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STRUCTURAL INSIGHTS INTO THE SIGNAL RECOGNITION PARTICLE

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■ **Abstract** The signal recognition particle (SRP) directs integral membrane and secretory proteins to the cellular protein translocation machinery during translation. The SRP is an evolutionarily conserved RNA-protein complex whose activities are regulated by GTP hydrolysis. Recent structural investigations of SRP functional domains and interactions provide new insights into the mechanisms of SRP activity in all cells, leading toward a comprehensive understanding of protein trafficking by this elegant pathway.

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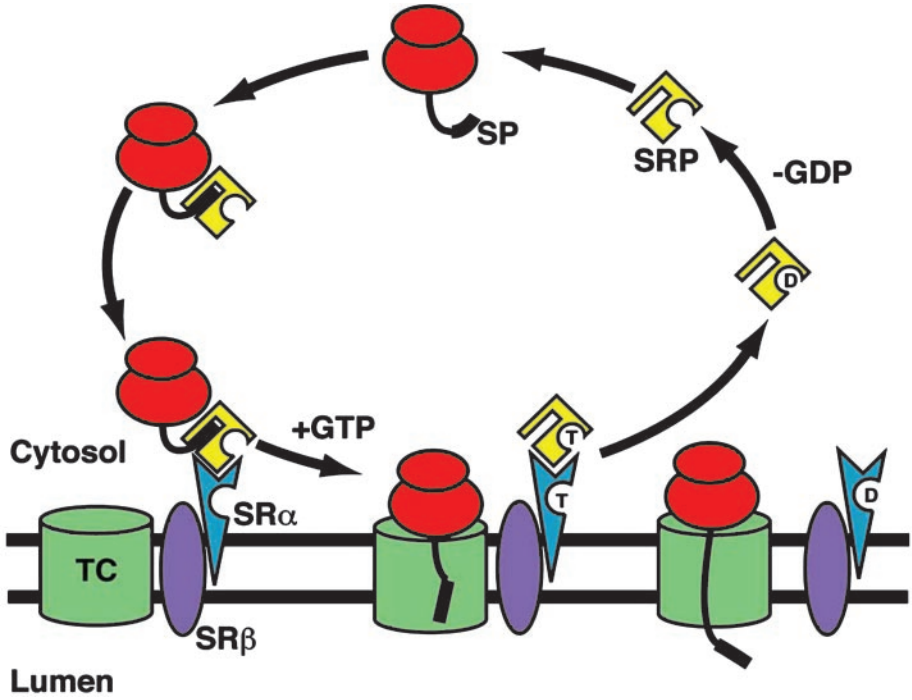


Figure 1 The eukaryotic SRP-dependent protein targeting cycle. A nascent polypeptide chain that is being actively translated by the ribosome (*red*) contains an amino-terminal signal sequence (SP) that is bound by the SRP (*yellow*), which arrests translation. The SRP-ribosome complex is targeted to the translocational complex (TC) embedded in the endoplasmic reticulum membrane via an interaction with the membrane-bound receptor complex SRα/SRβ. Following docking of the ribosome with the translocon, the signal sequence is released from the SRP, and the SRP is released from the SRP receptor in a GTP-dependent fashion.

INTRODUCTION

In all cells, the signal recognition particle (SRP) targets proteins destined for secretion or membrane insertion by binding to hydrophobic signal sequences at the N terminus of polypeptides as they emerge from ribosomes. In a process central to the ability of cells to communicate with other cells and the environment, the SRP recognizes ribosome-nascent chain complexes, docks with a specific membrane-bound SRP receptor, releases the ribosome-associated polypeptide into a translocon channel in the membrane, and dissociates from the SRP receptor primed for another cycle of protein targeting (Figure 1). GTP binding and hydrolysis by both the SRP and its receptor coordinate this process, suggesting that induced conformational changes enable ordered binding and

release of the signal peptide, the ribosome, the SRP receptor, and the translocon. Interestingly, all cytoplasmic SRPs are ribonucleoproteins that consist of one RNA molecule and up to six proteins, a subset of which share sequence, structural, and functional homology. Structural studies of SRP components and complexes, together with genetic and biochemical experiments, have provided significant insights into the mechanism of SRP-mediated protein trafficking as well as the pathway of SRP assembly (reviewed in 1–5). Here we discuss recent advances in understanding how both the bacterial and mammalian SRP particles form functional complexes and how they bind specifically to SRP receptor proteins and the ribosome. These data make possible future experiments to understand the specificity of signal peptide recognition and the coupling of nucleotide hydrolysis with structural dynamics that drive the protein translocation cycle.

MECHANISM OF COTRANSLATIONAL PROTEIN TRAFFICKING

All cells localize secretory and integral membrane proteins, whose biosynthesis begins in the cytoplasm, to specialized pores that enable cotranslational protein export. The SRP is an RNA-protein complex that provides an evolutionarily conserved mechanism for protein trafficking by recognizing the hydrophobic signal sequence found on the N terminus of targeted proteins.

Phylogenetic Conservation

In mammalian cells, the SRP includes one RNA molecule (7S RNA) and six proteins named according to their molecular weight: SRP72, SRP68, SRP54, SRP19, SRP14, and SRP9. SRP54 and one stem-loop, helix 8 of the 7S RNA, are conserved in archaea and eubacteria, and together with a second RNA helical region (helix 6) constitute the signal peptide binding domain (S domain) of the SRP. SRP54 also includes a GTPase domain and an N-terminal helical domain that play roles in communicating the peptide-bound state of the SRP to the SRP receptor, the ribosome, and the translocon pore. SRP19 induces a structural change in the S domain of 7S RNA required for SRP54 binding. SRP72 and SRP68 form a heterodimeric subcomplex that binds the middle segment of the 7S RNA, whereas SRP14 and SRP9 bind cooperatively to the end opposite helix 8 in the SRP RNA to form the Alu domain responsible for transient translational arrest during protein targeting.

In eubacteria, SRP comprises the smaller 4.5S RNA and an SRP54 homolog called Ffh (Fifty-four homolog). All SRP RNAs include the highly conserved binding site for the SRP54 protein, called domain IV in bacterial SRP RNA; however, the less well conserved Alu domain is missing in gram-negative bacteria. Both Ffh and 4.5S RNA are essential genes in *Escherichia coli*, and

SRP54 and Ffh have been shown to be functional homologs. Furthermore, a truncated form of 4.5S RNA, which includes just the Ffh binding site, supports growth in a 4.5S RNA-depleted strain, showing that the critical function of the RNA is contained within the peptide-recognition domain of the ribonucleoprotein complex.

The SRP receptor consists of a conserved protein, SRα in mammals and FtsY in bacteria, with GTPase activity, sequence, and structural homology to the GTPase domain of SRP54/Ffh. In mammalian cells, SRα binds a second subunit, SRβ, containing a single transmembrane region. SRβ is also a GTPase but has only distant homology to the GTPases of SRP54 and SRα. Interestingly, mutation of the SRβ GTPase domain, but not deletion of the transmembrane domain, disrupts signal recognition (SR) function in vivo (6). FtsY weakly associates with the bacterial inner membrane, perhaps through direct interaction with membrane phospholipids (7–11).

Structural Features of the Conserved SRP Components

Crystal structures of the GTPase domains of *Thermus aquaticus* Ffh and *E. coli* FtsY provided the first structural insights into GTPase function in SRP (12, 13). Ffh and FtsY each contain three domains, two of which, the N and G domains, are related at both the sequence and structural level and comprise the GTPase of each protein (Figure 2*a*). The G domain adopts a classical GTPase fold in which four conserved sequence motifs (I-IV) are organized around the nucleotide binding site. Motif II is part of a sequence unique to the SRP GTPases called the insertion-box domain (IBD) that extends by two strands the central β-sheet of the domain. The amino-terminal N domain, a four-helix bundle, packs against the G domain to form a contiguous unit referred to as the NG domain (Figure 2*b*). Side chains from the C-terminal end of the G domain contribute to the hydrophobic core of the N domain, an interface conserved in Ffh and FtsY that creates an axis about which the relative orientations of the N and G domains vary. Nucleotide-dependent changes in the relative N and G domain positions are proposed to enable N domain detection of the GTP-bound state of the G domain (14–16).

Crystal structures of full-length *T. aquaticus* Ffh as well as the human SRP54 M domain revealed an all-helical domain featuring a prominent hydrophobic cleft comprising helices αM1, αM2, and αM4 and an extended flexible loop, the "finger loop," connecting αM1 and αM2 (Figure 3) (17, 18). Adjacent to this cleft, helices αM3 and αM4 form a classical helix-turn-helix (HTH) motif that contains a conserved sequence of serine, arginine, and glycine residues essential for high-affinity binding to SRP RNA. The crystal structure of the *E. coli* Ffh M domain bound to the phylogenetically conserved region of SRP RNA revealed that the HTH motif binds the distorted minor groove of the RNA (Figure 4*a*) (19). In the protein-RNA complex, nucleotides in the asymmetric loop of the RNA wrap around the outside of the helix and make specific contacts to the M domain. Comparison of the structure to that of the unbound RNA shows that a

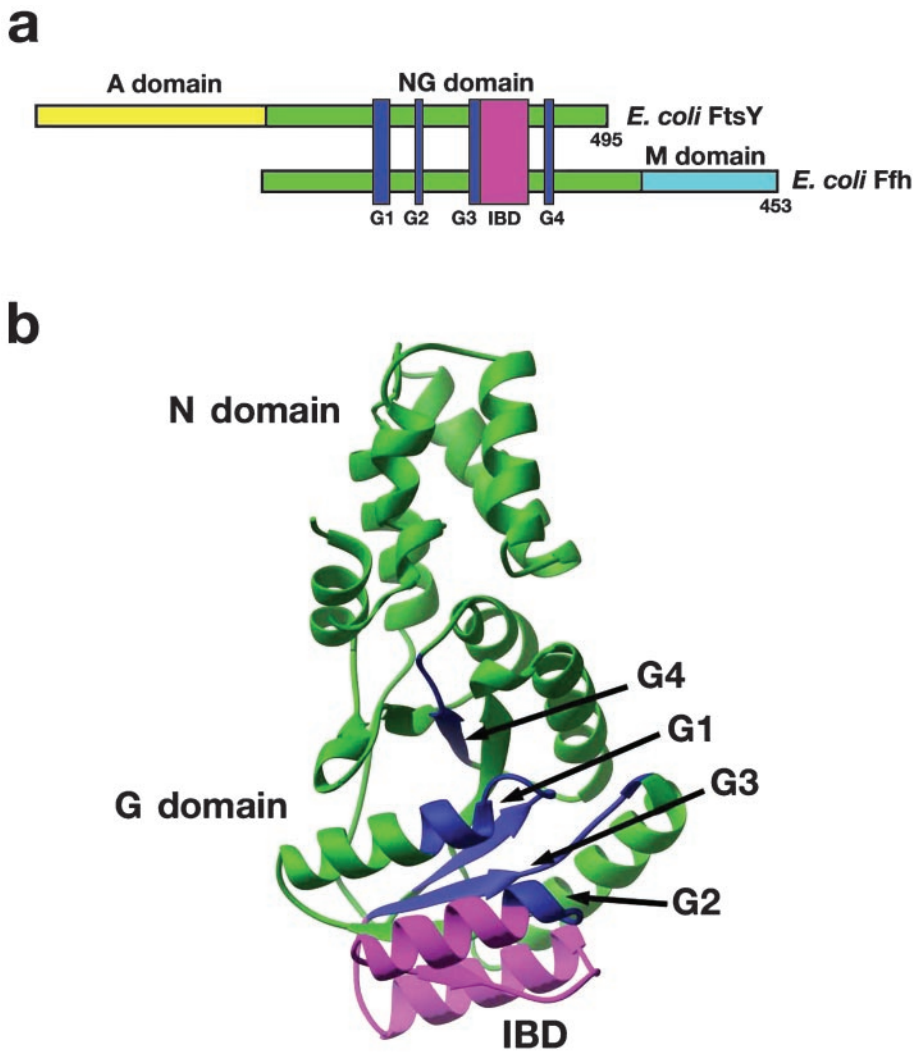


Figure 2 (*a*) Cartoon of the alignment of the *E. coli* FtsY and Ffh proteins. The figure emphasizes the conservation of the NG domain of each and includes the four conserved sequence motifs (G1-G4) and an insertion element (IBD that is unique to the SRP-associated ras-type GTPases. (*b*) Structure of the *E. coli* NG domain of the SRP receptor protein FtsY (PDB ID: 1FTS). The N domain represents the four helix bundle on the top and the ras-type GTPase domain (G domain) on the bottom. The four conserved sequence motifs in the Ffh/FtsY family of proteins (G1–G4) are highlighted in blue and the insertion-box domain (IBD) are highlighted in magenta.

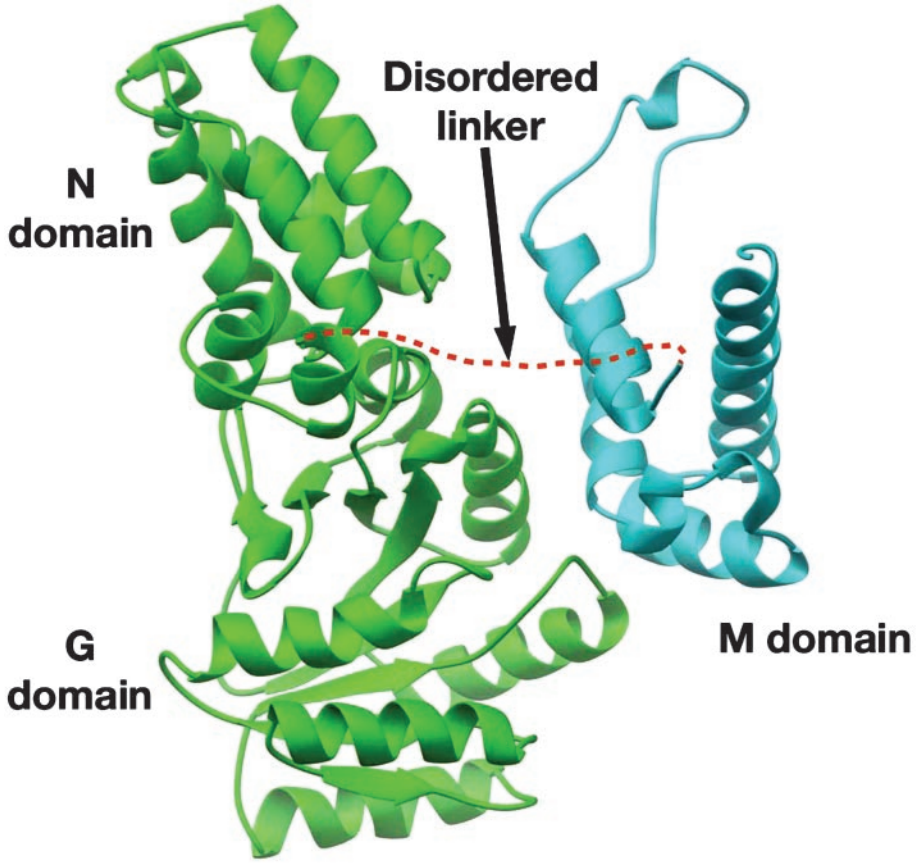


Figure 3 Crystal structure of the *Thermus aquaticus* Ffh protein (PDB ID: 2FFH). The two principal domains, the NG domain (*green*) and the M domain (*blue*), are shown. Ffh crystallized as a trimer of proteins in which a flexible linker between the two domains (residues 308–318) (shown as a *red dashed line*) was disordered, and thus the orientation of the two domains is ambiguous. Only one of the three possible pairs is shown.

significant conformational change is induced upon M domain binding, coupled to the ordered binding of metal ions and waters in the complex (19–23).

Role of GTP Hydrolysis

The protein targeting cycle is regulated by the coordinated action of GTPases, SRP54 and SRα/SRβ in eukaryotes and Ffh/FtsY in bacteria, that control signal peptide binding and release. In the GTP-bound state, SRP binds to a nascent signal peptide and the SRP receptor, leading to localization of the associated ribosome on the translocon. The GTPases in both SRP54/Ffh and SRα/FtsY are

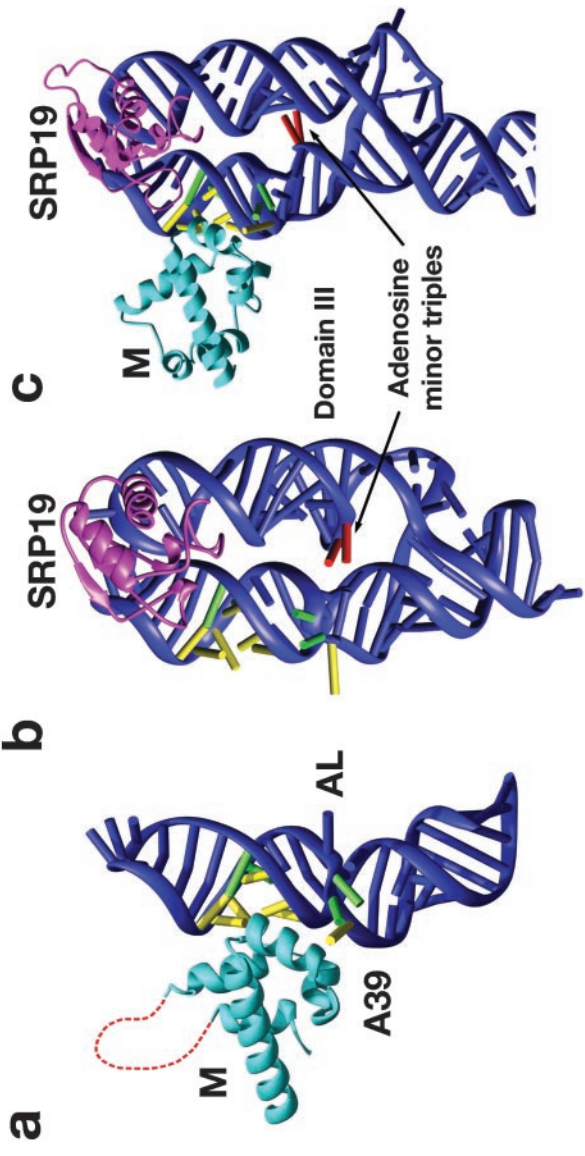


Figure 4 Structures of the signal recognition domain of the SRP from the three kingdoms of life. (*a*) Structure of the conserved domain of the *E. coli* 4.5S RNA (corresponding to domain IV of the eukaryotic SRP RNA) bound to Ffh M domain (PDB ID: 1DUL). A 33-amino acid segment between helices one and two of the M domain (*light blue*) was disordered in the structure (*dashed red line*). Nucleotides in the 4.5S RNA that are highly (*green*) and universally (*yellow*) conserved are highlighted along with A39 of the asymmetric internal loop (AL). (*b*) Structure of the *Methanococcus jannaschii* S domain RNA in complex with SRP19 (*magenta*) (PDB ID: 1LNG). Two adenosine residues in domain III, highlighted in red, contact the asymmetric loop of domain IV by forming A-minor base triples that potentially stabilize a conformation productive for M domain binding. (*c*) Structure of the ternary complex between the human S domain RNA/SRP19/SRP54 M domain (PDB ID: 1MFQ). The adenosines from the asymmetric loop that form A-minor triples with domain III are highlighted in red.

mutually stimulated upon complex formation and have been proposed to act as GAPs (GTPase activating proteins) for each other (24, 25). Following release of the signal peptide from SRP54/Ffh into the translocon, hydrolysis of SRP- and SR-bound GTP molecules causes dissociation of the SRP-SR complex and resumption of ribosome-catalyzed polypeptide synthesis. In eukaryotes, GTP hydrolysis by SRβ leads to dissociation of its complex with SRα. Key questions about the mechanism of SRP center on the role of GTP hydrolysis in coordinating and controlling the timing of these events in the cell.

The role of SRβ and why it is required in eukaryotic cells remains unclear, but some clues were provided by the crystal structure of SRβ bound to GTP and the N-terminal interaction domain of SRα (26). SRβ has nanomolar affinity for GTP (27), and in its GTP-bound state, it is catalytically inert when bound to SRα, requiring a GAP and a GEF (guanine-nucleotide exchange factor) to function as a GTPase switch for release of the SRα subunit (28). In the structure, an extensive intermolecular interface includes both polar and hydrophobic contacts with interdigitated side chains that produce a high-affinity complex (26). Recent data from a fluorescence nucleotide exchange assay show that the β subunit of the protein-conducting channel in the endoplasmic reticulum functions as the GEF for SRβ in yeast, and by analogy in other organisms as well (29). The nature of the SRβ GAP activity and how it correlates with ribosome and translocon binding and release await further biochemical and structural studies.

SIGNAL RECOGNITION PARTICLE ASSEMBLY

SRP comprises discrete domains corresponding to the translational arrest and peptide recognition functions of the particle. Results of structural and biochemical experiments highlight the topology of SRP as well as the hierarchy of interactions that result in a functional complex.

Structural and Functional Domains

Electron microscopy (EM) revealed that the eukaryotic SRP has an elongated rod-like structure (~240 x 60 Å) comprising 3 distinct regions (30, 31). The Alu domain and the S domain, responsible for translation arrest and signal sequence binding, respectively, lie at opposite ends of the complex. Connecting these functional centers is a low-mass region thought to be a flexible RNA linker. This global organization is consistent with simultaneous binding of the elongation arrest domain at the ribosomal subunit interface and binding of the signal recognition domain near the peptidyl exit site on the large ribosomal subunit. In crystal structures of the murine Alu domain, which include the Alu region of the SRP RNA bound to the SRP9/14 heterodimer, the RNA forms a U-turn that connects two helical stacks (32, 33). In contrast to earlier models, however, this structure does not resemble that of tRNA, implying that molecular mimicry is not

the mechanism of ribosome binding or elongation arrest. Part of the structural organization of the S domain of the SRP has recently been revealed in several crystal structures, which culminate with the human S domain RNA/SRP19/SRP54 M domain complex (34–36). The cryo-EM reconstruction of the mammalian SRP bound to the 80S ribosome is eagerly awaited to reveal the mechanism of simultaneous translational arrest and signal peptide binding by the SRP (89).

Assembly of the Signal Sequence Binding Domain

The universally conserved ribonucleoprotein core of the cytosolic SRP comprises the SRP54/Ffh protein and domain IV of the SRP RNA. In bacteria, these two components interact through the methionine-rich M domain of SRP54/Ffh to form a functional enzyme. The crystal structure of an *E. coli* SRP RNA/Ffh M domain complex (19) revealed contacts between two internal loops in the RNA and a series of strictly conserved amino acids in the M domain (Figure 4*a*). Strikingly, the crystal structure of a ternary complex between the human M-domain/SRP-19/S-domain RNA showed nearly identical architecture in the protein and RNA at this site of contact as well as an identical set of contacts between the two (36). Clearly, this interface remained constant over evolutionary time.

The first internal loop, symmetric in all SRP RNAs, contains six universally conserved nucleotides that form three noncanonical base pairs. Functional group mutagenesis showed that disruption of either of two of these pairs, a sheared G-G pair or a reverse Hoogsteen A-C pair, abolishes protein binding (22, 37); similarly, a single point mutation in this loop is lethal in *E. coli*. In contrast to the symmetric loop, the sequence of the asymmetric internal loop of the SRP RNA is more variable (38, 39). In both the *E. coli* and human SRP complexes, the asymmetric loop presents a 5′-side adenosine base to the M domain for extensive recognition by three universally conserved amino acids (19, 36). Because all phylogenetic variants of the SRP RNA have at least one adenosine on the 5′ side of the asymmetric loop, this set of contacts is probably universally conserved.

To overcome unfavorable electrostatic consequences of extruding the adenosine, a series of metal ions interact specifically with the major groove of the asymmetric internal loop (23). An extensive hydrogen bonding network between water, three cations, and the RNA, observed crystallographically (19), is essential for stability; removal of all metal cations from the binding reaction reduced the binding affinity of the complex by at least 10^6-fold (23). Although metal ions were observed throughout the major groove of the RNA, the metal ions in the asymmetric loop appear to stabilize the protein-RNA complex. For example, Mn^{2+} preferentially stabilizes the complex compared to Mg^{2+} by enabling formation of an additional A-A pair that likely further stabilizes the bound conformation (23). Additionally, Cs^+ enhances stability relative to other monovalent cations, in contrast to its behavior in other RNAs (40) and protein-RNA complexes (41), because Cs^+ binds at the site where the backbone comes into

close contact with itself and presumably alleviates unfavorable electrostatic interactions (23). Thus, the two metals that most stabilize the protein-RNA complex both affect the structure of the asymmetric internal loop.

Archaea and eukarya require the presence of a second protein, SRP19, for the efficient binding of SRP54 to the SRP RNA. This protein binds to another domain of the SRP RNA, domain III, and to the conserved GNRA tetraloop of domain IV (35) (Figure 4*b*). A solution structure of SRP19 alone (42), as well as crystal structures the S-domain RNA bound to SRP 19 (34, 35, 43), and SRP19/SRP54-M domain (36), show that cooperative assembly occurs through the stabilization of the bound form of the asymmetric internal loop motif via A-minor base triples (37, 44) with a second RNA helix (helix 6) (Figure 4*b*,*c*) (45–47). As in the *E. coli* structure, magnesium ions bind the asymmetric loop, presumably further stabilizing this region of the RNA. Thus, cooperative assembly of this RNP appears to be driven by the formation of new RNA-RNA contacts rather than protein-protein contacts, similar to the assembly of the central domain of the 30S ribosomal subunit (48, 49).

Although their interactions with SRP RNA are nearly identical, Ffh and SRP54 in the bacterial and human SRP complexes, respectively, use different strategies to stabilize the critical asymmetric loop. This begs the question as to why the SRP19/domain III-mediated assembly arose. Currently no other role for SRP19 in SRP function has been determined, suggesting that the primary role for this protein in the SRP is facilitating its assembly and stabilizing the intact particle. Thus, the domain III/SRP19 extension may be an evolutionary adaptation of the SRP to enhance or control the kinetics of assembly that cannot be achieved by metals alone.

THE SIGNAL RECOGNITION PARTICLE CYCLE

Nascent protein targeting involves a choreographed cycle of signal peptide binding and release coupled to GTP hydrolysis and interactions with the ribosome and translocon pore. Exciting recent advances in understanding GTPase activity and SRP-ribosome interactions have come from x-ray crystallography, cryo-electron microscopy and mutagenesis studies.

Signal Sequence Recognition

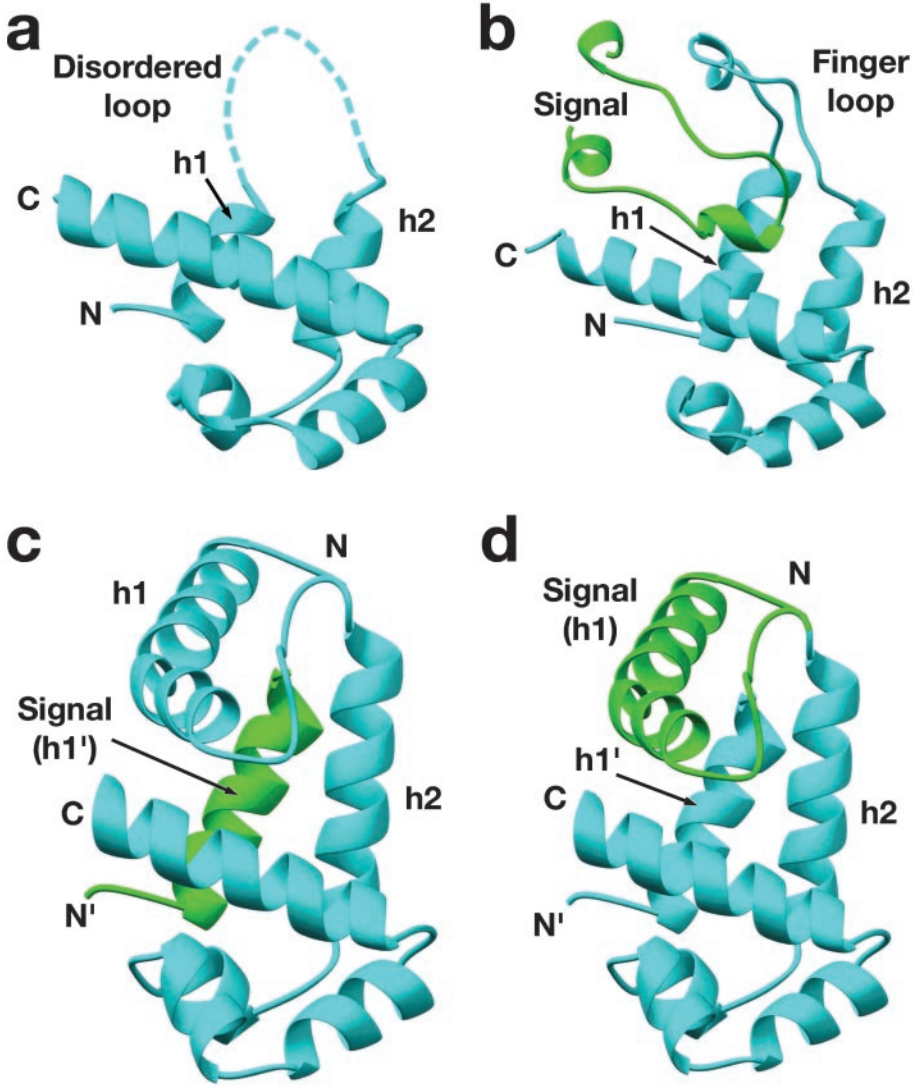
Though a number of structures of various domains of the SRP have revealed a wealth of information about protein-RNA, protein-protein, and protein-nucleotide interactions, few insights have emerged as to how the most critical ligand of all—the signal sequence of the protein to be targeted—is specifically recognized. A typical signal sequence comprises 9–12 large hydrophobic residues in a row (50) that adopt an α-helical conformation (51–53). In eukarya, these signals are typically found at the N terminus of the protein; however, in *E. coli*

the SRP-dependent signal is often a transmembrane helix within inner membrane proteins (54–57). The SRP appears to recognize any sequence that bears a critical level of hydrophobicity, though flanking basic residues are also important (52, 58). Currently unanswered is the fascinating question of how the SRP recognizes and productively binds almost any such hydrophobic α-helix.

Early studies of target recognition by the SRP suggested that nascent polypeptide chains bearing signal sequences cross-linked to SRP54/Ffh through the M domain and that the M domain was sufficient to mediate this interaction (59, 60). From these data, the "methionine bristle" hypothesis was proposed in which a flexible, methionine-rich pocket is used by the SRP to recognize almost any given signal sequence (61). Consistent with this hypothesis, structures of the M domain in the free and RNA-bound states show that the methionine-rich region of the protein, the presumed signal binding site, is conformationally flexible (Figure 5). In the structure of the *T. aquaticus* M domain (17), this region is involved in extensive crystal packing contacts with the signal binding site of an adjacent protein in the crystal lattice (Figure 5*b*). Consequently, it takes on a β-hairpin like structure, called the finger loop, that is partially inserted into a neighboring hydrophobic groove. Because authentic signal sequences are probably α-helical, the authors contend that the structure illustrates the inherent flexibility rather than the signal sequence binding mechanism of the finger loop. Underscoring its flexibility, this region of the M domain was entirely disordered in the *E. coli* M domain-4.5S RNA complex structure (Figure 5*a*) (19, 22). This may represent the true state of the signal recognition site in the SRP in solution, consistent with the conformational flexibility required for productive binding of heterogeneous targets.

Crystal structures of the human M domain present a different picture of how the signal sequence potentially interacts with the SRP. In the absence (18) and presence of SRP-RNA (36), the first helix of the M domain undergoes a "domain swap" with an adjacent protein (Figure 5*c*). This has been observed in the crystal structures of other proteins (62, 63) and suggests weak interactions between this helix and the rest of the M domain. The result of the domain swap is that helix one (h1′, Figure 5*c*) packs into a shallow, moderately hydrophobic groove of an adjacent molecule. Thus, in an alternative model of how the M domain may recognize signal sequences, helix 1′ (h1′) occupies nearly the same position as helix 1 in the *E. coli* and *T. aquaticus* structures, although helix 1 represents the signal peptide (Figure 5*d*).

These models for peptide recognition leave unanswered the question concerning the involvement of the NG domain and the RNA in target recognition by the SRP. Even though the M domain can bind the signal on its own or in complex with SRP RNA, the affinity of the interaction was improved by the NG domain, and the NG domain can be cross-linked to signal peptides in solution (64, 65). Whereas biochemical and crystallographic studies indicate that the NG and M domains of Ffh are loosely associated in the absence of a bound signal sequence and/or GTP (17, 66), a recent crystal structure of the intact SRP54 protein


a
Disordered loop
h1
C
h2
N
b
Signal
Finger loop
h1
C
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h2
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d
Signal (h1)
N
h1'
C
h2
N'

provides clues to interdomain communication that may occur upon signal binding (see below). It has also been proposed that the SRP RNA has a role in signal recognition via electrostatic interactions between the backbone of the RNA and positively charged residues adjacent to the hydrophobic sequence (19), a hypothesis supported by mutagenesis and in vivo experiments (58, 67). Although these data provide tantalizing clues to the mechanism of protein targeting by the SRP, a structure of the SRP-signal complex will be an important step toward an atomic-level understanding of SRP function.

Structure of the SRP54-RNA Complex

SRP54 is the only protein subunit conserved in all SRPs and controls communication with the SRP receptor, the ribosome, and the translocon. In the crystal structure of *T. aquaticus* SRP54, the linker region between the G and M domains was disordered and hence provided no information about the three-dimensional domain arrangement of SRP54 or its organization in complex with SRP RNA. Recent determination of structures of SRP54 from the archaeon *Sulfolobus solfataricus* alone and complexed with helix 8 of SRP RNA reveal the architecture of the complex and a hydrophobic contact between the M and N domains, suggesting a possible mechanism for interdomain communication (67a). The structures, solved by molecular replacement at a resolution of ~4 Å, reveal an L-shaped protein in which the NG domain represents the long arm and the M domain represents the short arm. Helix 8 of SRP RNA lies parallel to the long axis of the NG domain, giving the complex an overall U shape (Figure 6). Only one region of interaction, which involves a short stretch of hydrophobic contacts between the loop connecting helices αN3 and αN4 at the distal end of the N domain, the N-terminal region of αML, and the C-terminal region of the short α helix αM1b adjacent to the finger loop, is observed between the N and M domains (Figure 6). The high degree of evolutionary conservation of these

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Figure 5 Potential models of signal sequence recognition by the SRP54/Ffh M domain. (*a*) The hydrophobic, methionine-rich pocket in the *E. coli* M domain (PDB ID: 1DUL) is significantly disordered (*dashed line*), suggesting a conformationally dynamic binding site that can recognize a variety of sequences. (*b*) The conformation of the finger loop between helices one and two of the *T. aquaticus* M domain structure (PDB ID: 2FFH) is stabilized through its interaction with a significantly hydrophobic groove of an adjacent M domain in the crystal (note that the signal corresponds to the finger loop). (*c*) A model of signal recognition proposed from the structure of the human SRP M domain (PDB ID: 1QB2). Helix one (h1′) of an adjacent M domain in the crystal structure packs into a shallow, moderately hydrophobic groove formed between helices one and two (h1, h2). (*d*) An alternative model for signal sequence binding by the human M domain in which h1′ is considered part of the domain structure by virtue of its close superposition with helix 1 of the *E. coli* and *T. aquaticus* structures and helix 1 represents the signal sequence.

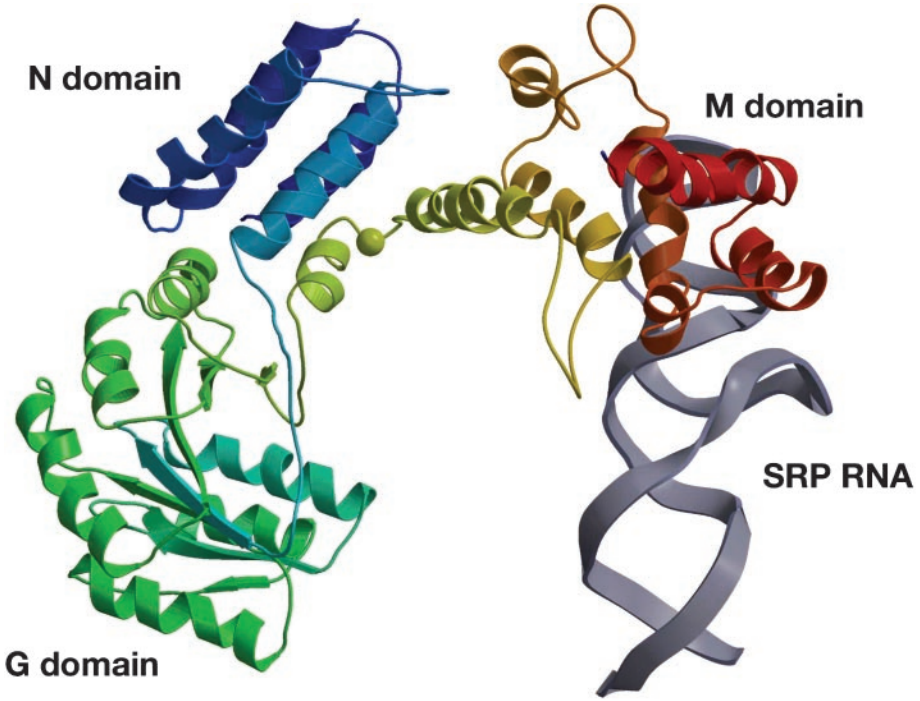

Figure 6 Crystal structure of a complex between *Sulfolobus solfataricus* SRP domain IV RNA and SRP54 (67a). The N domain of SRP54 (*blue*) contacts the N-terminal helix of the M domain (*red*), while the M domain forms a complex with the SRP RNA (*gray*) in a fashion similar to that of the bacterial and eukaryal variants. Figure courtesy of I. Sinning.

residues suggests a functional role of the contact, perhaps as a greasy hinge enabling interdomain flexibility in SRP54. Although the relative orientations of the NG and M domains are not affected by RNA binding, shape and charge complementarity between the phosphate backbone of the RNA minor groove and residues 121–126 of the G domain suggest a possible contact that might be induced at some stage in the SRP cycle; this would enable communication between the peptide binding and GTPase functionalities of the particle.

GTPase Stimulation in the SRP-SR Complex

Two central questions about the mechanism of SRP-mediated protein targeting are how GTPase activities in the SRP54/Ffh and SRα/FtsY proteins are enhanced upon heterodimerization of the SRP with its receptor, and how GTP hydrolysis is coupled to peptide binding and release. Crystal structures of the homologous GTPase domains of these two proteins, determined in their apo- and nucleotide-bound states, revealed a common two-domain NG fold comprising an α-helical

N domain packed against a G domain with a fold similar to those of other members of the GTPase superfamily. Structural similarity between the G domains of Ffh and FtsY and dimeric ATP-utilizing proteins led to a model for the Ffh-FtsY complex in which the G domains dimerize in an antiparallel orientation (68). Similar to other GTPases, four conserved motifs, I-IV, comprise the SRP GTPases and include residues directly involved in GTP binding and hydrolysis. In addition, the G domains of both Ffh and FtsY contain a subdomain, termed the insertion box domain (IBD), that extends the core α/β fold along the face distal to the N domain and was thought to provide the site of interaction in the targeting complex (12, 14, 69). Despite their similarities, the SRP GTPases exhibit several distinct properties relative to other GTPases, which include low affinity and rapid exchange of both GDP and GTP (70). GTP hydrolysis in an Ffh/FtsY complex is stimulated about 10-fold above that observed in either protein alone.

A significant advance in understanding GTPase activation has come from recent structure determinations of a complex between the Ffh and FtsY NG domains (70a, 70b). The two proteins associate longitudinally along the N and G domains, bringing the two active sites into direct contact to form a contiguous catalytic chamber that contains two bound GMPPCP molecules, two hydrated magnesium ions, and several waters. The contact region between the domains comprises most of the conserved sequence motifs of the SRP GTPases and buries $\sim$1800 Å^2 on each protein surface. Mutation of residues across the interface surface in FtsY disrupts GTPase activity in the complex, confirming the importance of the crystallographically observed interaction (70a). Formation of the Ffh-FtsY interface requires several conformational changes relative to the structures of the domains alone, including a rigid body motion of the N and G domains that translates the distal loops of the N domain by $\sim$11–12 Å. This movement is accompanied by a substantial shift in the position of the C-terminal helix, leading to speculation that the N domain functions as a sensor of targeting complex formation by triggering a change in the relative orientations of the M-domain and A-domain that are found on the C-terminal ends of the full-length Ffh and FtsY proteins, respectively. Conserved arginine sidechains in each protein that might serve as arginine fingers, analogous to the Ras/GAP structure, are oriented asymmetrically such that only the Arg in Ffh is positioned like the Arg supplied by the RasGAP. Possibly, the arrangement of arginines alternates within the chamber, perhaps contributing sequentially to hydrolysis of the two bound nucleotides.

Ribosome Interactions

In the mammalian system, SRP54 cross-links to two ribosomal proteins, L23a and L35, located near the polypeptide exit site on the ribosome (71). A similar interaction is observed in eubacteria, where Ffh/4.5S RNA complexes can be cross-linked to L23 (72, 73). Interestingly, protein L23 also cross-links to a chaperone protein, trigger factor (TF), though L23-SRP and L23-TF interactions

appear to be mutually exclusive. These data suggest that L23 may play a role in directing nascent polypeptides into the translocation machinery, though this hypothesis remains to be tested in vivo. The ribosome somehow induces a structural change in SRP54 that leads to increased GTP binding affinity, as well as increased affinity for the ribosome itself (74, 75). One possibility, suggested by the SRP54/RNA structure, is that this structural rearrangement corresponds to rotation of the NG domain with respect to the M domain that may occur upon signal peptide binding, with consequent opening of the finger loop similar to the conformation observed in the *T. aquaticus* Ffh structure (17). Signal peptide binding to the M domain might therefore result in a similar structural rearrangement in the NG domain interface as occurs upon GTP binding to the G domain, effectively linking signal sequence binding to the M domain with GTP binding to the G domain.

Nucleotide Exchange

The molecular mechanism of reciprocal GTPase activity in SRP and its receptor remains poorly understood. Recent evidence indicates that structural changes induced in the bacterial SRP receptor, FtsY, upon formation of the SRP-FtsY complex enhance nucleotide binding specificity in FtsY (76). Mutagenesis studies support a similar weak-binding affinity in the eukaryotic homolog SRα (77, 78). Why might this occur? One idea is that loosely bound GTP in free FtsY would prevent futile cycles of GTP hydrolysis in the substantial fraction of free FtsY in the cytosol (79). In this way, nucleotide hydrolysis would be coupled to binding of SRP and presumably to nascent signal peptides.

FUNCTIONS OF SIGNAL RECOGNITION PARTICLE RNA

Why does cellular SRP include an essential RNA, and what does the RNA contribute to SRP function as well as to other physiological activities in the cell? Most of the evidence to date addressing these questions comes from studies in the *E. coli* system. In vitro, the 4.5S RNA appears to stabilize the structure of the Ffh M domain, as indicated by circular dichroism and proteolysis experiments (66, 80), and studies on the kinetics of Ffh-FtsY complex formation show that 4.5S RNA enhances both association and dissociation of the complex (81, 82). Intriguingly, several lines of evidence support an additional role for 4.5S RNA in translation on the ribosome, because of the observation that the deleterious effects of 4.5S RNA depletion can be suppressed by mutations in translation factor EF-G or in the 16S or 23S ribosomal RNAs (83–86). Recent evidence implies that although the SRP RNA interaction with EF-G homologs is conserved in archaea, the essential activity of SRP RNA is in fact as part of the signal-sequence binding particle rather than on the ribosome (90). It is intriguing

to note that in chloroplasts the SRP RNA has apparently been replaced by a protein, indicating that study of this SRP may provide clues to the role of the RNA (87, 88).

FUTURE DIRECTIONS

With many structures of individual components of the SRP pathway now known, attention is focused on understanding how these molecules interact to enable efficient protein targeting. How signal peptide binding is achieved, how peptide binding and release is controlled, and how the SRP coordinates interactions with its receptor, the ribosome, and the translocon remain fascinating unanswered questions. A combination of genetic, biochemical, and structural approaches will be required to address these issues and fully illuminate the function of one of the most ancient of the cellular ribonucleoproteins.

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