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Molecular Mechanism of Co-translational Protein Targeting by the Signal Recognition Particle

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Abstract

The Signal Recognition Particle (SRP) is a key component of the cellular machinery that couples the ongoing synthesis of proteins to their proper localization, and has often served as a paradigm for understanding the molecular basis of protein localization within the cell. The SRP pathway exemplifies several key molecular events required for protein targeting to cellular membranes: the specific recognition of signal sequences on cargo proteins, the efficient delivery of cargo to the target membrane, the productive unloading of cargo to the translocation machinery, and the precise spatial and temporal coordination of these molecular events. Here we highlight recent advances in our understanding of the molecular mechanisms underlying this pathway, and discuss new questions raised by these findings.

Keywords

protein targeting; protein translocation; signal recognition particle; GTPases; signal sequence; molecular regulation

Proper localization of proteins to their correct cellular destinations is essential for the structure, organization, and function of all cells. Since Blobel's 'signal hypothesis', numerous pathways have been uncovered that transport proteins to various subcellular compartments. The co-translational targeting of proteins by the Signal Recognition Particle (SRP) is one of the most extensively studied protein targeting pathways, and has provided an excellent model system for in-depth mechanistic dissections to uncover the molecular basis of cellular protein localization. Here we highlight the key events in the SRP pathway and recent advances in our understanding of the molecular mechanisms underlying these events. For more comprehensive reviews of protein targeting and translocation, the readers are referred to the excellent earlier reviews (1-5).

Overview of SRP-dependent protein targeting

SRP comprises a universally conserved and essential component of the cellular machinery that targets translating ribosomes to cellular membranes, thus ensuring the proper biogenesis of membrane and secretory proteins. This process begins when a nascent polypeptide carrying a signal sequence emerges from the translating ribosome and is recognized by the SRP (Fig. 1A, blue). The ribosome•nascent chain complex (referred to as the RNC or cargo) is delivered to the target membrane via the interaction of SRP with the SRP receptor (SR; Fig. 1A, green). There, the cargo is transferred to the Sec61p (or secYEG in archaea and bacteria) translocon, which translocates the growing polypeptide across the membrane or

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integrates it into the membrane bilayer (Fig. 1A, right path). SRP and SR then dissociate from one another to enter subsequent rounds of targeting.

Mammalian SRP is a ribonucleoprotein complex comprised of six proteins and the 7S SRP RNA. It can be divided into two structurally and functionally dissectable domains: the S-domain, comprised of domains II–IV of 7S RNA and the SRP 19, 54, and 68/72 protein subunits, and the Alu domain, comprised of domain I of 7S RNA and the SRP 9/14 subunits (Fig. 1B). The most conserved subunit, SRP54, contains two structurally and functionally dissectable domains (Fig. 1C): a methionine-rich M-domain that binds the signal sequences and the SRP RNA (6-8), and a GTPase, NG-domain that interacts with the SR (9,10). Bacterial SRP consists of a SRP54 homologue, Ffh, in complex with a 4.5S RNA that contains the most conserved domain IV of the 7S RNA (Fig. 1B). Surprisingly, the much simpler bacterial SRP can replace its eukaryotic homologues to carry out efficient targeting of mammalian proteins into ER microsomes (11); this demonstrates the remarkable evolutionary conservation of SRP and shows that SRP54 and the SRP RNA comprise the functional core of SRP.

Eukaryotic SR is a heterodimeric complex of the SRα and SRβ subunits (12). SRα is a soluble protein and contains an NG-domain highly homologous to that of SRP54 (Fig. 1D, left). An X-domain in SRα binds to SRβ, a transmembrane protein, thus localizing SR to the ER membrane (Fig. 1D, left) (12,13). Intriguingly, SRβ also contains a GTPase domain whose GTP-bound state is required for binding SRα (13). GDP release from SRβ is accelerated by Sec61p, leading to the suggestion that the latter provides a nucleotide exchange factor for SRβ (14). Thus the membrane localization of SRα might not be an obligate event, but is rather subject to regulation by the GTPase cycle of SRβ. In bacteria, SR is a single protein FtsY which is highly homologous to SRα (Fig. 1D, right). An N-terminal A-domain allows FtsY to peripherally attach to the membrane through interactions with anionic phospholipids and with the translocon (15-17).

SRP is a key component of the cellular machinery that delivers endoplasmic reticulum (ER)-resident and secretory proteins to the mammalian ER membrane, or inner membrane proteins to the bacterial plasma membrane. Nevertheless, alternative pathways also mediate efficient protein secretion. The majority of bacterial secretory and outer-membrane proteins are targeted post-translationally by the chaperone SecB to the ATPase SecA, whose action drives the translocation of preproteins across the SecYEG translocon (Fig. 1A, middle path) (1). In yeast, the Sec62/63/71/72 system is the major pathway that mediates protein secretion. Additional targeting pathways likely exist that utilize other cellular machineries (Fig. 1A, left path). Despite the divergence of targeting machinery, the SRP pathway illustrates several key features of protein targeting: (i) the cellular destination of a protein is dictated by its ‘signal sequence’, which engages specific cellular targeting machineries; (ii) targeting factors cycle between the cytosol and membrane, and act catalytically to facilitate the delivery of cargo proteins to the translocation machinery; and (iii) targeting requires coordination of a series of molecular events including cargo loading / unloading, SRP-SR complex assembly / disassembly, and the productive handover of cargo to the translocation machinery. Not surprisingly, such coordination requires energy input, which is often provided by GTPase or ATPase modules in the targeting machinery. Although the components and molecular details differ, these features have been found in various targeting pathways and likely represent general principles of protein targeting.

Cargo Recognition

SRP signal sequences are characterized by a core of 8–12 hydrophobic amino acids that preferentially form an α-helix, but are otherwise highly divergent in length, shape, and

amino acid composition (18-20). This and the unusual abundance of methionine in the SRP54 M-domain led to the 'methionine bristle' hypothesis, in which the flexible sidechains of methionine provide a hydrophobic environment with sufficient plasticity to accommodate diverse signal sequences (21). In support of this model, an early crystal structure of *Thermus aquaticus* Ffh M-domain revealed a potential signal sequence binding groove comprised almost exclusively of hydrophobic residues (8). The first glimpse of a signal sequence bound to SRP was provided by a recent crystal structure of an SRP54-signal peptide fusion protein (Fig. 2, blue and red) (7). In this construct, the interaction of signal peptide occurs *in trans* with the SRP54 from another fusion protein (7). Nevertheless, this structure confirmed that the signal sequence binds in an α-helical conformation to the hydrophobic groove identified by Keenan *et al*, and illustrated important features of signal sequence recognition: (i) the hydrophobic core of the signal peptide helix interacts with helices lining the binding groove by a 4-4 'ridges-into-grooves' helical packing (Fig. 2); (ii) conformational adaptation of the signal sequence occurred upon binding in the groove, which could be a general feature for diverse signal sequences to be recognized by the SRP.

Nevertheless, isolated signal peptides bind SRP weakly, with equilibrium dissociation constants (K_d) in the micromolar range (22). In comparison, ribosomes bind the SRP with K_d values of 70 – 80 nM, and RNC's bearing strong signal sequences bind SRP with K_d's in the sub- to low-nanomolar range (23-25). Thus the ribosome provides an important driving force for recruitment of SRP, and acts synergistically with the signal sequence to allow high affinity SRP-cargo binding. The interaction site of SRP with the RNC was mapped by crosslinking (26) and cryo-EM analyses (27-29), which together showed that, in addition to interactions with the signal sequence, the NG-domain of SRP54 (or Ffh) interacts with ribosomal proteins L23 and to a lesser extent L35/L29, while the M-domain also contacts the 23S ribosomal RNA and the ribosomal protein L22 at the exit tunnel. Intriguingly, the sites at which SRP contacts the RNC largely overlaps with those of Sec61p/SecYEG (27,30), strongly suggesting that SRP and the translocon compete with one another for binding the cargo.

SRP and SR GTPases couple cargo recognition to efficient membrane delivery

After the initial recognition of cargo, rapid SRP-SR interactions must occur to deliver the cargo to the target membrane and then to transfer it to the translocation machinery. Moreover, a productive targeting reaction requires the targeting machinery to minimize non-productive cargo release, futile SRP-SR interactions, and premature disassembly of the cargo•SRP•SR targeting complex. These diverse and sometimes conflicting requirements are met by the ability of the SRP and SR GTPases to act as 'multi-state' regulators that undergo multiple distinct conformational changes in response to cues such as cargo binding and membrane localization (31-34). Each rearrangement provides a discrete point at which regulation can be exerted, thus ensuring the spatial and temporal precision of the different molecular events during protein targeting.

SRP and SR belong to a novel class of GTPases regulated by GTP-dependent dimerization (GAD), whose members also include the Septins, Toc proteins, human GBP1, MnmE, and the Dynamin family of GTPases (35). Unlike Ras-type GTPases that switch between 'on' and 'off' states depending on the bound nucleotide, these GTPases do not undergo substantial conformational changes between the apo-, GDP-, and GTP-bound states. Instead, a series of discrete conformational changes were observed during SRP-SR dimerization (Fig. 3A) (9,10,31,33,36). Beginning with a transient *early* intermediate held together primarily by interactions between the N-domains (Fig. 3A, step 1–2 and right panel) (33), extensive rearrangements occur in both GTPases to allow the formation of extensive

contacts between the NG-domains and direct interactions between the two GTP molecules across the dimer interface, thus giving a GTP-stabilized *closed* complex (Fig. 3A, step 3 and bottom panel) (9,10,31). Finally, a cooperative rearrangement of the Insertion Box Domain (IBD) loops in both proteins positions multiple catalytic residues adjacent to the bound GTP, leading to reciprocal GTPase activation (Fig. 3A, step 4 and left panel) (9,10,31), and GTP hydrolysis drives complex disassembly (Fig. 3A, step 5).

In the absence of biological cues, formation of a stable SRP-SR complex is extremely slow (22,37), due to the low stability of the *early* intermediate and the slow rate at which it rearranges to the *closed* complex (33). The cargo, by stabilizing the early intermediate and prolonging its lifetime, accelerates stable SRP-SR complex assembly 10^2–10^3-fold (25,34). Analogously, anionic phospholipids facilitate the rearrangement of SR into the *closed* conformation, and thereby accelerate stable SRP-SR complex assembly 160-fold (38). Thus cargo-loaded SRP or membrane-bound SR has a substantial kinetic advantage in forming a stable complex with their binding partner (Fig. 3B, step 2). This ensures rapid delivery of cargo to the membrane, and avoids futile interactions between the free SRP and SR.

Once at the membrane, the SRP needs to switch to a cargo-releasing mode and unload its cargo to the translocation machinery. Multiple studies suggested that the rearrangements of the SRP•SR complex from the *early* intermediate to the *closed* and *activated* states play essential roles in this functional switch. The interaction of cargo with the SRP weakens ~400-fold when the *early* targeting complex rearranges to the *closed* and *activated* states (34). Further, mutant GTPases that block the *closed* → *activated* rearrangement allow formation of a stable cargo•SRP•SR complex but block the engagement of cargo with the translocon (39). Finally, crosslinking and cryo-EM analyses showed that in the presence of SR and GTP analogues, the NG domain of SRP becomes mobile and detaches from ribosomal protein L23 (26,40). Together, these results demonstrate that simply bringing the cargo to the membrane in the vicinity of the translocon is not sufficient to initiate translocation; rather, elaborate rearrangements in the SRP•SR complex are required to drive the handover of cargo from the SRP to the translocon (Fig. 3B, steps 3–4).

The timing of GTP hydrolysis is crucial for productive protein transport, as the SRP must transfer its cargo to the translocon before GTP hydrolysis drives the irreversible disassembly of the SRP•SR complex (Fig. 3B, steps 4 vs. 5). Intriguingly, the cargo selectively stabilizes the SRP•SR complex in the *early* state and disfavors its subsequent rearrangements that lead to GTPase activation, thus delaying GTP hydrolysis (Fig. 3A, ⊥)(34,41). This effect, termed ‘pausing’, provides the targeting complex an important time window to search for the membrane translocon and prevents premature GTP hydrolysis that would lead to abortive reactions (Fig. 3B, red arrow d). As discussed below, delay of GTP hydrolysis also provides an important checkpoint to improve the fidelity of protein targeting.

A sequential model for substrate selection by the SRP

How the SRP or any protein targeting pathway faithfully selects the correct cargos has been a challenging question. Like other topogenic sequences that mediate protein localization, SRP signal sequences are highly divergent (18-20,42), and the SRP must be sufficiently flexible to accommodate diverse signal sequences (8,21). Nevertheless, the difference in signal sequences of substrates that engage SRP vs. alternative pathways is relatively minor (43). Thus despite its flexibility, SRP has evolved a strategy to remain highly specific to its substrates. Although it was thought that incorrect cargos lacking strong signal sequences are rejected because they do not bind tightly to the SRP (Fig. 3B, red arrow a), quantitative equilibrium measurements suggested otherwise. SRP displays appreciable affinities even for empty ribosomes and RNCs containing no signal sequences (23-25), and some RNCs

bearing non-SRP substrates bind SRP with high affinity (25). Thus the cargo binding step (Fig. 3B, step 1 and red arrow a), though important, could not provide the sole determinant for specificity.

A recent kinetic dissection demonstrated that the extensive molecular crosstalk between the cargo and the SRP/SR GTPases introduces additional fidelity checkpoints that help reject the incorrect cargos (25). Incorrect cargos could not mediate the formation of a stable and productive early intermediate, and are therefore more likely to exit the SRP pathway prematurely (Fig. 3B, red arrow b). Rearrangement of the *early* intermediate to the *closed* complex, which primes the cargo for unloading, is also 10-fold slower with the incorrect than the correct cargos (Fig. 3B, red arrow c). Finally, incorrect cargos could not effectively delay GTP hydrolysis, and are hence more likely to be rejected through premature GTP hydrolysis (Fig. 3B, red arrow d). A mathematical analysis based on the rate and equilibrium constants of the individual steps, the cellular SRP and SR concentrations, and a 3–5 second time constraint for co-translational protein targeting showed that all of these checkpoints are required to reproduce the experimentally determined pattern of substrate selection by the SRP (25). Thus the fidelity of substrate selection by the SRP is achieved through multiple checkpoints by using a combination of binding, induced fit, and kinetic proofreading mechanisms. Similar strategies have been demonstrated in tRNA synthetases (44), tRNA selection by the ribosome (45), and DNA and RNA polymerases (46,47) and may represent a general principle for complex biological pathways that need to distinguish between the correct and incorrect substrates based on minor differences.

Mammalian SRP: additional layers of complexity

Compared to its bacterial homologue, the mammalian SRP is significantly more complex (Fig. 1B) and adds additional opportunities for regulation. For example, SRP19 is required to organize the conformation of domains III and IV of the 7S RNA and thus enable the binding of SRP54 to the 7S RNA (48,49). Why the mammalian SRP requires this additional layer of allostery remains unclear. The SRP68/72 subunits have been implicated in controlling the interaction of SRP54 with the SR (50), but their precise function remain to be defined. Perhaps the most interesting aspect of mammalian SRP is the ‘Alu’ domain (Fig. 1B), which arrests translation elongation just after the signal sequence emerges from the ribosome. Early biochemical work found that SRP interacts with the ribosome at the step of EF-2 catalyzed translocation of tRNA (51), suggesting that it blocks the binding of elongation factors and thereby arrests translation. Consistent with this model, cryo-EM analysis showed that mammalian SRP forms an elongated, kinked structure, with its Alu domain reaching into the elongation factor binding site of the ribosome (27). A recent study further showed that, although elongation arrest is not a pre-requisite for protein targeting *in vitro*, abolishing this function *in vivo* leads to severe defects in protein targeting and mammalian cell growth (52). Together with the observation that the SRP could not target proteins when the nascent polypeptide exceeds a critical length (24,53), these results suggest that elongation arrest provides a crucial time window that allows the targeting complex to find and engage the translocon before the nascent chain loses translocation competence. Possibly, the larger size of mammalian cells compared to bacteria demands a longer time window for delivery of the cargo, and thus necessitated the evolution of this additional function.

Remaining questions and future perspectives

What comprises the molecular code of SRP signal sequences?

Early pioneering work identified a hydrophobicity core as the major determinant of signal sequences that mediate protein secretion (18,19). Subsequent work revealed additional

layers of complexity in that multiple targeting pathways mediate efficient protein secretion in bacteria and yeast, and signal sequences also specify the targeting pathway (see Fig. 1A) (20). Nevertheless, what and how information is encoded in signal sequences to confer specificity for the SRP pathway remained unclear. Although it was generally thought that a threshold level of hydrophobicity allows a signal sequence to specify the SRP pathway, recent work suggested that hydrophobicity is an important but not sufficient indicator for SRP-dependence (43). The difference between the hydrophobicity of SRP-dependent vs. SRP-independent signal sequences is small, making it difficult to define a hydrophobicity 'threshold' (43). Moreover, signal sequences with hydrophobicity above the apparent 'threshold' failed to engage the SRP (43). Apparently, additional molecular features of the signal sequence play important roles, including helical propensity (18), the presence of N-terminal basic residues (19,54), and additional properties that have yet to be identified (55). Crucial to our endeavor to 'decode' the signal sequence will be the availability of a more comprehensive catalogue of validated SRP-dependent vs. SRP-independent substrates, which would allow more systematic analyses of the different molecular features of signal sequences, and evaluation of their respective contributions to recognition by the SRP.

Signaling from inside the ribosome?

Previous models assumed that binding of the SRP or other cellular machineries to RNC occurs when signal sequences become exposed outside the ribosome. This view was initially challenged by the observation that the opening and closing of the Sec61p translocon is regulated by the nascent protein from inside the ribosome (56). More recent work showed that a signal sequence within the ribosome exit tunnel enhances the binding of SRP to the RNC (23,57) and helps recruit a regulatory protein RAMP4 to the Sec61p translocon (58). Further, in an alternative targeting pathway that delivers tail-anchored proteins to the ER, the Bat3 complex specifically binds the RNC when the C-terminal transmembrane segment of the nascent protein is inside the ribosome (59). Together, these results suggest that sequence or structural features of the nascent polypeptide inside the ribosome provides 'signals' that can be sensed and transmitted to the ribosome exit site and lead to the recruitment of different cellular factors. The nature of ribosome structural changes that underlie these signaling events, the mechanisms ensuring their specificity, and their precise roles in the respective cellular pathway are important questions for future studies.

How does the cargo communicate with the GTPases?

The finding that correct cargos induce extensive changes in the SRP and SR GTPases raises intriguing questions: given that the M- and NG-domains of SRP are connected by a flexible linker with no detectable interactions between them (Fig. 1C), how does the cargo communicate with the GTPases? Thus far, the SRP RNA, which binds to a helix-turn-helix motif close to the signal sequence binding groove (Fig. 2, magenta), provides the most likely candidate to mediate this communication. Recent studies found that a conserved electrostatic interaction between the tetraloop of the SRP RNA and basic residues on the SR provides a key contact that accelerates stable SRP-SR complex assembly (60). Comparison of the structures of free (8,61,62) with cargo-bound SRP (28,29) and with that of the cargo•SRP•SR early complex (63) suggest that, whereas the free SRP exists in a variety of 'latent' conformations in which the RNA tetraloop is not well positioned, a correct cargo pre-organizes the M- and NG-domains of SRP in a conformation that optimizes the electrostatic interaction of the RNA tetraloop with the incoming SR. Supporting this model is the finding that the SRP RNA only accelerates SRP-SR complex assembly in the presence of correct cargos (S.S., unpublished results).

Nevertheless, the above may be a highly simplistic model that leaves a number of observations unexplained. Signal peptides and detergents mimicking signal peptides could

partially enable the SRP RNA to stimulate SRP-SR complex assembly (22), suggesting that signal sequence binding alone could induce alterations in the SRP RNA even without the scaffolding effect from the RNC. Mutation of conserved residues in the linker connecting the SRP's M- and NG-domains abolishes the ability of the SRP RNA to stimulate complex assembly (64), suggesting that this region is not a passive linker but rather plays an active role in mediating the M-G domain communication. The precise mechanism by which signal sequence binding triggers rearrangements in the M-domain, the SRP RNA, and the linker region and in turn stimulates the assembly of the GTPases remains to be defined. Further, how the cargo delays GTP hydrolysis in the SRP•SR complex and how activation of the GTPase complex is coupled to the unloading of cargo remain elusive. These will be exciting questions for future investigations.

What is the mechanism of cargo handover to the translocation machinery?

The mechanism by which the RNC is transferred from the SRP to the Sec61/SecYEG translocon has remained the least understood aspect of the targeting reaction, and the role of the membrane translocon in this handover process is especially intriguing. As the SRP and the Sec61/SecYEG complex must compete for overlapping bindings sites on the RNC, in the simplest scenario the translocon serves as a thermodynamic 'sink' that traps any RNCs that have dissociated from the SRP and prevents them from rebinding. Nevertheless, the findings that rearrangement of the SRP•SR complex to the *closed* and *activated* states is essential for detachment of the SRP from the ribosome (39,40) and that the SR can directly interact with the translocon (14,15) raise the possibility that the translocon plays a more active role in the cargo handover process. We speculate that the interaction of SR with the membrane translocon could drive the rearrangement of the GTPase complex to the *closed* and *activated* states (Fig. 3, steps 3–4), such that the cargo handover process occurs in a concerted fashion that allows the membrane delivery of cargo to be efficiently coupled to their unloading and translocation. It will be challenging but rewarding to test and distinguish between these models, and to elucidate the precise mechanisms by which the action of the translocation machinery is coupled to those of the GTPases to complete the protein targeting reaction.

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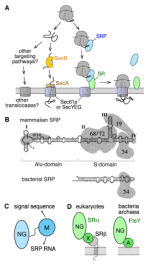

Figure 1.

Overview of the pathway and components of SRP-dependent protein targeting. (**A**) Multiple pathways deliver proteins to the ER or plasma membrane, with the SRP pathway mediating the co-translational targeting of translating ribosomes whereas the SecA/B and alternative pathways mediating the post-translational targeting of proteins. (**B**) Overall structure of the mammalian (upper) and bacterial (lower) SRP and binding sites for the SRP protein subunits. The Alu- and S-domains of SRP and Domains I–IV of the 7S SRP RNA are indicated. Adapted from reference (5). (**C**) Domain structure of the SRP54 (or Ffh) protein. (**D**) Domain organization of the eukaryotic (left) and bacterial (right) SRP receptor.

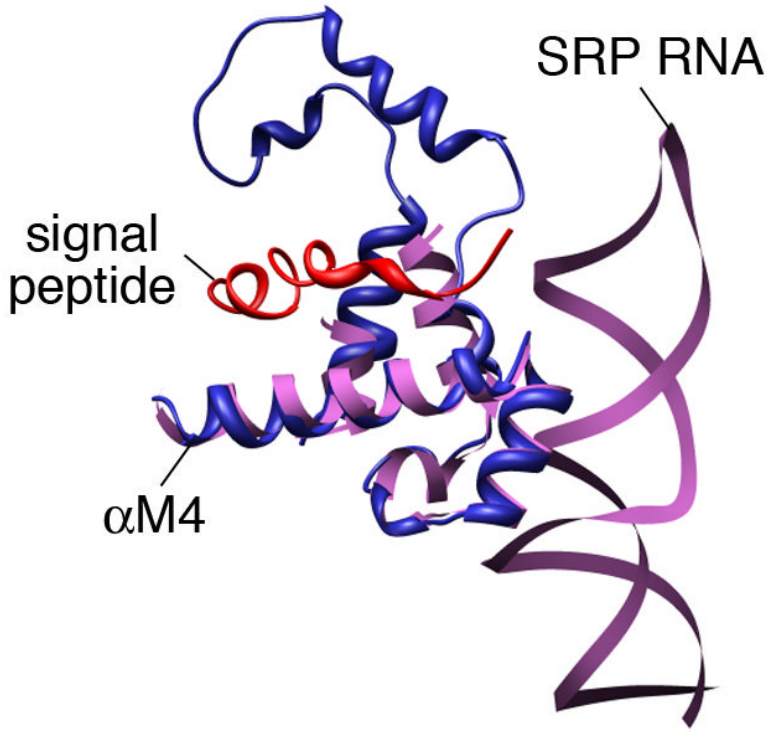

Figure 2.
A structural model of the SRP54 M-domain bound to the signal sequence and the SRP RNA. The crystal structure of the SRP54 M-domain (blue) in complex with a signal peptide (red; PDB ID 3KL4) was superimposed onto the structure of the Ffh M-domain in complex with a fragment of the SRP RNA (magenta; PDB ID 1DUL).

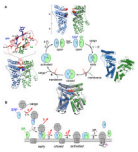

Figure 3.

Conformational changes in the SRP and SR GTPases ensure the efficiency and fidelity of protein targeting. The steps are numbered to be consistent between parts (A) and (B). T and D denote GTP and GDP, respectively, (**A**) A series of discrete rearrangements occur during the SRP-SR interaction and can be regulated by the cargo and membrane translocon. ⊥ denotes the effect of cargo in disfavoring the rearrangements to the *closed* and *activated* states. Top panel: the crystal structures of free Ffh (*blue*; 1JPJ) and FtsY (*green*; 1Q9B) NG-domains bound to GMPPNP (*spacefill*). The IBD loops in both proteins are highlighted in *red*. Right panel: molecular model of the early intermediate (63) with Ffh and FtsY in *blue* and *green*, respectively. Bottom panel: G-domain superposition of the co-crystal structure of the Ffh-FtsY NG domain complex (1RJ9; Ffh and FtsY in *blue* and *green*, respectively) with those of the free proteins (*grey*). Left panel: Co-crystal structure of the Ffh-FtsY NG domain complex (1RJ9) highlighting the IBD loops (*red*) and catalytic interactions in the GTPase active site (zoom-in), with the GMPPCP molecules from Ffh and FtsY in *blue* and *green*, respectively, active site Mg^{2+} in *magenta*, nucleophilic waters (W) in *blue*, and the side chains of catalytic residues in the IBD loops in *red*. (**B**) GTPase rearrangements provide multiple regulatory points during protein targeting. Step 1, a cargo with a signal sequence (magenta) enters the pathway upon binding SRP. Step 2, SRP associates with SR to form a targeting complex, which is stabilized by the cargo in the *early* conformation. Step 3, association of SR with phospholipids is proposed to drive rearrangement to the *closed* state, during which SRP weakens its affinity for the cargo. Step 4, interaction of SR with the translocon could further allow the SRP•SR complex to rearrange to the *activated* state, which drives the handover of cargo from the SRP to the translocon. Step 5, GTP hydrolysis drives the disassembly and recycling of SRP and SR. At each step, the cargo can be either retained in (black arrows) or rejected from (red arrows) the SRP pathway.