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Molecular Insights into Signal Recognition Particle-dependent Protein Targeting

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The majority of secretory and integral membrane proteins destined to insert into or traverse across the biological membranes are synthesized as precursor proteins with an N-terminal signal sequence that carries targeting information. In eukaryotes, these proteins are recognized by signal recognition particle (SRP) cotranslationally while being synthesized on the ribosomes and are targeted to endoplasmic reticulum (ER) membrane. In prokaryotes, presecretory or membrane proteins are targeted to the plasma membrane either post-translationally by the SecB pathway or co-translationally by the SRP pathway. The mammalian SRP is composed of six polypeptides (SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72) and a ~300 nt long 7S RNA. SRP binds to the signal sequences that emerge from ribosome and mediates the translational arrest of nascent chain. The SRP receptor, located at the ER membrane, recognizes the SRP-ribosome nascent chain complex and mediates the transfer of the translating ribosome to the translocation site in the membrane. Subsequently, translation is resumed, SRP and SRP receptor are detached from the ER membrane, followed by SRP dissociation from the receptor. In prokaryotes, the SRP pathway in an analogous manner targets protein precursors to the translocation sites of the cytoplasmic membrane. The components of *Escherichia coli* SRP cycle such as 4.5S RNA, Ffh and the SRP receptor, FtsY, share significant structural and functional conservation with their eukaryotic homologues. Thus, the basic mechanism for SRP-mediated protein targeting appears to be evolutionarily conserved, although there are notable differences between eukaryotic and prokaryotic SRP pathways.

Key Words: Protein targeting, Signal recognition particle, SRP RNA, SRP54, 4.5S RNA, Ffh, FtsY, GTPase

Introduction

Protein transport across the membrane can occur by two pathways, co-and post-translational targeting. In eukaryotes, the secretory or membrane proteins are first translocated across the endoplasmic reticulum (ER), and subsequently transported to various organelles or to the plasma membrane by vesicles. The co-translational targeting across the ER membrane involves the signal recognition particle (SRP) that recognizes N-terminal signal sequences of the ribosome-bound nascent chains. In prokaryotes, protein targeting to the plasma membrane is mediated either by SecB (an *Escherichia coli* cytosolic chaperon) or by SRP, depending on the nature of the signal sequence (reviewed in Wolin, 1994). SecB or other chaperones such as heat shock proteins interact with the nascent peptide either co-translationally or post-translationally, whereas the SRP

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Abbreviations: SRP, signal recognition particle; *ER,* endoplasmic reticulum; *RNC,* ribosome nascent chain; *IBD,* insertion box domain

pathway appears to be specialized mainly for the co-translational targeting of inner membrane proteins. In post-translational targeting, the synthesis of polypeptide chains is completed before being transported and this pathway is best characterized in *Saccharomyces cerevesiae* and *E. coli* (reviewed in detail, Wickner et al. 1991, Rapoport et al. 1996). In both pathways, the polypeptides are first transferred to translocation sites in the membrane through a protein-conducting channel, viz. the Sec61 and SecYEG complex of eukaryotes and prokaryotes, respectively (reviewed in Rapoport et al. 1996). The basic mechanism of protein transport across the ER membrane or plasma membrane in *E. coli* is evolutionarily conserved as they share homologous components and the signal sequences are functionally interchangeable between prokaryotes and eukaryotes.

Hydrophobic N-terminal signal sequences trigger the translocation of proteins across, and their integration into the membrane. Signal sequences generally begin within 10 amino acids from the N-terminus of the secretory proteins (Blobel & Dobberstein 1975, Inouye & Beckwith 1977) and are cleaved by signal peptidase after translocation. They are between 20 and 30 residues in length and do not show any sequence homology when compared to one another. These sequences can be divided into three characteristic regions: the N-terminal region with positively charged polar residues, the central hydrophobic core, and an uncharged, polar C-terminal region (von Heijne 1985). The hydrophobic core is formed by approximately 10-15 (not less than 6) residues, preferably leucines and alanines.

This review focuses on the molecular events and biochemical role of individual components of the SRP-mediated protein targeting in eukaryotes and prokaryotes. We also briefly dwell on the earlier work (reviewed in Lutcke 1995) while discussing the recent developments on the structure and function studies on SRP and other key players in the SRP cycle. Finally, we will discuss the similarities, differences and functional conservation between prokaryotic and eukaryotic SRP pathways.

SRP-dependent Protein Targeting in Eukaryotes

The SRP-dependent protein targeting pathway is present in all eukaryotes, although the extent of its usage varies among organisms. For instance, *S. cerevisiae* mutants lacking the SRP or the SRP receptor are viable, whereas the same mutations are lethal in other yeast strains. Mammalian SRP is the best characterized of all SRPs. Therefore, in this review the mammalian SRP is taken as a prototype to describe the eukaryotic SRP pathway. The genetic and biochemical studies on yeast SRP will be included, wherever the pertinent information is not available for mammalian SRP.

Structural and Biochemical Properties of SRP and Its Components

The mammalian SRP is a ribonucleoprotein (11S) consisting of one, about 300 nt long 7S RNA and six proteins (Walter & Blobel 1980). The SRP proteins are named according to their apparent molecular masses (kDa): SRP9, SRP14, SRP19, SRP54, SRP68 and SRP72 (table 1). These proteins are attached to 7S RNA either as monomers (SRP19 and SRP 54) or heterodimers (SRP9/14 and SRP68/72) (figure 1). SRP spontaneously reassembles from its components *in vitro* (Walter & Blobel 1983). Scanning transmission electron micrographs revealed that SRP is a rod shaped, 22-24 nm long and 5-6 nm wide, particle consisting of three linearly arranged domains that are separated by flexible hinges (Andrews et al. 1985, 1987). Micrococcal nuclease cleaves SRP into two distinct ribonucleoprotein complexes, indicating that the middle portion of SRP consists of protein-free RNA (Gundelfinger et al. 1983, Siegel & Walter 1986). The translation arrest activity of SRP is associated with the *Alu* domain of 7S RNA and the SRP9/SRP14 proteins. The other subparticle, comprising the S domain of 7S RNA and proteins SRP19, SRP54, SRP68 and SRP72, recognizes the signal sequence and is involved in docking at the ER (reviewed in Walter & Johnson 1994, Lutcke 1995).

SRP 7S RNA: The mammalian 7S RNA is ~300-nt long and can be divided into four structural domains according to Poritz et al. (1988) (figure 1) or into 8 helices (Larsen & Zwieb 1991). SRP RNAs from different organisms differ in length and number of domains. There are more than 30 eukaryotic 7S RNA sequences available in the SRP database (Zwieb & Samuelsson 2000).

Table 1 *Components of SRP and SRP receptor from eukaryotes and prokaryotes.*

Component	Functional properties
Mammalian SRP	
7S RNA	~300 nt long RNA, divided into four domains (I-IV).
SRP9/SRP14	Heterodimer binds to 7S RNA at domain I, promotes elongation arrest.
SRP19	Binds to 7S RNA in monomeric form at domain III. It is an initiator of SRP assembly.
SRP54	It has binding sites for GTP/GDP, signal sequence. Binds to 7S RNA at domain IV only in the presence of SRP19. It is a central key player in the SRP cycle.
SRP68/SRP72	Heterodimer Binds to 7S RNA at domain II, promotes translocation activity.
Mammalian SRP receptor	
SRα/SRβ	Heterodimeric protein, constituting 68 kDa SRα and 30 kDa SRβ. Both the proteins are GTPases. Receptor recognizes SRP-RNCs at ER membrane.
***E. coli* SRP**	
4.5S RNA	~114 nt long RNA. Functionally equivalent to 7S RNA, it has binding site for only one protein (Ffh).
Ffh (P48)	50 kDa protein, functional homologue of SRP54.
***E. coli* SRP receptor**	
FtsY	54 kDa protein, a functional homologue of SRα

Mammalian and *E. coli* SRPs are taken as examples for eukaryotes and prokaryotes, respectively. Mammalian SRP proteins are named according to their apparent molecular mass.

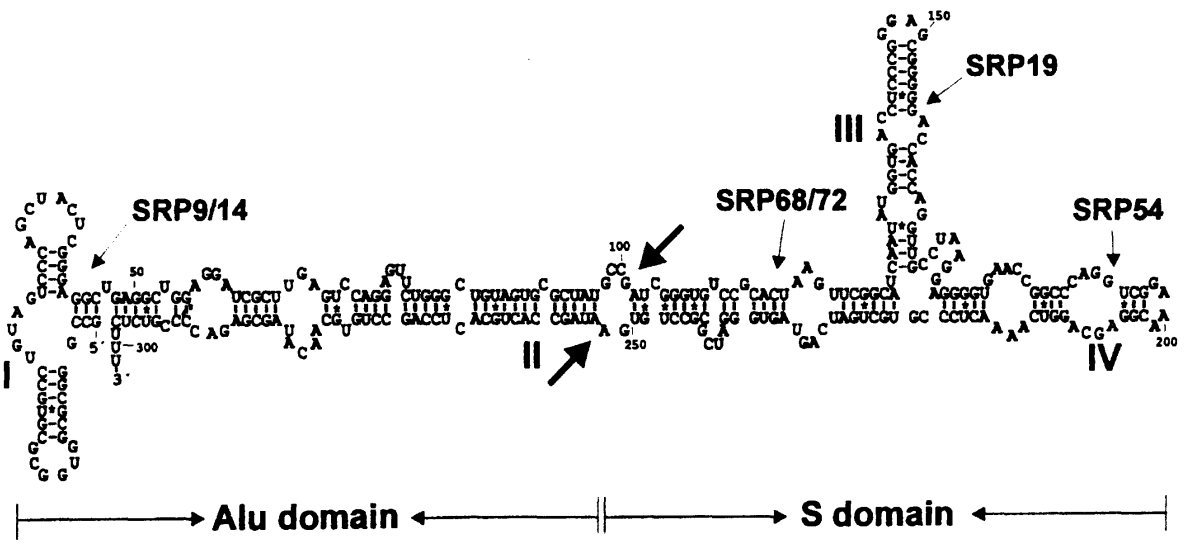

Figure 1 Model of the secondary structure of mammalian (*Homo sapiens*) SRP 7S RNA. The proposed interactions of SRP proteins to SRP RNA are indicated with thin arrows. The nomenclature used to define the domains of RNA is according to Poritz et al. (1988). Experimentally determined micrococcal nuclease cleavage sites (Gundelfinger et al. 1983), which separate the *Alu* domain from the S domain of SRP are shown in bold arrows.

Biochemical, sequence and secondary structure analyses indicated that the 7S RNA forms a highly base-paired secondary structure in which the *Alu*-like sequences form one domain and the S fragment another (Gundelfinger et al. 1983, 1984, Zwieb 1985). The *Alu* region of 7S RNA was shown to be important for translation arrest, suggesting that it might compete with the incoming tRNA due to its tRNA-like pseudoknot structure (reviewed in Bui & Strub 1999). Microinjection of fluorescently labelled 7S RNA into the nucleus resulted in rapid localization of RNA in the nucleolus followed by its appearance in the cytoplasm (Jacobson & Perderson 1998). The presence of the *Alu* region and domain IV of 7S RNA are necessary for this nucleolar localization. Green fluorescent protein fusions of SRP19, SRP62 and SRP78 proteins were also shown to be localized in the nucleolus (Politz et al. 2000). These results suggested that the nucleolus is the site where SRP is assembled.

SRP9/14: Proteins SRP9 and SRP14 form a heterodimer which binds specifically to the *Alu* domain of SRP RNA (Siegel & Walter 1988b, Strub et al. 1991); either protein alone does not bind, and the binding is independent of other proteins (Strub & Walter 1990). The *Alu* region of RNA and SRP9/14 proteins both are important for elongation arrest, and RNA or protein alone can not perform this function (Siegel & Walter 1985, 1986 and 1988b). The binding of SRP9/14 to the SRP *Alu* RNA is stochiometric (Walter and Blobel, 1983a) Mutational analysis indicated that the RNA binding region is formed by both the proteins (Bui et al. 1997). Crystal structure data revealed that SRP9 and SRP14 are structurally homologous and consist of the same α–β–β–β–α fold. This fold was named as *Alu* binding module (*Alu* bm) (Birse et al. 1997). The structural data suggested that the SRP *Alu* RNA binding heterodimer might have evolved from a homodimer by gene duplication. This view is supported by the finding that the *Alu* domain homologue of *S. cerevesiae* SRP consists of an SRP14 homodimer and a yeast-specific RNA structure, Adhy (*Alu* domain homologue in yeast) (Strub et al. 1999).

SRP19: SRP19 is the only SRP protein which binds to 7S RNA independent of other components (Lingelbach et al. 1988), and its binding is required for SRP54 to bind to 7S RNA (Hann et al. 1992, Romisch et al. 1990, Walter & Blobel 1983a, Zopf et al. 1990). However, SRP54 is capable of binding to SRP RNA which lacks domain III, suggesting that SRP19 might induce a conformational change in the 7S RNA such that it exposes the domain IV for the SRP54 binding (Romisch et al. 1990, Zopf et al. 1990). Mutational analysis on 7S RNA domains III and IV (helix 6 and 8) revealed that SRP19 binds to the tetranucleotide loop of domain III (helix 6) in a sequence specific manner and that it recognizes base pairs in the adjoining stem of domain IV (helix 8) tetraloop (Zwieb 1992, 1994).

SRP54: SRP54 binds to domain IV (helix 8) of 7S RNA. SRP54 consists of three domains, the N-terminal N domain, the central G domain with GTP/GDP binding motifs (G1, G2, G3 and G4), and the C-terminal methionine-rich M domain (Romisch et al. 1989, Bernstein et al. 1989) (figures 2, 3). Limited proteolysis cleaves SRP54 into a 33 kDa fragment comprising the NG domains, which retain GTPase activity, and a ~22 kDa fragment, the M domain, which binds the signal sequence and the SRP RNA (Romisch et al. 1990, Zopf et al. 1990). The M domain, which is rich in methionines and positively charged amino acids, is characteristic for all SRP54 homologues.

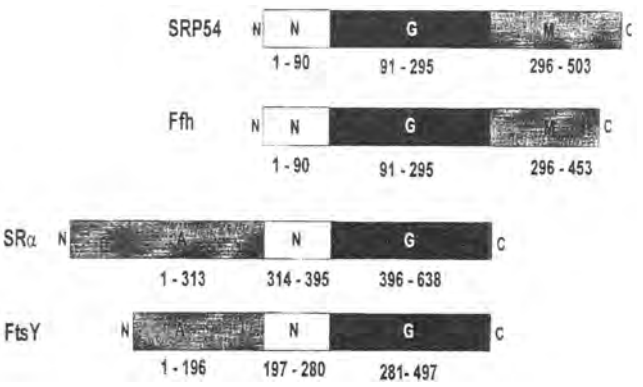

Figure 2 Domain structures of SRP54, SRα and their respective *Escherichia coli* homologues, Ffh and FtsY. N and G domains of Ffh and FtsY are indicated based on the crystal structures (Freyman et al. 1997, Montoya et al. 1997). N and G domains of SRP54 and SRα are identified based on sequence comparisons (Althoff et al. 1994). The M domain in SRP54 homologues and the acidic domain in SRα homologues are indicated based on the primary structures (Althoff et al. 1994, Romisch et al. 1990).

G1 motif (GX$_4$GKS/T)

E. coli **Ffh**	G	L	Q	G	A	G	K	T
M. jannaschii **Ffh**	G	I	Q	G	S	G	K	T
H. sapiens **SRP54**	G	L	Q	G	S	G	K	T
E. coli **FtsY**	G	V	N	G	V	G	K	T
M. jannaschii **SRα** homologue	G	I	N	G	T	G	K	T
H. sapiens **SRα**	G	V	N	G	V	G	K	S
H ras p21	G	A	G	G	V	G	K	S

G2 motif

E. coli **Ffh**	S	A	D	V	Y	R	P
M. jannaschii **Ffh**	A	A	D	T	Y	R	P
H. sapiens **SRP54**	C	A	D	T	F	R	A
E. coli **FtsY**	A	G	D	T	F	R	A
M. jannaschii **SRα** homologue	A	G	D	T	F	R	A
H. sapiens **SRα**	A	A	D	T	F	R	A
H ras p21	Y	D	P	T	I	E	D

G3 motif (DX$_2$G)

E. coli **Ffh**	D	T	A	G	R	L
M. jannaschii **Ffh**	D	T	A	G	R	H
H. sapiens **SRP54**	D	T	S	G	R	H
E. coli **FtsY**	D	T	A	G	R	L
M. jannaschii **SRα** homologue	D	T	A	G	R	Q
H. sapiens **SRα**	D	T	A	G	R	M
H ras p21	D	T	A	G	Q	E

G4 motif (N/T K/Q X D)

E. coli **Ffh**	T	K	V	D
M. jannaschii **Ffh**	T	K	L	D
H. sapiens **SRP54**	T	K	L	D
E, coli **FtsY**	T	K	L	D
M. jannaschii **SRα**	T	K	V	D
H. sapiens **SRα**	T	K	F	D
H ras p21	N	K	C	D

Figure 3 GTP binding consensus sequence motifs (G1-G4) in SRP-related GTPases. SRP54 and SRα homologues are selected from representative organisms of the three kingdoms of life (*Escherichia coli* for bacteria, *Methanococcus jannaschii* for archaea, *Homo sapiens* for eukaryotes). Consensus sequences are based on sequence comparisons of SRP-related GTPases available in the SRP database (Samuelsson & Zwieb 2000). GTP binding motifs (G1-G4) are compared to those of Ras as an example (Bourne et al. 1991).

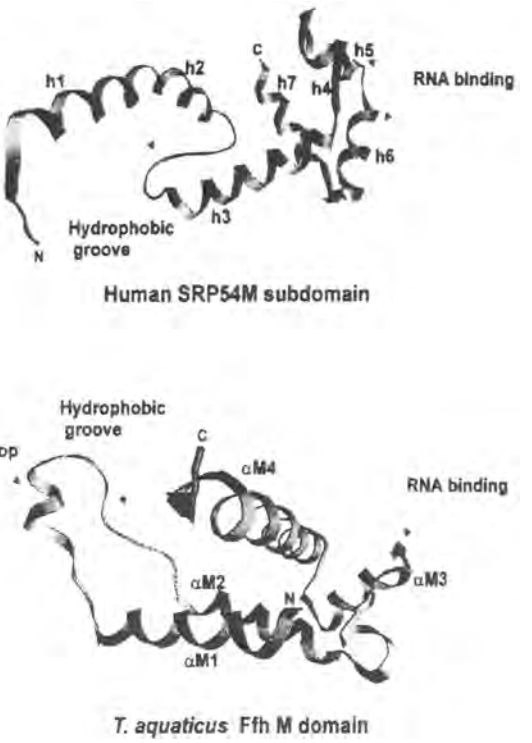

Figure 4 M domain structures of SRP54 and Ffh. Ribbon diagrams are drawn using the co-ordinates available in the Protein Data Bank for human SRP54M (Clemons et al. 1999) and *T. aquaticus* Ffh (Keenan et al. 1998). The numbering of helices, SRP RNA and signal sequence binding regions (hydrophobic groove) follow the respective reference.

The crystal structure of the conserved subdomain of human SRP54M (120 residues, positions 322 to 441) revealed that it consists of seven α-helices and a highly structured loop of 17 amino acid residues which is maintained between helix 2 and 3 (Clemons et al. 1999) (figure 4). A deep elongated groove is formed by helices 2, 3, 4 and connecting loops. The groove is the likely binding site for the signal sequence. It could accommodate a variety of signal sequences, including alpha-helical structures, as in the dimeric crystal structure of SRP54M helix 1 of one monomer locks into the hydrophobic groove of another.

Based on sequence conservation to other RNA binding proteins, it was predicted that the 7S RNA binding motif is present in the M domain (Althoff et al. 1994). *In vitro* studies indicated that the M domain alone is sufficient for 7S RNA binding and it is capable of binding

to other SRP RNA homologues, whereas the NG domain does not bind RNA (Lutcke et al. 1992, Romisch et al. 1990, Zopf et al. 1990). The crystal structure of human SRP54M suggests that helices 4, 5 ,6 and 7 constitute the RNA binding region (figure 4) (residues between 380-420). This proposal is supported by mutational data on SRP54 M (Gowda et al. 1998).

SRP68/72: SRP68/72 can be disassembled from SRP as a stable heterodimer in high ionic conditions and re-assembly requires only 7S RNA (Scoulica et al. 1987, Walter & Blobel 1983a). The binding of SRP72 follows SRP68 binding to 7S RNA. The binding region for SRP68/72 was mapped to the centre of the RNA, i.e. the S region of 7S RNA (Siegel & Walter 1988a). The affinity of SRP68/72 for binding to 7S RNA is about 7 nM, independent of the presence of SRP9/14 (Janiak et al. 1992). Mutation analysis revealed that the RNA binding activity of SRP68 is localized in the N-terminal part of SRP68 (Lutcke et al. 1993). The regions near the C termini of SRP68 and SRP72 were shown to be important for the association of the two proteins and hydrophobic interactions were assumed to play a role in this function (Lutcke et al. 1993). Alkylation of RNA-bound SRP68/72 resulted in SRP defective in either membrane insertion or translocation of nascent polypeptide, indicating that SRP68/72 has a role in the docking process (Siegel & Walter 1988a), possibly in the interaction with the SRP receptor at the membrane.

SRP Receptor

Membrane docking of the SRP-ribosome-nascent chain complex requires the presence of the SRP receptor (Gilmore et al. 1982a, Meyer et al. 1982, Gilmore & Blobel 1985). The mammalian SRP receptor is a heterodimeric protein consisting of the 68 kDaα subunit (SRα) and the 30 kDaβ subunit (SRβ) (Gilmore et al. 1982b, Tajima et al. 1986) (table 1). Proteolysis and carbonate extraction experiments showed that SRα is a peripheral and SRβ an integral membrane protein (Miller et al. 1995). SRα has significant sequence homology with the NG domain of SRP54 and comprises the four GTP binding consensus sequence elements characteristic for GTPase superfamily (figures 2, 3) (Althoff et al. 1994, Bourne et al. 1991, Bernstein et al. 1989, Romisch et al. 1989). The N-terminal region of SRα is required for the interaction with SRβ (Tajima et al. 1986, Hoffman & Gilmore 1988). Site-specific mutations in the consensus GTP-binding regions of SRα significantly affected GTP binding, and these mutants were defective in protein translocation, as they were incapable of forming stable complexes with SRP. These results indicated that protein translocation across the ER membrane requires a functional GTP binding site in SRα. cDNA cloning and sequence analysis of SRβ indicated that it is a type I integral membrane protein which is anchored in the membrane by an N-terminal transmembrane segment (Miller et al. 1995). The C-terminal GTPase domain is exposed to the cytoplasmic side of the membrane and is related to the ADP-ribosylation factor (ARF) subfamily of GTPases which function in vesicular trafficking. Genetic and biochemical experiments on *S. cerevisiae* SRβ showed that the GTPase domain, but not the transmembrane domain, was important for the function of the SRP receptor (Ogg et al. 1998).

SRP Cycle

In the functional cycle of eukaryotic SRP, four major steps can be distinguished: (i) Signal sequence recognition by SRP on ribosome nascent chain complexes, (ii) elongation arrest, (iii) binding of the SRP-ribosome nascent chain (SRP-RNC) complex to the SRP receptor at the ER membrane, (iv) dissociation of SRP from the ribosome, which is transferred to the translocon, and from the receptor to enter the next cycle (figure 5).

Signal sequence recognition: The binding of SRP to the translating ribosome occurs as soon as the hydrophobic signal sequence is exposed on the ribosome. This requires a length of the nascent protein of about 70 amino acids, i.e. 20-30 amino acids of the N-terminal signal sequence are followed by 30-40 amino acids that are bound inside the ribosome (Walter & Blobel 1981). The

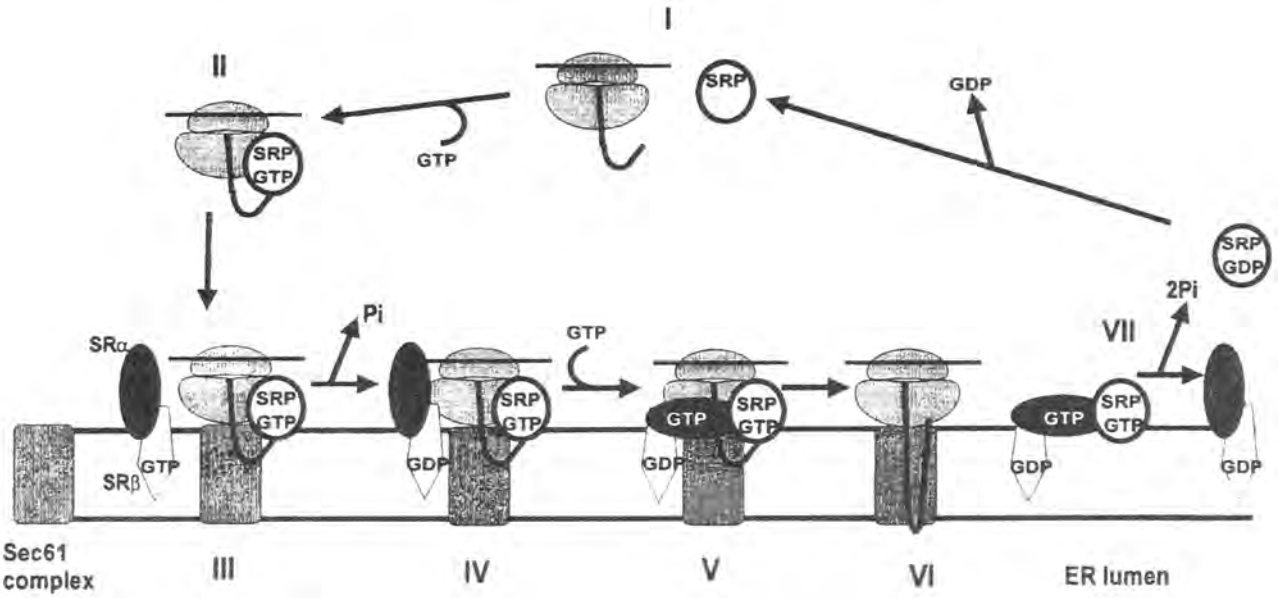

Figure 5 Model for SRP-mediated targeting of nascent proteins to the ER membrane. I. SRP recognizes the nascent chains that are exposed from the ribosome leading to elongation arrest. II. The presence of RNC stimulates the GTP binding to SRP while functioning as a "loading factor". III. SRP-RNCs contact the Sec61 complex. IV. This is followed by the ribosome interaction with GTP bound SRβ. V. Recognition of SRP-RNC complex by GTP bound SRP receptor. VI. Translation resumed and SRP, SRP receptor dissociates from the RNC. VII. GTP hydrolysis dissociates SRP from SRP receptor. Model adapted from Bacher et al. (1999)

presence of the signal sequence increases the affinity of SRP for nascent chain ribosomes several thousand-fold compared to nascent chains without signal sequence (Walter & Blobel 1981, 1983b).

Recently, it has been reported that ribosome binding promotes the GTP binding to SRP54 (Bacher et al. 1996). It was observed that in the presence of RNC the affinity of GTP increased by 10-fold (2 μM to 0.2 μM), whereas the GDP affinity remained unchanged (0.05μM). Based on these observations, it was proposed that SRP exchanges GDP with GTP upon interaction with RNC, as the concentration of GTP (100 μM) in mammalian cells is estimated to be 10 times higher than that of GDP (10 μM) (figure 5).

Elongation arrest: Elongation is halted upon SRP binding to the signal sequence emerging from the ribosome. This was shown for a heterologous system, i.e. mammalian SRP with wheat germ ribosomes (Walter & Blobel 1981, Meyer et al. 1982). In the homologous reticulocyte system, there was no arrest but rather a delay of preprolactin synthesis (Wolin & Walter 1989). Nevertheless, the delay in protein elongation may be sufficient to increase the length of the time that the nascent chain remains in the translocation competent state. SRP lacking the elongation arrest domain (*Alu* domain) was less efficient in protein translocation, indicating that elongation arrest is important, but not absolutely required, for translocation (Siegel & Walter 1985). The elongation arrest is released upon binding of the SRP-RNC complex to its receptor at the ER membrane (Meyer et al. 1982, Gilmore et al. 1982a,b).

Binding of SRP ribosome-nascent chain complex to SRP receptor at ER membrane: The The SRP-RNC complex binds to the ER membrane, and the SRβ subunit of the SRP receptor has been proposed to regulate the interaction of the SRP receptor with the ribosome, thereby allowing the SRα to scan for the membrane-bound SRP-RNCs (Bacher et al. 1996, 1999). The presence of GTP leads to the release of SRP from the RNC (Connolly & Gilmore 1989, Wiedmann et al. 1987) followed by tight association of SRP with the receptor (Connolly & Gilmore et al. 1991). The prerequisite for tight association of SRP with SRα is that both the proteins should be in the GTP bound form (Rapiejko & Gilmore 1992, 1997). After the release of signal sequence, the nascent chain is integrated into translocation channel (Sec61 complex) (Gorlich et al. 1992a,b,

High et al. 1991, 1993), and the SRP-SRP receptor complex dissociates from the translocation channel (Connolly et al. 1991).

The exact role of SRβ in SRP cycle was not clear for a long time. Recent biochemical experiments have shown that SRβ binds to GTP with high affinity (0.02 μM) and interacts with ribosomes (Bacher et al. 1999). Upon interaction of SRβ with RNC, the GTPase activity is enhanced, and the presence of RNC reduces the affinity of guanine nucleotides. Based on these results, it was proposed that the interaction of SRβ with RNCs in the SRP cycle (figure 5) might serve a proofreading function to ensure that correct RNCs are delivered for targeting.

Dissociation of SRP from the receptor

The dissociation of SRP from its receptor is induced by GTP hydrolysis (Cannolly et al. 1991, Miller et al. 1993). It was shown that SRP and SRP receptor reciprocally stimulate their GTPase activity when they interact at low salt conditions (Miller et al. 1993). The effect was not observed at high ionic strength, although GTP hydrolysis is stimulated with SRP and SRP receptor in the presence of proteoliposomes and the components of the translocation channel (Bacher et al. 1996). This could be due to the function of ribosome as a GTP loading factor for SRP54 in the presence of nascent chain bearing signal sequence, hence the GTP hydrolysis is not stimulated in the absence of translocation channel components.

Models of the Eukaryotic SRP-GTPase Cycle

Two different models were proposed for SRP-GTPase cycle (Rapiejko & Gilmore 1997, Bacher et al. 1996, 1999) which differ with respect to the function attributed to nucleotide-free forms or GTP-bound forms of SRP and its receptor in the SRP cycle. It has been shown that SRP-RNCs are capable of interacting with ER membrane in the absence of GTP, although the release of nascent chain from the SRP requires GTP or a non-hydrolyzable analogue, such as GMPPNP (Connolly et al. 1991). Upon SRP and SRα interaction, the GTP binding affinity of both proteins is increased about 10-fold, suggesting that SRα increases GTP binding to SRP and vice versa (Miller et al. 1993, Rapiejko & Gilmore 1997). Both SRP and SRα readily exchange guanine nucleotides prior to their interaction (Rapiejko & Gilmore 1997). These results suggested that SRP and SRα are targeted to the ER membrane in the empty form and, upon binding GTP, undergo a conformational change that leads to tight association, followed by the release of signal sequence. GTP hydrolysis dissociates SRP from the receptor, which then enters into a new cycle, and the GDP dissociates from both proteins due to its lower affinity. This "concerted switch model" does not address the role of SRβ.

According to the second model (figure 5), the ribosome works as GTP loading factor for SRP54 and hence SRP would be in the GTP-bound form prior to the interaction with the receptor, taking into account the concentration of GTP *in vivo*. In the absence of GTP, SRP-RNC complexes do not interact with ER membrane at physiological salt concentrations, suggesting that SRP interacts with the receptor in the GTP-bound form (Bacher et al. 1996). For the receptor, the mutational analysis suggested that SRα required being in the GTP bound form to interact with the SRP-RNC complex (Rapiejko & Gilmore 1992).

The two models differ with respect to the events prior to the association of SRP with the receptor, whereas the events following the association are depicted similarly. More kinetic and structural information on the various complexes formed during the SRP cycle is required to formulate a unifying model.

SRP-dependent Protein Targeting in Prokaryotes

The signal sequence hypothesis was proposed by Blobel and Dobberstein in 1975, and by 1980, the SRP and the SRP receptor had been characterized in the mammalian system. In *E. coli*, genetic screens for export mutants were successfully employed to dissect the SRP-independent SecB pathway (reviewed in Schatz & Beckwith 1990, Wickner et al. 1991, Pugsley 1993). However, similar approaches were unsuccessful in identifying SRP-like components in *E. coli*, probably because relatively small number of substrates for translocation were selected to screen these mutants, and these substrates follow the SecB dependent

protein transport, such as maltose binding protein and LamB (reviewed in Wolin et al. 1994). Recently, the application of genome-wide screening identified several polytopic inner membrane proteins as SRP substrates (Ulbrandt et al. 1997).

The discovery of the SRP pathway in *E. coli* awaited the determination of the protein sequence for mammalian SRP54. The first clue came from secondary structure analysis of SRP RNAs by Poritz et al. (1988) that *E. coli* 4.5S RNA contained a similar secondary structure to the highly conserved domain IV of SRP7S RNA. Subsequently, the cloning of the SRP54 gene and the database search identified a homologous protein in *E. coli* (P48; now called Ffh, fifty four homologue) (Bernstein et al. 1989, Romisch et al. 1989). Previously, the functional significance for the Ffh in *E. coli* was unknown. *In vivo*, Ffh and 4.5S RNA were shown to interact with one another that led to the proposal of SRP like particle in *E. coli* (Poritz et al. 1990, Ribes et al. 1990). FtsY, an *E. coli* protein homologous to mammalian SRα (Bernstein et al. 1989, Romisch et al. 1989), was shown to be the functional equivalent to the mammalian SRP receptor (Luirink et al. 1994). It was observed that inhibition of the SRP pathway in *E. coli* blocks the membrane insertion of several polytopic inner membrane proteins (MacFarlane & Muller 1995, de Gier et al. 1996, Ulbrandt et al. 1997). *In vitro* and *in vivo* studies showed that the SRP and Sec pathways converge at the translocon, and that the preprotein translocase (SecA and SecY, E, G) was required for SRP-dependent protein targeting (Valent et al. 1998, de Gier et al. 1998). However, in the SRP pathway, SecA and ATP were shown to be dispensable for the transfer and insertion of nascent chain into translocon (Scotti et al. 1999). During protein synthesis, polytopic membrane proteins were shown to be selected by SRP for co-translational membrane targeting, whereas the secretory proteins were directed to SeA/SecB-mediated post-translational targeting pathway by trigger factor (Beck et al. 2000).

E. coli SRP-structure and Function Studies

Compared to mammalian SRP, the composition of *E. coli* SRP is much simpler and it consists of an RNA (4.5S RNA) and only one protein (P48 or Ffh) (Portiz et al. 1990, Ribes et al. 1990) (table 1). Chimeric mammalian SRP in which SRP54 was replaced with Ffh could bind to the signal sequence and induce translational arrest (Bernstein et al. 1993, Hauser et al. 1995). *E. coli* SRP and its receptor, FtsY, functionally replaced their mammalian homologues in targeting nascent secretory proteins to microsomal membranes (Powers and Walter, 1997).

4.5S RNA: E. coli 4.5S RNA (114 nt) is one of the smallest members of the family of SRP RNAs (Larsen & Zwieb 1991). It forms an extended stem-loop structure which is homologous to the most highly conserved domain (domain IV) of SRP RNA (figure 6). 4.5S RNA homologues are found in other bacterial species, and the length varies from 270 nt (*Bacillus subtilis*) to 79 nt (*Mycoplasma mycoides*) (Althoff et al. 1994, Struck et al. 1988, Samuelsson & Guindy 1990). *E. coli* 4.5S RNA lacks domains I and III and most of domain II when compared to mammalian 7S RNA.

Recently, the solution structures of 4.5S RNA fragments from domain IV have been solved (Schmitz et al. 1999a,b). In these structures, the conserved nucleotides in the symmetric internal loop A are involved in various interactions. A47 and A63 form a cross-strand A/A-stack in the minor groove without base pairing with cross-strand C62 and C46, respectively. The displacement of the A/A stack permits the formation of hydrogen bonds between imino and amino protons of G61 with the phosphate group of A47. The imino group of G48 forms a cross-strand hydrogen bond with the phosphate of C62. This is facilitated by the C2'-endo conformation of the ribose of G48, as it

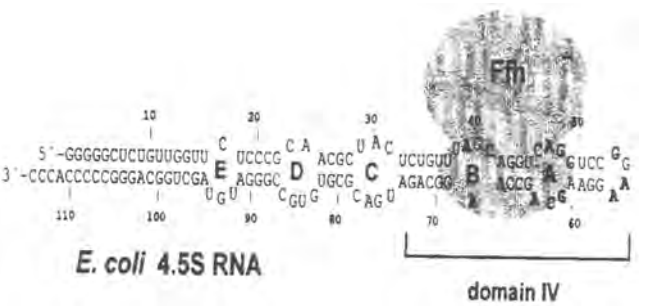

Figure 6 Secondary structure of *E. coli* 4.5S RNA. Phylogenetically conserved nucleotides are shown in bold letters. Internal loops are numbered from A-E. Figure adapted from Schmitz et al. (1999b). The putative Ffh binding region is shown based on chemical footprinting data (Lentzen et al. 1996) and mutational analysis (Wood et al. 1992).

stretches out the backbone (Schmitz et al. 1999a). The asymmetric internal loop B provides a hinge in the RNA molecule and it is uniquely structured and appears flexible. The conserved nucleotides do not form any nucleotide-specific interactions in loop B (Schmitz et al. 1999b).

Ffh (Fifty four homologue): Ffh, earlier known as P48, was identified as a homologue of SRP54 through sequence comparison studies. It shares 31% amino acid identity and 60% similarity with SRP54 (Bernstein et al. 1989, Romisch et al. 1989). Upon incubation of RNCs with *E. coli* cell extracts, Ffh was found to be cross-linked with nascent chains bearing signal sequences but not with proteins that lack signal sequences or contained non-functional signal sequences (Luirink et al. 1992). Like SRP54, it also has the N-terminal N domain, a central G domain with the GTP binding consensus sequences, and a C-terminal methionine- rich M domain (figure 2 and figure 3). The structure of the NG domain of Ffh from *Thermus aquaticus* revealed that the N domain forms a bundle of four antiparallel α-helices and that the G domain has a β/α fold that is structurally similar to Ras-type GTPases (Freyman et al. 1997) (figure 7). The presence of an insertion in the G domain (insertion box domain, IBD) with two helices and two strands (β2a-α1a-β2b-α1b) is characteristic for SRP-type GTPases. Another characteristic feature of this subfamily of GTPases is that in the apo form the nucleotide binding pocket is rather wide, indicating that a conformational change is required to bind the guanine nucleotide (Montoya et al. 1997). Asp135 of motif II forms a salt bridge with Arg 191 from motif III and Lys111 in motif I forms a hydrogen bond with Asp187 from motif III. This network of hydrogen bonds and salt bridge stabilizes the empty state (nucleotide free form) of the protein (Freyman et al. 1999).

The crystal structure of M domain of Ffh from *Thermus aquaticus* revealed it consists of four amphipathic α-helices which are organized around a hydrophobic groove (Keenan et al. 1998) (figure 4). The groove is 25 Å in length, 15 Å in width and 12 Å in depth and is formed by αM1, αM2, αM4 and the finger loop. It is comprised of only hydrophobic residues (11 Leu, 3 Phe, 3 Met, 2 Val, and 2 Ile) which are similar to that observed in human M domain. The RNA binding helix-turn-

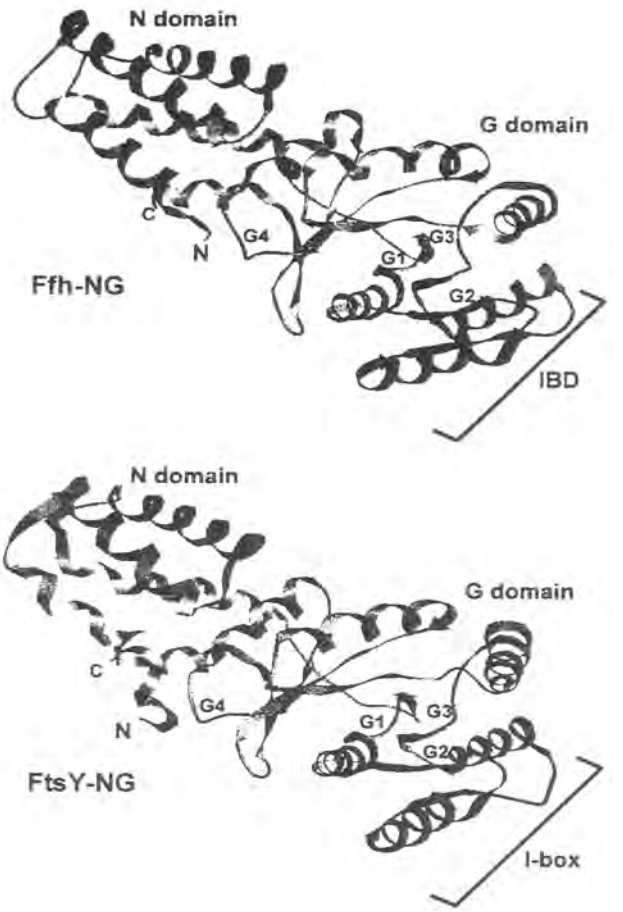

Figure 7 Crystal structures of the NG domains of Ffh and FtsY. The co-ordinates available in the Protein Data Bank were used to draw the ribbon diagrams of Ffh (Freyman et al., 1997) and FtsY (Montoya et al., 1997). The I-box insertions are indicated in black. Guanine nucleotide binding motifs are indicated as G1, G2, G3 and G4.

helix motif is constituted by 21 residues, ranging from 383-404 residues (from αM3 to αM4) in *T. aquaticus* Ffh. It was suggested that this HTH motif could be superimposed on the HTH motif of *lac* repressor with a rms deviation of 0.55 Å (Keenan et al. 1998). Though there are similarities between the human SRP54 M domain and *T. aquaticus* M domain structures, there are notable differences with respect to the hydrophobic groove that presumably binds the signal sequence. In Ffh, the groove is reported to be wide and short and binds a loop in contrast to SRP54M, where the groove is deep and elongated and binds alpha helices (Keenan et al. 1998, Clemons et al. 1999) (figure 4).

Interaction of Ffh with 4.5S RNA: Immuno precipitation experiments using Ffh specific antibodies

suggested that Ffh forms a complex with 4.5S RNA which was one of the first evidences for the existence of an SRP like particle in *E. coli* (Poritz et al. 1990, Ribes et al. 1990). The K_d for this association is in the nM range as monitored by nitrocellulose filter binding assay (Schmitz et al. 1996, Jagath et al. unpublished results). Mutational analysis in *B. subtilis* Ffh suggested that the SRP RNA binding region is located between 364-432 residues that includes positively charged motif 398RRKRIAKGSG407 (sequence from *B. subtilis* Ffh) (Kurita et al. 1996). This is in concurrence with the RNA-binding HTH motif observed in the crystal structure of *T. aquaticus* Ffh (Keenan et al. 1998). Conserved Arg residues in the HTH motif (398 and 401 residues) and the conserved G-S/C-G motif (residues 405-407) were shown to be important for RNA binding (Kurita et al. 1996). The 43-mer deletion mutant of 4.5S RNA consisting of the conserved domain IV (figure 6) or 28 mer that comprises only the internal loop A and the terminal tetraloop are capable of binding to Ffh with the affinity of 20 nM and 250 nM, respectively, indicating that the main contacts for Ffh are probably located in the symmetric internal loop A. Site specific mutations of phylogenetically conserved bases in the internal loop A affected the Ffh binding *in vitro* as well as *in vivo* (Wood et al. 1992). However, mutations in the tetraloop region or in the adjoining stem did not have any influence on the Ffh binding (Wood et al. 1992, Jagath et al. unpublished results). These results are compatible with the NMR structure of 4.5S RNA domain IV as discussed above. Chemical and enzymatic probing experiments revealed that the apical tetraloop region is not protected, in agreement with the mutational analysis (Lentzen et al. 1996). Hydroxyl radical probing experiments yielded an asymmetric pattern of backbone reactivity and this led to the proposal that the RNA molecule might be bent at the asymmetric internal loop B (Lentzen et al. 1996). A bent has in fact been observed in the solution structure of 4.5S RNA domain IV (Schmitz et al. 1999b).

Crystal structure of the E. coli SRP core: The crystal structure of the complex of domain IV of 4.5S RNA with an M-domain fragment of *E. coli* Ffh (amino acids 328 to 432) has been solved recently (Batey et al. 2000). The structure of the M domain in the complex is similar to that of Ffh from *T. aquaticus* and of human SRP54, although density for the finger loop is not observed in the crystal. In contrast, compared with unbound domain IV RNA, the structure of the RNA is changed significantly in the complex, in line with previous fluorescence data (Lentzen et al. 1994). Four nucleotides of the asymmetric loop are completely extruded from the helix, and three of these bases are stacked to form a unique surface by wrapping around the outside of the helix. This facilitates positioning the conserved A39 base for contacts with the protein. A large cavity is created upon this extrusion filled with cations and water molecules that help to bridge the conserved residues at the RNA-protein interface. The RNA-protein interface is formed by interactions from helices 2b (Glu386) and 3 (Arg398 and Arg401) in the M-domain and A47-C62 pair from the symmetric internal loop and A39 from the asymmetric internal loop. A39 also connects internal loops A and B by hydrogen bonding. Based on the structure data, the molecular surface responsible for signal sequence binding was proposed to be contributed by protein (two-third) and RNA backbone (one-third) (Batey et al. 2000). The combination of hydrophobic and electrostatic contacts in the signal sequence binding region observed in the structure probably would fit into the structural organization of signal sequence in which the core hydrophobic region is flanked by positively charged residues and neutral polar residues (see Introduction).

Kinetics of guanine nucleotide binding to Ffh and SRP: Ffh is a GTPase like its eukaryotic homologue SRP54 (Miller et al. 1994). *E. coli* Ffh has a significant intrinsic GTPase activity compared to that of SRP54 (Jagath et al. unpublished results). In order to understand the mechanism by which guanine nucleotides bind to Ffh/SRP, we have studied the binding of guanine nucleotides to Ffh and SRP in the presence and absence of *E. coli* ribosomes using fluorescent mant [2′(3′)-O-(N methylanthraniloyl)] derivatives of GDP and GTP (Jagath et al. 1998). These studies showed that the association and dissociation rate constants for both nucleotides are about 1 $\mu M^{-1} s^{-1}$ and 10 s^{-1}, respectively. The dissociation rate constant is about 100,000 times higher than that observed for Ran and Ras-type GTPases, whereas it is similar to

Table 2 *Guanine nucleotide binding to SRP-type GTPases and comparison with other GTPases*

Protein	Nucleotide	k_{off} (s^{-1})	K_d (M)
Ffh	GTP	7.6	1.2×10^{-6}
Ffh	GDP	3.7	1.3×10^{-6}
FtsY	GTP	15.0	13.0×10^{-6}
FtsY	GDP	5.0	1.8×10^{-6}
Ran	GTP	11×10^{-5}	3.8×10^{-11}
Ran	GDP	1.5×10^{-5}	6.0×10^{-12}
Ran/RCC1	GTP	19.0	-
Ran/RCC1	GDP	21.0	8.0×10^{-7}
EF-Tu	GTP	3×10^{-2}	1.5×10^{-7}
EF-Tu	GDP	2×10^{-3}	2.2×10^{-9}
EF-Tu/EF-Ts	GTP	100	1.0×10^{-7}
EF-Tu/EF-Ts	GDP	2000	5.0×10^{-6}

The dissociation rate constants and K_d values for GTP/GDP binding to above described GTPases are taken from the available literature. Ffh (Jagath et al. 1998), FsY (Jagath et al. 2000), Ran and Ran/regulator of chromosome condensation (RCC1) (Klebbe et al. 1995), EF-Tu and EF-Tu/EF-Ts (Romero et al. 1985, Pingoud et al. 1990)

the values observed for Ran in the presence of the exchange factor RCC1 (table 2). These results suggest that SRP-type GTPases do not require any exchange factors to dissociate the GDP after GTP hydrolysis (Jagath et al. 1998).

Structure of Ffh NG domain in complex with GDP-mechanism of GDP release from SRP: The structures of Ffh NG domain as Mg^{2+}GDP complex or with GDP (without Mg^{2+} ion) and their structural comparison with the apo form of NG domain were recently reported (Freyman et al. 1999). In the Mg^{2+}GDP complex, the structure around the guanine binding site was found to be similar to that observed in other GDP-bound GTPases (Kjeldgaard & Nyborg 1996, Sprang 1997). The β-phosphate is found to be curled under the P loop and the Mg^{2+} ion is co-ordinated by four water molecules, a phosphate oxygen, and the hydroxyl group of Thr 112, a typical feature of GDP-bound GTPases (Kjeldgaard & Nyborg 1992, Tong et al. 1991, Scheffzek et al. 1995). In the Mg^{2+}-free GDP complex, the β-phosphate is turned away from the P loop, a new contact of GDP with Gln144 is observed, and a salt bridge is formed between Asp135 and Arg191, which appears to stabilize the structure. This structure looks like a state intermediate between the Mg^{2+}GDP complex and apo form of the NG domain. In the Mg^{2+}GDP structure, the distal end of the N domain is shifted by 2.5 Å compared to that observed in the apo structure. The N and G domains are connected by a highly conserved, flexible hydrophobic interface that allows N and G domains to move during the binding and release of nucleotide. Thereby, the nucleotide occupancy of the G domain could be signalled to the N domain. Based on these results, Freyman et al. (1999) proposed a mechanism for the nucleotide release from SRP in which the active side chain hydrogen bond network, Gln144, the closing loop and flexibility of interface between the N and G domains are suggested to play an important role.

SRP Receptor from *E. coli*

The data base search using SRP54 amino acid sequence revealed that Ffh and FtsY proteins share significant similarity with their mammalian SRP cycle components, SRP54 and SRα in their NG domain region (Bernstein et al. 1989, Romisch et al. 1989) (figure 2). FtsY is required for cell viability and its depletion causes accumulation of certain proteins, leading to the

(Gill & Salmond 1990, Luirink et al. 1994). The proteins that are accumulated upon FtsY depletion are also accumulated by overexpression of Ffh. This could be explained, as the SRP receptors are being titrated away by overexpression of Ffh leading to functional imbalance (Luirink et al. 1994). FtsY was shown to be localized in both cytosol as well as at the cytoplasmic membrane (Luirink et al. 1994). FtsY, like its eukaryotic homologue SRα, consists of three domains, the N-terminal acidic domain, the N domain, and the G domain (Bernstein et al. 1989, Romisch et al. 1989) (figure 2). FtsY was shown to be associated with the cytoplasmic membrane through the acidic domain (Zelzany et al. 1997). The crystal structure of the NG domain of FtsY is similar to that of the Ffh NG domain (Montoya et al. 1997) (figure 7). The N domain (residue 197-280) is formed by a bundle of four α-helices, and the G domain (residues 291-495) has the characteristic Ras-type GTPase fold with an I box insertion in the effector loop region similar to that in Ffh. The interface between N and G domains is formed by hydrophobic interactions. The four consensus GTP binding elements are present but scattered (figure 7), in keeping with the observation that guanine nucleotide binding induces a conformational change of FtsY (Jagath et al. 2000). Recently, FtsY was shown to be important for the membrane targeting of *E. coli* ribosomes (Herskovits & Bibi 2000).

Guanine nucleotide binding studies: The interaction of GTP/GDP with FtsY was studied by Trp fluorescence using mutant FtsY(Trp343) in which two tryptophans (12 and 128) were replaced with phenylalanine. These results suggest that the environment of Trp343 changes upon binding GTP/GDP (Jagath et al. 2000) as the Trp fluorescence increased by 15-20%. The affinity of GDP (1.6 μM) is higher than that of GTP (13 μM) and dissociation rate constants are in the range of 5-15 s^{-1}, similar to those observed for Ffh, thus obviating the need for an exchange factor (table 2). Similar results were reported for the isolated NG domain of FtsY (Moser et al. 1997).

Interaction of SRP with FtsY

SRP and FtsY moderately stimulate each other's GTPase activities like their eukaryotic homologues SRP54 and SRα (Miller et al. 1994, Powers & Walter 1995). In the mammalian system, the mutual GTPase stimulation of SRP and SRP receptor was highly dependent on the buffer conditions and could be observed only in the presence of other components, such as RNCs and liposomes (Bacher et al. 1996). Recently it was shown that phospholipids stimulate the GTPase activity of FtsY (de Leeuw et al. 2000), indicating that the full stimulation of the GTPase may require the presence of all components of the targeting complex.

SRP interacts with FtsY in a GTP-dependent manner; in the presence of GDP the formation of the complex is not observed (Jagath et al. 2000, Kusters et al. 1995, Miller et al. 1994). SRP induces conformational changes in the I-box region of FtsY (Jagath et al. 2000), indicating that it may be the SRP binding site. The association rate constant for SRP and FtsY complex formation is rather low, about 0.2 $\mu M^{-1}s^{-1}$, which indicates that a conformational rearrangement has to take place during complex formation. In the absence of 4.5S RNA, Ffh alone is not capable of inducing the conformational changes in the FtsY (Jagath et al. 2000). However, recent studies by Peluso et al. (2000) showed that Ffh induces the conformational changes in the I-box region of N-terminal truncated FtsY, suggesting an important role for N-terminal domain of FtsY. The GTPase activity studies on *Mycoplasma mycoides* Ffh and FtsY homologs showed that enhanced GTP hydrolysis was observed for the mixture of Ffh and FtsY or NG domain of Ffh and FtsY in the absence of SRP RNA, indicating a direct complex formation between Ffh and FtsY (Macao et al. 1997). Rapid reaction kinetics showed that the association of SRP-FtsY complex is about 200 times faster than that of Ffh-FtsY complex. The dissociation rates are 3.3×10^{-3} s^{-1} and $1.2 \times 10^{-5} s^{-1}$ for Ffh-FtsY and SRP-FtsY complexes, respectively, suggesting that 4.5S RNA also enhances the dissociation of Ffh and FtsY complex by 200 times (Peluso et al. 2000). All the biochemical and kinetic studies indicate that the 4.5S RNA plays a modulatory role in Ffh-FtsY complex formation.

Role of the conserved tetraloop region of 4.5S RNA: GNRA and UNCG tetraloops are the most frequently occurring hairpins in RNAs (Woese et al. 1990). Solution structures for the both types of tetraloops are available, and they consist of diloop conformation as the 1st and 4th nucleotides of the loops form a hydrogen bond (Cheong & Varani 1990, Heus & Pardi 1991). The domain IV of SRP RNA is terminated by a tetraloop with GNRA (GGAA or GAAA) consensus sequence except in the case of yeast (*S. cerevesiae*) and plant SRP RNAs. It was shown earlier in fission yeast (*Schizosaccharomyces pombe*) SRP RNA that the GAAA tetraloop can be replaced with a UUCG tetraloop with only moderate effects on viability (Selinger et al. 1993). In contrast, in the case of *E. coli* 4.5S RNA, the UUCG tetraloop substitution completely abolishes the interaction of SRP with the receptor, FtsY, *in vitro* and blocks cell growth (Jagath et al., unpublished results).

Thermodynamic studies on RNA hairpin tetraloops by Antao and Tinoco (1991, 1992) revealed that UUCG tetraloops with C-G loop-closing pair is more thermostable compared to that of GNRA and G-C loop-closing base pair of UUCG tetraloops. The disruption of C-G loop-closing base pair or interchanging with G-C base pair in the 4.5S RNA tetraloop indeed partially restored the function of UUCG tetraloop (Jagath et al. unpublished results). That similar mutations in *S. pombe* RNA caused growth defects could be due to impaired binding of SRP19 which, based on results obtained for human SRP (Zwieb 1994), is likely to interact with the stem adjacent to the tetraloop.

These results suggest that binding of FtsY to SRP require structural flexibility in the tetraloop region of 4.5S RNA. Since FtsY does not interact with the RNA directly and presumably binds to the Ffh moiety of SRP, the results indicate that structural details of the RNA influence the structure of Ffh in a way that the interaction with FtsY is affected.

SRP Cycle in *E. coli*

The available evidence suggests the following model for the bacterial SRP cycle (figure 8). The nucleotide

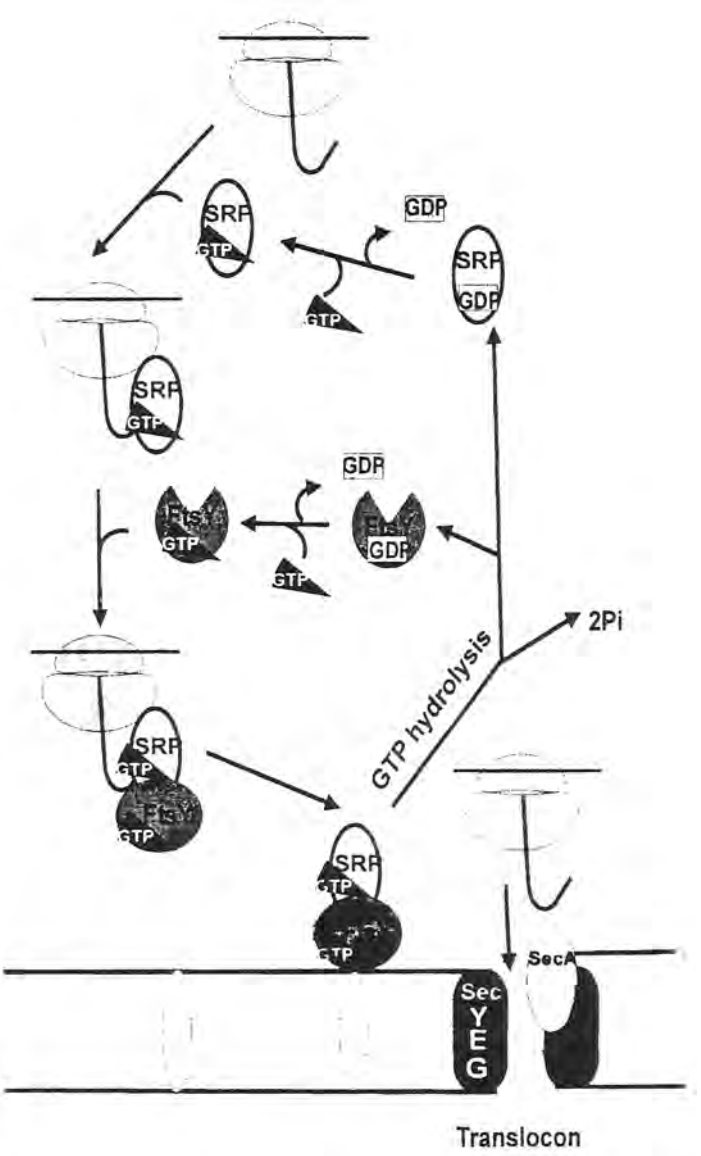

Figure 8 SRP-dependent protein targeting in *E. coli*. The ribosome nascent chains bearing hydrophobic signal sequences are recognized by SRP in the GTP bound form. The SRP-RNC complex recognizes FtsY, and the ternary complex is targeted to membrane. The RNC is released from SRP and the nascent chain is integrated into the translocon. GTP hydrolysis drives the dissociation of SRP from FtsY. Spontaneous exchange of GDP for GTP on both SRP and FtsY inititiates a new cycle.

binding properties suggest that both SRP and FtsY enter the cycle in the GTP bound form (Jagath et al. 1998, Jagath et al. 2000). SRP binds to RNCs exposing a signal sequence. There is no evidence for an arrest of elongation caused by SRP binding to RNC, in keeping with the fact that *E. coli* SRP, unlike mammalian SRP, does not contain the elongation

arrest domain (*Alu* domain). Subsequently, the SRP-RNC complex binds to the SRP receptor, FtsY. It is not known whether this interaction takes place in the cytosol or at the membrane. The events at the membrane, that is the transfer of the RNC to the translocon and the timing of GTPase activation is not known. Recently, a further stimulation of the GTPase of FtsY by membrane phospholipids has been reported (de Leeuw et al. 2000), indicating that the contact with the membrane may be important for the stimulation of the GTPase of FtsY; whether there is a similar effect on Ffh is not known. In the presence of FtsY, the nascent chains are released from SRP and delivered to the translocon (SecY, E, G) in a GTP dependent manner (Valent et al. 1998, de Gier et al. 1998). GTP hydrolysis leads to the dissociation of the SRP-FtsY complex and the recycling of SRP.

Structural and Functional Conservation of the SRP Pathway

The signal sequences play an important role in determining the route for targeting whether it follows an SRP dependent or independent pathway. Recently, it has been demonstrated in *E. coli* that Lep (leader peptidase) and Lep-inv use the SRP pathway for membrane insertion. The wild type M13 procoat protein integrates into the membrane in a SRP-independent fashion. A hybrid construct (H1-procoat) was prepared in which hydrophobic core of procoat signal sequence was replaced with first transmembrane segment of Lep signal sequence, thus increasing the hydrophobicity of the signal sequence. This H1-procoat protein follows the SRP-dependent pathway unlike the wild-type procoat protein (de Gier et al. 1998). Similar studies were performed *in vitro* using dog pancreas microsomes that Lep and Lep-inv were targeted to the ER membrane in an SRP-dependent fashion, whereas the procoat assembles into ER membrane in an SRP-independent manner (Dierks et al. 1996, Watts et al. 1983, Wickner 1988). Thus, the basic mechanism of membrane protein assembly appears to be evolutionarily conserved from prokaryotes to eukaryotes. SRP54, SRα and domain IV of SRP RNA are highly conserved and found in every organism (Althoff et al. 1994). It was also shown that *E. coli* SRP components are capable of interacting with their eukaryotic counterparts, and vice versa, suggesting the evolutionary conservation of SRP pathway between prokaryotes and eukaryotes (reviewed in Lutcke 1995, Powers & Walter 1997).

Even though the basic mechanism for SRP dependent pathway in eukaryotes and prokaryotes seems to be conserved, there are quite a few differences between the prokaryotic and eukaryotic SRP pathways. So far, an equivalent for SRβ has not been found in prokaryotes, and *in vitro* experiments using purified translocon components, Sec Y, E and G suggested the minimal requirements for SRP-dependent protein targeting (Scotti et al. 1999). This also indirectly rules out the existence of SRβ in *E. coli*. SRP RNAs differ in their size and presence of various domains among different organisms. In *E. coli*, only the conserved domain IV and part of domain II are present (Poritz et al. 1988). Domain I, which is responsible for the elongation arrest observed in the mammalian and yeast systems, is lacking in prokaryotes. Similarly, an SRP19 homologue was not found in *E. coli*, in keeping with the absence of its binding site, domain III, in *E. coli* 4.5S RNA.

Conclusions and New Advances in Understanding the SRP Cycle

This review summarizes the structural and biochemical insights that led to have understanding of the various events in the SRP cycle. The basic machinery with which SRP interacts and targets the nascent chains to the ER membrane in eukaryotes or plasma membrane in prokaryotes seems to be established. Also, some of the intermediate steps in the cycle are being elucidated. The crystal structures of purified proteins, fragments and binary complexes of some of the SRP components are available, and these provided some of the molecular clues for the mode of action. The first step in the SRP cycle, i.e. signal sequence recognition, needs an understanding at molecular level as SRP54 or Ffh are capable of accommodating a variety of signal sequences and the M-domain is responsible for signal sequences binding in addition to SRP RNA binding. Crystal structures of Ffh, human SRP54M and core domain of *E. coli* SRP gave some insights into this, but crystal structure of SRP with signal sequence would provide more definitive answers in understanding this biological process. Also, it is important to have the crystal structure of these binary and ternary complexes with full length proteins as there is cross-talk between N, G and M-domains (Zheng & Gierasch 1997).

The next step in the eukaryotic SRP cycle is the translational arrest function that is absent in the *E. coli*. *In vitro* studies clearly established this step and also recent structural studies advanced our knowledge on this activity (reviewed in Bui & Strub 1999). But the importance of translation arrest *in vivo* needs to be confirmed. The *in vivo* translocation system in *S. cerevisiae* may provide more conclusive answers into this phenomenon (Johnsson & Varshavsky 1994).

The molecular events taking place during the docking step are not clear. In the presence of GTP, several events have been observed; RNCs dissociate from the ribosome, SRP is docked to membrane then binds to receptor tightly due to the conformational rearrangements in both receptor and SRP. However, the sequence of the events has not been dissected. The SRP cycle is controlled by three interacting GTPases, SRP54, SRα and SRβ. The mechanisms by which they control the SRP cycle, at which step GTP is added and utilized, have been strongly debated. There are two GTPases (Ffh and FtsY) in *E. coli* SRP cycle but the molecular mechanisms involved in this SRP-GTPase cycle are also not well studied. Therefore, it is necessary to have more structural information and to conduct biochemical experiments in more complete systems to answer the open, challenging questions in the eukaryotic and prokaryotic SRP cycles.

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