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THE SIGNAL RECOGNITION PARTICLE

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■ **Abstract** The signal recognition particle (SRP) and its membrane-associated receptor (SR) catalyze targeting of nascent secretory and membrane proteins to the protein translocation apparatus of the cell. Components of the SRP pathway and salient features of the molecular mechanism of SRP-dependent protein targeting are conserved in all three kingdoms of life. Recent advances in the structure determination of a number of key components in the eukaryotic and prokaryotic SRP pathway provide new insight into the molecular basis of SRP function, and they set the stage for future work toward an integrated picture that takes into account the dynamic and contextual properties of this remarkable cellular machine.

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INTRODUCTION

The signal recognition particle (SRP) is a molecular machine that coordinates targeting of nascent secretory and membrane proteins to the translocation apparatus of the cell during translation. SRP and its membrane-associated receptor (SR) were first identified in mammalian cells in the early 1980s (1–3). Extensive biochemical analysis of their function using in vitro translation and translocation extracts led to a general description of the mechanism of SRP-mediated protein targeting to the endoplasmic reticulum (ER) membrane in eukaryotes. Identification of SRP and SR homologs in bacteria, and later, analysis of the entire genomes of numerous organisms from each of the three domains of life, revealed that components of the SRP pathway are universally conserved. Moreover, genetic and biochemical analysis of the SRP pathway in prokaryotes showed that the salient features of the molecular mechanism of SRP-dependent protein targeting are also conserved. Advances in the structure determination of a number of key components in the eukaryotic and prokaryotic SRP pathway are providing new insight into the molecular basis of SRP function. Almost twenty years have passed since the mammalian SRP and its receptor were first identified as factors required for targeting of nascent secretory and membrane proteins to the ER during translation. Here we discuss recent biochemical, genetic, and structural studies of SRP and its receptor in an attempt to present a unified picture of SRP-dependent protein targeting. We also attempt to identify the many intriguing questions that remain unanswered.

COTRANSLATIONAL PROTEIN TARGETING

Mechanism

When ribosomes translate proteins destined for either secretion or membrane integration, they are directed to the ER (or plasma membrane in prokaryotes) by SRP (1, 3). This cotranslational targeting is generally divided into two distinct steps: (*a*) recognition of a signal sequence and (*b*) association with the target membrane. The SRP cycle (Figure 1) is initiated when SRP binds to the hydrophobic signal sequence of the nascent chain as it emerges from the ribosome. Signal sequence binding by SRP transiently arrests elongation (in eukaryotes) and targets

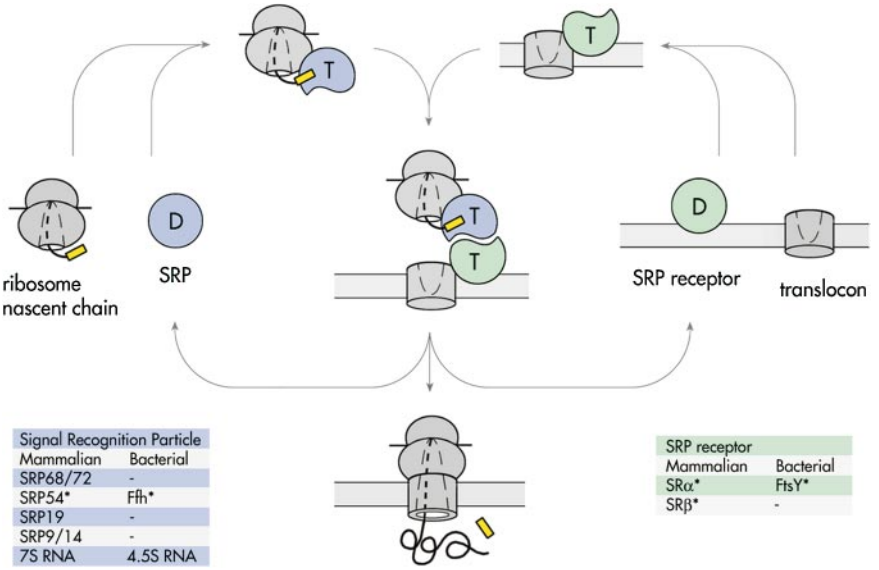

Figure 1 Cotranslational targeting by SRP. In this model, GTP binding and hydrolysis by the SRP GTPases is modulated, in part, by the ribosome and the translocon. SRP (blue) and SR (green) are labeled D in the GDP-bound state and T in the GTP-bound state. The signal sequence (yellow) of the nascent chain is indicated and the target membrane (horizontal gray bar) represents the eukaryotic ER membrane and the prokaryotic plasma membrane. Although many of the states in the model have experimental support, some steps in the pathway are poorly understood. For example, a potential role of the empty state of the SRP GTPases is not yet understood, and therefore is not included in the model. Likewise, little is known about the events leading to release of the signal sequence after the ribosome–nascent chain–SRP complex is recruited to the membrane. The individual molecular components of SRP and SR are listed, and proteins containing a GTPase domain are indicated with an asterisk. Adapted from (13a).

the ribosome–nascent chain–SRP complex to the membrane via a GTP-dependent interaction with the SRP receptor (2, 4, 5). This interaction releases SRP from the ribosome–nascent chain complex (6) and translation resumes, with the ribosome now engaged to the protein translocation channel, or translocon (7–9), through which the nascent chain is directed. GTP hydrolysis by the SRP–SRP receptor complex (10) dissociates the complex, allowing SRP to initiate a new round of targeting (11). SRP thus provides strict coupling between protein synthesis and translocation, thereby ensuring that the nascent polypeptide cannot fold or misfold in the cytosol. Cotranslational targeting, therefore, has conceptual advantages over post-translational targeting modes (12, 13) that are likely to impose many more constraints on passenger proteins in order to retain them in a translocation-competent state.

Evolutionary Conservation

The key components of SRP-dependent protein targeting are conserved (Figure 1), as homologs of SRP and SR have been identified in all living cells analyzed to date. Mammalian SRP is a ribonucleoprotein complex comprising six polypeptides (named by their apparent molecular weight: SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) and one RNA (termed 7SL RNA or SRP RNA) (1,3). Signal sequence recognition is mediated by the SRP54 subunit (14,15), which also binds directly to SRP RNA to form the "business end" of the particle. In addition, SRP54 is a GTPase. The prokaryotic homolog of SRP54, called Ffh or P48 (16,17), shows very similar properties to its mammalian counterpart and has been used extensively to characterize the SRP pathway. Like SRP54, Ffh is a GTPase that binds to signal sequences and is also an RNA binding protein that interacts with prokaryotic SRP RNA (termed 4.5S RNA in *Escherichia coli*) (18). The 4.5S RNA lacks however, a domain present in mammalian SRP RNA, the Alu domain, which is required for the elongation arrest activity of mammalian SRP (19,20). In fact, Ffh and 4.5S RNA are the only subunits of the ribonucleoprotein complex in the bacterial cytosol and function as a streamlined, or minimal, SRP.

Mammalian SRP receptor is composed of two distinct subunits (2,4,21). The α-subunit (SRα) is a GTPase and is closely related in sequence, structure, and function to the GTPase domain of SRP54. SRα associates with the β-subunit of SR (SRβ), an integral membrane protein of the ER. SRβ contains a single transmembrane region. Interestingly, SRβ is also a GTPase, although its sequence is only distantly related to those of SRP54 and SRα; SRβ is most closely related to GTPases of the Sar1 and ARF families (22). Mutations in the GTPase domain of SRβ (but, perhaps surprisingly, not deletion of its membrane-spanning region) disrupt SR function in vivo (23). Bacterial SR (like prokaryotic SRP) is streamlined, consisting of a single polypeptide, FtsY, which, like its mammalian homolog SRα, is a GTPase. FtsY is either soluble or loosely associated with the bacterial inner membrane (24–26). The phylogenetic differences in SR structure point to a eukaryote-specific role for SRβ. What this role is, and what the additional needs of a eukaryotic cell are that require SRβ, remain poorly understood.

The original proposal that Ffh and FtsY function in prokaryotic protein secretion met with skepticism, in part because neither of the components was initially identified as a secretory mutant in genetic screens (27,28). Consequently, substantial effort has been directed toward demonstrating a functional role for these components. It has now been shown that depletion of either 4.5S RNA or Ffh most strongly affects the insertion of integral membrane proteins (29–32), whereas such depletions only weakly impair secretion of soluble periplasmic proteins (18,26,33). Similarly, depletion of FtsY affects the insertion of inner membrane proteins (34). The failure to identify components of the SRP pathway in screens for secretion mutants is likely a result of the ability of the prokaryotic post-translational targeting pathway (12,35) to relatively efficiently target proteins that would otherwise utilize the SRP-dependent pathway.

Functional conservation between prokaryotic and eukaryotic cotranslational targeting is best demonstrated by the fact that Ffh and FtsY efficiently substitute for SRP54 and SRα in eukaryotic protein translocation assays in vitro (36). This complementation is remarkable, given that specific interactions of SRP and its receptor with the ribosome and the membrane translocation machinery are thought to be important for the cotranslational targeting pathway (37–39). One possibility is that SRP actually functions more autonomously than presently thought, interacting only with a signal sequence and the SRP receptor during targeting. Alternatively, interactions of SRP and SR with the ribosome and the translocon (although not experimentally well defined) may be highly conserved. In either case, it seems clear from these results that the basic mechanism of cotranslational protein targeting is conserved from bacteria to mammals.

PROTEIN TARGETING BY THE SIGNAL RECOGNITION PARTICLE GTPases

Background

During protein targeting by SRP, two—and in the case of eukaryotes, three—GTPases interact to confer unidirectionality to the reaction. Sequence homology of these SRP GTPases to other GTPases is largely confined to discrete sequence motifs that constitute the GTP binding site. Distinct conserved features of the SRP54 and SRα sequences suggest, however, that they constitute a new subfamily in the GTPase superfamily (40). This observation poses the exciting questions of why, mechanistically, an otherwise unique GTPase module is repeated twice in the same cellular pathway, and how such a system might have evolved. Neither question has a satisfactory answer yet.

The Signal Recognition Particle GTPase Cycle

GTP binding—but not GTP hydrolysis—is required for the initial steps of the targeting reaction (11); thus GTP binding is thought to accompany targeting, and GTP hydrolysis to cause release of SRP from its receptor (Figure 1). GTP hydrolysis by SRP is stimulated significantly by interaction with SR. Indeed, the bacterial GTPases Ffh and FtsY interact directly and act as regulatory proteins for each other. In vitro experiments taking advantage of an FtsY mutation that changes its substrate specificity from GTP to XTP demonstrate that FtsY stimulates GTP hydrolysis by SRP in a reaction requiring XTP, and that, reciprocally, SRP stimulates XTP hydrolysis by FtsY in a reaction requiring GTP (41). Thus, these two GTPases act as GTP-dependent GTPase-activating proteins (GAPs) for each other, a reciprocally symmetric interaction that is unique among known GTPases.

Additional components are capable of modulating the enzymatic properties of SRP and SR during the targeting reaction. For example, GTP binding by SRP is thought to be stimulated by the ribosome (37). Thus, ribosome-bound SRP is

likely in its GTP-bound form prior to its interaction with SR. In contrast, signal sequence binding to SRP is thought to inhibit GTP binding and hydrolysis (42, 43). Taken together, these observations are reconciled by a model in which signal sequence binding to SRP inhibits GTP hydrolysis (but not GTP binding) until the ribosome–nascent chain complex is transferred to the translocation apparatus in the membrane (Figure 1).

As with SRP, the GTPase activity of SR is also modulated by other components. Ribosomes appear to interact in a GTP-dependent manner with the β-subunit of SR, leading to stimulation of GTP hydrolysis by SRβ (38). Interestingly, recent data suggest that SRβ must be in its GTP-bound form in order to interact with SRα (44). These data suggest that localization of SRα to the ER membrane might be regulated indirectly by the ribosome. In bacteria, which lack an SRβ homolog, localization of SR may be mediated by anionic phospholipids in the membrane, via an interaction that also stimulates its GTPase activity (24, 45). In addition, recent data suggest that the translocon may regulate the GTP hydrolysis cycle of the SRP-SR complex (39).

The current model is that the ribosome and translocon together regulate the intrinsic enzymatic activities of the SRP and SR as part of a proofreading mechanism to ensure efficient, unidirectional targeting of proteins to the membrane. In this view, SRP and SR function as molecular matchmakers, SRP picking up ribosomes with exposed signal sequences in the cytosol, and SR picking up empty translocons in the plane of the membrane. As a result of these interactions, SRP and SR would both be set to the GTP-bound state and hence display high affinity for one another, which in turn would facilitate transfer of the nascent chain from SRP to the translocon and allow the establishment of a functional ribosome–membrane junction. Despite considerable progress and the general appeal of this model, most of the precise details of the SRP GTPase cycle remain elusive.

A Uniquely Conserved GTPase Module

Crystal structures of the GTPase domains from *Thermus aquaticus* Ffh (46) and from *E. coli* FtsY (47) represent a significant advance in our understanding of the SRP GTPases (Figure 2). Ffh and FtsY each contain three domains, two of which, termed the N and G domains, are related at both the sequence and structural levels and comprise the GTPase of each protein. The G domain (Figure 2, green and orange) adopts a classical GTPase-fold (48), in which four conserved sequence motifs (I–IV) are arranged around the nucleotide-binding site. One of these motifs (II) is contained in a unique insertion within the G domain (termed the insertion-box domain, or IBD; Figure 2, orange) that extends the central β-sheet of the domain by two strands and is characteristic for the SRP GTPase subfamily.

The amino-terminal N domain (Figure 2, blue) is a four-helix bundle that packs tightly against the G domain to form a single structural unit, commonly referred to as the NG domain. The N domain is splayed apart at one end, and side chains from the C-terminus of the G domain complete the packing of its hydrophobic core. This interface is conserved in both Ffh and FtsY, and it serves as a hinge

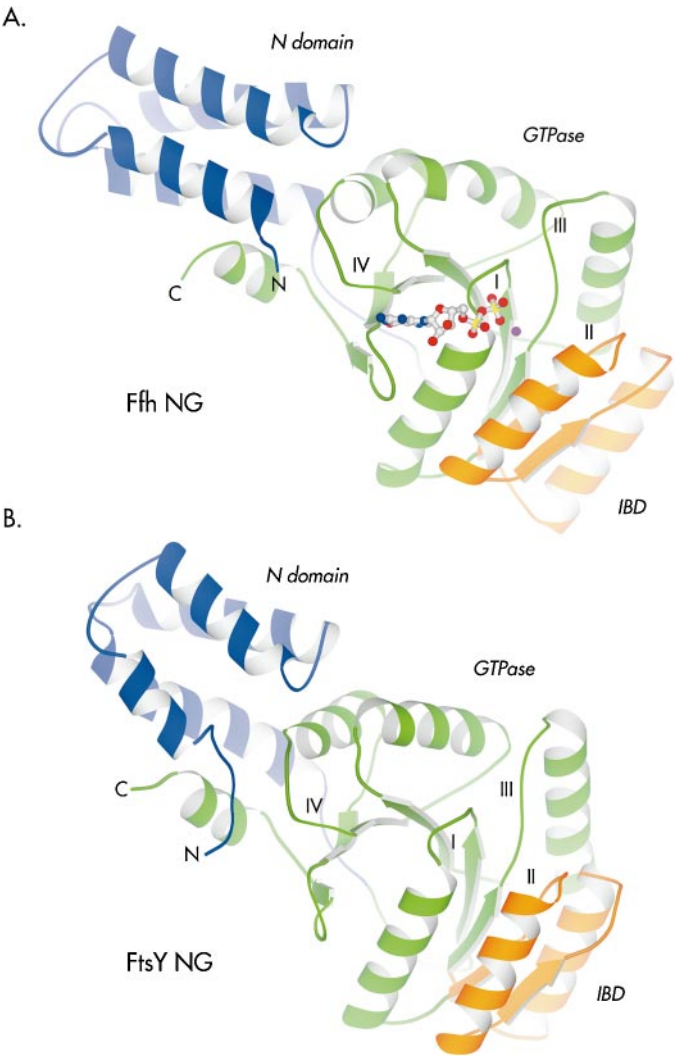

Figure 2 The SRP GTPase fold. (*A*) Mg^{2+} GDP-bound form of the NG domain from *T. aquaticus* Ffh (46, 50). (*B*) Apo form of the NG domain from *E. coli* FtsY (47). The N-terminal N domain (blue) packs tightly against the Ras-like GTPase fold (green). The conserved IBD (orange) is unique to the SRP family of GTPases. The four conserved GTPase sequence motifs are indicated (I–IV). The apo structure of the Ffh NG domain from the archaeon *Acidianus ambivalens* (49) is structurally similar to the NG domains shown here.

about which the relative orientation of the N and G domains varies (49,50). The location of the N domain with respect to the G domain suggests that it is responsive to nucleotide-dependent conformational changes in the SRP GTPases that are distinct from the classical phosphate switch (51). Conserved GTPase motif IV of the G domain, which interacts with the guanine base of bound nucleotide, is adjacent to the conserved N/G interface. This proximity suggests that one function of the N domain is to sense or regulate the GTP-binding state of the G domain.

The Signal Recognition Particle–Receptor Interaction

SRP and SR interact primarily through their respective NG domains. This is supported by the observation that SRP RNA and the third domain of SRP54/Ffh (the M domain) are not required for stimulation of GTPase activity in vitro (52,53). Conversely, reconstituted SRP lacking its NG domain fails to target ribosome–nascent chain complexes to the membrane (54). Furthermore, the NG domain of FtsY can target SRP to the membrane even when fused to an unrelated membrane protein (55).

It is likely that, as with other GTPases, significant conformational changes occur as the SRP GTPases cycle through different stages of nucleotide occupancy. SRP and SR interact in their GTP-bound states but have only low affinity for each other when empty or bound to GDP. Moreover, GTP hydrolysis by SRP and SR is dramatically increased upon formation of the complex, relative to the rate of hydrolysis by the individual components. Nucleotide-dependent changes in the structure of the interacting NG domains are likely to be responsible for these different properties.

The unique IBD of the SRP subfamily of GTPases may play an important role in mediating the SRP-SR interaction. In Ras-like proteins, regions of conserved motifs II and III undergo large conformational changes during the GTPase cycle. Not surprisingly, these regions are also the targets of GAP proteins that stimulate the hydrolysis of GTP. In the SRP GTPases, motif II is located within the IBD (Figure 2) and is too far from the active site in the apo state to catalyze hydrolysis (46,47). One function of the IBD may be to sequester residues within motif II away from the active site prior to formation of the SRP-SR complex. In this way, the GTP-dependent formation of the complex could be coupled to conformational changes within motif II that lead to reciprocal stimulation of GTP hydrolysis by the enzymes (56).

An additional layer of complexity in the SRP-SR interaction is conferred by SRP RNA, which enhances the GTPase activity displayed by the components (42,43). Recently, it was found that SRP RNA dramatically increases the rate at which GTP-loaded SRP and SR associate and dissociate; that is, it promotes complex formation in a catalytic fashion without significantly stabilizing the complex (53). This observation suggests, unexpectedly, that SRP RNA could exert kinetic control on the protein targeting reaction, and thus that the RNA itself could be a target for regulation. Even subtle changes in RNA conformation—such as those that might be induced by signal sequence binding (see below) or interaction with the ribosome

(or both)—could have a dramatic effect on the ability of the SRP to interact with its receptor on a physiologically relevant timescale.

Nucleotide Exchange

In contrast to most GTPases, SRP GTPases have a relatively weak affinity for GDP. The existence of a stable empty state in the structures of the apo-NG domains from Ffh and FtsY is consistent with biochemical evidence in the eukaryotic system suggesting that the apo state is a functionally significant (albeit transient) intermediate (42, 57). After releasing the signal sequence to the translocon, the SRP-SR complex hydrolyzes GTP and SRP dissociates from the receptor. After hydrolysis, most GTPases rely on a guanine-nucleotide exchange factor (GEF) to dissociate GDP, and thereby reset the GTPase switch (40, 51). In contrast, purified complexes of SRP54-SRα and Ffh-FtsY are capable of multiple rounds of GTP hydrolysis in the absence of additional components (42, 43). Thus, it appears that an external GEF is not required by the SRP GTPases (41).

In support of this idea, kinetic analysis of GDP binding to the NG domain of *E. coli* FtsY (58) and to full-length Ffh (59) indicates that the relatively low affinity of these proteins for nucleotide is due primarily to a high dissociation rate. This observation led to the intriguing suggestion that SRP GTPases contain an intrinsic nucleotide exchange activity, and that the IBD might confer this property upon them (58). However, the crystal structures of the *T. aquaticus* Ffh NG domain bound to Mg^{2+}-GDP and GDP reveal no large conformational changes in response to nucleotide release. Instead, the structures suggest that the observed low affinity for GDP results from the concerted action of several elements of the protein structure that act to disrupt the interaction of bound nucleotide with conserved sequence elements in the active site (50). Thus, stabilization of the nucleotide-free state, a hallmark of GEF activity, appears to be achieved intrinsically in the case of the SRP GTPases.

The GTPases of SRP-dependent protein targeting present a fascinating puzzle, as both the biochemical logic and the structural nature of the interaction between the proteins remain to be understood. That GTP hydrolysis can function to provide unidirectionality to the interactions in the targeting reaction provides a basis for rationalizing the mechanism. However, it remains unclear why a mechanism utilizing hydrolysis of two—or, in eukaryotes perhaps even three—GTP molecules has evolved. This question is particularly fascinating because two of the GTPases are structurally related and act as mutual GTPase activators, suggesting that there is an intrinsic symmetry in their interaction.

SIGNAL SEQUENCE RECOGNITION

Background

Cotranslational targeting of a protein to the ER (or the plasma membrane in prokaryotes) is initiated when SRP binds to a hydrophobic signal sequence present

at the N terminus of the nascent chain. These sequences typically comprise ~20–30 residues organized into three general regions (60): (*a*) a short, positively charged N-terminal region, (*b*) a central hydrophobic core, and (*c*) a more polar C-terminal region that includes a cleavage site for signal peptidase (61).

Given their function as specific cellular "address codes," signal sequences are surprisingly divergent. Instead of sharing a specific consensus sequence, they display common physicochemical properties. Two features in particular appear to be necessary for entry into the cotranslational targeting pathway. First, the central core of the signal sequence must possess a certain threshold level of hydrophobicity (62). Introduction of even a single charged amino acid side chain into the hydrophobic core renders that sequence nonfunctional for SRP-mediated targeting. Second, signal sequences must be capable of adopting an α-helical conformation. In hydrophobic environments, isolated signal peptides possess significant helical structure (63, 64). Perturbing this conformation by introducing helix-breaking residues into the sequence disrupts its ability to function as a cotranslational targeting signal (65). Interestingly, after release from SRP, signal sequences bind to the mammalian translocation channel in an α-helical conformation (66). Beyond hydrophobicity and helical conformation, however, little restriction is placed on either the length or the primary sequence of a functional signal sequence.

Owing to their remarkable degeneracy, SRP must be capable of binding to a wide variety of signal sequences. Cross-linking and functional studies implicate the M domain of SRP54/Ffh as the primary site of signal sequence binding to SRP (67–69). The M domain derives its name from the fact that it contains a high percentage of methionine (Met) residues, a feature that is conserved from bacterial to mammalian SRP (17). In *E. coli*, about 16% of the amino acids in the M domain are Met; this frequency is more than 6 times greater than that at which Met typically occurs in proteins (70). That this abundance is conserved suggests that Met is fundamentally important to the structure and function of the M domain. The hydrophobic Met side chain is flexible, because it is unbranched and because of the unique conformational properties of its thioether linkage (71). This feature, in combination with secondary structure prediction, led to the proposal that Met and other conserved hydrophobic residues in the M domain are arranged such that their flexible side chains form "bristles" lining a hydrophobic binding site (17). These bristles would confer sufficient plasticity to allow SRP to recognize a wide variety of signal sequences.

A Dynamic and Hydrophobic Groove in the M Domain of Ffh

Experimental support for the Met-bristle hypothesis comes from the crystal structure of full-length Ffh from *T. aquaticus* (72). The structure reveals that the M domain is constructed from amphipathic α-helices arranged around a small hydrophobic core (Figure 3*A*). The most prominent structural feature of the domain is a large exposed hydrophobic groove, constructed from three helices (αM1, αM2, and αM4) and a long flexible loop (the "finger loop", connecting αM1 and αM2).

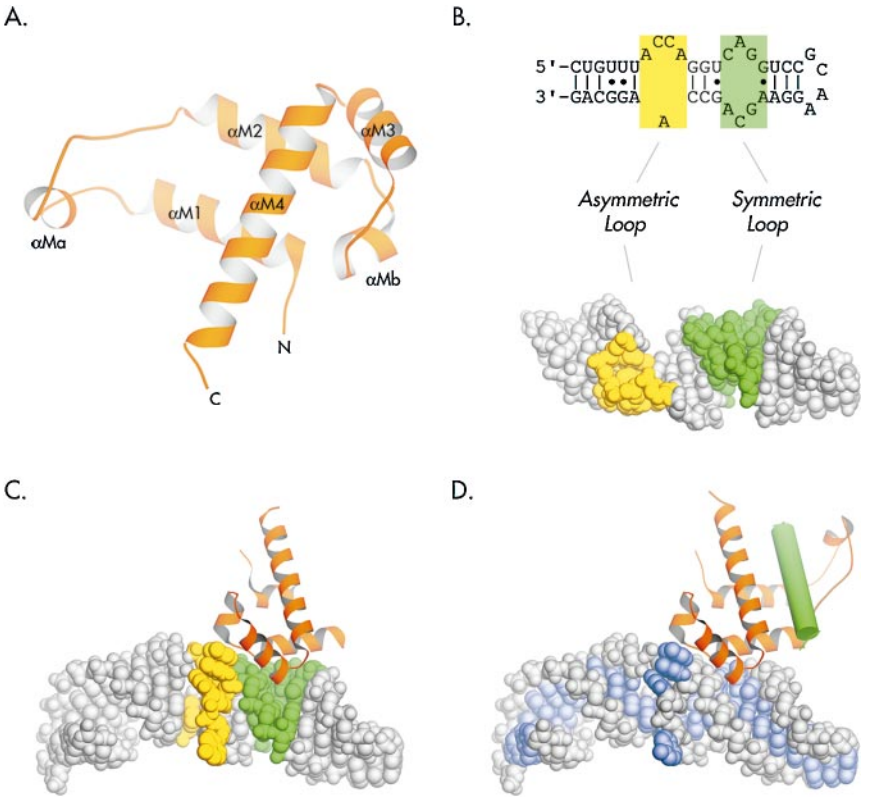

Figure 3 The ribonucleoprotein core of the SRP. (*A*) Apo form of the M domain from full-length *T. aquaticus* Ffh (72). The loop connecting helices αM1 and αM2 (the finger loop) is a flexible motif that forms part of the putative binding groove for the hydrophobic signal sequence. (*B*) Solution structure of the apo form of domain IV from *E. coli* SRP RNA (83). Also shown is a secondary structural representation of the domain IV sequence. Nucleotides within the symmetric and asymmetric loops are highlighted in green and yellow, respectively. (*C*) The M domain-domain IV complex from *E. coli* Ffh (74). (*D*) Modeling the *T. aquaticus* M domain onto the structure of the *E. coli* complex reveals that a signal sequence (green cylinder) bound in the hydrophobic groove of the M domain could simultaneously contact the backbone of domain IV RNA (gray). Nucleotide bases are shown in blue.

The dimensions of the M domain groove are compatible with binding a signal sequence in an α-helical conformation. Strikingly, the surface of the groove is lined almost entirely with side chains from phylogenetically conserved hydrophobic residues. In *T. aquaticus*, which lives at 75°C, many of the conserved Met residues found in SRP54/Ffh are replaced by branched hydrophobic residues (Leu, Val and Phe), presumably because increased thermal motion at elevated temperatures

eliminates the need for the more flexible Met side chain in the thermophilic protein. Modeling the location of Met residues from *E. coli* Ffh onto the structure of the *T. aquaticus* Ffh M domain reveals that the vast majority of these residues map to the groove. Thus, a groove lined with flexible hydrophobic side chains is a conserved feature of the M domain, in support of the hypothesis that the capacity of SRP to recognize many signal sequences is conferred by plasticity of the binding site.

In addition to flexibility conferred by hydrophobic side chains within the M domain groove, main chain flexibility may also be functionally important for signal sequence binding. Analysis of three different (but related) crystal forms of *T. aquaticus* Ffh (72) reveal that the finger loop adjusts its conformation in response to slight shifts in crystal packing. In the crystal structure of a fragment of the human M domain, the finger loop region (and helix αM1) is completely rearranged to form a well-ordered M domain dimer (73), although the physiological relevance of this arrangement remains to be determined. In the structure of the *E. coli* M domain (bound to a fragment of SRP RNA), the finger loop is completely disordered (74). Taken together, these structures indicate that the finger loop is a highly flexible motif and suggest the attractive idea that it adjusts its conformation in response to signal sequence binding and release. Although the precise molecular details of the signal sequence–SRP interaction remain to be determined, it now seems clear that the structural elements forming the M domain groove provide the necessary flexible and hydrophobic environment to accommodate many different signal sequences.

Compared to prokaryotic Ffh, the C terminus of eukaryotic SRP54 extends beyond helix αM4 in the M domain by as many as $\sim$100 residues. Proximity of this extension to the hydrophobic groove of the M domain, and the continued abundance of Met residues in the extension, implicates this region in signal sequence binding. This is borne out experimentally; deletion of a portion of the C terminus from mammalian SRP54 abolishes cross-linking to signal sequences in vitro (68). It is appealing to envision that the longer eukaryotic C terminus might increase the hydrophobic surface area available for signal sequences binding. Yet, the hydrophobic cores of eukaryotic signal sequences are shorter on average than the transmembrane regions thought to act as SRP-pathway-directed signal sequences in prokaryotic inner membrane proteins. Thus, why the C terminal extension is present in eukaryotic SRP54 but not in prokaryotic Ffh remains a mystery.

The Ribonucleoprotein Core of the Signal Recognition Particle

The M domain also mediates the high-affinity interaction of SRP54/Ffh with SRP RNA (67, 69). Adjacent to its putative binding groove for the signal sequence, the M domain folds into a well-ordered small globular structure in which two helices (αM3 and αM4) are arranged in a classical helix-turn-helix (HTH) motif (72) (Figure 3*A*)—an arrangement found in many DNA-binding proteins (75). Deletion mutants of the M domain from *Bacillus subtilis* Ffh define a region spanning helices αM2 to αM4 that is necessary (and sufficient) for binding SRP RNA (76). Within this region, the HTH motif contains a highly conserved, positively charged

sequence motif that includes arginine, serine, and glycine residues that are essential for high-affinity binding to SRP RNA (77).

An SRP-like component is also present in chloroplasts where, together with an organellar FtsY homolog, it targets proteins for translocation across the thylakoid membrane (78–80). Surprisingly, despite possessing the conserved HTH sequence motif in its M domain, chloroplast SRP contains no known RNA subunit. Instead, the chloroplast SRP54 homolog is associated with two copies of a 43-kilodalton protein (78) of unknown function that may replace a functional role of the RNA in cytosolic SRP. Proteins targeted to the thylakoid membrane are first imported into chloroplasts from the cytosol; thus, this is the only known system in which SRP must clearly work posttranslationally. Consistent with the view that one important role of SRP RNA is to interact with the ribosome, SRP RNA may be dispensable in chloroplasts because chloroplast SRP operates exclusively in a posttranslational mode.

The binding site for the M domain on SRP RNA is an ~50-nucleotide motif termed domain IV. It consists of three short helical segments connected by a symmetric and an asymmetric internal loop and bounded on one end by a tetraloop (Figure 3*B*). Sequences within the two internal loops are highly conserved; indeed, it was the realization that this sequence conservation exists that first suggested that SRP operates in prokaryotes (20). Nucleotides within the internal loops are protected from chemical and enzymatic modification upon binding to Ffh (81). Together, Ffh and domain IV define the minimal ribonucleoprotein core that is both necessary and sufficient for the function of prokaryotic SRP in vivo (74).

The solution structure of isolated domain IV (82, 83) (Figure 3*B*) and its crystal structure (84) reveal that the two internal loops are constructed from distinctly different architectures. The symmetric loop is highly ordered in the absence of its binding partner, and it contains a number of unusual base interactions between phylogenetically conserved nucleotides. As a result, its minor groove is shallower than normally observed with A-form RNA. In contrast, the asymmetric loop is flexible. This confers significant variation to the relative orientation of the double-helical regions flanking the asymmetric loop, as was first inferred from enzymatic and chemical protection experiments with 4.5S RNA (81).

An exciting advance in our understanding of the structure and function of SRP comes from the crystal structure of the *E. coli* Ffh M domain bound to domain IV of 4.5S RNA (Figure 3*C*) (74). In this structure, the HTH motif of Ffh binds to a distorted minor groove of the RNA helix. Comparison of the unbound and bound conformations reveals that domain IV undergoes a dramatic structural rearrangement upon formation of the complex. In the protein-RNA complex, the asymmetric loop of domain IV is held in an ordered and highly unusual conformation, with four of the five bases that make up the loop fully exposed on the outside of the RNA helix. In contrast, bases in the symmetric loop undergo only relatively minor rearrangements upon binding to the M domain. Although the two loops are separated in the secondary structure by a double-helical segment of three base pairs, in the complex the bases from the two loops form a virtually contiguous surface to which the protein binds.

This unusual surface presented to Ffh requires the HTH motif to contact the RNA in a manner quite different from that observed for classical HTH proteins that bind DNA. DNA-binding proteins project amino acid side chains from a single helix (the "recognition helix", corresponding to helix αM4 in the M domain) deep into the major groove of the DNA (85). In contrast, the M domain presents two helices (αMb and αM3) to the minor groove of the RNA, making numerous direct and solvent-mediated contacts with the distorted surface of the RNA. In this manner, the protein—which itself undergoes no large conformational rearrangements upon binding—brings the symmetric and asymmetric loops together to form a highly specific and stable protein-RNA interface (compare Figure 3*B* and 3*C*).

The intimate structural relationship between the M domain and its RNA implies that they are functionally intertwined. In the crystal structure of the complex, regions of the M domain that form the proposed binding site for the signal sequence are disordered. Yet, modeling the structure of the *T. aquaticus* M domain onto domain IV reveals that the binding pocket for the signal sequence extends onto a ledge of RNA backbone formed by the helical segment between the symmetric loop and the terminal tetraloop of domain IV (Figure 3*D*). This juxtaposition suggests that signal sequences may bind to both the protein and RNA components of SRP—their hydrophobic core interacting with the methionine-lined pocket of the M domain and their polar N- or C-terminal region contacting the RNA backbone (74). Thus, binding of signal sequences might be accompanied by conformational changes in the SRP RNA. In this way, the RNA might directly participate in determining the ligand-bound state of SRP and possibly its ability to interact with upstream (ribosome) or downstream (SRP receptor) components of the targeting pathway (53).

Understanding the mechanism of signal sequence binding and release by SRP presents a fascinating challenge. That SRP provides a dynamic, hydrophobic environment to bind a wide variety of signal sequences is now clear. However, fundamentally related questions remain a mystery. How, for instance, does allosteric communication between the NG and M domains of SRP affect signal sequence binding (42, 68, 86, 87)? And what is the event that triggers release of the signal sequence once SRP is docked to its receptor? This question is particularly interesting in light of the fact that multiple components—the GTPase domain of SRP, SRP RNA, SR, or perhaps even the translocon itself—may contribute to the process. Our full understanding of the rules governing the binding and release of signal sequences awaits a more precise structural and functional description of these components at the molecular level.

ELONGATION ARREST

Background

Protein targeting by SRP is thought to be obligatorily cotranslational (with the exception of chloroplast SRP, described above). In eukaryotes, recognition of the

signal sequence by SRP slows synthesis of the nascent chain while the ribosome–nascent chain complex is targeted to the ER (88). This process, termed elongation arrest, has so far been described only in eukaryotes, although archaean and some prokaryotic SRP RNAs also contain Alu-like sequences (see below) that are required for this activity in mammalian SRP. Elongation arrest is thought to increase translocation efficiency by lengthening the amount of time that SRP can maintain the nascent chain in a translocation-competent state. This was first demonstrated in vitro by adding microsomal membranes at various times to synchronized translation reactions (89). More recently, experiments in yeast have identified mutations in the Alu domain of SRP that confer a defect in the coupling of protein translation and translocation, demonstrating the importance of elongation arrest activity in vivo (90).

The Alu Domain

Elongation arrest is mediated by the Alu domain of eukaryotic SRP, comprising two protein subunits (termed SRP9 and SRP14) bound to the Alu sequences of SRP RNA. *E. coli* SRP lacks an elongation arrest activity, and to date, no homologs of SRP9 or SRP14 have been identified in either the archaebacterial or prokaryotic kingdoms. SRP9 and SRP14 exist as an obligate heterodimer, termed SRP9/14, and may be fused together into a single polypeptide and still function indistinguishably from the wild-type proteins in elongation arrest (91).

Although the two proteins possess only limited sequence homology, the crystal structure of the SRP9/14 heterodimer from *Mus musculus* reveals that SRP9 and SRP14 are structurally homologous, each comprising an α-β-β-β-α fold (Figure 4*A*) (92). Indeed, in yeast, two copies of SRP14 are thought to form a homodimer that takes the place of the SRP9/14 heterodimer in higher eukaryotes (90). SRP9 and SRP14 associate in a psuedo twofold symmetric arrangement to form a single structural unit defined by a distinctive saddle shape arising from the formation of a continuous six-stranded β-sheet (Figure 4*A*). The heterodimer interface is composed mainly of residues from the β1 strand and α2 helix of each protein, as well as the C-terminal region of SRP14. Biochemical analysis of SRP9/14 mutants reveals that while deletion of either the α1 or the α2 helix of SRP14 disrupts dimerization, deletion of the α2 helix of SRP9 has little effect (93). Thus, despite their structural similarity and symmetric interaction, the two proteins make different contributions to formation of the physiological dimer.

SRP9/14 binds with subnanomolar affinity to the Alu sequences of SRP RNA (94, 95). The Alu RNA comprises two domains: a 5′ domain that includes the first 47 nucleotides of SRP RNA and a 3′ domain of 39 nucleotides that includes the last 17 nucleotides of SRP RNA (Figure 4*B*). The 5′ domain consists of a three-way junction in which two helical hairpins are connected to a third helical stem by a conserved U-turn motif. The 5′ domain contains the most highly conserved nucleotides within the Alu RNA and is capable of binding to SRP9/14 by itself

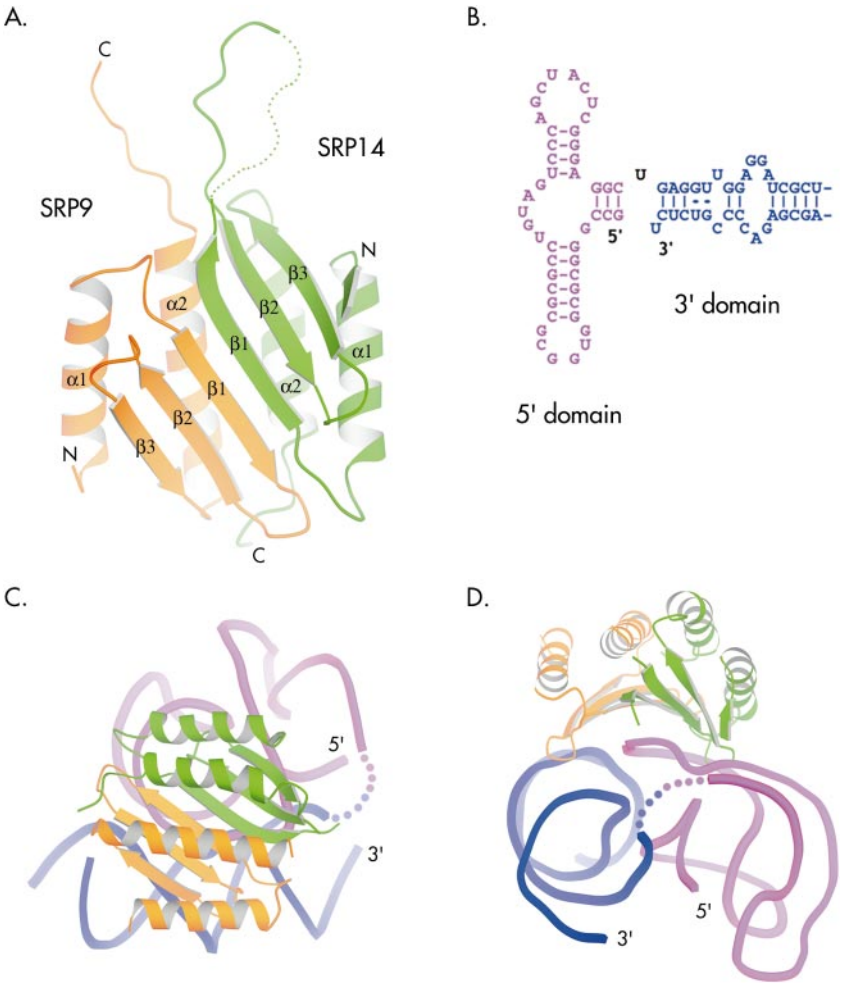

Figure 4 The SRP Alu domain. (*A*) The SRP9/14 heterodimer is a single functional unit formed by the association of two polypeptide chains, SRP9 (orange) and SRP14 (green) (92). The loop connecting β1 and β2 in SRP14 is disordered in the crystal and is indicated as a dotted line. (*B*) Secondary structure of the SRP Alu RNA, the binding site of SRP9/14. The 5′ domain (magenta) and the 3′ domain (blue) are linked together in a flexible manner. (*C*) Model of the Alu domain (96). Interdomain flexibility about the linker region (dotted line) allows formation of a compact ribonucleoprotein complex. (*D*) Alternative view of the Alu domain emphasizing the interaction between the saddle-shaped surface of SRP9/14 and both domains of the Alu RNA. The 5′ domain is capable of binding to SRP9/14, even in the absence of the 3′ domain.

(96). The 3′ domain consists of two helical segments connected by an internal asymmetric loop, and it also contributes to binding the SRP9/14 heterodimer, as suggested by chemical footprinting of the protein complex on Alu RNA (94).

The crystal structure of the 5′ domain bound to SRP9/14 reveals a compact RNA fold consisting of two interacting helical stacks connected by the central U-turn motif (96). SRP9/14 binds to the core of the RNA fold including the central U-turn, but the proteins bind asymmetrically, with the majority of protein-RNA contacts made to the concave β-sheet surface of SRP14. These interactions are mediated primarily by positively-charged side chains that directly contact the phosphate backbone of the RNA, and there are no base-stacking interactions with aromatic side chains. It is likely that the 5′ domain-SRP9/14 complex by itself is biologically significant. This notion is based on the observation that the structural integrity of the SRP9/14 binding site within the 5′ domain is required for efficient transcription of SRP RNA (97). Thus, the compactly folded 5′ domain-SRP9/14 complex may affect the processing and/or assembly of SRP, possibly by interacting directly with components of the transcriptional machinery.

The Alu RNA is unusual in that it is formed from the extreme 5′ and 3′ ends of the much larger SRP RNA. Chemical footprinting experiments suggest that regions of both the 5′ and 3′ domains contact SRP9/14 (94). Tethering the 5′ and 3′ ends of SRP RNA together blocks high affinity binding to SRP9/14, unless the ends are joined by an extended linker sequence (98). Taken together, these data suggest that flexibility between the 5′ and 3′ domains is required so that the RNA can undergo a conformational change that permits binding to SRP9/14.

The low-resolution crystal structure of a modified Alu RNA comprising both the 5′ and 3′ domains bound to the SRP9/14 heterodimer provides a structural model of the entire Alu domain (96). In the crystal, interactions are formed between adjacent complexes, and these are interpreted in the context of monomeric SRP by invoking the 5′–3′ interdomain flexibility evident from biochemical studies. According to this model, the 3′ domain, which forms a double helical structure, packs against the 5′ domain and makes extensive contacts with the concave β-sheet surface of SRP9 (Figure 4*C–D*). The model rationalizes how SRP14 mutants deficient in binding SRP9 regain the ability to form stable complexes with SRP9 in the presence of SRP RNA (93) and is consistent with chemical footprinting experiments (94) and with comparative sequence analysis of the Alu RNA (99). Thus, assembly of the Alu domain is likely to involve a dramatic conformational rearrangement in the Alu RNA that leads to the formation of the compact and functional ribonucleoprotein complex.

Interaction with the Ribosome

Eukaryotic SRP must perform two distinct functions simultaneously during the initial stage of cotranslational targeting—binding to the signal sequence and pausing translation by the ribosome. Structures of the ribosome (100, 101) and of

ribosome-translocon complexes (102, 103) define a channel through which the nascent polypeptide chain exits the ribosome. Consistent with this assignment, SRP can be cross-linked to ribosomal proteins L23 and L29, located near the exit site for the nascent chain (M. Poole and B. Dobberstein, personal communication). The site at which the Alu domain interacts with the ribosome to pause translation, however, is unknown. It has been proposed that the Alu domain interacts with the ribosome near the A-site, located ~160 Å from the putative polypeptide exit site (93, 104). Electron microscopy reveals that mammalian SRP possesses a rod-like shape of approximately 60 × 240 Å (105), consistent with a model in which one end of SRP binds to the nascent chain at the polypeptide exit site, while its other end (the Alu domain) binds near the ribosomal A-site to pause translation.

Beyond a phenomenological description, relatively little is known about the elongation arrest activity of the eukaryotic SRP. The functional rationale, biochemical mechanism, and structural basis of this activity remain to be understood. The components of the ribosome that the Alu domain interacts with are unknown, and the structure of the Alu domain, which shows no obvious similarity to known structures of the translational machinery, provides few additional clues. A number of intriguing questions remain: Why is elongation arrest a conserved feature in eukaryotes but dispensable in at least some prokaryotes? What leads to release of elongation arrest after targeting has occurred? And how does SRP interact with the ribosome to simultaneously carry out activities for both elongation arrest and signal-sequence binding? High-resolution structural analysis of ribosome-SRP complexes, as well as genetic analysis of the yeast SRP (90), should soon provide us with answers to at least some of these questions.

FUTURE PERSPECTIVE

The tremendous advances in our understanding of cotranslational protein targeting over the past twenty years are the direct result of a combination of genetic, biochemical, and structural analyses. Yet many of the molecular details of this process remain a mystery. SRP is an ancient ribonucleoprotein machine that functions in the context of other cellular complexes to direct proteins to the cell membrane. It accomplishes this task, in part, by coordinating cycles of binding and release of the nascent chain in the right molecular context and at the right time. The many inputs to the machine—namely, the ribosome, signal sequences, nucleotide, the SRP receptor, and the translocon—all modulate its activity. Understanding this process and its regulation requires that we move beyond a static description of the biochemical and structural properties of the individual components (much of which is now in hand) toward an integrated picture that takes into account the dynamic and contextual properties of this remarkable cellular machine.

LITERATURE CITED

1. Walter P, Blobel G. 1980. *Proc. Natl. Acad. Sci. USA* 77:7112–16
2. Gilmore R, Walter P, Blobel G. 1982. *J. Cell Biol.* 95:470–77
3. Walter P, Blobel G. 1982. *Nature* 299: 691–98
4. Meyer DI, Dobberstein B. 1980. *J. Cell Biol.* 87:503–8
5. Gilmore R, Blobel G, Walter P. 1982. *J. Cell Biol.* 95:463–69
6. Connolly T, Gilmore R. 1989. *Cell* 57: 599–610
7. Johnson AE, van Waes MA. 1999. *Annu. Rev. Cell Dev. Biol.* 15:799–842
8. Matlack KE, Mothes W, Rapoport TA. 1998. *Cell* 92:381–90
9. Pohlschroder M, Prinz WA, Hartmann E, Beckwith J. 1997. *Cell* 91:563–66
10. Miller JD, Walter P. 1993. *Ciba Found. Symp.* 176:147–59
11. Connolly T, Rapiejko PJ, Gilmore R. 1991. *Science* 252:1171–73
12. Muller M, Koch HG, Beck K, Schafer U. 2000. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107–57
13. Hann BC, Walter P. 1991. *Cell* 67:131–44

13a. Walter P, Johnson AE. 1994. *Annu. Rev. Cell Biol.* 10:87–119

14. Kurzchalia TV, Wiedmann M, Girshovich AS, Bochkareva ES, Bielka H, Rapoport TA. 1986. *Nature* 320:634–36
15. Krieg UC, Walter P, Johnson AE. 1986. *Proc. Natl. Acad. Sci. USA* 83:8604–8
16. Romisch K, Webb J, Herz J, Prehn S, Frank R, et al. 1989. *Nature* 340:478–82
17. Bernstein HD, Poritz MA, Strub K, Hoben PJ, Brenner S, Walter P. 1989. *Nature* 340:482–86
18. Poritz MA, Bernstein HD, Strub K, Zopf D, Wilhelm H, Walter P. 1990. *Science* 250:1111–17
19. Siegel V, Walter P. 1986. *Nature* 320:81–84
20. Poritz MA, Strub K, Walter P. 1988. *Cell* 55:4–6
21. Tajima S, Lauffer L, Rath VL, Walter P. 1986. *J. Cell Biol.* 103:1167–78
22. Miller JD, Tajima S, Lauffer L, Walter P. 1995. *J. Cell Biol.* 128:273–82
23. Ogg SC, Barz WP, Walter P. 1998. *J. Cell Biol.* 142:341–54
24. de Leeuw E, Poland D, Mol O, Sinning I, ten Hagen-Jongman CM, et al. 1997. *FEBS Letters.* 416:225–29
25. Ladefoged SA, Christiansen G. 1997. *Gene* 201:37–44
26. Luirink J, ten Hagen-Jongman CM, van der Weijden CC, Oudega B, High S, et al. 1994. *EMBO J.* 13:2289–96
27. Beckwith J. 1991. *Science* 251:1161–62
28. Bassford P, Beckwith J, Ito K, Kumamoto C, Mizushima S, et al. 1991. *Cell* 65:367–68
29. Macfarlane J, Muller M. 1995. *Eur. J. Biochem.* 233:766–71
30. de Gier JW, Mansournia P, Valent QA, Phillips GJ, Luirink J, von Heijne G. 1996. *FEBS Lett.* 399:307–9
31. Ulbrandt ND, Newitt JA, Bernstein HD. 1997. *Cell* 88:187–96
32. Tian H, Boyd D, Beckwith J. 2000. *Proc. Natl. Acad. Sci. USA* 97:4730–35
33. Phillips GJ, Silhavy TJ. 1992. *Nature* 359:744–46
34. Seluanov A, Bibi E. 1997. *J. Biol. Chem.* 272:2053–55
35. Valent QA, Scotti PA, High S, de Gier JW, von Heijne G, et al. 1998. *EMBO J.* 17:2504–12
36. Powers T, Walter P. 1997. *EMBO J.* 16: 4880–86
37. Bacher G, Lutcke H, Jungnickel B, Rapoport TA, Dobberstein B. 1996. *Nature* 381:248–51
38. Bacher G, Pool M, Dobberstein B. 1999. *J. Cell Biol.* 146:723–30

39. Song W, Raden D, Mandon EC, Gilmore R. 2000. *Cell* 100:333–43
40. Bourne HR, Sanders DA, McCormick F. 1990. *Nature* 348:125–32
41. Powers T, Walter P. 1995. *Science* 269: 1422–24
42. Miller JD, Wilhelm H, Gierasch L, Gilmore R, Walter P. 1993. *Nature* 366:351–54
43. Miller JD, Bernstein HD, Walter P. 1994. *Nature* 367:657–59
44. Legate KR, Falcone D, Andrews DW. 2000. *J. Biol. Chem.* 275:27439–46
45. de Leeuw E, te Kaat K, Moser C, Menestrina G, Demel R, et al. 2000. *EMBO J.* 19:531–41
46. Freymann DM, Keenan RJ, Stroud RM, Walter P. 1997. *Nature* 385:361–64
47. Montoya G, Svensson C, Luirink J, Sinning I. 1997. *Nature* 385:365–68
48. Sprang SR. 1997. *Annu. Rev. Biochem.* 66:639–78
49. Montoya G, Kaat K, Moll R, Schafer G, Sinning I. 2000. *Struct. Fold. Des.* 8:515–25
50. Freymann DM, Keenan RJ, Stroud RM, Walter P. 1999. *Nat. Struct. Biol.* 6:793–801
51. Bourne HR, Sanders DA, McCormick F. 1991. *Nature* 349:117–27
52. Macao B, Luirink J, Samuelsson T. 1997. *Mol. Microbiol.* 24:523–34
53. Peluso P, Herschlag D, Nock S, Freymann DM, Johnson AE, Walter P. 2000. *Science* 288:1640–43
54. Zopf D, Bernstein HD, Walter P. 1993. *J. Cell Biol.* 120:1113–21
55. Zelazny A, Seluanov A, Cooper A, Bibi E. 1997. *Proc. Natl. Acad. Sci. USA* 94:6025–29
56. Jagath JR, Rodnina MV, Wintermeyer W. 2000. *J. Mol. Biol.* 295:745–53
57. Rapiejko PJ, Gilmore R. 1997. *Cell* 89:703–13
58. Moser C, Mol O, Goody RS, Sinning I. 1997. *Proc. Natl. Acad. Sci. USA* 94: 11339–44
59. Jagath JR, Rodnina MV, Lentzen G, Wintermeyer W. 1998. *Biochemistry* 37: 15408–13
60. von Heijne G. 1985. *J. Mol. Biol.* 184:99–105
61. Paetzel M, Dalbey RE, Strynadka NC. 1998. *Nature* 396:186–90
62. Valent QA, Kendall DA, High S, Kusters R, Oudega B, Luirink J. 1995. *EMBO J.* 14:5494–505
63. McKnight CJ, Briggs MS, Gierasch LM. 1989. *J. Biol. Chem.* 264:17293–97
64. Bruch MD, McKnight CJ, Gierasch LM. 1989. *Biochemistry* 28:8554–61
65. Rothe C, Lehle L. 1998. *Eur. J. Biochem.* 252:16–24
66. Plath K, Mothes W, Wilkinson BM, Stirling CJ, Rapoport TA. 1998. *Cell* 94:795–807
67. Romisch K, Webb J, Lingelbach K, Gausepohl H, Dobberstein B. 1990. *J. Cell Biol.* 111:1793–802
68. Lutcke H, High S, Romisch K, Ashford AJ, Dobberstein B. 1992. *EMBO J.* 11:1543–51
69. Zopf D, Bernstein HD, Johnson AE, Walter P. 1990. *EMBO J.* 9:4511–17
70. McCaldon P, Argos P. 1988. *Proteins* 4:99–122
71. Gellman SH. 1991. *Biochemistry* 30:6633–36
72. Keenan RJ, Freymann DM, Walter P, Stroud RM. 1998. *Cell* 94:181–91
73. Clemons WM Jr, Gowda K, Black SD, Zwieb C, Ramakrishnan V. 1999. *J. Mol. Biol.* 292:697–705
74. Batey RT, Rambo RP, Lucast L, Rha B, Doudna JA. 2000. *Science* 287:1232–39
75. Steitz TA, Ohlendorf DH, McKay DB, Anderson WF, Matthews BW. 1982. *Proc. Natl. Acad. Sci. USA* 79:3097–100
76. Kurita K, Honda K, Suzuma S, Takamatsu H, Nakamura K, Yamane K. 1996. *J. Biol. Chem.* 271:13140–46
77. Althoff S, Selinger D, Wise JA. 1994. *Nucleic Acids Res.* 22:1933–47
78. Tu CJ, Schuenemann D, Hoffman NE. 1999. *J. Biol. Chem.* 274:27219–24

79. Kogata N, Nishio K, Hirohashi T, Kikuchi S, Nakai M. 1999. *FEBS Lett*. 447:329–33
80. Franklin AE, Hoffman NE. 1993. *J. Biol. Chem*. 268:22175–80
81. Lentzen G, Moine H, Ehresmann C, Ehresmann B, Wintermeyer W. 1996. *RNA* 2:244–53
82. Schmitz U, James TL, Lukavsky P, Walter P. 1999. *Nat. Struct. Biol*. 6:634–38
83. Schmitz U, Behrens S, Freymann DM, Keenan RJ, Lukavsky P, et al. 1999. *RNA* 5:1419–29
84. Jovine L, Hainzl T, Oubridge C, Scott WG, Li J, et al. 2000. *Struct. Fold. Des*. 8:527–40
85. Brennan RG. 1992. *Curr. Opin. Struct. Biol*. 2:100–8
86. Newitt JA, Bernstein HD. 1997. *Eur. J. Biochem*. 245:720–29
87. Zheng N, Gierasch LM. 1997. *Mol. Cell*. 1:79–87
88. Walter P, Blobel G. 1981. *J. Cell Biol*. 91:557–61
89. Siegel V, Walter P. 1988. *EMBO J*. 7:1769–75
90. Mason N, Ciufo LF, Brown JD. 2000. *EMBO J*. 19:4164–74
91. Bovia F, Bui N, Strub K. 1994. *Nucleic Acids Res*. 22:2028–35
92. Birse DE, Kapp U, Strub K, Cusack S, Aberg A. 1997. *EMBO J*. 16:3757–66
93. Bui N, Wolff N, Cusack S, Strub K. 1997. *RNA* 3:748–63
94. Strub K, Moss J, Walter P. 1991. *Mol. Cell. Biol*. 11:3949–59
95. Janiak F, Walter P, Johnson AE. 1992. *Biochemistry* 31:5830–40
96. Weichenrieder O, Wild K, Strub K, Cusack S. 2000. *Nature* 408:167–73
97. Emde G, Frontzek A, Benecke BJ. 1997. *RNA* 3:538–49
98. Weichenrieder O, Kapp U, Cusack S, Strub K. 1997. *RNA* 3:1262–74
99. Zwieb C, Muller F, Larsen N. 1996. *Fold. Des*. 1:315–24
100. Cate JH, Yusupov MM, Yusupova GZ, Earnest TN, Noller HF. 1999. *Science* 285:2095–104
101. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. 2000. *Science* 289:920–30
102. Menetret J, Neuhof A, Morgan DG, Plath K, Radermacher M, et al. 2000. *Mol Cell*. 6:1219–32
103. Beckmann R, Bubeck D, Grassucci R, Penczek P, Verschoor A, et al. 1997. *Science* 278:2123–26
104. Siegel V, Walter P. 1988. *Proc. Natl. Acad. Sci. USA* 85:1801–5
105. Andrews DW, Walter P, Ottensmeyer FP. 1985. *Proc. Natl. Acad. Sci. USA* 82: 785–89

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