Structure and function of Signal Recognition Particle (SRP)

Henrich Lütcke & Bernhard Dobberstein
Zentrum für Molekulare Biologie Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Signal recognition particle (SRP), a cytoplasmic ribonucleoprotein particle (for reviews see [35, 19]) serves as an adapter between ribosomes synthesizing secretory or membrane proteins and the translocation sites in the membrane of the rough endoplasmic reticulum (RER). SRP binds to the hydrophobic core of a signal sequence when it emerges from a ribosome. SRP then inhibits the further elongation of the nascent polypeptide, until it interacts with the docking protein (DP or SRP receptor), its receptor in the RER membrane [18]. The signal sequence is then released from SRP and is inserted into a proteinaceous translocation site in the RER membrane (for review, see [24]. SRP dissociates from its receptor and re-joins the pool of cytoplasmic SRP.

Assembly of SRP

The mammalian SRP is composed of a 7S RNA (or: SRP RNA) to which six polypeptides of 9, 14, 19, 54, 68, and 72 kDa are attached either as monomers (SRP19 and SRP54) or as heterodimers (SRP9/14 and SRP68/72). SRP can
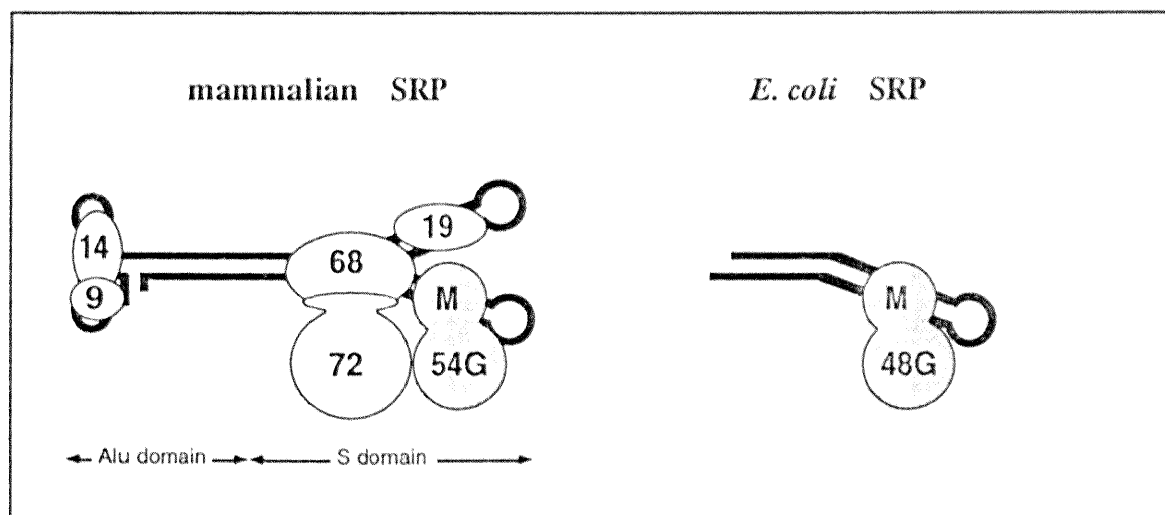

Fig. 1. SRP from mammalian and *E. coli* cells. The RNAs (7S RNA and 4.5S RNA, respectively) are depicted as thick lines. The proteins are indicated by circles and identified by their molecular masses (kDa). For SRP54 and its *E. coli* homologue p48, the GTPase (G) and the methionine-rich (M) domains are indicated.

be divided into two domains using nuclease (see Fig. 1). One domain (Alu domain) consists of the SRP9/14 heterodimer attached to the Alu sequences at the 5′ and 3′ ends of the 7S RNA [7]. The other domain (S domain) consists of the remaining four largest proteins attached to the central so-called S fragment of the 7S RNA [7].

Purified SRP can be disassembled into its RNA and protein components and can be reconstituted from the isolated constituents into an active particle [34]. The isolated heterodimeric proteins, SRP9/14 and SRP68/72, cannot be separated into their respective polypeptide subunits under non-denaturing conditions. When the SRP proteins are expressed *in vitro* from their cDNAs and used to reconstitute SRP, they reveal different requirements for the assembly with 7S RNA.

SRP9 and SRP14 associate with each other in the absence of 7S RNA and only subsequently can bind to 7S RNA [31]. Thus, the formation of an RNA binding site depends on the formation of the heterodimer.

In contrast, SRP68 binds to 7S RNA in the absence of SRP72 via a highly positively charged region near the N-terminus [17]. SRP72 only assembles into a complex with 7S RNA in the presence of SRP68. The two *in vitro* synthesized proteins do not, or do very inefficiently, associate with each other in the absence of 7S RNA. C-terminal regions of both the SRP68 and the SRP72 are essential for the association of the two proteins with each other in the presence of 7S RNA. Thus, SRP68 serves as an adapter for the assembly of SRP72 into a complex with the 7S RNA [17; see Fig. 1)].

The two monomeric proteins, SRP19 and SRP54, bind directly to 7S RNA [14, 27]. SRP19 mediates the assembly of SRP54 with SRP RNA. This was found with the mammalian components *in vitro* [27] and with the homologous yeast components *in vivo* (in *S. cerevisiae*; [8]). No requirement for SRP19 has been found in the *in vitro* assembly of mammalian SRP54 with the smaller 7S RNA homologues of *E. coli* (4.5 S RNA) and *B. subtilis* (6S RNA) which lack

the stem and loop of the 7S RNA to which SRP19 binds [12, 38].

Signal sequence binding

SRP54 is the signal sequence binding protein of SRP. It can be separated proteolytically into two fragments. The C-terminal fragment is highly positively charged and unusually rich in methionine residues and hence called M domain or SRP54M [1, 26]. SRP54M is necessary and sufficient for binding SRP54 to the SRP RNA [27, 16]. The positive charges have been proposed to line the hydrophilic faces of four amphipathic helices [1] and to mediate the binding to the SRP RNA [10].

The signal sequence binding site of SRP has also been mapped to SRP54M [37, 10]. SRP54M alone or reconstituted into SRP specifically interacts with a signal sequence exposed on a ribosome [16, 37], albeit with lower affinity than intact SRP54 or SRP [37, Bacher and Dobberstein, unpublished observations]. A signal sequence binding pocket has been proposed to be formed by the hydrophobic faces of the amphipathic helices predicted to be present in SRP54M. The pocket would be lined by many methionine residues which, by virtue of their linear hydrophobic side chains, could readily adapt to the various signal sequences [1].

The proximity of the binding sites for signal sequences and for the SRP RNA within SRP54M suggests that the information about the binding of a signal sequence by SRP54M may be relayed via the 7S RNA to other regions of SRP. This in turn could effect subsequent activities like the inhibition of elongation.

The N-terminal domain of SRP54 (SRP54N + G or SRP54G) contains a predicted GTPase domain (similar to small GTPases) with an N-terminal extension [1, 26]. SRP54N + G may be close to SRP54M since the alkylation of SRP54N + G with N-ethyl maleimide (NEM) prevents the binding of a signal sequence to SRP54M [16]. This, and the finding that the binding of a signal sequence to SRP54M is

inefficie
gests th
by whic
require
binding
plexes.
comple

The SI

Once S
nascen
the furt
involve
tution
alkylat
unable
less, si
entire
the co
protein
intact

The
inhibit
the RI
[20] ar
domai
The in
plex o
interac

Docki

When
nascer
RER
is rele
Sec61
site [
brane
(DP)

DP
dispo
unit
Muta

inefficient in the absence of SRP54N + G, suggests that SRP54N + G may control the affinity by which SRP54M binds a signal sequence. No requirement for GTP has been detected for the binding of SRP to ribosome/nascent chain complexes. However, the release of SRP from such complexes requires GTP [2].

The SRP-mediated pausing of elongation

Once SRP has bound to a signal sequence in a nascent polypeptide, it can retard or even stop the further elongation [34]. This activity of SRP involves the Alu domain of SRP [28]. Reconstitution of SRP which omits SRP9/14, or includes alkylated SRP9/14, renders the resulting particles unable to inhibit the elongation [30]. Nevertheless, such particles, and even SRP lacking the entire Alu domain, are still capable of promoting the co-translational translocation of a secretory protein *in vitro*, albeit with lower efficiency than intact SRP [29].

The mechanism by which the elongation is inhibited is unknown. It has been proposed that the RNA in the Alu domain resembles a tRNA [20] and may thus exert its inhibitory effect in the domain of the ribosome to which tRNAs bind. The inhibition of elongation lasts until the complex of SRP, ribosome and nascent polypeptide interacts with the DP in the RER membrane [4].

Docking of SRP to the RER membrane

When SRP in the complex with ribosome and nascent chain interacts with the membrane of the RER in the presence of GTP, the signal sequence is released from SRP54 and makes contact with Sec61p, a protein component of the translocation site [11, 6]. The component in the RER membrane to which SRP binds is the docking protein (DP) or SRP receptor.

DP is composed of a largely cytoplasmically disposed α- and a membrane-integrated β-subunit [13, 32] both of which bind GTP [2]. Mutational analysis of DPα suggests that GTP is

bound by DPα upon interacting with the complex of ribosome, nascent polypeptide and SRP and that this may trigger the release of the signal sequence from SRP as well as an increased affinity between SRP and DP [23]. Upon hydrolysis of GTP, the interaction between DP and SRP becomes sensitive to salt extraction [3].

Using reconstituted SRP, components have been identified which are necessary for mediating the targeting of a nascent secretory protein to microsomal membranes [30]. Selective alkylation of SRP68/72 in SRP allowed the modified SRP to still bind to a complex of ribosome and nascent polypeptide and to arrest the further elongation but prevented the interaction of the complex with the membrane [30]. Furthermore, the SRP68/72-alkylated SRP displayed a reduced affinity for DP. SRP lacking SRP54N + G likewise failed to promote targeting and to form a stable complex with DP [36]. Indeed, SRP54 appears to interact directly with DP, as a GTPase activity was greatly enhanced when both proteins were incubated in a purified system in the presence of SRP RNA [22]. Thus it appears that molecules required to mediate a stable interaction of SRP with the membrane, involve on the one hand SRP54 and SRP68/72, and on the other DP and possibly other membrane components.

The release of the signal sequence from SRP

Most recently, the reconstitution and functional characterization of membrane vesicles from detergent solubilized and fractionated microsomes has led to the identification of essential components of the translocation machinery in the RER membrane [5, 6]. One such component essential for the translocation of model polypeptides was identified as the mammalian Sec61p [6]. This protein had previously been identified as the major RER membrane protein in contact with different signal sequences, once they had been released from SRP [11]. When membrane vesicles lacking sec61p were added to a translocation assay, the signal sequence remained bound to

SRP54 as if no membranes had been added [D. Görlich and T. Rapoport, pers. communication]. The previously observed translocation defect of such vesicles may therefore be due to the failure of SRP54 to release the signal sequence if no downstream acceptor for the signal sequence is available.

SRP in other organisms

Homologues of the SRP RNA have been described in eubacteria, archaebacteria, yeasts, plants, and mammals (for a compilation, see [12]), and particles homologous to SRP have been shown to function in *E. coli* [21] and *S. cerevisiae* [9].

The SRP of *E. coli* contains the 48 kDa homologue of SRP54 (p48 or Ffh) which is found in a complex with the 4.5S RNA [25, 22, cf. Fig. 1]. No other protein has been detected in the particle in addition to p48. P48 interacts with the signal sequence of nascent preprolactin *in vitro* [15] and, in *E. coli*, is essential for growth and the efficient export of some proteins [21]. The SRP of *S. cerevisiae* consists of one RNA molecule (scR1) and several proteins, one of which is the SRP54 homologue [9]. Like in *E. coli*, disruption of the SRP in *S. cerevisiae* differentially affects the secretion of different proteins. The disruption of the gene encoding SRP54 severely inhibits but does not completely abolish growth [9]. Thus, it appears that SRP is important for protein secretion in possibly all organisms.

SRP, a signal sequence-specific chaperone

SRP in its minimal form may be represented by the *E. coli* SRP. By binding to the hydrophobic core of a signal sequence it maintains it in a conformation competent for translocation. Thus, the minimal SRP can be considered a signal sequence-specific chaperone. Other chaperones (e.g. SecB, DnaJ/DnaK, GroEL/GroES, SecA) interact with the mature portion of secretory (pre)proteins and thereby may cooperate with

SRP in the post-translational translocation of proteins.

References

1. Bernstein HD, Poritz MA, Strub K, Hoben PJ, Brenner, S & Walter P (1989) Nature 340: 482–486
2. Connolly T & Gilmore R (1989) Cell 57: 599–610
3. Connolly T, Rapiejko PJ & Gilmore R (1991) Science 252: 1171–1173
4. Gilmore R & Blobel G (1983) Cell 35: 677–685
5. Görlich D, Hartmann E, Prehn S & Rapoport TA (1992a) Nature 357: 47–52
6. Görlich D, Prehn S, Hartmann E, Kalies K-U & Rapoport TA (1992b) Cell 71: 489–503
7. Gundelfinger ED, Krause E, Melli M & Dobberstein B (1983) Nucleic Acids Res. 11: 7363–7374
8. Hann BC, Stirling CJ & Walter P (1992) Nature 356: 532–533
9. Hann BC & Walter P (1991) Cell 67: 131–144
10. High S & Dobberstein B (1991) J. Cell Biol. 113: 229–233
11. High S, Görlich D, Wiedmann M, Rapoport TA & Dobberstein B (1991) J. Cell Biol. 113: 35–44
12. Larsen N & Zwieb C (1991) Nucleic Acids Res. 19: 209–213
13. Lauffer L, Garcia PD, Harkins RN, Coussens L, Ullrich A & Walter P (1985) Nature 318: 334–338
14. Lingelbach K, Zwieb C, Webb JR, Marshallsay C, Hoben PJ, Walter P & Dobberstein B (1988) Nucleic Acids Res. 16: 9431–9442
15. Luirink J, High S, Wood H, Giner A, Tollervey D & Dobberstein B (1992) Nature 359: 741–743
16. Lütcke H, High S, Römisch K, Ashford AJ & Dobberstein B (1992) EMBO J. 11: 1543–1551
17. Lütcke H, Prehn S, Ashford AJ, Remus M, Frank R & Dobberstein B (1993) J. Cell Biol. 121: 977–985
18. Meyer DI & Dobberstein B (1980) J. Cell Biol. 87: 503–508
19. Nunnari J & Walter P (1992) Curr. Op. Cell Biol. 4: 573–580
20. Okada N (1990) J. Mol. Evol. 31: 500–510
21. Phillips GJ & Silhavy TJ (1992) Nature 359: 744–746
22. Poritz MA, Bernstein HD, Strub K, Topf D, Wilhelm H & Walter P (1990) Science 250: 1111–1117
23. Rapiejko PJ & Gilmore R (1992) J. Cell Biol. 117: 493–503
24. Rapoport TA (1992) Science 258: 931–936
25. Ribes V, Römisch K, Giner A, Dobberstein B & Tollervey D (1990) Cell 63: 591–600
26. Römisch K, Webb J, Herz J, Prehn S, Frank R, Vingron M & Dobberstein B (1989) Nature 340: 478–482
27. Römisch K, Webb J, Lingelbach K, Gausepohl H & Dobberstein B (1990) J. Cell Biol. 111: 1793–1802
28. S
29. S
30. S
31. S
32. T

28. Siegel V & Walter P (1985) J. Cell Biol. 100: 1913–1921
29. Siegel V & Walter P (1986) Nature 320: 81–84
30. Siegel V & Walter P (1988) Cell 52: 39–49
31. Strub K & Walter P (1990) Mol. Cell. Biol. 10: 777–784
32. Tajima S, Lauffer L, Rath VL & Walter P (1986) J. Cell Biol. 103: 1167–1178
33. Walter P & Blobel G (1981) J. Cell Biol. 91: 557–561
34. Walter P & Blobel G (1983) Cell 34: 525–533
35. Walter P, Gilmore R & Blobel G (1984) Cell 38: 5–8
36. Zopf D, Bernstein HD, Johnson AE & Walter P (1990) EMBO J. 9: 4511–4517
37. Zopf D, Bernstein HD & Walter P (1993) J. Cell Biol. 120: 1113–1121
38. Zwieb C (1991) Nucleic Acids Res. 19: 2955–2960

of

r,

ce

A

o-

B

6:

3:

&

9:

ch

C,

eic

&

er-

&

87:

4:

6
H

17:

ol-

ron

&