RNA gymnastics in mammalian signal recognition particle assembly

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More than one third of the cellular proteome is destined for incorporation into cell membranes or export from the cell. In all domains of life, the signal recognition particle (SRP) delivers these proteins to the membrane and protein traffic falls apart without SRP logistics. With the aid of a topogenic transport signal, SRP retrieves its cargo right at the ribosome, from where they are sorted to the translocation channel. Mammalian SRP is a ribonucleoprotein complex consisting of an SRP RNA of 300 nucleotides and 6 proteins bound to it. Assembly occurs in a hierarchical manner mainly in the nucleolus and only SRP54, which recognizes the signal sequence and regulates the targeting process, is added as the last component in the cytosol. Here we present an update on recent insights in the structure, function and dynamics of SRP RNA in SRP assembly with focus on the S domain, and present SRP as an example for the complex biogenesis of a rather small ribonucleoprotein particle.

The signal recognition particle (SRP) is universally conserved and plays a central role in co-translational protein transport.1,2 The discovery of SRP as a ribonucleoprotein complex presents an example of the serendipity of discovery. Already in 1970, studies on oncornavirus described a 7S RNA (later 7SL RNA) that was derived from the infected host cell rather than from the virus.3 This 7S RNA associated with polyribosomes in a sub-stoichiometric fashion and somehow seemed to participate in the translation process.4 In the 1980s, SRP was first reported as an 11S protein complex in canine pancreatic tissue5 (Fig. 1A). SRP assembly starts in the nucleolus,8 but is completed with the addition of SRP54 in the cytosol.9 Here, we focus on the structure, function, and dynamics of mammalian SRP RNA in respect to ribonucleoprotein particle (RNP) assembly. SRP RNA has been found in all domains of life, but the size and secondary structure elements vary significantly between the phylogenetic groups, however with a number of generally conserved features.10,11 Long SRP RNA (7S RNA, in contrast to short bacterial 4.5S RNA) folds into a terminal Alu domain and an S domain of about equal sizes of 150 nucleotides.12,13 The S domain harboring the 4 larger proteins recognizes SRP targets through the presence of an N-terminal signal sequence as soon as it emerges from the ribosomal tunnel.5,6 With the discovery of the 7S RNA as part of the protein complex, SRP was then defined as the signal recognition particle.7

While in the past 30 y the analysis of SRP structure and function has come a long way, the biogenesis of mammalian SRP remained enigmatic. The mammalian SRP consists of a single copy of SRP RNA with about 300 nucleotides that forms the assembly platform for 6 proteins (named according molecular mass: SRP9/14, SRP19, SRP54, SRP68/72)5 (Fig. 1A). SRP assembly starts in the nucleolus,8 but is completed with the addition of SRP54 in the cytosol.9 Here, we focus on the structure, function, and dynamics of mammalian SRP RNA in respect to ribonucleoprotein particle assembly. SRP RNA has been found in all domains of life, but the size and secondary structure elements vary significantly between the phylogenetic groups, however with a number of generally conserved features.10,11 Long SRP RNA (7S RNA, in contrast to short bacterial 4.5S RNA) folds into a terminal Alu domain and an S domain of about equal sizes of 150 nucleotides.12,13 The S domain harboring the 4 larger proteins recognizes SRP targets through the presence of an N-terminal signal sequence as soon as it emerges from the ribosomal tunnel exit.14-16 The Alu domain (including SRP9/14) imposes an elongation arrest or retardation of translation17-19 by binding into the elongation factor binding site.20-24 The structure of mammalian SRP has been determined by cryo-EM studies in the context of the ribosome – nascent chain complex (RNC),25,26 but is not available on its own. In the past 15 years, sub-structures of mammalian SRP have been determined, but it was only this year that the mechanism of the

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S domain remodeling RNA-binding domain of the SRP68 protein was described, which we will now integrate in the current scheme of SRP assembly. SRP RNA is transcribed by RNA polymerase III and is only slightly processed. Its 3’ end consists of a modifiable oligo (U) tract like other RNA polymerase III transcripts. In yeast this region was recently shown to bind to the stabilizing La protein homolog and to the nuclear RNA quality machinery including...
TRAMP-stimulated exosomal degradation. The 3′ end of human SRP RNA is shortened to a single uridine and an adenine is added. While the cellular control shortens to a single uridine and an adenine, it does not give rise to defined electron density when bound to the RNC. The connection seems to form a flexible hinge necessary for adapting SRP to the ribosomal surface. S domain RNA folding depends on the SRP19 and SRP68 proteins. SRP19 comprises a single, monomeric RNA binding domain (RBD), while SRP68 comes as a large solenoidal heterodimer together with SRP72, the structure of which so far is unknown. A significant portion of SRP68/72 seems to be flexible, as it does not give rise to defined electron density when bound to the RNC.  

Chemical probing data and mutational analyses revealed the primary binding site for SRP19 to involve the distal end of helix 6 and its closing GNAR tetraloop with an unusual conservation of an adenine at the third position. SRP68 localizes to the 3-way junction connecting helices 5, 6 and 8. SRP72 binds to helix 5 adjacent to SRP68 and was described to stabilize an RNA “kink-turn” at the 5e-loop, however, no structure of this interaction is yet available. Interestingly, SRP68 and SRP72 have been implied in SRP export and also SRP independent functions. Mammalian S domain RNA by itself is flexible and its structure could not be determined until now. First atomic insights in RNA structure and in the process of SRP assembly came from the structure of human SRP19 bound to helix 6. SRP19 is a flexible protein with a β topology that adopts a stable fold upon RNA binding. It binds to a widened major groove and the phosphoribose backbone of the GNAR tetraloop (Fig. 1B, second panel) leaving the conserved adenine solvent exposed. However, crystal packing immediately suggested a plausible model for its strict conservation by the formation of RNA-RNA tertiary interactions, which could be subsequently confirmed by all structures including the complete S domain RNA (for human SRP). Some of its mysteries could be resolved recently by structure determination of the ternary complex of human SRP68-RBD, S domain RNA and SRP19. The purely α-helical SRP68-RBD has similarity to a tetratricopeptide repeat (TPR) fold (Fig. 1B, third panel). It comprises an extended positively charged patch for RNA binding but does not resemble any classical RNA-binding domain. SRP68-RBD binds as a rigid body to the 3-way junction of SRP RNA helices 5, 6, and 8 mainly via the phosphoribose backbone. Three α-helices constitute a concave surface with one α-helix being accommodated in the T-loop structure and one α-helix protruding deeply into the major groove of helix 5 at the 5f-loop, thus significantly stabilizing the RNA 3-way junction. Like for SRP19, SRP68-RBD binding remodels the complete S domain RNA. SRP68-RBD induces a kink of helix 5 in respect to the coaxially stacked helix 8 by about 20° away from helix 6 with the hinge point locating proximal to the asymmetric loop of helix 8. It is important to note, that the SRP68-mediated kink in SRP RNA is maintained upon interaction of SRP with the RNC and that the kink is essential for establishing a specific contact between SRP RNA and rRNA (Fig. 1D). This interaction provides an explanation for the deleterious effect of SRP68 depletion on translocation. In addition to these long-range RNA rearrangements, the insertion of an α-helix (a so-called arginine-rich motif (ARM)) into the
major groove causes a groove opening that coincides with the remodeling of the 5f-loop (Fig. 1C) (see also51). Like the asymmetric loop in helix 8, the asymmetric 5f-loop switches from a protein-free inward “closed” conformation41 to a protein-bound “open” conformation exposing 2 conserved purines bases (human: A231, G232). Intriguingly, the corresponding region in bacterial 4S RNA is known to be responsible for SRP GTPase activation and thus is critically involved in the control of the targeting process.52,53 Therefore, the principle of SRP GTPase stimulation by exposed purine bases might be conserved also in mammalian SRP. However, this has not been shown so far and further experiments are necessary.

With the incorporation of SRP19, SRP9/14, and SRP68/72, nucleolar assembly is complete and the pre-SRP is exported (not well investigated) to the cytosol where SRP54 is added as the last component. SRP54 is the key-driver of SRP and comprises a methionine-rich M domain for signal sequence and RNA binding, and an SRP GTPase domain (SRP54-NG) for regulation (Fig. 1B, fourth panel). Binding of the M-domain (SRP54-M; “closed” conformation without signal54) to helix 8 of the SRP RNA induces the formation of a stacked platform by the asymmetric loop and involves the read-out of the modified minor groove of the most highly conserved SRP RNA sequence formed by 5 non-canonical base pairs of the symmetric loop.55 SRP is now ready to bind to an RNC, and to accommodate a hydrophobic signal sequence emerging from the ribosomal exit tunnel (“open” conformation of the M domain50) (Fig. 1D). RNC binding again results in rearrangements, e.g. involving a conserved hinge region in SRP54.16 Protein targeting proceeds by the interaction of SRP54-NG with its twin in the SRP receptor (SRo-NG, not shown)52,56. The gymnastics described here for the SRP RNA during SRP assembly is then continued in an elaborate choreography involving the RNC-SRP complex, the membrane associated SRP receptor (SR) and the translocation machinery.

Disclosure of Potential Conflicts of Interest

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