Signal Recognition Particle: An Essential Protein-Targeting Machine

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Abstract

The signal recognition particle (SRP) and its receptor compose a universally conserved and essential cellular machinery that couples the synthesis of nascent proteins to their proper membrane localization. The past decade has witnessed an explosion in in-depth mechanistic investigations of this targeting machine at increasingly higher resolutions. In this review, we summarize recent work that elucidates how the SRP and SRP receptor interact with the cargo protein and the target membrane, respectively, and how these interactions are coupled to a novel GTPase cycle in the SRP·SRP receptor complex to provide the driving force and enhance the fidelity of this fundamental cellular pathway. We also discuss emerging frontiers in which important questions remain to be addressed.

Contents

INTRODUCTION

Proper localization of proteins to their correct cellular destinations is essential for sustaining order and organization in all cells. Roughly 30% of the proteome is initially destined for the eukaryotic endoplasmic reticulum (ER) or the bacterial plasma membrane. Although the precise number of proteins remains to be determined, it is generally recognized that the majority of these proteins are delivered by the signal recognition particle (SRP), a universally conserved protein-targeting machine (1–4). Thirty years ago, the components and pathway for SRP-dependent protein targeting were first elucidated in mammalian cells through in vitro reconstitutions in cell extracts (5–9). The identification of the SRP homolog in prokaryotes a decade later further highlighted the salient, universally conserved features of this pathway (10–12). The biochemical accessibility of the bacterial SRP system has enabled in-depth mechanistic investigations of this pathway, allowing us to understand its underlying molecular mechanism at unprecedented depth and resolution.

SRP: signal recognition particle

OVERVIEW OF SRP-DEPENDENT PROTEIN TARGETING

With the exception of the chloroplast SRP (see Chloroplast SRP: A Unique Posttranslational SRP, below), SRP-mediated protein targeting is a strictly cotranslational process that begins when a nascent polypeptide destined for the ER or plasma membrane emerges from the ribosome (**Figure 1*a***). The N-terminal signal sequence on the nascent polypeptide serves as the signal that allows the ribosome·nascent chain complex (termed the RNC or cargo) to engage the SRP and, through interaction with the SRP receptor (SR), to be delivered to the vicinity of the Sec61p (or SecYEG in prokaryotes) translocon at the target membrane (**Figure 1*a***). There, the RNC is transferred to the Sec61p/SecYEG machinery, which either integrates the nascent polypeptide into the lipid bilayer or translocates it across the membrane to enter the secretory pathway. Meanwhile, the SRP and SR dissociate from each other to mediate additional rounds of targeting (**Figure 1*a***).

The size and composition of the SRP vary widely across species. Surprisingly, the bacterial SRP and SR, though highly simplified compared with those in eukaryotes, can replace their mammalian homologs to mediate

efficient targeting of mammalian proteins to the ER (11, 13). This demonstrates the remarkable evolutionary conservation of the SRP pathway and shows that the bacterial machinery can represent the functional core of the SRP necessary and sufficient for protein targeting. This provides a useful starting point for mechanistic dissections.

The bacterial SRP contains the universally conserved SRP54 protein (called Ffh in bacteria) bound to the 4.5S SRP RNA. Ffh has two structurally and functionally distinct domains (**Figure 1*b***, left): a methionine-rich M-domain that recognizes the signal sequence and binds, with picomolar affinity, to the SRP RNA (14–16), and a special GTPase or NG domain that interacts with a highly homologous NG domain in the SR (**Figure 1*b***) (17, 18). The bacterial SR, called FtsY, also contains an N-terminal acidic A domain that allows this receptor to peripherally associate with the membrane (19, 20).

The cotranslational SRP pathway minimizes the aggregation or misfolding of nascent proteins before they arrive at their cellular destination and is therefore highly advantageous in the targeted delivery of membrane and secretory proteins. Nevertheless, an increasing number of posttranslational protein-targeting pathways have been identified (**Figure 1*a***, left). The best characterized thus far is the bacterial SecB/A system, which delivers bacterial secretory and outer-membrane proteins to the SecYEG complex and, through the ATPase cycles of SecA, drives the translocation of substrate proteins across the SecYEG translocon (1, 2). In yeast, the Sec62/63/71/72 system is a major pathway that mediates protein secretion (21, 22). Additional targeting pathways have been found, including the twin-arginine-translocase (Tat) system, heat shock protein 70 (Hsp70)-dependent pathways, and most recently the guided entry of tail-anchored proteins (GET) pathway (**Figure 1*a***, left path) (1, 2, 23–26).

Despite the divergence of targeting machineries, the SRP pathway illustrates several key features that are general to almost all protein-targeting processes: (*a*) the cellular destination of a protein is dictated by its signal sequence, which allows it to engage a specific targeting machinery; (*b*) targeting machineries cycle between the cytosol and membrane, acting catalytically to bring cargo proteins to translocation sites at the target membrane; and (*c*) targeting requires the accurate coordination

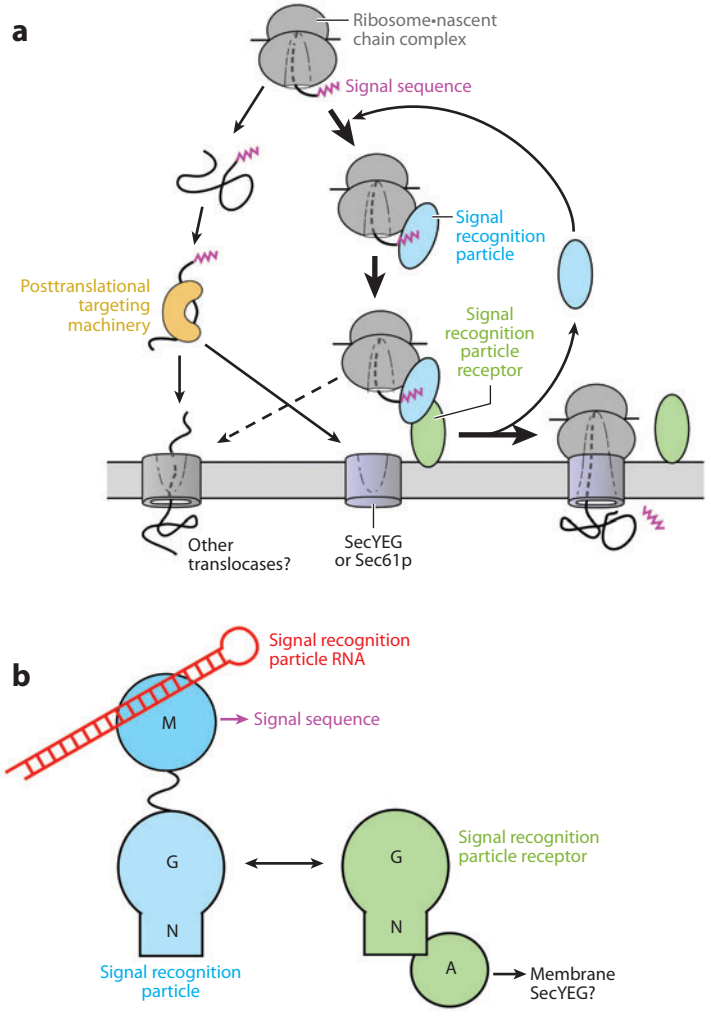


Figure 1

Overview of the pathways and components of the signal recognition particle (SRP). (*a*) Multiple pathways deliver newly synthesized proteins to the endoplasmic reticulum or plasma membrane, with the SRP pathway mediating the cotranslational targeting of translating ribosomes (*right*) and posttranslational targeting machineries mediating the targeting of proteins released from the ribosome. (*b*) Domain structures of the ribonucleoprotein core of the SRP, which is composed of the SRP54 (or Ffh) protein and the SRP RNA (*left*) and the bacterial SRP receptor (*right*).

Protein targeting: the process of delivering a newly synthesized protein to specific organelles in the cell

of multiple dynamic events, including cargo loading/unloading, targeting complex assembly/disassembly, and the productive handover of cargo from the targeting to the translocation machinery. Not surprisingly, such molecular choreography requires energy input, which is often harnessed by GTPase or ATPase modules in the targeting machinery. Below, we discuss recent advances in our understanding of the molecular mechanisms that underlie these key events in the SRP pathway.

MOLECULAR INTERACTIONS AND REGULATION OF THE SRP CORE

Cargo Recognition by the SRP

Timely recognition of signal sequences by the SRP is essential for proper initiation of cotranslational protein targeting. Signal sequences that engage the SRP are characterized, in general, by a core of 8–12 hydrophobic amino acids that preferentially adopts an α-helical structure (27, 28). Cross-linking and phylogenetic analyses have implicated the M domain of Ffh/SRP54 in binding the signal sequence (29–31). The unusually high methionine content of this domain further led to a methionine bristle hypothesis, in which the flexible methionine side chains provide a hydrophobic environment with sufficient plasticity to accommodate a variety of signal sequences (10). In support of this model, crystallographic analyses of Ffh (16) and SRP54 signal-peptide fusions (15, 32) showed that the signal sequence binds to a groove in the Ffh/SRP54 M domain composed almost exclusively of hydrophobic residues. Two different modes of signal-peptide docking were observed (15, 32); this is probably owing to the different signal sequences used in the two studies and supports the notion that signal-sequence interaction with the M domain is quite flexible. A conserved, flexible finger loop connects the α1 and α2 helices that line the bottom of the signal sequence binding groove. This loop may act as a flexible flap that closes upon the signal sequence (16, 33, 34), although there is currently no direct evidence for this model. Intriguingly, mutations in this loop disrupt the interaction between the SRP and SR GTPases and the unloading of RNC to the translocon (35, 35a), suggesting that it plays a role beyond that of facilitating signal-sequence recognition. How the fingerloop promotes these downstream steps in the pathway remains to be determined.

Despite these interactions, the SRP binds isolated signal sequences weakly, with dissociation constants (K_d) in the micromolar range (36, 37). In comparison, RNCs containing no signal sequences or even empty ribosomes bind the SRP with K_d values of 80–100 nM (38–40). Thus, the ribosome makes a significant contribution to the recruitment of the SRP. The binding site of the SRP on the ribosome was identified by cross-linking studies (41, 42) and cryoelectron microscopy (cryo-EM) reconstructions of the RNC·SRP complex in both the eukaryotic and bacterial systems (43–45). Together, these results show that basic residues on the tip of the Ffh N domain contact ribosomal proteins L23 and, to a lesser extent, L29 (L23a and L35 in eukaryotes, respectively) in the vicinity of the ribosomal exit site (**Figure 2*a***). In the cryo-EM structure, the M domain also contacts ribosomal RNAs and perhaps ribosomal proteins L22 and L24, although these contacts need to be verified biochemically. These ribosomal contacts, together with the interaction of the Ffh/SRP54 M domain with the signal sequence, allow the SRP to bind its correct cargos with sub- to low-nanomolar affinity (38–40, 46).

Membrane Localization of the SRP Receptor

Bacterial SR is a single protein, FtsY, that lacks a bona fide transmembrane (TM) domain. The results of microscopy (47, 48), cell fractionation (49), and in vitro binding experiments using synthetic liposomes (19, 50, 51) indicate that the interaction of FtsY with the bacterial inner membrane is weaker and more dynamic compared to those of integral membrane proteins. Although the N-terminal A domain may mediate FtsY's membrane association, recent studies

Signal sequence: a transferable, *cis*-acting element on the nascent polypeptide that engages protein-targeting machineries and mediates proper localization of the protein

RNC: ribosome·nascent chain complex

SR: signal recognition particle receptor

Translocon: a protein complex that mediates the translocation or integration of proteins in the membrane bilayer; used interchangeably with translocation machinery and translocase

GTPase: guanosine 5′-triphosphate (GTP) hydrolase

ATPase: adenosine 5′-triphosphate (ATP) hydrolase

Twin-arginine-translocase (Tat): a system composed of TatA, TatB, and TatC proteins that can transport folded proteins across the membrane

Heat shock protein 70 (Hsp70): a family of ubiquitously expressed, 70-kDa molecular chaperones that facilitate protein folding and biogenesis

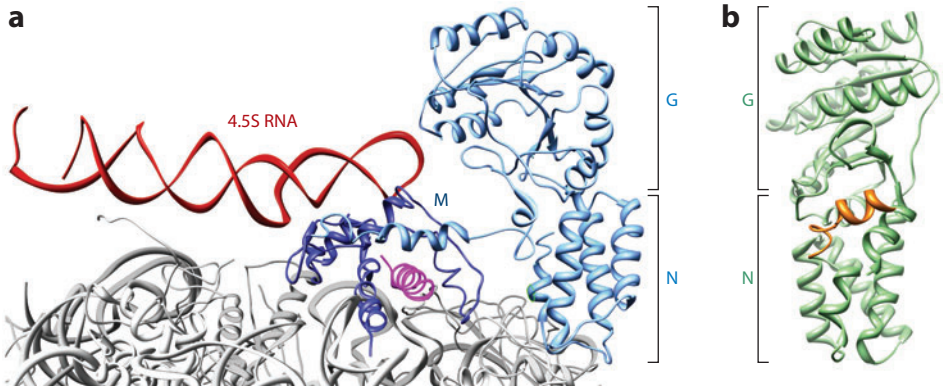


Figure 2

(*a*) A molecular model for interaction of the bacterial signal recognition particle (SRP) with the translating ribosome [*gray*; Protein Data Bank (PDB) 2J28], derived from cryoelectron microscopy reconstruction and docking of the crystal structures of individual protein fragments as described in Reference 44. The M and NG domains of the SRP are in dark and light blue, respectively, the SRP RNA is in red, and the signal sequence is in magenta. (*b*) Crystal structure of the bacterial FtsY (NG+1) construct (PDB 2QY9; *green*) highlighting its lipid-binding helix at the N terminus (*orange*). Adapted from Reference 51.

show that FtsY(NG+1), in which only Phe196 immediately preceding the NG domain is retained, is sufficient to sustain lipid binding of FtsY and cotranslational protein targeting in vivo and in vitro (19, 51–53). Similar observations were made with the chloroplast FtsY homolog (54). Comparison of the crystal structure of FtsY(NG+1) with that of FtsY-NG (19, 55) showed that Phe196 induced folding of an amphiphilic α-helix rich in basic residues at the junction between the A and N domains, and the induced α-helix provides FtsY's primary lipid-binding motif (**Figure 2*b***, orange).

This structure, together with in vitro binding studies, also showed that FtsY preferentially binds the anionic phospholipids phosphatidylglycerol (PG) and cardiolipin (CL) (19, 50, 51). This preference is corroborated by experiments in vivo in which overexpression of genes involved in PG and CL biosynthesis rescued an FtsY mutant defective in lipid binding (56). Anionic phospholipids also preferentially interact with and activate the SecYEG machinery (57) and the SecA ATPase (58, 59) and stimulate the integration and export of membrane and secretory proteins (60–62). Together, these observations suggest that sites of bacterial inner membrane enriched in anionic phospholipids could constitute active zones for protein targeting and translocation, an attractive hypothesis that awaits to be tested.

In addition to lipid interactions, a direct interaction of FtsY with SecYEG would provide an attractive mechanism to more precisely localize the targeting complex to the translocon. Recent cross-linking and copurification studies provided evidence for this interaction (63, 64). Subsequent cross-linking and mutational studies further showed that the A domain of FtsY and the cytosolic loops of SecYEG connecting TMs 6–7 and TMs 8–9 (termed C4 and C5 loops in prokaryotes and L6/7 and L8/9 loops in eukaryotes) participate in this interaction (20, 64, 65). Nevertheless, several puzzling observations remain. Given the low sequence conservation of the FtsY A domain and its dispensability for cotranslational targeting, it is unclear to what extent this domain helps facilitate the targeting reaction. The NG domain of FtsY may also interact with SecYEG (65), but direct evidence for this interaction remains to be obtained. Most importantly, the SecYEG C4 and C5 loops that interact with FtsY are also crucial for its interaction with the ribosome (65),

Guided entry of tail-anchored proteins (GET): a pathway that mediates the posttranslational targeting of tail-anchored membrane proteins to the endoplasmic reticulum membrane

Cotranslational targeting: a mode of protein targeting in which the nascent protein is delivered while still attached to the translating ribosome

suggesting that the interaction of FtsY with SecYEG is transient and must be broken to allow for stable docking of RNC onto the SecYEG machinery. The timing, mechanism, and precise roles of the FtsY-SecYEG interaction remain challenging questions for future studies.

Eukaryotic SR is a heterodimeric complex consisting of the α and β subunits (66). SRα is a soluble protein highly homologous to FtsY. Instead of the A domain, SRα contains an N-terminal X domain that dimerizes with SRβ, an integral membrane protein, thus localizing SRα to the ER membrane (67). SRβ also contains a GTPase domain that, unlike the two GTPases in the SRP and FtsY/SRα described below, is most homologous to the ADP ribosylation factor family of GTPases (67, 68). Intriguingly, stable SRα-β association requires SRβ to be bound with GTP (67), and the Sec61β subunit of the Sec61p complex could accelerate GDP dissociation from SRβ (69), suggesting that Sec61β potentially serves as a nucleotide exchange factor that maintains SRβ in the GTP-bound state active for binding SRα. A split ubiquitin assay demonstrated the direct interaction of SRβ with the yeast Sec61p homolog, Ssh1p, in vivo (70); disruption of this interaction impairs cotranslational protein targeting and cell growth (71). These results suggest functional interactions of the eukaryotic SR with the Sec61p translocon that parallel findings with the bacterial FtsY, showing that the membrane localization of the eukaryotic SR may be subject to more complex regulation.

P-loop GTPase fold: the most populous protein fold in nucleotide hydrolases that uses the binding and hydrolysis of GTP to regulate cellular functions

Switch II loop: a structural segment in Ras-type signaling GTPases that interacts with effector proteins and moves upon GTP hydrolysis

Regulation of Protein Targeting by the SRP and SRP Receptor GTPases

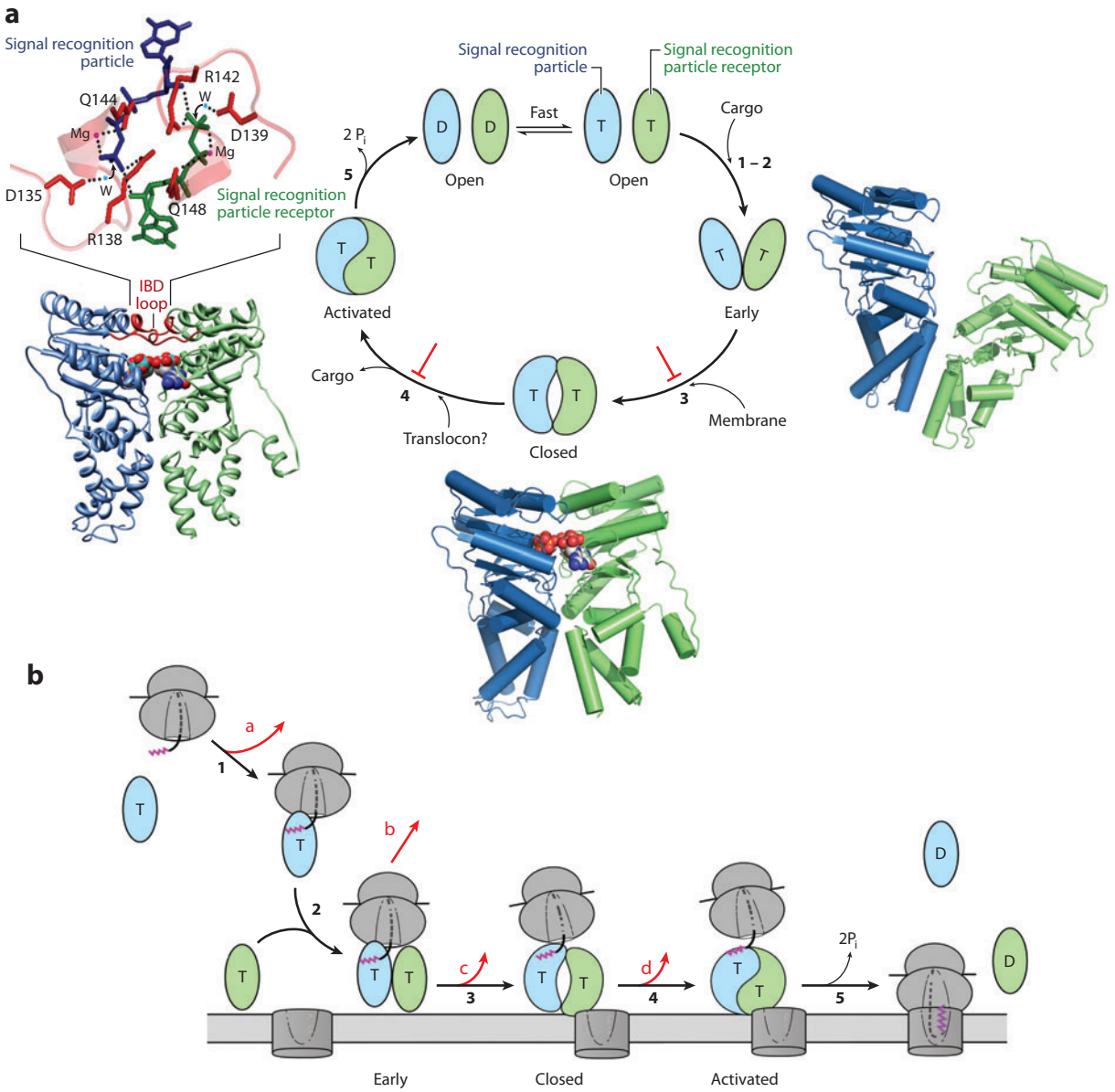
At the membrane, the SRP and SR meet and interact with each other through their GTPase modules. Both proteins contain a central G domain that shares homology with the classic P-loop GTPase fold (55, 72). Unique to the SRP and SR GTPases is an additional β-α-β-α insertion box domain (IBD) in which a flexible IBD loop (red in **Figure 3*a***) contains multiple catalytic residues and provides an equivalent of the switch II loop in Ras-type GTPases (55, 72). In addition, a four-helix bundle preceding the GTPase fold forms the N domain, which together with the G domain composes a structural and functional unit termed the NG domain (**Figure 2**). Unlike classic signaling GTPases that exert regulation by switching between a GTP-bound, active state and a GDP-bound, inactive state (73, 74), the SRP and SR represent a novel class of GTPases whose activities are regulated by nucleotide-dependent dimerization cycles (75). Members of this family also include FlhF, MinD, MnmE, the septins, Toc proteins, human guanylate binding protein-1, and the dynamin family of GTPases (75–78). In the past decade, mechanistic studies of the

Figure 3

Conformational changes in the signal recognition particle (SRP) and SRP receptor (SR) GTPases ensure the efficiency and fidelity of protein targeting. The steps are numbered to be consistent between panels (*a*) and (*b*). The Ffh and FtsY NG domains are in blue and green, respectively. T and D denote GTP and GDP, respectively. (*a*) (*Middle*) A series of discrete rearrangements drives the SRP•SR GTPase cycle and is regulated by the cargo and target membrane. ⊥ denotes the pausing effect of cargo in disfavoring the conformational rearrangements. (*Right*) molecular model of the early intermediate [Protein Data Bank (PDB) 2XKV]. (*Bottom*) Cocrystal structure of the Ffh-FtsY NG domain complex in the closed/activated conformation (PDB 1RJ9). The two GTP analogs are in a space-filling model. (*Left*) Zoom of the composite active site formed at the dimer interface required for GTPase activation, with the GMPPCP molecules from Ffh and FtsY in blue and green, respectively, active site Mg^{2+} in magenta, nucleophilic waters (W) in blue, and catalytic residues in the insertion box domain (IBD) loops in red. Adapted from Reference 84. (*b*) GTPase rearrangements provide the driving force and ensure the fidelity of protein targeting. In Step 1, a ribosome•nascent chain complex with a signal sequence (*magenta*) binds the SRP. In Step 2, the cargo-loaded SRP forms a stabilized early targeting complex with FtsY. In Step 3, membrane association of FtsY drives rearrangement to the closed state, which weakens the SRP's affinity for the cargo. In Step 4, interaction of SR with SecYEG may drive GTPase rearrangements to the activated state required for cargo handover. In Step 5, the cargo is unloaded from the SRP onto SecYEG, and GTP hydrolysis drives the disassembly and recycling of the SRP and SR. At each step, the cargo can be either retained in (*black arrows*) or rejected from (*red arrows*) the SRP pathway. Adapted from Reference 40.

bacterial SRP and SR GTPases have elucidated the biological logic and regulatory mechanism for these twin GTPases, which could provide general principles for understanding other members of this GTPase family.

Free Ffh and FtsY exhibit minor structural differences in the apo, GDP-, and GTP-bound states (55, 72, 79–82). Even with GTP bound, both GTPases by themselves are in an inactive open conformation, exhibiting fast nucleotide dissociation and exchange rates as their nucleotide-binding pocket is wide open (**Figure 3*a***) and low basal GTPase activity as their catalytic loops are not correctly positioned (83). Their GTPase cycle is driven by a series of conformational changes during their dimerization that culminate in reciprocal GTPase activation (**Figure 3**; 84). GTPase assembly is



initiated with a transient early intermediate, which forms rapidly but is highly unstable (K_d ~ 4–10 μM; **Figure 3**, step 2) (85). This intermediate lacks stable contacts between the G domains of Ffh and FtsY and is primarily stabilized by electrostatic attractions between their N domains (**Figure 3*a***, right panel) (86, 87). Subsequent GTP-dependent rearrangements, primarily involving readjustments at the intramolecular G-N domain interface (17, 18, 88, 89) and removal of an inhibitory N-terminal helix (90–92), lead to the formation of a stable closed complex in which extensive stereospecific interactions form between the G domains of both proteins (K_d ~ 16–30 nM; **Figure 3*a***, step 3 and lower panel). Two pairs of hydrogen bonds form across the dimer interface through the 3′-OH of one GTP and the γ-phosphoryl oxygen of the other; these further stabilize the GTPase dimer (17, 18). The final GTPase activation step involves local rearrangements of the IBD loops, which must be brought into close proximity to the GTP molecules to form an activated complex (**Figure 3*a***, step 4). Each IBD loop provides at least three catalytic residues (Asp135, Arg138, and Gln148 in Ffh and their homologous residues in FtsY) that coordinate the nucleophilic water, the γ-phosphoryl oxygen, and the active site Mg^{2+}, forming a composite active site conducive to hydrolyzing GTP (**Figure 3*a***, left panel) (17, 18, 89). Following hydrolysis, the SRP•FtsY complex is much less stable and quickly disassembles (**Figure 3*a***, step 5; 83, 93).

Importantly, each of the GTPase rearrangements during the dimerization and activation of the SRP and FtsY provides a discrete regulatory point at which they can sense and respond to the presence of the RNC and target membrane, thus allowing the loading of cargo on the SRP to be tightly coupled to its delivery to the membrane. For example, with free SRP and FtsY, assembly of a stable closed complex is extremely slow (k_{on} ~10^2–10^3 $M^{-1}s^{-1}$) (36, 83, 94) and insufficient to sustain the protein-targeting reaction. The RNC, by stabilizing the early intermediate more than 100-fold and preventing its premature disassembly, accelerates stable SRP•FtsY assembly 10^3-fold (95). Analogously, phospholipid membranes, by helping to preorganize FtsY into the closed conformation, accelerate GTPase assembly 160-fold (51, 96, 97). These allosteric regulations ensure the rapid delivery of cargo to the membrane and minimize futile cycles of interactions between the free SRP and SR.

Intriguingly, the RNC also disfavors the rearrangement of the GTPase complex to the closed and activated states and delays GTPase activation in the targeting complex (40, 95). This generates a highly stabilized early targeting intermediate in which the RNC is predicted to bind the SRP with picomolar affinity while GTP hydrolysis is paused (95). These effects are highly beneficial in preventing abortive reactions at early stages of targeting; however, they pose serious challenges for the cargo unloading and GTPase activation events at later stages. Multiple observations strongly suggest that the resolution to this problem lies in part in the subsequent GTPase rearrangements to the closed and activated states, which help switch the targeting complex from a cargo-binding to a cargo-releasing mode. The interaction of cargo with the SRP is predicted to weaken ~400-fold when the early targeting complex rearranges to the subsequent conformational states (95). Further, mutant GTPases that block the closed → activated rearrangement appear to block the engagement of cargo with the translocon (98). Finally, cross-linking and cryo-EM analyses showed that in the presence of SR and GTP analogs, the NG domain of the SRP becomes mobile and detaches from L23 (42, 99). Importantly, anionic phospholipid membranes can induce these late GTPase rearrangements (**Figure 3**, step 3; 51), suggesting an attractive mechanism to spatially couple the membrane delivery of RNCs to their subsequent unloading.

Collectively, these results provide a coherent picture of how the unusual GTPase cycle of the SRP and SR provides exquisite spatial and temporal coordination of protein targeting (**Figure 3*b***). GTPase assembly is minimized in the absence of biological cues but is

initiated when the SRP is loaded with RNCs bearing strong signal sequences (**Figure 3*b***, steps 1–2). But in the absence of the target membrane, the RNC·SRP·SR complex is primarily stalled in the early conformational stage, in which the cargo is tightly bound to the SRP and GTP hydrolysis is delayed. Interaction of FtsY with phospholipid membranes helps relieve this pause and induce the GTPase rearrangements into the closed/activated states, in which the interaction of the ribosome with the SRP is weakened and the RNC can be more readily released from the targeting complex (step 3). What ultimately drives the completion of the cargo handover event and reactivates GTP hydrolysis (steps 4–5) is still unclear, although the SecYEG translocon provides an attractive candidate. Finally, GTP hydrolysis drives the disassembly and recycling of the SRP and SR, allowing them to initiate new rounds of protein targeting.

Fidelity of the SRP: Binding, Induced Fit, and Kinetic Proofreading

Like other topogenic sequences that mediate protein localization, SRP signal sequences are highly divergent (27, 28, 100, 101), and the SRP must be sufficiently flexible to accommodate this diversity. Nevertheless, the SRP must also remain highly specific to its substrates to minimize the mislocalization of proteins, which would be detrimental to cells. How the SRP or any protein-targeting machinery faithfully selects its correct substrates has been a challenging question. Although previous work has focused on the observation that the SRP binds weakly to the incorrect cargos bearing no or weak signal sequences (**Figure 3*b***, red arrow a), quantitative biophysical measurements show that the SRP binds with substantial affinity to the incorrect cargos or even the empty ribosome (K_d ~ 80–100 nM; 38–40). Given the cellular SRP concentration (~400 nM in bacteria), it appears unlikely that the discrimination in the cargo-binding step is sufficient to reject all the incorrect cargos.

A quantitative dissection of the individual molecular events in the bacterial SRP pathway (**Figure 3*b***) demonstrates that the multiple conformational rearrangements in the SRP·FtsY GTPase complex provide a series of additional checkpoints to further reject the incorrect cargos (40). These include: (*a*) formation of the early intermediate, which is stabilized more than 100-fold by the correct, but not incorrect, cargos (**Figure 3*b***, red arrow b); (*b*) rearrangement of the early intermediate to the closed complex, which is ~10-fold faster with the correct rather than the incorrect cargos (**Figure 3*b***, red arrow c); and (*c*) GTP hydrolysis by the SRP·FtsY complex, which is delayed ~8-fold by the correct cargo to give the targeting complex a sufficient time window to identify the membrane translocon. In contrast, GTP hydrolysis remains rapid with the incorrect cargo ($t_{1/2}$ < 1 s), which could abort the targeting of incorrect cargos (**Figure 3*b***, red arrow d). A mathematical simulation based on the kinetic and thermodynamic parameters of each step strongly suggests that all these fidelity checkpoints are required to reproduce the experimentally observed pattern of substrate selection by the SRP (40).

These results support a novel model in which the fidelity of protein targeting by the SRP is achieved through the cumulative effect of multiple checkpoints by a combination of mechanisms, including cargo binding, induced SRP-SR assembly, and kinetic proofreading through GTP hydrolysis. Additional discrimination could come from the SecYEG machinery, which further rejects the incorrect cargos (102). Analogous principles have been demonstrated in the DNA and RNA polymerases (103, 104), the spliceosome (105), tRNA synthetases (106), and tRNA selection by the ribosome (107) and may represent a general principle for complex biological pathways that need to distinguish between correct and incorrect substrates based on minor differences.

SRP RNA: A Central Regulator of the SRP

Besides the SRP54 (or Ffh) protein, the SRP RNA is the only other universally conserved

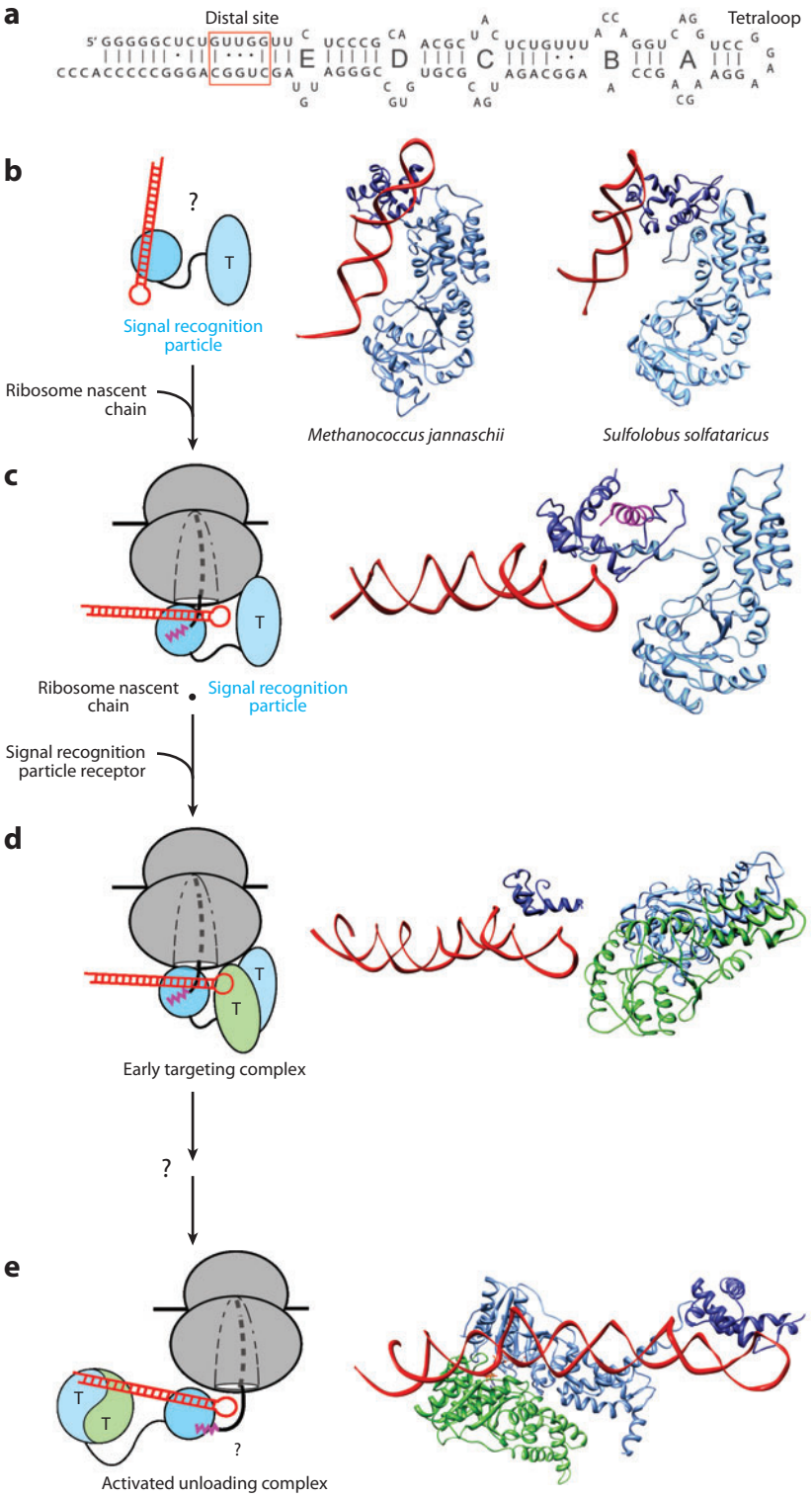
and essential component of the SRP (108). However, its precise roles in protein targeting have remained enigmatic. In early biochemical reconstitutions of the mammalian SRP, the SRP RNA appeared to be nothing more than a scaffold that holds different SRP protein subunits together (**Figure 5**). The discovery of the bacterial SRP RNA (109), which binds a single protein Ffh, implied a much more active role for this RNA. Recent biochemical and structural studies strongly support this view and show that the SRP RNA can mediate global reorganization of the SRP in response to cargo binding and provide additional interactions with the SR, thus mediating the molecular communication between the cargo and the SRP/SR GTPases during protein targeting.

The bacterial 4.5S SRP RNA contains the most conserved domain IV of the SRP RNA and forms an elongated hairpin structure capped by a highly conserved GGAA tetraloop at one end (**Figure 4*a***). Two internal loops, A and B, mediate binding of this RNA to a helix-turn-helix motif in the M domain of Ffh with picomolar affinity (14, 110). In contrast, the orientation of the M domain/RNA complex relative to the Ffh NG domain exhibits a high degree of variability. Crystallographic analyses and structural mapping studies have generated at least four different structures or structural models of the SRP, each exhibiting a distinct interdomain arrangement (see **Figure 4*b*** for two examples; 16, 33, 34, 111–113). Collectively, these observations suggest that apo-SRP could exist in a variety of global conformations, likely due to the 30 amino acid–long flexible linker connecting the M and NG domains of Ffh.

Upon binding the RNC, the SRP undergoes a global conformational change (**Figure 4*c***; 44, 45, 114). The bidentate interaction of the RNC with Ffh reorients its M and NG domains such that the SRP RNA now lies parallel to the ribosome surface with its GGAA tetraloop positioned adjacent to the FtsY-interacting surface on the Ffh NG domain (**Figure 4*c***). This is important, as the RNA tetraloop is required for rapid assembly of a stable SRP•FtsY complex (83, 85, 94, 115, 116). More recent kinetic and phylogenetic analyses (117), hydroxyl radical footprinting experiments (118), and cryo-EM analysis (86) identified a key electrostatic interaction between the SRP RNA tetraloop and conserved basic residues surrounding Lys399 on the lateral surface of FtsY (**Figure 4*d***). This interaction stabilizes the otherwise highly labile early intermediate, thus accelerating stable SRP•FtsY assembly 10^2–10^3-fold (85, 117). Importantly, the RNA tetraloop or FtsY Lys399 exerts these stimulatory effects only when the SRP is bound to RNCs bearing strong signal sequences (117, 119) and, to a lesser extent, to a signal peptide

Figure 4

RNA-mediated global reorganization of the signal recognition particle (SRP) couples the GTPase cycle to the cargo loading and unloading events during protein targeting. (*a*) Secondary structure of the *Escherichia coli* 4.5S SRP RNA. The internal loops A–E, the GGAA tetraloop, and the distal site near the 5′, 3′ end of this RNA are denoted. (*b*) The free SRP exists in a variety of latent conformations in which the SRP RNA tetraloop is not positioned to contact the SRP receptor (SR). Two representative structures of the SRP from *Methanococcus jannaschii* [*left*; Protein Data Bank (PDB) 2V3C] and *Sulfolobus solfataricus* (*right*; PDB 1QZW) are shown. (*c*) Binding of the ribosome•nascent chain complex (RNC) induces the SRP into a more active conformation, in which the SRP RNA tetraloop is properly positioned to interact with the G domain of the incoming SR to form a stabilized early targeting complex, as shown in panel *d*. Both panels show the molecular model derived from cryoelectron microscopy reconstructions of the RNC•SRP or RNC•SRP•FtsY early complex; the ribosome is not shown for clarity. (*e*) GTPase activation is potentially coupled to relocalization of the SRP•SR NG-domain complex to the distal end of the SRP RNA, a conformation that is more conducive to cargo unloading (PDB 2XXA). The structures in panels *b* and *c* are aligned with respect to the SRP54 NG domain, and the structures in panels *c*–*e* are aligned with respect to the SRP RNA. Color codings are the same as in **Figure 2**.



or signal peptide mimics (36). Combined with structural analyses (32, 45, 86, 99), a coherent model emerges in which the RNC optimizes the conformation of the SRP so that the SRP RNA tetraloop is prepositioned to interact with the incoming FtsY, thus allowing rapid recruitment of the SR to be achieved specifically for the correct cargos (**Figure 4*b–d***).

Intriguingly, neither the SRP RNA tetraloop nor FtsY Lys399 affects the equilibrium stability of the SRP·FtsY complex in the closed/activated states (94, 117), suggesting that their interaction is highly transient and occurs only during the early intermediate stage of GTPase dimer assembly. A recent crystallographic study using full-length 4.5S RNA (120) revealed a completely different configuration of the SRP·FtsY complex in which a closed/activated GTPase complex docks at a distinct site near the 5′,3′ end of the SRP RNA ~100 Å away from the tetraloop end (**Figure 4*a***, distal site, and **Figure 4*e***). Mutations of the distal site compromised GTPase activation in the SRP·FtsY complex, supporting the importance of this alternative RNA-GTPase interaction (120). Recently, single-molecule fluorescence microscopy experiments directly demonstrated that the Ffh·FtsY NG-domain complex, after initial assembly near the RNA tetraloop, relocalizes to the opposite end of the SRP RNA, where its GTPase activity is fully activated (121). In the context of the protein-targeting reaction, this movement is highly attractive as it removes the GTPase complex from the ribosome exit site, generating a conformation that allows the RNC to be more easily released from the targeting complex and the SecYEG complex to more readily access the ribosome exit site (**Figure 4*e***). In addition, the unloading of cargo could be tightly coupled to GTPase activation in such a mechanism. Though still at an early stage, these models are supported by the observation that the movement of the GTPase complex to the SRP RNA distal site is directly activated by the SecYEG translocation machinery (120a).

Transition from the Targeting to Translocation Machinery

At the end of the protein-targeting reaction, the RNC must be unloaded from the SRP·FtsY complex onto the heterotrimeric SecYEG (or Sec61p) translocation machinery. We refer the readers to References 2, 121–123 for more comprehensive reviews of this machinery. In the context of the cotranslational targeting reaction, studies in recent years have provided rich structural information to explain how the translocon interacts with the RNC and potentially interfaces with the SRP-targeting machinery. A crystal structure of *Methanococcus jannaschii* SecYEβ (124) showed that TMs 1–10 of SecY form an hourglass-shaped pore in this channel. Lining one side of this pore are TMs 2b and 7, which form the lateral gate where hydrophobic signal sequences and TMs in the nascent polypeptide bind and subsequently enter the lipid bilayer (125–127). Cryo-EM reconstructions of the RNC·SecYEG complex (or its eukaryotic homologs) at increasing resolution (128–131), combined with biochemical and genetic studies (132, 133), further identified the highly conserved basic residues in the C4 and C5 (or L6/7 and L8/9) loops of SecY as the key motifs that mediate interaction with ribosomal proteins L23 and L35 at the exit site.

Remarkably, the binding sites of the SecYEG/Sec61p complex on the translating ribosome overlap extensively with those of the SRP (**Figure 2*a***). This raises challenging questions as to how the RNC is handed over from the targeting to translocation machinery without nonproductive loss of the translating ribosome. The most productive mechanism for the cargo transfer event is probably through a concerted pathway in which the two contacts of the SRP with the RNC, those with the L23/L35 ribosomal proteins and with the signal sequence, are sequentially dissolved and replaced by those of the SecYEG machinery. Several observations described earlier, including the loss of density for the Ffh-FtsY NG-domain complex in cryo-EM reconstructions of the targeting complex (99), the ability of the NG-domain complex

to relocalize to the SRP RNA distal end (120, 120a), and the requirement of GTPase rearrangements for detachment of SRP from the ribosome (95, 98) provide clues that support such a mechanism. The ability of the SR to directly interact with the SecYEG/Sec61p complex (64, 65, 69–71) further raises the possibility that the translocon plays an active role in the cargo handover process. Nevertheless, the cargo handover event remains the least understood aspect of the cotranslational targeting reaction. The fate of the signal sequence in this cargo handover event, their timing relative to one another and to the hydrolysis of GTP, and the molecular forces that drive this step remain challenging questions for future investigations.

EUKARYOTIC SRP

Mammalian SRP: Additional Layers of Complexity

Compared with its bacterial homolog, the mammalian SRP is significantly larger and more complex, comprising six proteins and a 7S SRP RNA (**Figure 5**). It can be divided into two distinct domains: the S domain, comprising domains II–IV of the 7S RNA and the SRP 19, 54, and 68/72 protein subunits, and the Alu domain, comprising domain I of the 7S RNA and the SRP 9/14 subunits (**Figure 5**). The increased complexity adds additional layers of nuance and regulation for the mammalian SRP, many of which await to be elucidated.

For example, the mammalian SRP54 subunit binds the 7S RNA weakly by itself. Indeed, premature binding of SRP54 could cause the two RNA-binding loops for SRP19 to misfold, disrupting the native assembly of the SRP (134, 135). In vivo, assembly of the mammalian SRP goes through an ordered pathway in which all the SRP proteins except SRP54 are imported to the nucleus to bind the SRP RNA; the partially assembled SRP is then exported to the cytoplasm for SRP54 binding, thus completing its assembly (109, 136–138; see Reference 139 for a more complete review of SRP assembly). In vitro reconstitutions showed that prebinding of SRP19 to the 7S RNA is required for loading the SRP54 subunit onto the SRP RNA (8, 140). Crystallographic analyses showed that SRP19 bridges the two tetraloops in both domains III and IV (or helices 6 and 8) of the 7S RNA and preorganizes the internal loops in domain IV into a conformation required for stable SRP54 binding (141–146; see Reference 147 for a more complete review). Why the mammalian SRP requires this additional layer of allostery during its assembly remains unclear.

In addition, although much is known about the binding sites of SRP68/72 on the 7S RNA (148–153), the structure and precise function of the SRP68/72 subunits remain to be determined. Chemical-probing experiments have suggested that SRP68/72 cooperates with SRP19 to preorganize the 7S RNA into a conformation competent for SRP54 binding by exposing the SRP54 binding sites on the 7S RNA (144, 154). These subunits have also been implicated in controlling the interaction of SRP54 with the SR (155). Direct evidence for both of these models remains to be obtained.

The most interesting aspect of the mammalian SRP, aside from the core functions, is the Alu domain (**Figure 5**) that arrests translation elongation just after the signal sequence emerges from the ribosome. Early biochemical work found that the SRP interacts with the ribosome during elongation factor 2–catalyzed translocation of tRNA (156), suggesting that the SRP competes with elongation factors for binding. Recent biochemical and cross-linking studies further show that SRP9/14 electrostatically interacts with ribosomal RNA via at least two stretches of basic residues and also contacts ribosomal proteins at the interface between the large and small ribosomal subunits (157, 158). Consistent with this notion, cryo-EM analysis showed that mammalian SRP forms an elongated, kinked structure in which the Alu domain reaches into the elongation factor binding site at the ribosome subunit interface (**Figure 5*b***; 43). Although the elongation arrest activity is not a prerequisite for protein targeting in vitro, deletion of SRP9/14 in vivo results in severe defects in protein targeting and

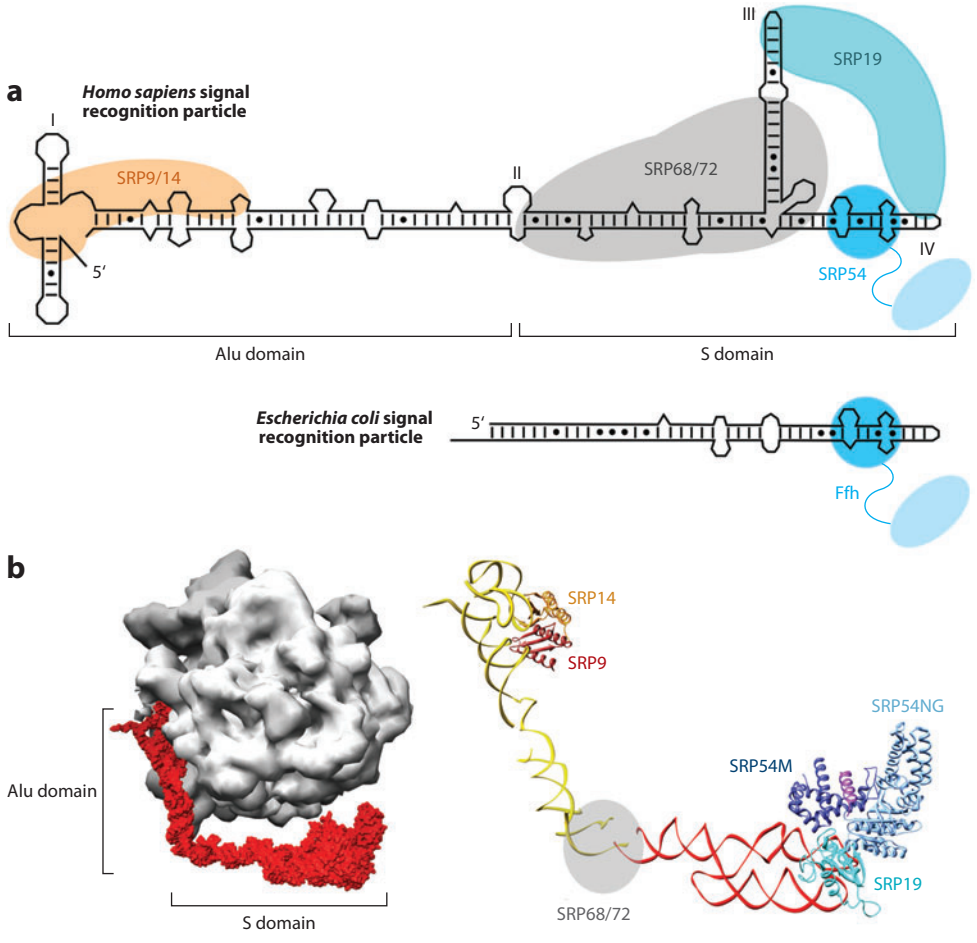


Figure 5

Organization of the mammalian signal recognition particle (SRP). (*a*) Comparison of the RNA secondary structure and composition of the mammalian and bacterial SRP. (*b*) Cryoelectron microscopy (cryo-EM) reconstruction of the mammalian SRP bound to the ribosome•nascent chain complex (*left*; EMD-1063) and molecular model of the mammalian SRP derived from the cryo-EM and docking of the crystal structures of the individual proteins (*right*; Protein Data Bank 1RY1). The SRP54 M and NG domains are in dark and light blue, respectively, SRP19 is in cyan, SRP9 is in brown, SRP14 is in orange, and the SRP68/72 complex, which lacks a crystal structure, is represented as a gray sphere. The S and Alu domains of the SRP RNA are in red and yellow, respectively.

mammalian cell growth (159). Together with the observation that the SRP could not target proteins when the nascent polypeptide exceeds a critical length (39, 160), these results suggest that elongation arrest provides a crucial time window that allows the targeting complex to engage the translocon before the nascent chain loses translocation competence. The precise mechanism and degree of elongation arrest by the Alu domain and how it communicates and/or cooperates with the S domain during the targeting reaction remain to be elucidated.

Chloroplast SRP: A Unique Posttranslational SRP

The cotranslational nature of the SRP pathway is universally conserved except for in the chloroplast in green plants, in which a unique

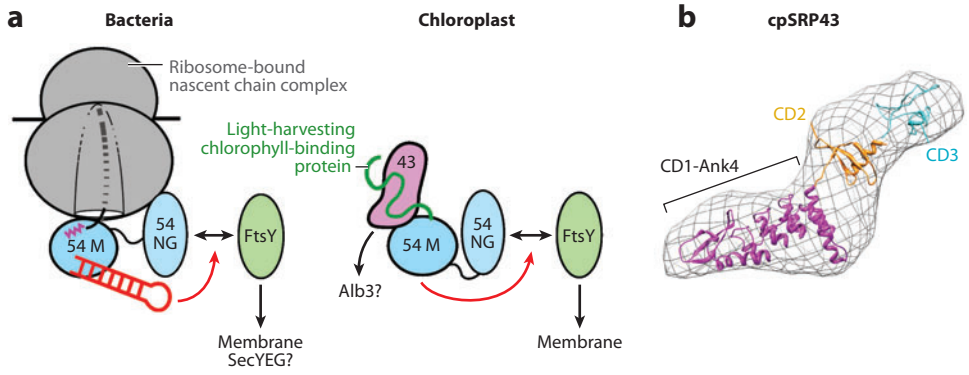


Figure 6

(*a*) Similarity and differences between the bacterial (*left*) and chloroplast (*right*) signal recognition particle (SRP) systems. The SRP54 M and NG domains, FtsY, and the SRP RNA are colored as in **Figure 2**. The light-harvesting chlorophyll-binding protein (LHCP) is in green, and cpSRP43 is in magenta. The red arrows denote the stimulatory effects of the SRP RNA (*left*) and the cpSRP54 M domain (*right*) on assembly of the GTPase complex. (*b*) A molecular model of cpSRP43, obtained from small angle X-ray reconstructions of its 3D shape (envelope; 183) and rigid-body docking of the structures of the chromodomain (CD) 1-ankyrin (1–4)-CD2 [Protein Data Bank (PDB) 3UI2] and CD3 (PDB 1X3P) fragments.

posttranslational SRP pathway has evolved. Instead of delivering RNCs as its cargo, the chloroplast SRP (cpSRP) is dedicated to the delivery of the light-harvesting chlorophyll *a*,*b*–binding proteins (LHCPs) from the chloroplast stroma to the thylakoid membrane (**Figure 6**) (161, 162). Analogous to the cytosolic SRP, the cpSRP pathway is mediated by close homologs of the SRP54 and SR GTPases, cpSRP54 and cpFtsY, respectively (**Figure 6**) (162–165). However, the cpSRP54 M domain lost the ability to bind the otherwise universally conserved SRP RNA (166). Instead, a unique SRP subunit in chloroplasts, cpSRP43, binds a C-terminal extension in the cpSRP54 M domain to form the cpSRP (**Figure 6**) (167, 168). As detailed below, these changes likely reflect adaptation of the cpSRP system to the posttranslational targeting of LHCPs. In addition, another pool of cpSRP43-free cpSRP54 was found in stroma, which together with cpFtsY mediates the cotranslational targeting of some of the chloroplast-encoded membrane proteins, such as D1 (169). We refer the readers to References 170–172 for comprehensive reviews of the cpSRP. Here, we focus on valuable lessons that came from comparison of the cpSRP with the classic cytosolic SRP in recent years.

How does the cpSRP bypass the otherwise strictly conserved SRP RNA? In cytosolic systems, a major function of the SRP RNA is to accelerate the interaction between the SRP and FtsY GTPases and thus ensure the rapid delivery of cargo. Kinetic analysis in the cpSRP system showed that, even in the absence of the SRP RNA, the cpSRP and cpFtsY GTPases interact 400-fold faster than their bacterial homologs (173). Subsequent crystallographic (174, 175) and biochemical cross-complementation (176) analyses revealed two key molecular mechanisms underlying this phenomenon: (*a*) Compared with bacterial FtsY, the conformation of the cpFtsY NG domain more closely resembles that in the closed SRP•FtsY complex, which may allow cpFtsY to bypass some of the rearrangements required for stable GTPase assembly (174, 175), and (*b*) more importantly, the cpSRP54 M domain functionally mimics the SRP RNA, accelerating its interaction with cpFtsY 100-fold and allowing them to achieve an interaction rate that matches the RNA-catalyzed interaction between their bacterial homologs (**Figure 6**, red arrows) (176). It is probable that, analogous to the cytosolic SRP system, the interaction between the cpSRP and cpFtsY GTPases is

Light-harvesting chlorophyll *a*,*b*–binding proteins (LHCPs): proteins that form the antenna complex on photosynthetic centers in green plants

Posttranslational targeting: a mode of protein targeting in which a fully synthesized nascent protein is delivered after release from the ribosome

Chromodomain: chromatin organization modifier domain, a highly conserved protein domain in eukaryotes often involved in chromatin remodeling

Ankyrin repeat: a 33-residue protein motif that folds cooperatively with neighboring repeats and is one of the most common protein interaction motifs

AAA+ disaggregases: a family of ATPases associated with diverse cellular activities that mediate ATP-dependent remodeling of protein aggregates

regulated by upstream and downstream components of the pathway, such as the substrate protein or the target membrane (177); these allosteric regulations and their roles in the cpSRP pathway remain to be uncovered.

The cpSRP43 subunit is responsible for substrate recognition and enables the cpSRP to adapt to the challenge of posttranslational protein targeting. Unlike in the cotranslational pathway, cpSRP must handle fully synthesized, highly hydrophobic LHCPs that are prone to aggregation and misfolding in aqueous environments. Early work found that LHCPs are effectively chaperoned in the stroma, where they form a soluble transit complex with the cpSRP (162, 164, 178–180), although substrate capture by the cpSRP may require additional factors, such as LTD at the chloroplast envelope (181). Recent biochemical dissections showed that cpSRP43 is necessary and sufficient for binding with high affinity to LHCPs and maintaining them in a soluble, translocation-competent state (182, 183). cpSRP43 is composed of a unique combination of protein-interaction motifs, with three chromodomains (CDs) (184, 185) and four ankyrin repeats (Ank1–4) sandwiched between CD1 and CD2 (**Figure 6*b***) (172, 186). The ankyrin repeat domain specifically recognizes L18, a relatively polar 18-amino acid motif between TM2 and TM3 of LHCP (180, 187, 188). Crystallographic analyses further showed that the CD1-Ank4 fragment of cpSRP43 folds into an elongated horseshoe structure (**Figure 6*b***), in which a groove across the concave surface of Ank2 to 4 binds a highly conserved DPLG turn in the L18 peptide (189), enabling specific recognition of LHCPs by cpSRP43. As a molecular chaperone, cpSRP43 likely also interacts with and shields the hydrophobic TMs in LHCPs, although the molecular basis of these interactions remains to be deciphered. Finally, recent work found that even after LHCPs had already aggregated, cpSRP43 can resolubilize the aggregate and return them to soluble fractions in vitro (182, 183). This disaggregase activity was unexpected, as cpSRP43 lacks ATPase domains and hence must use a mechanism distinct from that of the well-studied AAA+ disaggregases (190). This finding demonstrated the capability and diversity of chaperone function during posttranslational membrane protein targeting. The molecular basis underlying cpSRP43's disaggregase activity and its precise roles in LHCP biogenesis in vivo remain to be determined.

At the thylakoid membrane, the cpSRP and cpFtsY deliver LHCPs to the Alb3 translocase (see more discussion of this translocase in SRP-Dependent Targeting to Other Translocons, below). Recently, biochemical studies (191, 192) and in vivo complementation analyses (193, 194) showed a direct interaction between cpSRP43 and the C-terminal stromal domain of Alb3. The molecular mechanism underlying this interaction and its precise roles in the targeting and integration of LHCP remain unclear. Nevertheless, this interaction is highly attractive, as it provides a mechanism to accurately localize the targeting complex to the Alb3 translocase and to couple the membrane delivery of LHCP to its subsequent integration. Lessons learned from this system could be leveraged to help understand the mechanism of cargo unloading in the cytosolic SRP pathway.

NEW FRONTIERS

Molecular Code of the Signal Sequence

Early pioneering work has identified a hydrophobic core as the major determinant of signal sequences that mediate protein secretion, facilitated by basic amino acids at the N terminus in some cases (27, 28). The propensity to adopt α-helical structures in apolar media has also been identified as an important determinant of the signal sequence (195, 196). However, subsequent work revealed additional layers of complexity. First, multiple pathways mediate protein secretion in bacteria and yeast, and signal sequences also specify the targeting pathway (**Figure 1*a***) (101). Second, although a threshold level of hydrophobicity

in signal sequences was generally thought to specify the SRP pathway, more recent studies in bacteria (197) and yeast (198) indicated that the correlation between hydrophobicity and SRP-dependent targeting is poor, and signal sequences with hydrophobicity above the apparent threshold failed to engage the SRP (197). Third, special N-terminal extensions of a strong SRP signal sequence, such as those found in the bacterial autotransporter EspP, can allow nascent proteins to escape the SRP pathway (40, 199). Apparently, additional molecular features of the signal sequence, including helical propensity (195, 196), the presence of N-terminal basic residues (28, 200), and other properties, play important roles that have yet to be identified. How the information from all the different features is integrated to compose the molecular code that specifies the SRP remains unclear. Crucial to the effort to decode the signal sequence will be the availability of a more comprehensive catalog of validated SRP-dependent versus SRP-independent substrates, which would allow more systematic analyses of the molecular features of signal sequences and evaluation of their respective contributions to recognition by the SRP.

The Crowded Ribosome Exit Site

Accumulating data now indicate that the ribosome exit site is a crowded environment where multiple protein biogenesis factors interact. As a newly synthesized protein emerges from the ribosomal exit tunnel, it interacts with a host of cellular factors that facilitate its folding, localization, maturation, and quality control. These include molecular chaperones such as trigger factor (TF) in bacteria, Hsp70 (DnaK/J in bacteria), and the nascent chain–associated complex (NAC) in yeast; modification and processing enzymes such as methionine aminopeptidase (or peptide deformylase in bacteria), N-acetyltransferase, and arginyl transferase; and protein-targeting and translocation machineries such as the SRP and SecYEG (1, 201, 202). Even posttranslational targeting factors, such as SecA (203) and the Bag6 complex (204), were recently reported to interact with the RNC. Many of these factors, including the SRP, SecYEG, TF, and SecA, contact the ribosome via the same protein, L23 (or Rpl25 in eukaryotes) (205), and recognize hydrophobic sequences on the nascent polypeptide. It is currently unclear whether and how these factors compete or cooperate with one another for binding the translating ribosome (198, 206–211). Further, the molecular mechanisms by which a nascent protein is sorted among different cotranslational factors and committed to the correct biogenesis pathway remain key questions for future investigations.

Nascent chain–associated complex (NAC): a heterodimeric complex that binds eukaryotic ribosomes in close proximity to the emerging nascent protein

Signaling from Inside the Ribosome

Most previous models assumed that binding of the SRP or other cellular machineries to the RNC occurs when signal sequences become exposed outside the ribosome. This view was initially challenged by the observation that the opening and closing of the Sec61p translocon are regulated by TMs in the nascent protein from inside the ribosome (212). More recently, multiple biochemical and cross-linking studies showed that, even when a signal sequence is still within the ribosome and has not emerged outside the exit tunnel, its presence enhances the binding of SRP to the RNC (38, 213) and helps recruit a regulatory protein, RAMP4, to the Sec61p translocon (214). Further, in the GET pathway, the Bag6 complex is specifically recruited to the RNC when the C-terminal TM of the nascent protein emerges inside the ribosome (204). These results suggest that sequence or structural features of the nascent polypeptide inside the ribosome provide signals that can be transmitted to the ribosome and lead to the recruitment of cellular factors. The nature of the structural changes in the ribosome that underlie these signaling events, the mechanisms ensuring the specificity of these signals, and their precise roles in the respective cellular pathway are important questions for future studies.

SRP-Dependent Targeting to Other Translocons

Although SecYEG (or Sec61p) is a central protein-conducting channel where many co- and posttranslational pathways converge, membrane insertion of a subset of proteins requires the translocase YidC, a member of the YidC/Oxa1/Alb3 family of proteins that facilitate the insertion and assembly of membrane proteins (see References 215–217 for more comprehensive reviews). Although some of YidC's functions are carried out through cooperation with the SecYEG machinery (218), increasing evidence shows that YidC can act independently of SecYEG to mediate the insertion of several proteins, including phage-procoat proteins (219, 220), the mechanosensitive channel MscL (221, 222), and subunit c of the F_1F_0 ATP synthase (223, 224). In many studies, the targeting of MscL and the F_1F_0 subunits to YidC appears to depend on the cotranslational SRP/FtsY machinery (225–227). As noted earlier, the cpSRP targets LHCPs to Alb3, the YidC homolog in chloroplasts. The structure (228, 229) and mechanism of YidC as an independent membrane protein insertase, how it interacts with the ribosome and the nascent polypeptide, and how it interfaces with the SRP-targeting machinery remain to be determined. The decision-making process that allows the SRP to route a subset of its substrate proteins to the YidC instead of the SecYEG translocon also needs elucidation and will likely reveal additional layers of nuance and regulation in this pathway.

Translation-Independent Targeting of Membrane Proteins

Although targeted delivery of membrane proteins based on signals embedded in the nascent polypeptide has been long established, a recent study provided evidence for an alternative pathway(s) that localizes proteins to the target membrane in a translation-independent manner based on *cis*-acting elements in the TM domain–encoding sequences of the mRNA (230). Codons for hydrophobic amino acids in the TM domains were hypothesized to be highly enriched in uracil content, which could provide a distinctive signature for these mRNAs to enable their recognition and targeted delivery to the membrane (231). The components, pathways, and mechanisms of translation-independent targeting of membrane proteins and the contribution of these pathways to proper membrane protein localization within cells remain open questions.

SUMMARY POINTS

1. The SRP and SR catalyze the cotranslational delivery of membrane and secretory proteins to translocation machineries on the target membrane.
2. Signal sequences allow nascent proteins to engage the correct cellular biogenesis machinery and thus be directed to their proper cellular destination.
3. The SRP recognizes its cargos through bidentate interactions with the signal sequence and the ribosome. Likewise, the SR localizes to the target membrane through bidentate interactions with the phospholipid membrane and the SecYEG/Sec61p translocon.
4. Two homologous GTPases in the SRP and SR use a unique GTPase cycle to drive and regulate the capture, delivery, and unloading of cargo during protein targeting. They represent a growing class of dimerization-activated GTPases.
5. The fidelity of substrate selection by the SRP is achieved through a combination of binding, induced fit, and kinetic proofreading mechanisms.

6. The SRP RNA orchestrates global reorganization of the SRP, which enables rapid SRP-SR GTPase assembly in response to cargo binding.
7. The eukaryotic SRP contains an additional Alu domain that arrests translation elongation, which may provide a longer time window for the SRP to complete the targeting reaction in larger eukaryotic cells.
8. The chloroplast SRP is dedicated to the delivery of fully synthesized LHCPs and has evolved unique molecular strategies to meet the challenges of posttranslational membrane protein targeting.

FUTURE ISSUES

1. How is the translating ribosome productively handed over from the targeting to the translocation machinery?
2. What are the molecular codes that comprise the signal sequence?
3. How are nascent proteins sorted among the myriad of protein biogenesis factors at the ribosome exit site and committed to the correct biogenesis pathway?
4. Does a nascent polypeptide inside the ribosome tunnel signal the ribosome to recruit specific factors, and if so, how?
5. How does the SRP route a subset of its substrates to the YidC or other membrane translocases instead of to SecY/Sec61p?
6. Does information embedded in the mRNA direct proteins to the membrane, and if so, how?

DISCLOSURE STATEMENT

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NOTE ADDED IN PROOF

Although it has long been recognized that the cpSRP in higher green plants has lost the SRP RNA, a recent study by Trager et al. (232) identified multiple species in green and blue algae, as well as in lower green plants, in which the SRP RNA is still an integral part of the cpSRP. The cpSRP from these species likely represents evolutionary intermediates via which the ancient cotranslational SRP evolved to the RNA-less cpSRP for posttranslational targeting of proteins.

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