

Protein Targeting across Evolution: SRP in the Three Domains of Life

Comparing signal recognition particle components provides insights into both conserved and divergent elements

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For a particular cell to properly carry out its functions, its proteins need to be correctly located within or outside the cell. In the case of prokaryotes, proteins can be found in the cytoplasm, periplasm (if present), secreted beyond the cellular confines, or embedded in the membrane(s) surrounding the cytoplasm. Regardless of the eventual location of any particular protein, biosynthesis of all proteins begins on cytoplasmic ribosomes.

How do extracytoplasmic proteins cross the hydrophobic phospholipid bilayer surrounding the cytoplasm? Crossing this barrier is a problem not only for secreted proteins that are designed for aqueous environments, but also for membrane-spanning proteins that transfer loops of hydrophilic amino acid residues to the outer face of such membranes. Of course, such problems are not unique to prokaryotes. For instance, during the first step on the eukaryotic secretory pathway, proteins insert into or traverse the membrane of the endoplasmic reticulum (ER).

At least three key steps are involved in moving proteins across biological membranes. Thus, such proteins need to be correctly recognized, targeted to specialized translocation sites along those membranes, and then transported into or across those barriers. In all three domains of life—the *Eukarya*, *Bacteria*, and *Archaea*—the recognition and targeting phases of the translocation of extracytoplasmic proteins can be mediated by the signal recognition particle (SRP) pathway.

SRP Pathway of *Eukarya*—Coordinating Protein Translation, Translocation

First elucidated in mammals by Nobel laureate Gunter Blobel at Rockefeller University in New

York, N.Y., Bernhard Dobberstein, now at the University of Heidelberg, Germany, and Peter Walter, now at the University of California, San Francisco, the SRP pathway of higher *Eukarya* remains the best-understood version of this system. This SRP pathway delivers signal sequence-bearing nascent polypeptides to ER membranes (Fig. 1). The signal sequence corresponds to a cleavable N-terminal extension that is responsible for a protein being recognized by the SRP pathway, yet which is not found in the mature protein.

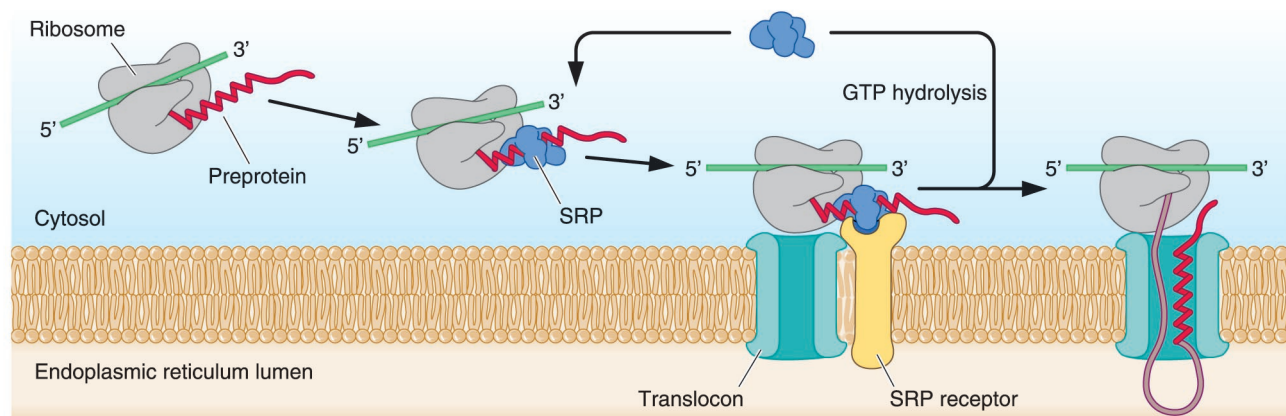
Once the signal sequence and approximately 40 amino acid residues of the mature domain of a nascent polypeptide chain exit the ribosome, the signal sequence is recognized by SRP. Such binding in turn leads to an interaction between SRP and the ribosome, resulting in a slowing down or arrest of continued protein translation. Next, the trimeric complex of the ribosome, SRP, and nascent protein are targeted to the ER membrane via the affinities of SRP for the membrane-associated SRP receptor and of the ribosome for the membranous Sec61 $\alpha\beta\gamma$ protein translocation complex, or “translocon.” Once the ternary complex docks at the ER membrane, a series of GTP hydrolyses leads to release of the SRP and transfer of the signal sequence to the Sec61 α translocon subunit. Now, with the brake on protein translation, i.e. SRP, gone, biosynthesis and concomitant translocation of the nascent polypeptide chain can continue directly into the translocon pore. Finally, at a later stage, the signal sequence is released by the enzyme signal peptidase.

SRP is central to this pathway. In higher *Eukarya*, SRP consist of a 7S RNA moiety to which six distinct polypeptides are attached (Fig. 2).

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FIGURE 1



The SRP pathway of higher *Eukarya*. Proteins destined for translocation across the ER membrane are synthesized as preproteins bearing signal sequences (red) on cytoplasmic ribosomes (light green). The newly exposed signal sequence is recognized by SRP (blue), which in turn binds the ribosome to inhibit continued translation. The ribosome-nascent chain-SRP complex is then targeted to the ER membrane via the affinities of SRP for its receptor (purple) and the ribosome for the translocon (dark green). A series of GTP hydrolysis steps ensues and SRP is released. With SRP no longer arresting translation, protein synthesis continues directly into the pore of the translocon. The released SRP now seeks out a new ribosome translating a signal sequence-bearing preprotein to reinitiate the cycle.

SRP9 and SRP14 form a heterodimer that is implicated in translation arrest. SRP68 and SRP72 also form a heterodimer, yet its role remains poorly understood. SRP19 is involved in SRP assembly, while SRP54 is responsible for signal sequence recognition, serves as the GTPase moiety of the particle, and mediates binding to the SRP receptor. In *Eukarya*, the membrane-associated SRP receptor is composed of the peripheral α subunit and the integral β subunit. Like SRP54, both receptor subunits possess GTPase activity, although many other details of the SRP pathway GTPase cycle remain to be worked out.

The SRP Pathway of Bacteria—a Role in Membrane Protein Insertion

In 1989, about 10 years after the eukaryal SRP system was discovered, researchers identified bacterial homologues of the eukaryal SRP54 and SRP receptor α subunit proteins. Although the genes encoding these proteins proved essential for bacterial cell growth, they did not at first appear to be involved in secreting proteins, particularly because most bacterial proteins are secreted after, not during, their synthesis. Of late, however, several research groups, including that of Harris Bernstein at the National Institutes of Health in Bethesda, Md., showed that the bac-

terial SRP pathway is involved in inserting a subpopulation of proteins into the plasma membrane.

The SRP pathway of bacteria apparently is far simpler than its eukaryal counterpart. In *Escherichia coli*, for example, SRP consists of a 4.5S RNA to which a single polypeptide, the SRP54 homologue Ffh, is attached (Fig. 2). However, as one examines SRP across the bacterial kingdom, some embellishment of this rudimentary particle can be detected. In *Bacillus subtilis*, SRP consists of a longer piece of RNA to which an additional protein, HBSu, is bound. The SRP receptor is also simpler in the bacterial system. Among bacterial species, the two-component receptor of the eukaryal SRP is replaced by the single FtsY protein. The homologue of the eukaryal SRP receptor α subunit, FtsY seemingly modulates between its cytosolic and membrane-associated forms.

Originally thought to operate much like the eukaryal SRP pathway, evidence now suggests that the bacterial SRP pathway functions differently. For instance, FtsY, the bacterial SRP receptor, targets translating ribosomes to the plasma membrane prior to the involvement of SRP, according to Eitan Bibi at the Weizmann Institute of Science in Rehovot, Israel. Indeed, it is not known how FtsY interacts with the bacte-

rial membrane. Other components of the bacterial SRP pathway may also behave in a manner unique to bacteria. For example, it is not known how and when nascent chains are delivered to the Sec translocation apparatus of the bacterial plasma membrane, according to Joen Luirink at the Vrije University in Amsterdam, the Netherlands, Mathias Müller at Freiberg University in Freiberg, Germany, and their respective collaborators, who are investigating these and other phenomena of the bacterial SRP system.

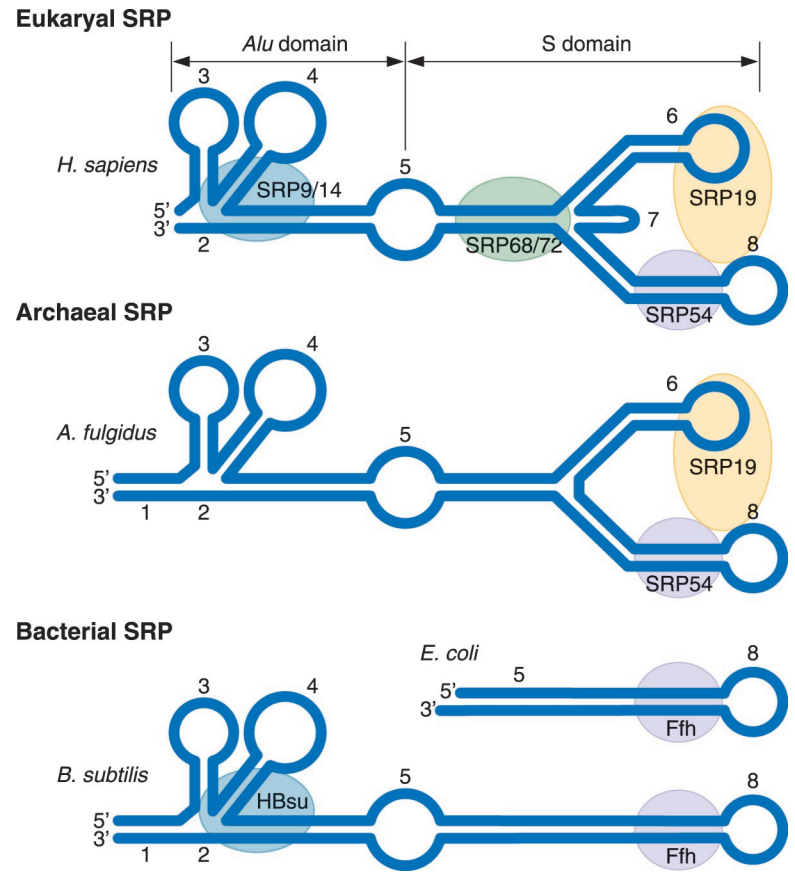
Archaeal SRP—a Mosaic of Eukaryal, Bacterial, and Archaeal Features

Pioneering 16S-ribosomal RNA secondary structure analyses led Carl Woese of the University of Illinois, Urbana-Champaign, to redraw the universal tree of life into three separate and distinct domains consisting of the *Eukarya*, *Bacteria*, and *Archaea*. Originally thought to be found only in challenging physical environments, the microorganisms within *Archaea* also are major denizens of many “normal” environments. *Archaea* are attracting ever-increasing attention, in part because so many archaeal organisms embody a hybrid of archaeal-specific traits with selected eukaryal and bacterial traits.

Indeed, this biological mosaic is evident when one examines the SRP system of *Archaea* (Fig. 2). At first glance, the archaeal SRP closely resembles its eukaryal homologue, albeit simpler. The archaeal SRP includes a 7S RNA molecule that assumes a secondary structure much like that seen in the eukaryal particle, as well as two of the six polypeptides found in the eukaryal SRP, namely SRP19 and SRP54. In contrast, the SRP receptor appears to be common to both prokaryal domains. Like *Bacteria*, *Archaea* also contain the FtsY protein.

Delving closer, however, one detects components of the archaeal SRP pathway that appear to be unique to this life form. Archaeal SRP RNA, for instance, contains regions not found in the eukaryal molecule, yet lacks others found in eukaryal SRP RNA. Archaeal SRP19 and FtsY proteins are smaller than their counterparts in the other domains. Furthermore, the archaeal SRP seems to assemble in a distinctive manner.

FIGURE 2



SRP in the three domains of life. Schematic representations of SRP from *Eukarya* (*Homo sapiens*), *Archaea* (*Archaeoglobus fulgidus*), and *Bacteria* (*Escherichia coli* and *Bacillus subtilis*) are presented. In the different SRP RNA molecules (black lines), phylogenetically supported regions of Watson-Crick and G-U base pairs are depicted by parallel lines, while regions containing non-Watson-Crick pairs are shown as bulges. The 5' and 3' ends of each RNA molecule are shown, as is the numbering of the SRP RNA helices. SRP9/14 is shown in blue, SRP68/72 is shown in green, SRP19 is shown in orange, and SRP54 is shown in purple. The *Alu* and S domains of the particle are also indicated.

Using a Comparative Approach To Elucidate SRP Pathways

With SRP pathway components available from each of the three domains of life, researchers are comparing SRP systems across evolutionary lines. This approach enables them to develop a better understanding of the assembly, structure, and function of this targeting pathway than would be possible if they were limited to examining each domain separately.

Consider the *Alu* domain that acts during translation arrest, an early step in the eukaryal SRP cycle. Whether this phenomenon is medi-



ated by the protein component of the *Alu* domain (the SRP9/14 heterodimer in higher eukaryotes or the SRP14 homodimer of yeast), by its RNA component, or by both is not known (Fig. 2). Indeed, our views regarding SRP RNA have changed considerably. Once thought to serve merely as a scaffolding to which various SRP proteins bind, SRP RNA now appears to play an active role in SRP function. Some researchers argue that this RNA is principally responsible for SRP function, with various SRP proteins acting to fold it properly. If so, could SRP RNA be responsible for mediating translation arrest, possibly interacting with ribosomes in a tRNA-like manner? The answer might come from examining the archaeal SRP. In archaeal SRP, the *Alu* domain is apparently composed solely of RNA; homology-based searches of sequenced genomes have failed to identify archaeal homologues of either SRP9 or SRP14. While it remains to be shown that translation arrest plays a role in an archaeal SRP pathway, addressing this point offers an opportunity to elucidate the respective roles of protein and RNA during this step.

Matters may not, however, be so simple. The SRP of the gram-positive bacterium *B. subtilis* includes an SRP RNA molecule that assumes a secondary conformation similar to that of the archaeal SRP, yet also contains a novel protein subunit in its *Alu* domain (Fig. 2). This histone-like HBSu protein does not share significant sequence similarity with either SRP9 or SRP14, but does share substantial homology with the SRP9/14 heterodimer at the structural level. Again, while SRP-mediated translation arrest has not been addressed in *B. subtilis*, examining this point will surely elucidate the phenomenon. Finally, the possibility that *Archaea* also contain structural homologues of SRP9, SRP14, or HBSu cannot be discounted. Unfortunately, current analytical tools do not allow accurate structural predictions from purely genomic sequence information.

Examining Broadly Conserved Components of the SRP Pathway

Just as these comparisons reveal how SRP components vary across biological domains, such an approach also can uncover conserved features within the SRP pathway. For example, SRP54 is an essential component of SRP in all three domains of life. Furthermore, all known SRP54

proteins can be divided into two domains—the NG-domain, which is involved in guanidine nucleotide interaction and binding to the SRP receptor, and the M domain, which binds to SRP RNA and the signal sequence. The conserved nature of SRP is also reflected in reconstitution experiments in which SRP54 from a given domain can recognize SRP RNA or signal sequences taken from a representative of either one of the other two domains of life.

It is surprising, therefore, that SRP54 from *Eukarya* cannot bind eukaryal SRP RNA in the absence of SRP19. In studying assembly of the archaeal SRP, Christian Zwieb at the University of Texas Health Sciences Center at Tyler and I find that, in contrast to SRP19-dependent SRP54-RNA interaction in *Eukarya*, SRP54 in *Archaea* can bind to the asymmetric loop of SRP RNA helix 8 in the absence of SRP19. In *Archaea*, SRP19 stimulates SRP54 binding. It seems that that upon binding SRP19, SRP RNA undergoes a conformational change in which helices 6 and 8 are brought into close proximity. This conformational change is particularly sensed in the asymmetric loop of helix 8, which becomes primed for SRP54 binding. In the case of the eukaryal SRP, such an SRP19-induced conformational change is a necessary prerequisite for any binding of SRP54.

A comparative approach of SRP pathway components may also be useful in understanding FtsY, the prokaryal SRP receptor and homologue of the eukaryal SRP receptor α subunit. Like SRP54, FtsY can be functionally divided into two distinct portions, the A domain and the NG domain. The NG domain interacts with SRP54 and, like the SRP54 NG domain, interacts with guanidine nucleotides through a series of conserved sequence motifs.

The similarities of the SRP54 and FtsY NG domains suggest that the two proteins likely arose from an ancient gene duplication event, and indeed, SRP54 and FtsY protein comparisons are used in rooting phylogenetic trees. The FtsY A domain is implicated in the binding of FtsY to the plasma membrane via clusters of positive charges located at the extreme N-terminal region of the domain. These positive charges are thought to interact with the negatively charged headgroups of membrane phospholipids. Indeed, the N-terminal sequences of various archaeal and bacterial FtsY A domains contain positively charged arginine and lysine residue clusters.

In the case of halophilic archaea that live in high salt concentrations often nearing saturation, different FtsY-membrane interactions may be at play. Here, the number of positively charged residues at the N-terminal portion of the A domain is much lower than in other prokaryotes. Given that the cytoplasm of halophilic archaea contains molar salt concentrations, electrostatic interactions between the A domain and phospholipid headgroups may be compromised. Moreover, other archaeal FtsY proteins may similarly rely on this alternative mode of membrane attachment that likely occurs in the haloarchaea. This novel mode of membrane association may also arise because of the unusual, ether-based chemistry of archaeal membrane phospholipids.

The Evolution of SRP

Comparing SRP across evolutionary lines can also be used to infer the origins of this ribonucleoprotein complex. Conceivably the precursor of SRPs contained 7S RNA, SRP19, and SRP54. As *Bacteria* evolved and developed alternative protein-targeting pathways, the bacterial SRP may have simplified. In contrast, with the development of various intracellular organelles and an enhanced need for precise protein targeting, the eukaryal SRP likely grew more complex, incorporating additional subunits. Furthermore, archaeal SRPs may contain additional

protein subunits that could either represent novel components or correspond to structural homologues of SRP elements from other domains of life.

Conceivably, the ancestral SRP contained only RNA, later evolving to incorporate SRP54. Over time, other subunits were likely incorporated in response to changing needs. For instance, SRP19 began to appear in some, but seemingly not all *Archaea*. *Eukarya*, requiring a more complex SRP, may have enlisted additional subunits with specialized functions and compartmentalized SRP assembly.

Recent SRP structural studies in several laboratories, including those of Irmigard Sinning at the University of Heidelberg and Stephan Cusack at the European Molecular Biology Laboratory, both in Germany, enhance our understanding of SRP at the molecular and mechanistic levels. Continued progress in this arena will certainly lead to further insight into the workings of the SRP system across evolution. Moreover, the continued development of improved model systems for studying the bacterial and archaeal SRP pathways will advance our understanding of protein targeting in the prokaryotes. Finally, the availability of an ever-increasing number of genomic sequences, together with improved genome analysis tools, could lead us to previously unidentified SRP components—keeping us on course for a fuller comprehension of the SRP pathway.

ACKNOWLEDGMENT

Work in my laboratory is supported by the Israel Science Foundation (grant #291/99).

SUGGESTED READING

- Batey, R. T., R. P. Rambo, L. Lucast, B. Rha, and J. A. Doudna. 2000. Crystal structure of the ribonucleoprotein core of the signal recognition particle. *Science* 287:1232–1239.
- Bhuiyan, S. H., K. Gowda, H. Hotokezaka, and C. Zwieb. 2000. Assembly of archaeal signal recognition particle from recombinant components. *Nucleic Acids Res.* 15:1365–1373.
- De Leeuw, E., K. te Kaat, C. Moser, G. Menestrina, R. Demel, B. de Kruijff, B. Oudega, J. Luirink, and I. Sinning. 2000. Anionic phospholipids are involved in membrane association of FtsY and stimulate its GTPase activity. *EMBO J.* 19:531–541.
- Eichler, J., and R. Moll. 2001. The signal recognition particle of archaea. *Trends Microbiol.* 9:130–136.
- Herskovits, A. A., A. Seluanov, R. Rajsbaum, C. M. ten Hagen-Jongman, T. Henrichs, E. S. Bochkareva, G. J. Phillips, F. J. Probst, T. Nakae, M. Ehrmann, J. Luirink, and E. Bibi. 2001. Evidence for coupling of membrane targeting and function of the signal recognition particle (SRP) receptor FtsY. *EMBO Rep.* 2:1040–1046.
- Keenan, R. J., D. M. Freymann, R. M. Stroud, and P. Walter. 2001. The signal recognition particle. *Annu. Rev. Biochem.* 70:755–775.
- Lu, Y., H. Y. Qi, J. B. Hyndman, N. D. Ulbrandt, A. Teplyakov, N. Tomasevic, and H. D. Bernstein. 2000. Evidence for a novel GTPase priming step in the SRP protein targeting pathway. *EMBO J.* 20:6724–6734.
- Tozik, I., Q. Huang, C. Zwieb, and J. Eichler. 2002. Reconstitution of the signal recognition particle of the halophilic archaeon *Haloflex volcanii*. *Nucleic Acids Res.* 30:4166–4175.
- Wild, K., O. Weichenrieder, K. Strub, I. Sinning, and S. Cusack. 2002. Towards the structure of the mammalian signal recognition particle. *Curr. Opin. Struct. Biol.* 12:72–81.
- Zwieb, C., and J. Eichler. 2002. Getting on target: the archaeal signal recognition particle. *Archaea* 1:27–34.