Published in final edited form as:

Nature. 2012 December 13; 492(7428): 189–191. doi:10.1038/492189a.

All clear for ribosome landing

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Protein sorting within a cell is not an easy undertaking. The synthesis of many proteins destined to leave the cell or to become part of a cellular membrane must be coupled with their transport across or into the appropriate membrane. A universal RNA–protein complex called the signal recognition particle (SRP) plays an essential role in this process by interacting with ribosomes, the cell’s protein-synthesizing factories, while they are engaged in protein translation. SRP then escorts the translating ribosomes to a cellular organelle known as the endoplasmic reticulum or, in bacteria, to the cytoplasmic membrane, where they dock onto a transport channel called the Sec complex, or SecYEG in bacteria. SRP must presumably be displaced from the ribosome for docking to happen, but the mechanism of this molecular exchange has been an enigma. On page 271 of this issue, Shen *et al.*¹ provide compelling evidence that a remarkable conformational change driven by the RNA component of SRP enables the ribosome to deliver its protein cargo efficiently.

Most membrane and secreted proteins that emerge from translating ribosomes carry a signal peptide — a sequence at their amino terminus that earmarks them for export from the cytoplasm. SRP recognizes and binds to this peptide and then interacts with a membrane receptor (FtsY) before releasing its nascent-protein cargo and transferring the ribosome to SecYEG (Fig. 1). This co-translational mode of protein targeting ensures that proteins destined to leave the cytoplasm begin their journey before they aggregate or fold into a structure that cannot fit through the SecYEG channel.

Although SRP has undergone dramatic expansions and contractions during evolution, even the simplest bacterial SRPs contain two highly conserved core elements — a protein (Ffh) that binds to the signal peptide and an elongated, roughly 100-nucleotide RNA that forms a ‘hairpin’. Despite this relative simplicity, SRP must not only distinguish signal peptides from the bulk of nascent polypeptides, but also release them at the right place and time.

Many aspects of the protein-targeting cycle mediated by SRP are well understood. For instance, it is known that the signal-peptide-recognition and RNA-binding activities of Ffh both reside in a single domain, called the M domain. Also, both Ffh and FtsY contain a GTPase domain that hydrolyses the nucleotide GTP to GDP and thereby acts as a regulatory switch. In the presence of a signal peptide, SRP RNA catalyses an interaction between the GTPase domains of Ffh and FtsY, and the binding of GTP brings the two domains into close proximity^{2,3}. Subsequently, structural rearrangements in the GTPase domains promote GTP hydrolysis, disassembly of the SRP–FtsY complex and dissociation of SRP from the

ribosome⁴. Moreover, in the bacterium *Escherichia coli*, FtsY seems to recruit SecYEG to ensure that nascent polypeptides are released at the correct location⁵.

Despite much progress, however, it has remained unclear how the transfer of ribosome–nascent-protein complexes to SecYEG is coordinated with the enzymatic activity of the GTPase domain and the recycling of the SRP–FtsY complex. To complicate matters, SRP and SecYEG bind to overlapping sites on L23, a ribosomal protein located at the point where nascent polypeptides emerge from a tunnel in the large subunit of the ribosome⁶. SRP must therefore be reoriented without prematurely releasing its ribosome–nascent-protein cargo.

A clue as to how the transfer might occur emerged from a crystal structure of the SRP–FtsY complex⁷ that captured the interacting GTPase domains bound to a sequence near the distal end of the SRP RNA hairpin. This configuration was unexpected, because previous work⁸ had shown that the complex is stabilized by bonds between the FtsY GTPase and a 4-nucleotide turn (a tetraloop) at the top of the hairpin. The structure⁷ suggested that the two domains might travel together to a remote ‘landing pad’ more than 100 Å away after first interacting at the tetraloop. Such spectacular molecular gymnastics would be possible only because the two domains of Ffh are connected by a flexible linker.

To obtain direct evidence for this putative large-scale movement and to glean its functional significance, Shen *et al.* attached a dye to the FtsY GTPase domain and a different dye to the distal end of SRP RNA. They then measured the distance between the dyes throughout the SRP cycle using an innovative method called single-molecule fluorescence resonance energy transfer (smFRET). In this way, they could obtain precise positional information about individual Ffh–FtsY GTPase complexes.

The authors made several intriguing observations. They not only showed that the Ffh–FtsY GTPase complex relocates to the far end of the SRP RNA hairpin, but also obtained evidence that the complex undergoes a lengthy ‘search process’ before landing at its new destination. This migration is stimulated by SecYEG and requires a conformational change within the GTPase complex that primes it for GTP hydrolysis. The relocation of the complex is also required for both GTP hydrolysis and effective delivery of the nascent protein to SecYEG. Taken together, these results suggest that the GTPase complex is brought to the brink of activation, whereupon its relocation to the distal end of the RNA exposes the nascent protein’s exit site on the ribosome and allows the ribosome to dock onto SecYEG (Fig. 1c, d). The relocation also stimulates GTP hydrolysis by the complex and SRP recycling.

Although Shen and colleagues’ work provides notable mechanistic insights, it also raises several questions. For one, how does SecYEG trigger the reorientation of the Ffh–FtsY GTPase complex and the subsequent hydrolysis of GTP? Also, in the likely event that the signal peptide remains bound to the M domain of Ffh upon relocalization of the GTPase complex, what catalyses cargo release? One possibility is that the movement of the complex reduces the affinity of Ffh for the signal peptide by repositioning another segment of Ffh that has been proposed⁹ to participate in substrate recognition. Finally, how can these results be

reconciled with the observation¹⁰ that the distal end of SRP RNA is not required for a cell to survive? It is conceivable that, even in the absence of a distal binding site, transient displacement of the GTPase complex from the tetraloop allows the translating ribosomes to be transferred to SecYEG, albeit with reduced efficiency.

Perhaps the most striking implication of Shen and co-workers' study is that the two ends of the SRP RNA hairpin have distinct functions at different stages of the targeting cycle (Fig. 1). We take it for granted that proteins can be multifunctional, but much less is known about the capabilities of the many RNAs found in nature. As the characterization of RNA proceeds, the message that even a small nucleic-acid sequence can encode considerable functional information is certainly worth bearing in mind.

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Figure 1 |. Handover of translating ribosomes.

a. The signal recognition particle (SRP) has a protein (Ffh) component and an RNA component that forms a hairpin structure. Ffh recognizes and binds to signal peptides on nascent proteins during protein translation. **b.** The GTP-bound GTPase domain of Ffh (labelled GTP) subsequently interacts with the GTPase domain of FtsY — a receptor that resides in the endoplasmic reticulum or the bacterial cytoplasmic membrane. In bacteria, FtsY recruits the SecYEG protein-translocation channel. **c.** Shen *et al.*¹ report that SecYEG stimulates a dramatic relocalization of the two interacting GTPases from the tetraloop region of SRP RNA to an evolutionarily conserved distal sequence. **d.** Reorientation of the GTPase complex facilitates both the docking of the translating ribosome onto SecYEG and hydrolysis of the two GTP nucleotides to GDP. GTP hydrolysis stimulates the recycling of SRP and FtsY.