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Targeting proteins to membranes: structure of the signal recognition particle

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In all three kingdoms of life, co-translational targeting of secretory and membrane proteins to the prokaryotic plasma membrane or eukaryotic endoplasmic reticulum is mediated by a ribonucleoprotein complex, the signal recognition particle (SRP), and its membrane-associated receptor (SR). SRP binds to signal sequences of nascent proteins as they emerge from the exit tunnel of the ribosome. The resulting targeting complex, composed of the SRP and the ribosome–nascent chain complex (RNC), then docks with the SR in a GTP-dependent manner. Passing through a complex series of conformational states, SRP and SR deliver the RNC to the translocon, which in turn mediates protein translocation across or integration into the membrane. The core structural and mechanistic principles of SRP-dependent protein targeting are universally conserved. Recent structural investigations combining X-ray crystallography and cryo-electron microscopy have provided new insights into three essentials steps of the SRP-dependent protein targeting cycle: the assembly and interaction of the SRP ribonucleoprotein core, the GTP-dependent SRP–SR association, and the interaction between SRP and the ribosome.

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Introduction: the SRP-mediated targeting cycle

Evolutionarily related signal recognition particles (SRPs) and their cognate membrane-associated receptors (SRs) mediate the co-translational targeting of membrane and secretory proteins in all cells [1,2]. Signal sequences specify unidirectional protein translocation across or integration into membranes. A typical signal sequence consists of a stretch of 9–12 large hydrophobic residues that is either a transient or permanent part of the protein to be translocated [3,4]. These sequences are likely to adopt α-helical conformations when bound or in transit. If sufficiently hydrophobic but not too long, signal sequences can be recognized by the SRP as they emerge from ribosomes. Upon signal sequence recognition, SRP promotes a pause in translational elongation in eukaryotes, presumably to keep the nascent chain as short as possible before insertion into the translocation pore.

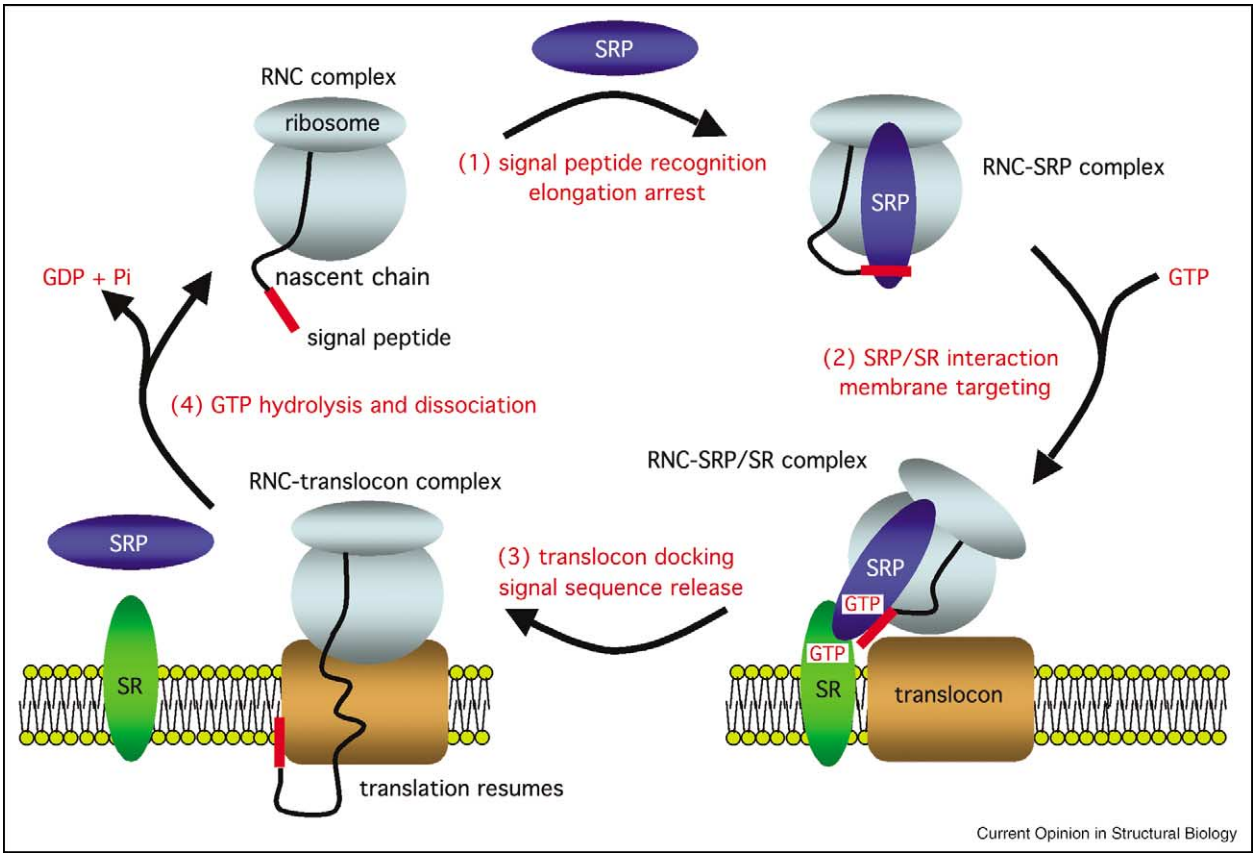
Both SRP and SR contain GTPase domains. Their signal-bound, GTP-dependent association into a tight complex is crucial for targeting the ribosome–nascent chain complex (RNC) to the protein translocation apparatus at the membrane. SRP–SR complex formation leads to reciprocal activation of GTP hydrolysis by both GTPases, which, only in complex, form a unique 'dual active site' in which the O3′ of one GTP becomes a key component of the active site of the other GTPase. This reciprocity is thought be coupled to and thus to govern docking, release of the RNC to the translocon and SRP recycling (Figure 1). Thus, SRP and SR act as molecular matchmakers that deliver RNCs that synthesize selected subsets of proteins to the translocon. Recent structures and biochemical analysis provide new insights into the mechanism of SRP-mediated protein targeting [5,6].

This review gives an overview of the structural organization of the SRP throughout the diverse phyla, showing their evolutionary conservation, and focuses on the dynamic nature of the SRP-mediated protein targeting cycle. A recent cryo-EM structure of the eukaryotic SRP bound to the ribosome reveals an integrated picture of the interaction between SRP and the active ribosome. Also, the recent X-ray structure of the universally conserved heterodimeric core of the SRP–SR targeting complex reveals the crucial role of GTP for targeting complex assembly and unidirectionality of the targeting cycle. Combined with a recent structure of the protein translocation pore, these new insights raise fascinating questions about the molecular mechanisms of protein translocation through biological membranes.

Architecture and assembly of SRPs in the different kingdoms of life: eukarya and archaea compared to bacteria

Metazoan SRPs consist of six proteins (SRP54, SRP19, SRP68, SRP72, SRP9, SRP14) and a 300-nucleotide RNA (SRP RNA) [7–9]. SRP can be divided into the Alu and S domains, which define the two major functional units of the SRP particle (Figure 2a). The S domain contains well-characterized SRP19 and SRP54, [10,11•,12••,13••] and

Figure 1



The SRP-mediated co-translational protein targeting cycle. A nascent polypeptide with a signal peptide emerges from the ribosome and is recognized by the SRP, causing elongation arrest (1). The RNC–SRP complex is then targeted to the membrane through GTP-dependent interactions between the SRP and its SR (2). At the membrane, after ribosome docking to the translocon, the signal sequence is released from the SRP (3) and, following GTP hydrolysis, the SRP–SR targeting complex dissociates (4).

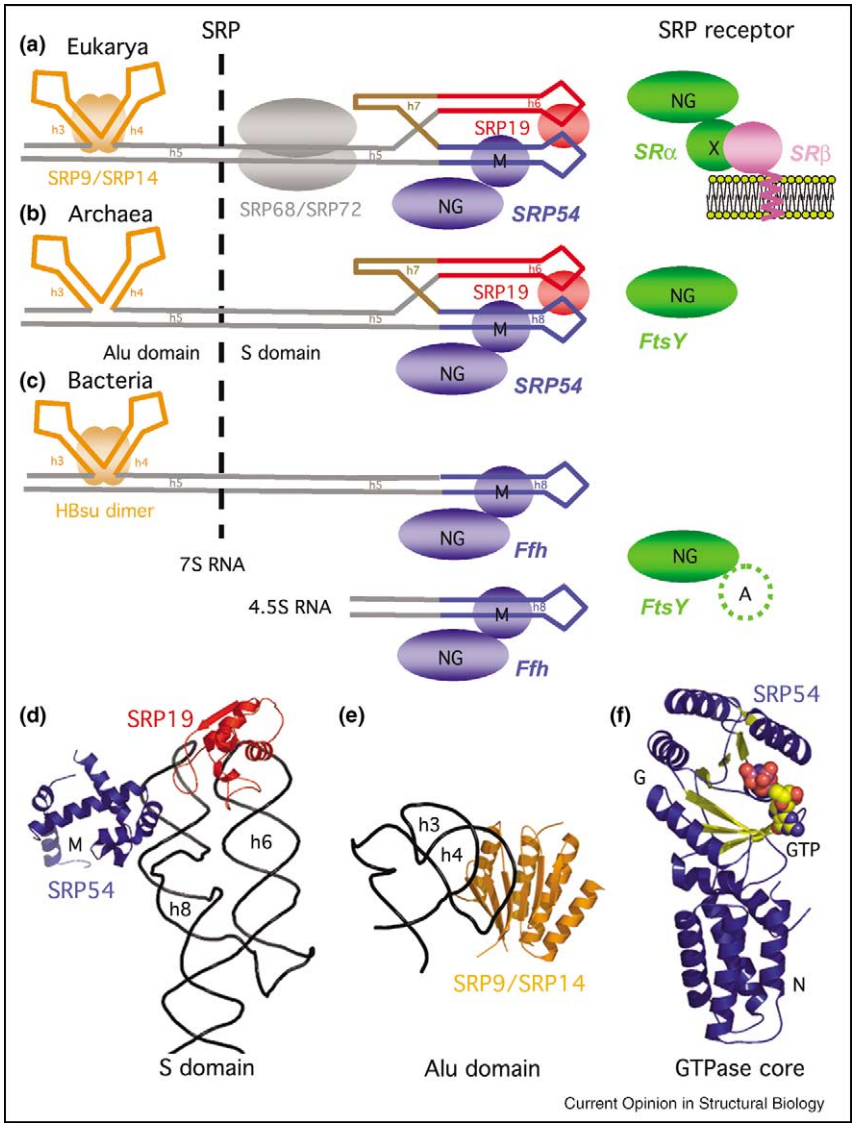
the so far less-characterized heterodimer SRP68/SRP72, and functions in signal sequence recognition and SR interaction. The Alu domain contains the heterodimer SRP9/SRP14 [14,15] and contributes to elongation arrest mediated by eukaryotic SRP (Figure 2e).

Archaeal SRPs contain an SRP RNA of similar size and fold to that in eukaryotes. To date, the structures of only two protein subunits, SRP19 and SRP54, have been described (Figure 2b). Bacterial SRPs are further streamlined, and contain shorter RNAs (e.g. a 110-nucleotide 4.5S RNA in *Escherichia coli*) and a single protein homologous to SRP54 (termed Ffh) (Figure 2c). *In vitro*, bacterial SRP and SR can efficiently replace their mammalian counterparts [16], indicating that these simpler components contain all of the essential features required for co-translational protein targeting. The increased complexity of the archaeal and eukaryotic components therefore most likely provides additional regulatory features.

In eukaryotes, SRP assembly and biogenesis is a hierarchical process [9,17] that involves the initial binding of SRP19 to SRP RNA. The primary role of SRP19 is architectural; binding induces conformational changes in the RNA, which in turn promote SRP54 binding [17–19]. The structure of the S domain shows that SRP19 and SRP54 sit at the tip of a compact RNA core formed by the parallel association of helices 6 and 8 of the SRP RNA (Figure 2d). In bacterial SRPs, in which SRP19 is absent, the folded RNA structure is alternatively stabilized by ions [20,21].

In the S domain, SRP54 is responsible for signal sequence recognition and RNC targeting to the membrane. SRP54 contains three domains, termed the N, G and M domains. The C terminal, methionine-rich M domain contains the signal-sequence-binding site and provides the primary contact to SRP RNA. It is connected to the N and G domains through a flexible linker [22]. The N-terminal N domain (a four-helix bundle) and the G domain (a ras-like GTPase fold) are structurally and functionally coupled (NG domain). This arrangement is universally conserved among all SRP-type GTPases [23], including the SRs, although it is not found in any other GTPase family. SRP-type GTPases

Figure 2



The SRP and its receptor through evolution. Schematic representations of the architecture of the SRPs and SRs from **(a)** eukarya, **(b)** archaea and **(c)** bacteria. The GTPases (Ffh/SRP54 and FtsY/SRα) are indicated in bold. The principal RNA helices present in eukaryotic and archaeal SRP RNA (~300 nucleotides), and in bacterial 4.5S RNA (~115 nucleotides) are labeled h2 through h8. In SRP54/Ffh, the M domain is responsible for signal peptide and RNA recognition. The NG domains of SRP and SR are closely related. Some bacterial FtsY contain an extra N-terminal A domain, which is proposed to be involved in association with the membrane. The eukaryotic SR is composed of two subunits: the regulatory subunit SRβ (containing an N-terminal transmembrane anchor) and SRα. SRα is subdivided into two domains: an N-terminal SRx domain involved in GTP-dependent SRα–SRβ heterodimerization and the NG domain. Some Gram-positive bacteria, such as *Bacillus subtilis*, retain a long SRP RNA with an Alu-like domain to which a dimeric protein (HBsu) is bound, akin to SRP9/SRP14 in eukaryotic SRP. The main structural domains of SRP. **(d)** Structure of the human S domain, with the SRP19 (PDB codes 1L1W, 1L9A and 1LNG) and SRP54M (PDB code 1MFQ) subunits bound, respectively, to helices h6 and h8 of the SRP RNA. **(e)** Structure of the human Alu domain (PDB codes 1914, 1E8O and 1E8S), with the SRP9/SRP14 heterodimer bound to helices h3 and h4 of the SRP RNA. **(f)** Structure of the conserved NG domain of all SRP-type GTPases (PDB codes 1FTS, 1FFH, 1NG1, 2NG1, 3NG1, 1JPJ, 1JPN, 1O87 and 1J8M).

also contain an insertion box domain (IBD) that is unique to this GTPase subfamily (Figure 2f) [24–28].

The SRs are similarly phylogenetically conserved. In bacteria and archaea, SRs are single-subunit proteins, also named FtsY because the *E. coli* SR maps to an Fts operon [29]. Bacterial SRs are peripherally associated with membranes (Figure 2b,c). By contrast, eukaryotic SRs are composed of two subunits: SRα, the homolog of FtsY, and SRβ, a membrane-anchoring subunit that contains yet another GTPase domain of unknown function (Figure 2a).

Interaction with the translation machinery: ribosome and SRP

High-resolution X-ray structures of subcomplexes of eukaryotic, archaeal and bacterial SRPs, low-resolution EM structures of free SRP [30,31] and cryo-EM structures of SRP bound to RNCs [32••] imply quite detailed structural and functional models for how the SRP–ribosome–SR interaction encodes correct targeting (Figure 3a). Comparison of the free and ribosome-bound mammalian SRP structures delineates major conformational changes and structural flexibility in SRP that bring it into its functionally critical state for targeting.

SRP binds to ribosomes whether or not a signal sequence is present; however, its affinity for ribosomes with emerging signal peptide is greater. Thus, SRP is thought to sample many RNCs efficiently for the presence of a signal sequence before identifying its correct 'signal'. Cross-linking localizes several anchoring points for SRP on the ribosome. Cross-links between residues of ribosomal proteins L23 and L35 (positioned in close proximity to the exit site of the protein tunnel through which the nascent chain traverses the large ribosomal subunit) and the N domain of SRP54 imply a zone of protein–protein contacts [33,34], and cross-links between SRP RNA and rRNA suggest SRP RNA–rRNA contacts [35]. Both are functionally important. The binding area for the Alu domain on the ribosome overlaps with the elongation factor binding site at the interface between the two ribosomal subunits [36], thus explaining how SRP modulates translation elongation (Figure 3b).

The structural basis of signal sequence binding and recognition remains mysterious. Several M domain structures have been solved [20,21,37,38] but, in spite of many attempts, no complex between SRP54 and a cognate signal sequence has yet been reported. The structures reveal that the M domain contains a deep groove that is bounded by a finger loop on one side and is lined by flexible sidechains of conserved hydrophobic residues (Figure 3c). The hydrophobic groove with structural plasticity is a conserved feature of M domains and may explain how SRP can bind signal peptides of diverse lengths and sequences. The conformational flexibility of the so-called 'finger loop' of the M domain may also play a role in the transition between the closed (empty) and open (signal-bound) states of the M domain. Signal sequences often contain positively charged residues that may play an as yet unrecognized role in SRP–signal peptide recognition through additional electrostatic interactions with the SRP RNA backbone.

Crystal structures of archaeal *Sulfolobus solfataricus* (*Ssol*) SRP54 in its free state and in complex with helix 8 of SRP RNA [39•] revealed intrinsic flexibility between protein domains, and suggest that the hinge region linking the G and M domains (Figure 3d) is involved in interdomain communication. Hydrophobic contacts between residues of the M and N domains could participate in communicating the occupancy state of the signal-binding pocket to the G domain. Biochemical data suggest that signal peptide binding might also involve the NG domain [40]. Thus, signal peptide binding could regulate the GTPase activity of SRP54 [41]. Analysis of the eukaryotic RNC–SRP and *Ssol* SRP structures further suggests that the flexible linker region plays a central role in a sequence of conformational changes [42]. It has been suggested that transfer of the signal sequence from the ribosome to the M domain results in an increased GTP affinity [43] that renders SRP competent for targeting of the RNC through specific interaction with the SR and subsequent docking of the RNC to the translocon. However, although this model is appealing on teleological grounds, at physiological concentrations, SRP is likely to be already occupied by GTP.

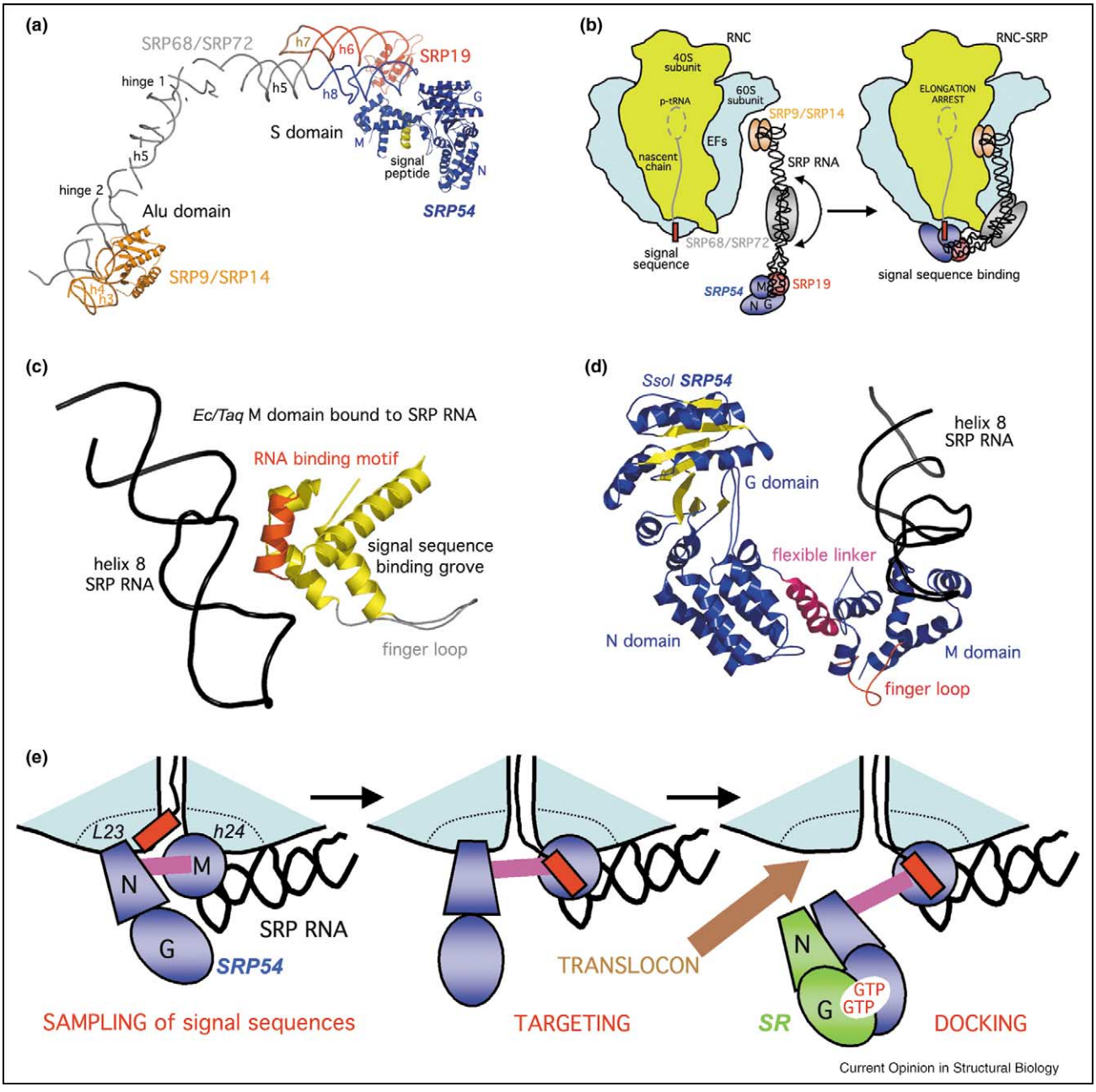
Targeting to the membrane: the GTP catalytic cycle in protein targeting

Despite their similarities with conventional GTPases such as ras, SRP-type GTPases exhibit unique properties, including low affinity for, and rapid exchange of GDP and GTP. There is no known requirement for a guanine nucleotide exchange factor (GEF). Moreover, activation of SRP-type GTPases is triggered after SRP–SR complex formation between the two homologous GTPase domains of SRP54 and SRα [44]. In the SRP–SR complex, each GTPase acts as a GTPase-activating protein (GAP) for the other GTPase [45]; there is no known requirement for an extrinsic GAP.

How does SRP–SR direct correct targeting? Two recent X-ray structures [46••,47••] revealed structural insights into the mechanism of interaction of the GTPases in the SRP–SR complex. Both GTPases undergo conformational changes and associate as a head-to-head, quasi-twofold symmetrical heterodimer [48,49], burying an extensive interaction surface of 3200 $Å^2$. Conserved residues from the N and G domains of both proteins contribute to the interface. A conserved ALLEADV sequence in the N domain and a conserved loop in the IBD define the edges of the heterodimer interface (see Figure 4a,b). The highly cooperative formation of the complex aligns the two GTP molecules in a shared composite active site, in which each GTP is contacted exclusively by sidechains contributed by the same GTPase to which it is bound.

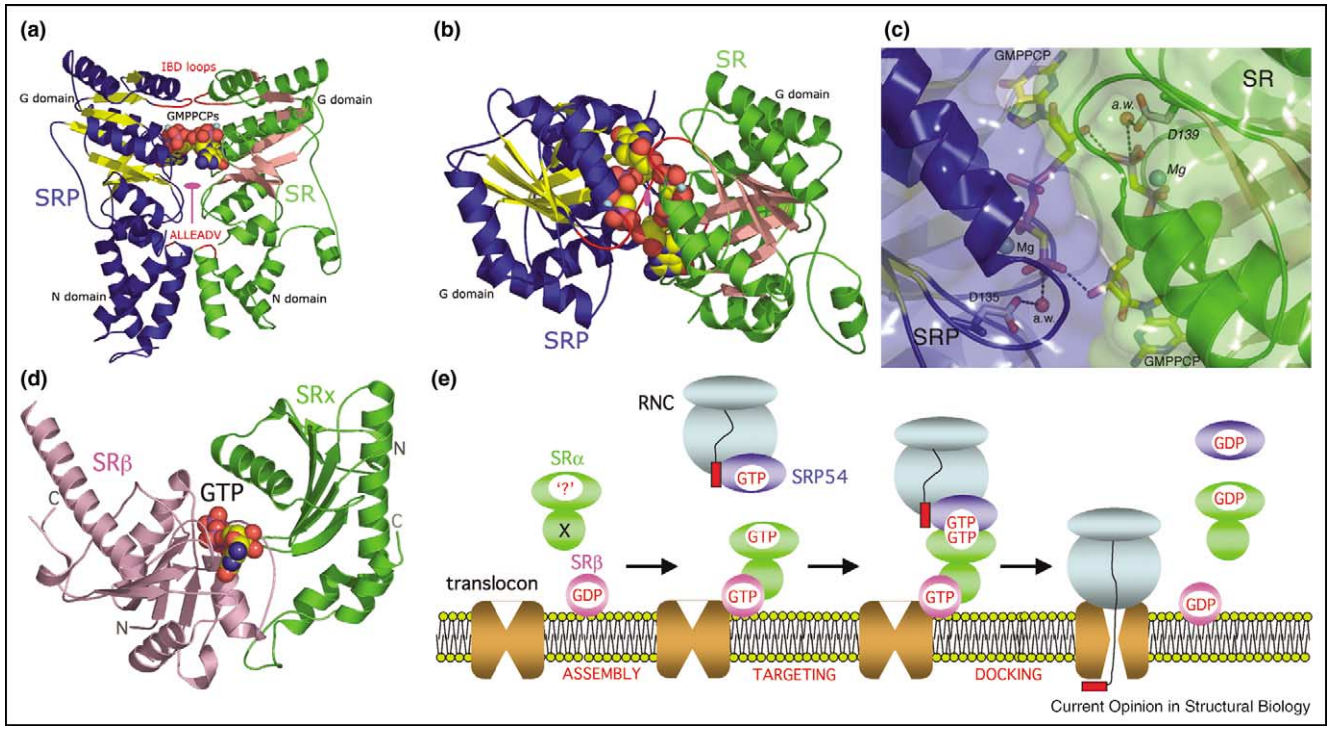
The catalytically important interaction that spans the interface occurs between the two bound GTPs: the 3′-OH of one GTP is hydrogen bonded to the γ-phosphate of the other (Figure 4c) and vice versa. This circle of interactions between the twinned GTPs is severed twice upon hydrolysis, leading to complex dissociation after cargo delivery. Extensive biochemical analysis supports

Figure 3



The structure of mammalian SRP and its dynamic interactions with the ribosome. **(a)** Molecular model of the eukaryotic SRP based on the fitting of X-ray structures of eukaryotic, archaeal and bacterial SRP subcomplexes in the 12 Å resolution cryo-EM density map (PDB code 1RY1). Mouse and human SRP9/SRP14, *M. jannaschii* SRP19 and *M. jannaschii* SRP54 are displayed. The approximate location of the SRP68/SRP72 heterodimer is shown based on unassigned electron density in the cryo-EM maps. **(b)** Model of the eukaryotic SRP binding to an RNC. Free SRP is likely to adopt an extended conformation. SRP54 binds next to the peptide tunnel exit site and scans for signal peptides. Upon binding to the signal sequence emerging from the ribosome, SRP undergoes a conformational change (localized around the hinge 1 region near the SRP68/SRP72 heterodimer) and adopts the kinked conformation observed in the cryo-EM study. This promotes competitive binding of the Alu domain at the entry site for elongation factors (EF) and causes elongation arrest. **(c)** Signal peptide recognition by the M domain of SRP. The M domain of *T. aquaticus* Ffh has been superimposed on the structure of the *E. coli* ribonucleoprotein core (PDB codes 1DUL and 2FFH). The putative hydrophobic binding site for the signal peptide is shown (yellow). The finger loop adopts a variety of conformations in the several known structures and may play a role in the signal-binding process. **(d)** Conformational flexibility of SRP54/Ffh (PDB codes 2FFH, 1QZW and 1QZX). The structure of *Ssol* SRP54 bound to helix 8 of SRP RNA is shown with the N, G and M domains. The flexible linker between the G and M domains is shown in pink. **(e)** A model of the SRP conformational changes on the RNC. The exit tunnel of the 60S subunit of the ribosome is schematized with the reported cross-linking sites between SRP and ribosome. As the ribosome binds to SRP, it induces a conformational change that allows SRP to scan and sample for emerging signal peptides. Further conformational changes are transmitted to the GTPase core, resulting in SR binding. The association between the two NG domains partially exposes the translocon-binding site on the ribosome (brown arrow), initiating the docking of the RNC to the membrane.

Figure 4



The SRs and the universally conserved GTPase core of the SRP–SR targeting complex. **(a,b)** Structure of the *T. aquaticus* FtsY–Ffh NG complex (PDB codes 1OKK and 1RJ9). The two twinned SRP GTPases associate in a parallel way to form a quasi-symmetric heterodimer with an extensive surface that includes both N and G domains. Interaction of SRP (Ffh) with SR (FtsY) brings the two half active sites and their respective GTPs in close apposition to form the shared active site at the center of the two associated G domains. **(c)** Close-up view of the composite active site, with the twinned substrates, the essential and strictly conserved catalytic residues, the attacking waters (aw) and the magnesium cofactors. **(d)** Eukaryotic SR is composed of two subunits: SRα and SRβ. The structure of the yeast SRβ–SRx heterodimer (PDB code 1NRJ) shows the GTP-dependent interaction between the SRβ subunit (homologous to the Arf ras-like GTPases) and the SRx domain of the SRα subunit. The NG domain of SRα, which is responsible for the interaction with the SRP, is not present in this structure. **(e)** A speculative eukaryotic SR cycle. GTP-primed SRβ binds the SRx domain of the SRα subunit. The SRP–RNC is targeted to the membrane via the GTP-dependent interaction between SRP and SRα, and docked on the translocon. It is unknown whether SRβ hydrolyzes GTP during each round of targeting and how this hydrolysis is regulated. The translocon might function as a nucleotide exchange factor for SRβ.

the importance and functional significance of the extensive interaction surface, and the role of the 3′-OH groups in association, reciprocal activation and catalysis [46••,50•]. The IBD motifs rearrange and each contribute three strictly conserved residues, which are of central importance to catalysis. Two of these residues contact the γ-phosphate and stabilize the transition state, whereas the catalytic aspartate residue positions a water molecule for nucleophilic attack, which will then break the β–γ phosphate bond (Figure 4c). It is possible that an additional arginine residue has to be repositioned to complete the active site [47••,50•].

These structures provide the first insight into how the SR–SRP interaction drives SRP-dependent protein targeting. The structures explain the coupling of SRP–SR complex formation and its subsequent disassembly, and suggest a unique activation mechanism for the SRP family of GTPases. The extensive interactions, both from active site residues and from the twinned substrates, explain why complex formation is GTP dependent and how GTP hydrolysis leads to complex dissociation.

Distinct classes of mutations have been isolated that inhibit specific steps of SRP–SR complex formation and activation, suggesting an ordered series of discrete conformational steps during formation of the active SRP–SR complex [6,50•]. Each step could potentially provide a control point in the targeting reaction by the action of additional components, thus ensuring the binding and release of cargo at the appropriate place and time.

In eukaryotes, an additional level of complexity arises from the heterodimeric structure of SR, in which the additional GTPase SRβ is an essential subunit. The association between the two subunits, SRα and SRβ, is GTP dependent, as shown by the recent structure of the yeast SRα–SRβ heterodimer [51••] (Figure 4d). SRβ has been suggested to coordinate signal sequence release from SRP with ribosome binding to the translocon

[52,53] (Figure 4e). As such, SRβ might represent an additional layer of regulation in the transfer of signal sequences from the SRP to the translocon. Alternatively, SRβ may use its GTPase domain to control recruitment and activation of SRα in a way that more globally controls the translocation capacity of the cell according to need.

Conclusions

Several essential questions concerning SRP-dependent protein targeting remain to be answered. How is signal sequence recognition by SRP accomplished? We have not yet obtained a structure of such a complex. Furthermore, SRP RNA and signal sequence regulate different steps of the GTP catalytic cycle; SRP RNA stimulates the SRP–SR interaction [54] and dissociation of the signal sequence from the SRP appears to be a prerequisite to GTP hydrolysis [55]. Comparison of the SRP–RNC and RNC–translocon cryo-EM structures shows that both SRP and the translocon bind next to the exit site of the ribosomal protein tunnel [32••,42,56,57] in a mutually exclusive way, indicating that targeting must coordinate SRP release with translocon binding. The mechanisms by which SRP facilitates the transfer of the nascent chain and the docking of the ribosome onto the translocon remain unknown [58,59••]. The structural flexibility of SRP appears to be crucial for it to act as an adaptor between the ribosome and the translocon.

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