Structure and function of the signal recognition particle (SRP)

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Signal recognition particle (SRP) is a ribonucleoprotein complex which mediates the targeting of nascent secretory (Walter and Blobel, 1980), lysosomal (Erickson et al. 1983) and membrane proteins (Katz et al. 1977; Sakaguchi et al. 1984; Rottier, et al. 1985 Lipp and Dobberstein, 1986;) to the endoplasmic reticulum membrane (ER). The steps involved in this process have been elucidated using in vitro systems that faithfully reproduce the translocation of nascent proteins across membranes (Blobel and Dobberstein, 1975). The steps can be described as follows (see Fig. 1.): Translation of mRNA coding for a secretory protein is initiated on free ribosomes in the cytoplasm (1). Most secretory proteins are synthesized with a N-terminal extension called a signal sequence. Upon emergence from the ribosome, this sequence is recognized by SRP (2). At this stage SRP can arrest or retarde (Walter and Blobel, 1981; Lipp et al., 1987) further elongation until contact is made with the receptor for SRP in the ER membrane, the docking protein (Meyer et al. 1982) or SRP receptor (Gilmore et al. 1982; Gilmore and Blobel, 1983) (3). Elongation resumes and the nascent chain is translocated across the membrane. On the luminal side of the membrane the signal sequence of most presecretory proteins is cleaved by signal peptidase (4). For further detail on the initial events of protein transport through the secretory pathway see recent reviews by Hortsch and Meyer (1986) and Walter and Lingappa (1986).

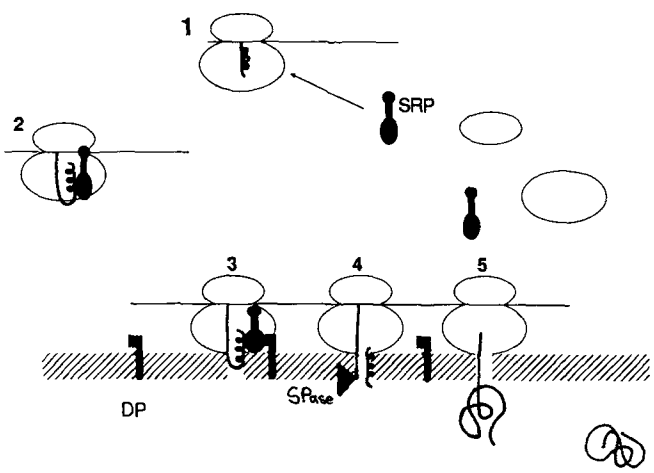

Figure 1
Steps in the translocation of a secretory protein across the membrane of the ER.
1. Start of translation on a free cytoplasmic ribosome. 2. SRP interacts with the signal sequence emerging from the ribosome. 3. Ribosomes with bound SRP contact the DP in the ER membrane. 4. The nascent polypeptide chain is translocated across the membrane and the signal sequence is cleaved off. 5. When translation is completed, the secretory protein accumulates in the lumen of the ER. The ribosomes dissociate from the membrane and can start a new round of translation.

Structure of the signal recognition particle (SRP)

SRP is a small 11S cytoplasmic ribonucleoprotein particle. It has been purified to homogeneity

from a salt extract of dog pancreas rough microsomal vesicles (Walter and Blobel, 1980). From wheat germ a SRP-like component has also been isolated and functionally characterized (Prehn et al. 1987). When tested in a wheat germ cell-free translation system, SRP is required for translocation of newly synthesized proteins into salt-extracted microsomes. When analyzed by electron microscopy, SRP is an elongated rod-shaped particle, 5-6 nm wide and 23-24 nm long (Andrews et al. 1985). SRP consists of a 7SL RNA and six nonidentical polypeptide chains of 9, 14, 19, 54, 68, and 72 kDa (Walter and Blobel, 1980; Walter and Blobel, 1982) (Fig. 2). Heterodimers are formed by the 9/14 and the 68/72 kDa proteins (Walter and Blobel, 1983; Scoulica et al., 1987).

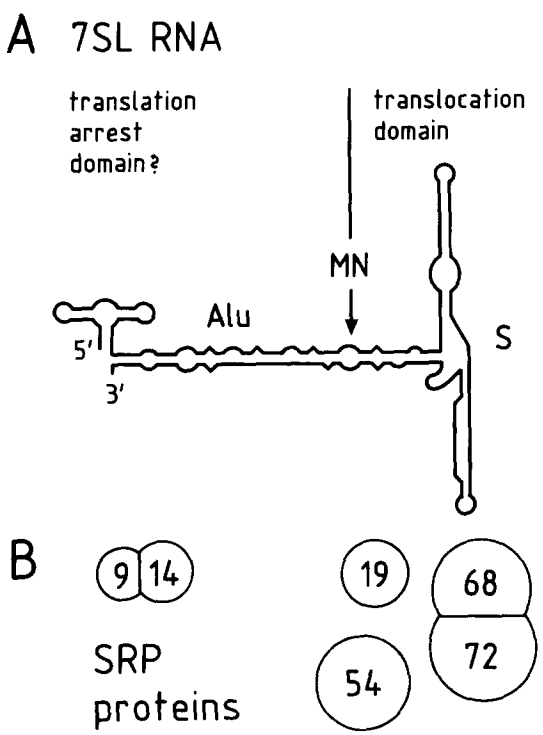

Figure 2
Structural components of the SRP
A: Alu: Alu repetitive sequences at the 5' and 3' end of 7SL RNA; S: central segment specific for 7SL RNA; MN: site for cleavage by micrococcal nuclease. (redrawn from Zwieb, 1985).
B: The 9/14 kDa heterodimer binds to the Alu containing part of 7SL RNA and the 19, 54, and 68/72 kDa proteins to the central S segment. The molecular weight of the SRP proteins is given in kilodalton.

7SL RNA

The 7S RNA of SRP, also called 7SL, comprizes about 300 nucleotides. The segments 100 nucleotides from the 5'end and 40 nucleotides from the 3'end are homologous to Alu sequences which are highly repetitive elements of the human genome. The central S fragment of 160 nucleotides is unique for the 7SL RNA (Ullu et al. 1982). The secondary structure of the 7SL RNA has been established using specific nucleases and the compensatory base change approach (Gundelfinger et al. 1984; Zwieb, 1985). The basic structural feature of 7SL RNA is a central rod which is formed by the nucleotides at positions 48 to 118 and 233 to 299. It is flanked by two small stem-loop structures at one end and two larger loop structures at the other. The small stem-loops are formed by the 44 nucleotides at the 5'end and the two larger ones by nucleotides 119 to 232 (see Fig. 2). The small stem-loops and most of the rod are formed by the Alu segments, the larger loops by the central S segment (Zwieb, 1985).

Limited digestion of the 7S RNA in SRP with micrococcal nuclease (MN) leads to two subparticles, one containing the paired Alu segments from the 5'and 3'end of the RNA and one the central S fragment. The 9 and 14 kDa proteins are bound to the Alu segments and the 19, 54, 68 and 72 kDa proteins to the central S segment (Gundelfinger et al. 1983) (Fig. 2). The large SRP subparticle containing the S segment can still promote translocation of secretory proteins across microsomal membranes, but it does no longer cause an elongation arrest in the synthesis of presecretory proteins (Siegel and Walter, 1985). These findings suggest that the Alu-like RNA in SRP and the 9/14

kDa proteins confer elongation-arresting activity to the particle (Siegel and Walter, 1986) (Fig. 2). This also shows, that the elongation arrest is not a prerequisite for protein translocation across the ER membrane (Siegel and Walter, 1985).
The human genome is rich in sequences that are structurally related to the 7SL RNA. The 7SL gene family consists of four 7SL genes, 500 7SL pseudogenes that are truncated at one or both ends of the 7SL sequence and 500 000 Alu sequences (Ullu and Weiner, 1984). 7SL genes are transcribed by RNA polymerase III. The 7SL RNA promoter resides internal to the 5'Alu-like part of the 7SL gene (Ullu and Weiner, 1985). Ullu and Tschudi (1984) suggested that Alu sequences were derived from 7SL RNA by a deletion of the central 7SL-specific sequence.

SRP proteins

The proteins in SRP are required for the membrane translocation activity of the particle, since alkylation with N-ethylmaleimide inactivates the particle (Walter and Blobel, 1980). Similarly, antibodies against the 54, 68 and 72 kDa proteins have been shown to neutralize SRP activity in vitro (Walter and Blobel, 1983).
SRP can be disassembled into its native RNA and protein components by unfolding the particle with EDTA and separation on polycationic matrixes. Isolated SRP proteins are inactive in promoting translocation of secretory proteins across ER membranes. However, when combined with 7SL RNA in the presence of magnesium, the proteins associate stoichiometrically with 7SL RNA and form fully active SRP (Walter and Blobel, 1983). A stepwise removal of the proteins from the 7SL RNA has been achieved by incubating SRP in 2 M KCL. During this treatment, heterodimers of the 9/14 kDa and of the 68/72 kDa proteins are released from the RNA (Scoulica, et al. 1987). In reconstitution studies it has been shown that the 9/14, the 19 and the 68/72 kDa proteins bind directly to the 7S RNA whereas the 54 kDa protein requires for its binding to the RNA the presence of the 19 kDa protein (Walter and Blobel, 1983).
SRP seems to interact via its 54 kDa polypeptide with the signal sequence of nascent polypeptides. Using a photocrosslinking approach the signal sequence of nascent preprolactin was found to be bound to the 54 kDa SRP protein (Kurzchalia et al. 1986, Krieg et al., 1986, Wiedman et al. 1987).

The domain structure of the SRP proteins has been investigated using mild elastase treatment and protein-specific antibodies. A 55 kDa domain was cleaved from the 72 kDa protein and a 35 kDa domain from the 54 kDa protein and both were released from the particle. Release of these domains led to inactivation of the particle (Scoulica et al., 1987).

Function of SRP

The functions of SRP have up to now only been determined in cell-free systems. It was found, that SRP is required for the translocation of proteins across microsomal membranes and that SRP can arrest elongation of secretory proteins. The SRP-mediated translation arrest has only been observed in a heterologous cell-free system containing components from plant and animal. No arrest could be found when homologous cell-free systems were used (Meyer, 1985). It is conceivable that in the intact cell SRP retards rather than completely blocks the synthesis of presecretory proteins. A biological function of such a mechanism could be a tight coupling of translation with membrane translocation (Walter and Blobel, 1981). Another possible function for SRP might be that it maintains the nascent polypeptide chain in a translocation competent form. Nascent polypeptides that remain translocation competent throughout their synthesis would not necessarily require SRP and DP. This indeed was found to be the case for certain small polypeptides that could be translocated across the ER membrane in the absence of SRP and DP (Schlenstedt and Zimmermann, 1987).

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