de la Recherche (CNRS)*, the University of Lorraine and the “*Association pour la recherche contre la Sclérose Latérale Amyotrophique* » (ARSLA).

FIGURE LEGENDS

Figure 1. Hypothetical model of mammalian SRP biogenesis

During the biogenesis of mammalian SRP, a pre-complex containing 7SL RNA and SRP9, SRP14, SRP68, SRP72 and SRP19 proteins may be assembled in the nucleolus where 7SL RNA is processed. The pre-SRP is then exported into the cytoplasm and the mature SRP is then formed by SRP54 binding with the help of SMN complex. See text for details, references and discussion about this model.

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