Annu. Rev. Cell Biol. 1994. 10:87–119
Copyright © 1994 by Annual Reviews Inc. All rights reserved

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

SIGNAL SEQUENCE RECOGNITION AND PROTEIN TARGETING TO THE ENDOPLASMIC RETICULUM MEMBRANE

Peter Walter
Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143–0448

Arthur E. Johnson
Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019

KEY WORDS: signal recognition particle, SRP, SRP receptor

CONTENTS

INTRODUCTION 88
OVERVIEW OF SIGNAL RECOGNITION PARTICLE-DEPENDENT PROTEIN TARGETING 88
STRUCTURE OF THE SRP AND SRP RECEPTOR 90
Nomenclature 90
SRP RNA 90
SRP Structure and Assembly 93
SRP Protein Subunits 96
SRP Receptor 99
MECHANISM OF SRP-DEPENDENT PROTEIN TARGETING 100
Selection of Signal Sequences 100
Elongation Arrest 102
Targeting 105
Beyond Targeting 108
ALTERNATIVE TARGETING ROUTES 109
SRP-independent Targeting 109
Modular Translocons? 112

0743-4634/94/1115-0087$05.00

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

INTRODUCTION

In this review, we attempt a timely survey of issues concerning protein translocation across the endoplasmic reticulum membrane, with a strong focus on the initial events that lead to the selection and proper delivery of proteins to this membrane system. Many new questions are raised by recent discoveries: it is now known that targeting can occur by multiple pathways and that the molecular machines that catalyze targeting and translocation are conserved in all cell types examined (from bacteria to mammalian cells). It is desirable to integrate the information from these different organisms into a coherent picture because we feel that the similarities—as well as the differences—found over such vast evolutionary distances will illuminate the fundamental principles that govern the inner workings of these components. Although this philosophy may cause an occasional oversimplification, we believe that it sets a useful conceptual framework to guide future experimental investigation. We focus here on recent developments and open questions, and do not intend this review to be comprehensive. Where appropriate, reference to more detailed reviews is given in the text.

OVERVIEW OF SIGNAL RECOGNITION PARTICLE-DEPENDENT PROTEIN TARGETING

Translocation of soluble proteins across the endoplasmic reticulum (ER) membrane or integration of membrane proteins into the ER membrane are the first steps in the processes that deliver proteins to the secretory pathway and thereby initiate their journey to the outside of the cell, to the plasma membrane, or to the intracellular organelles that comprise the endomembrane system (Palade 1975). In mammalian cells, the synthesis of these proteins takes place on ribosomes that are bound to the rough ER membrane, and protein translocation and integration occur simultaneously with ongoing protein synthesis, i.e. cotranslationally. Because all other proteins are thought to be synthesized on ribosomes that are free in the cytosol, a mechanism must exist that mediates the selective attachment to the ER membrane of the ribosomes that synthesize proteins destined for secretion or for integration (Blobel & Dobberstein 1975).

In vitro assays have identified the signal recognition particle (SRP) and the SRP receptor (also known as the docking protein) as components required to target ribosomes to the ER membrane, and a detailed model describing their function has been proposed (Walter et al 1984; Walter & Lingappa 1986; Nunnari & Walter 1992; Gilmore 1993; Rapoport 1992; Sanders & Schekman 1992) (see Figure 1). The process is initiated when a signal sequence in the nascent protein chain emerges from the ribosome and is recognized by the SRP. This interaction causes the SRP to bind tightly to the ribosome, which

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

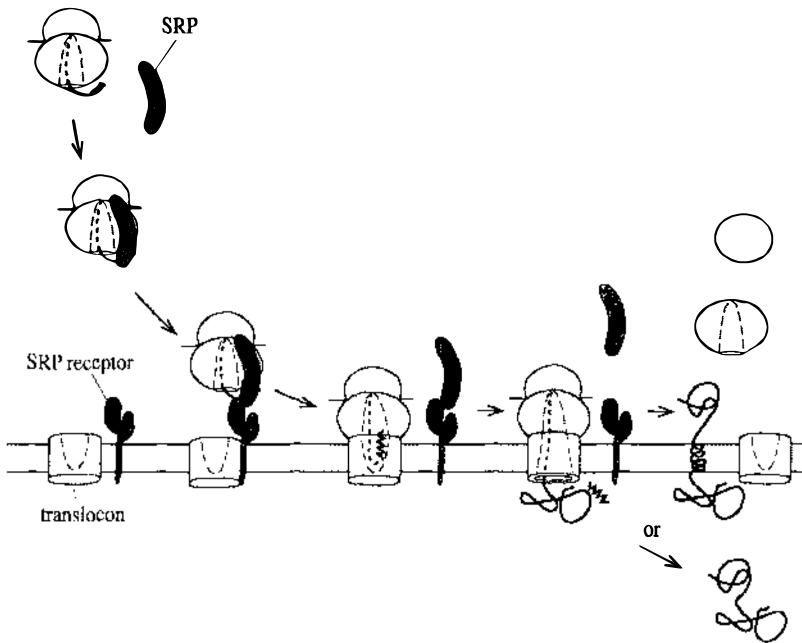


Figure 1 SRP-dependent protein targeting. SRP binds to the signal sequence of a nascent polypeptide emerging from the ribosome to form the targeting complex in which elongation is arrested. SRP in the targeting complex then binds to the SRP receptor in the ER membrane. This interaction leads to the formation of the ribosome-translocon junction and translation elongation resumes. SRP and SRP receptor then dissociate from each other and can engage in another round of targeting. After targeting, the signal sequence is initially in an aqueous compartment, formed by the ribosome and translocon components, that is sealed off from both the cytosol and the ER lumen. As the nascent chain grows, the translocation pore opens and allows the passage of the nascent protein across the membrane. In most cases, the signal sequence is removed on the lumenal side of the ER membrane by signal peptidase (not shown). Soluble proteins are released into the lumen of the ER. Transmembrane segments of nascent membrane proteins function as "stop-transfer" sequences. They must be recognized in the translocation pore, stop the translocation process, and then trigger the pore to open on one side to release the membrane protein laterally into the membrane.

effects a pause ("elongation arrest") in the translation of the nascent protein. The resulting complex consists of the ribosome, the nascent chain with its signal sequence, and the SRP, and it is herein referred to as the targeting complex. Interaction of the SRP in the targeting complex with the SRP receptor, an ER membrane protein, releases the SRP from the ribosome and signal sequence and allows translation to continue. Concomitantly, the ribosome becomes bound to other components in the ER membrane. These components, collectively termed a translocon (Walter & Lingappa 1986), catalyze the transfer of the growing protein chain across the membrane, presumably through a

gated aqueous pore (Crowley et al 1994; Simon & Blobel 1991). SRP and SRP receptor act catalytically in this process; they are not part of the ribosome-translocon junction that mediates the transfer of the protein chain across the lipid bilayer. Rather, they function to direct the ribosome to the correct intracellular membrane and are then released from the ribosome.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

STRUCTURE OF THE SRP AND SRP RECEPTOR

Nomenclature

Mammalian SRP contains one 7S RNA molecule, originally termed the 7SL RNA and referred to herein as SRP RNA, and six different polypeptides with molecular masses of 9, 14, 19, 54, 68, and 72 kd (Walter & Blobel 1980, 1982) that are designated SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72, respectively. The SRP receptor consists of two subunits with molecular masses of 72 and 30 kd that are are designated SRα and SRβ (Tajima et al 1986). There is now good evidence that SRP and SRP receptor homologues exist in all organisms (see, e.g. Dobberstein 1994; Althoff et al 1994). We refer to the RNA in each SRP species as SRP RNA and identify the SRP proteins in different organisms by the name of the corresponding mammalian protein wherever there is sequence and functional homology, even if the molecular mass of the non-mammalian protein differs from that of its mammalian homologue. The *Escherichia coli* SRP54 is also known as Ffh or p48, and the *E. coli* SRP RNA is known as 4.5S RNA.

SRP RNA

SRP RNA is the central component of SRP: functionally it may mediate SRP's association with the ribosome and the SRP receptor, and structurally it provides the backbone onto which the SRP proteins assemble to form the SRP. The predicted secondary structure of the 300-nucleotide mammalian SRP RNA suggests an elongated conformation formed by extensively base-paired helices (Figure 2) (Ullu et al 1982). Two different ways are used to divide the SRP RNA structure into domains. First, four domains, designated domains I-IV (Figure 2A), are defined based on the distinct elements of the secondary structure: domain I is a variable structure at the 5′ end of the molecule, while domain II is the main stem that ends by bifurcating into two stem-loop structures defined as domains III and IV (Poritz et al 1988). The second division is based on the finding that mammalian SRP RNA is homologous for about 100 nucleotides from its 5′ end and for about 50 nucleotides from its 3′ end with the highly repetitive Alu DNA family (Ullu et al 1982). Although they are positioned on either end of the SRP RNA sequence, the 5′ and 3′ Alu sequences form a structurally contiguous domain ("Alu domain") in the folded

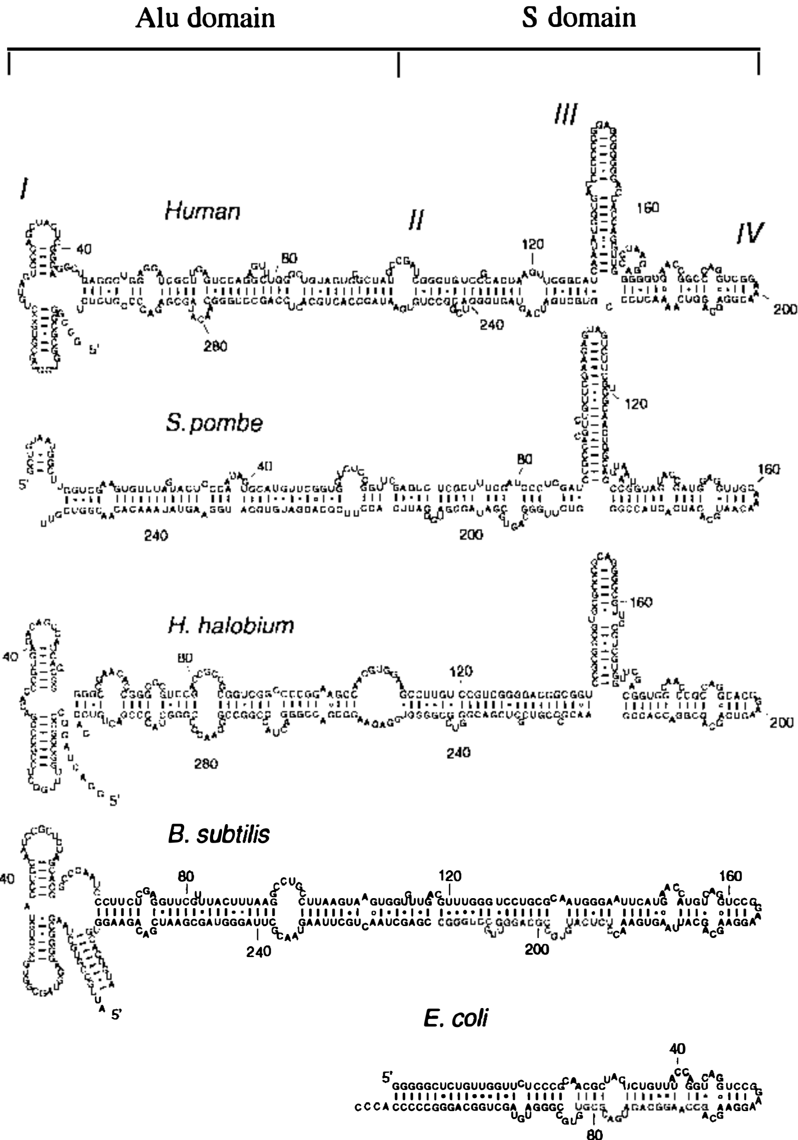


Figure 2 Secondary structures of SRP RNAs. RNAs from all three kingdoms are shown. Note the similarities both in the position and in the primary sequence of the bulges in domain IV. For a complete listing of sequences and secondary structures refer to a databank on SRP RNAs compiled by Larsen & Zwieb (1993).

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

SRP RNA. The Alu domain therefore consists of domain I and about half of domain II. Interestingly, SRP RNA is thought to be the evolutionay progenitor of Alu sequences, which may have arisen by reverse transcription from SRP RNA (Ullu & Tschudi 1984). In contrast, the central 150 nucleotides of SRP RNA comprise a unique sequence. The structural domain formed by this central RNA portion is termed the S domain (Siegel & Walter 1986; Ullu et al 1982). As discussed below, Alu and S domains define structurally and functionally distinct parts of SRP.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

The secondary structure of SRP RNAs has been confirmed by phylogenetic comparison of SRP RNA sequences from a wide variety of organisms (Larsen & Zwieb 1991, 1993) and by experimental approaches that include nuclease digestion (Gundelfinger et al 1984), chemical modification (Andreazzoli & Gerbi 1991), and mutagenesis (Selinger et al 1993a; Zwieb 1991). The overall shape and dimensions of the secondary structure are conserved in eukaryotes and archeae despite a remarkable evolutionary drift in the sequences of the SRP RNAs. There are only very short regions of sequence conservation, and an unexpectedly large number of mutations in SRP RNA do not affect its function in vivo (Liao et al 1992). Therefore, it appears that the secondary and most likely tertiary structural features of SRP RNA are important for SRP assembly and function. The only known SRP RNA that has not yet been folded to fit the consensus structure is from the yeast *Saccharomyces cerevisiae*. This SRP RNA is about twice as long as the mammalian RNA (Felici et al 1989; Hann & Walter 1991), and it remains to be determined experimentally which portions of the sequence correspond to the known domains. Eubacterial SRP RNAs can be thought of as truncated versions of eukaryotic SRP RNA that lack domain III in the case of the *Bacillus subtilis* RNA and that lack domain III and the Alu-domain in the case of the *E. coli* RNA (Poritz et al 1988; Struck et al 1988) (Figure 2).

The most conserved sequence motif of SRP RNA is found in domain IV (Figure 2) and consists of a tetra loop and two bulges that are found in conserved positions and that contain short stretches of highly conserved nucleotide sequences. This motif was originally recognized as the most characteristic feature of all SRP RNAs (Poritz et al 1988; Struck et al 1988) and led to the discovery that 4.5S RNA is the *E. coli* SRP RNA homologue. The two conserved bulges in domain IV provide the binding site for SRP54 (Samuelsson 1992; Selinger et al 1993a; Wood et al 1992; L-S Kahng & P Walter, unpublished), whereas the conserved tetra loop at the end of domain IV can be replaced by a different sequence without affecting SRP function (Selinger et al 1993b). Hence, despite the conservation of its sequence, the tetra loop does not seem to be a target for base-specific contacts. A short conserved sequence motif in domain I has been implicated in the binding of SRP9 and SRP14 (Strub et al 1991).

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

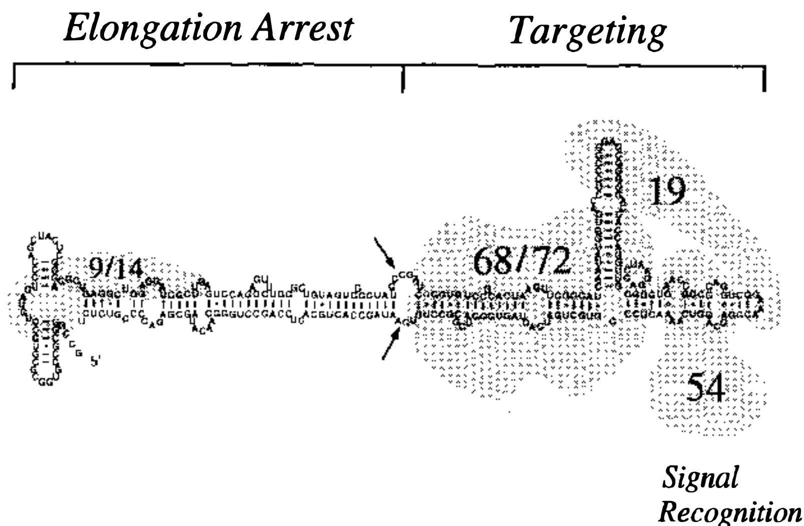
SRP Structure and Assembly

The overall shape of mammalian SRP resembles an elongated rod, 240 Å long × 60 Å wide, in electron micrographs (Andrews et al 1985), and electron-dense SRP RNA appears to extend throughout the length of the rod (Andrews et al 1987). Such an extended structure may reflect a requirement for SRP to span a considerable physical distance in order to perform its multiple functional roles; signal sequence recognition occurs near the nascent chain exit site in the large ribosomal subunit, while elongation arrest is likely to be mediated via interactions about 160 Å away near the peptidyltransferase center of the ribosome (Berneabeau et al 1983). As discussed below, the signal recognition and elongation arrest activities of SRP map to SRP54 and to the Alu domain of SRP RNA, respectively, and are predicted to reside on opposite ends of the particle.

The protein subunit binding sites on the SRP RNA were determined by footprinting experiments (Siegel & Walter 1988b; Strub et al 1991) and are schematically depicted in Figure 3. These studies took advantage of the finding that SRP can be dissociated into its individual protein and RNA subunits when EDTA is added to remove magnesium ions. SRP subunits can then be purified and reassembled under the appropriate conditions to form an active SRP (Siegel & Walter 1985; Walter & Blobel 1983). SRP68 and SRP72 bind to SRP RNA as a stable heterodimer (designated SRP68/72), as do SRP9 and SRP14 (SRP9/14) (Siegel & Walter 1985; Walter & Blobel 1983). Both SRP54 and SRP19 bind to the SRP RNA individually, but SRP54 will not form a salt-stable complex with the SRP RNA unless SRP19 is also added (Poritz et al 1990; Walter & Blobel 1983; Miller et al 1993). A requirement for SRP19 to stabilize SRP54 binding has also been observed in vivo; a mutation in SRP19 in the yeast *S. cerevisiae* causes SRP54 to fall off the particle (Hann et al 1992). While the details of this intriguing interaction remain to be determined, it appears that binding of SRP19 to SRP RNA removes a destabilizing influence of domain III on the SRP54/domain IV interaction. In agreement with this hypothesis, mammalian SRP54 and its *E. coli* homologue form a stable complex with *E. coli* SRP RNA, which lacks domain III, in the absence of any additional protein (Römisch et al 1990; Zopf et al 1990; L-S Kahng & P Walter, unpublished).

The assembly of multicomponent ribonucleoprotein particles requires a number of specific, and probably ordered, protein-RNA and protein-protein interactions to obtain a complete functional particle. This has been amply demonstrated, for example, in many studies of ribosomal subunit assembly, where the association of ribosomal proteins with rRNA occurs in a specific order and the binding of proteins to the complex is often cooperative (e.g. Nomura et al 1969). An understanding of such interactions not only provides

A Mammalian SRP



B *E. coli* SRP

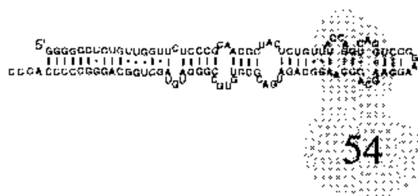


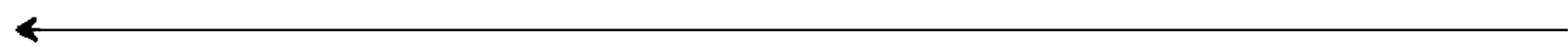
Figure 3 Domain structure of SRP. (*A*) The approximate positions of the SRP protein subunits on mammalian SRP RNA are shown. The binding sites of SRP19 and SRP68/72 were determined by enzymatic footprinting using α-sarcin, a nuclease that cleaves both single- and double-stranded RNA (Siegel & Walter 1988b). The SRP19 binding site was confirmed by SRP RNA fragmentation studies (Zwieb 1991). The binding site for the SRP9/14 heterodimer was determined using chemical footprinting (Strub et al 1991). These locations also agree with the enzymatic splitting of the SRP RNA into two halves in which the Alu domain co-purifies with SRP9/14 and the S domain is found with SRP68/72, SRP54, and SRP19 (Siegel & Walter 1986). The position of SRP54 is inferred from the binding site of its prokaryotic homologue on *E. coli* SRP RNA (*B*), which was determined by mutagenesis (Wood et al 1992), footprinting, and SELEX (L-S Kahng et al, unpublished) experiments and from mutagenesis studies in the yeast *S. pombe* (Selinger et al 1993a).

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

a recipe for particle formation, but may also provide clues about molecular interactions within the particle during its functional cycle.

The binding of SRP subunits may also occur in an ordered fashion. SRP proteins bind to the SRP RNA with a high affinity: the K_d for the SRP RNA complexes with SRP9/14 and with SRP68/72 are < 0.1 and 7 nM, respectively (Janiak et al 1992), and the K_d for *E. coli* SRP54/SRP RNA complex is 5 nM (BS Watson et al, unpublished) as determined by equilibrium binding assays using fluorescently labeled RNAs. The large difference in these K_d values may be important for SRP assembly. Because SRP9/14 binds with high affinity to the 5′ end of the SRP RNA, this association may serve to nucleate the folding of the SRP RNA and thereby ensure that SRP assembly is initiated properly. This is consistent with what has been observed during the assembly of the much larger bacterial ribosomal subunits, where the ribosomal proteins that nucleate their assembly also bind near the 5′ ends of the 16S and 23S rRNAs presumably because this portion of the rRNA is exposed first during RNA synthesis (Nowotny & Nierhaus 1988).

It also appears that SRP assembly is cooperative. Active SRP assembles even in the presence of an excess of naked SRP RNA (Walter & Blobel 1983), and the addition of SRP19 and SRP54 stabilizes the binding of SRP68/72 to the SRP RNA (J-C Chen & A Johnson, unpublished). In contrast, the binding affinity of SRP68/72 for a SRP RNA was unaffected by the presence of SRP9/14, which indicates that the binding of the heterodimers is noncooperative in the absence of SRP54 and SRP19 (Janiak et al 1992). SRP68/72 and SRP9/14 therefore associate randomly and independently with SRP RNA to form non-interacting domains in the particle, consistent with the substantial separation of their binding sites shown in Figure 3. It remains to be shown whether the assembly of the entire particle is cooperative in the presence of SRP19 and SRP54 or whether the cooperativity is restricted to the assembly of proteins on the S domain of SRP RNA.



In interpreting this cartoon, two main caveats must be kept in mind. First, the indicated limits of the protein binding sites in the S domain are not precise because the footprinting data obtained enzymatically do not have as high a resolution as those obtained chemically for SRP9/14. Moreover, the binding site for mammalian SRP54 has not been delineated experimentally, but is inferred from studies of the yeast and bacterial homologues. Second, it is possible that the conformation of the SRP RNA is sensitive to the presence of one or more of the SRP proteins, the SRP receptor, or other components of the system. Such conformational changes upon protein binding have been observed using both chemical modification (Andreazzoli & Gerbi 1991) and fluorescence (Janiak et al 1992) techniques.

The structural arrangement has been dissected in terms of domains with specific functions (Siegel & Walter 1988d). SRP54, SRP68/72, and SRP9/14 are involved in the three primary SRP functions: signal sequence recognition, targeting, and elongation arrest, respectively, as discussed in the text. The nucleolytic sites that allowed the isolation of an SRP subparticle containing only the S domain of SRP RNA are indicated by the arrows; this subfragment is active in signal recognition and targeting.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

SRP Protein Subunits

The sequences of all mammalian SRP proteins have been determined (Bernstein et al 1989; Herz et al 1990; Lingelbach et al 1988; Lütcke et al 1993; Römisch et al 1989; Strub & Walter 1989, 1990). All six proteins have a high abundance of basic amino acids and in this aspect resemble ribosomal proteins. Otherwise, with the notable exception of SRP54 (see below), they have no characteristic sequence motifs or significant sequence similarity to other known proteins. Homologues to mammalian SRP subunits have also been identified in the yeast *S. cerevisiae* (Amaya et al 1990; Hann et al 1989; Stirling & Hewitt 1992; J Brown & P Walter, unpublished). Based on their sequence, SRP72, SRP68, SRP19, and SRP14 are only loosely conserved over this evolutionary distance (about 20–30% sequence identity), but are still clearly recognizable as homologues (Stirling & Hewitt 1992; Brown & Walter, unpublished). In contrast, SRP54 is highly conserved (47% sequence identity) (Hann et al 1989), which suggests that this protein plays a central role in SRP function that results in many more constraints on its structure. SRP54 is also the only known protein subunit of the *E. coli* SRP, and it is remarkably 31% identical to its mammalian counterpart.

No procedure is yet available to separate purified heterodimeric SRP9/14 and SRP68/72 protein complexes into their individual proteins without causing irreversible denaturation. With the availability of cDNA clones for mammalian SRP9, SRP14, SRP68, and SRP72, however, it became possible to produce these subunits individually by in vitro translation and to assess their RNA-binding properties and domain structures. Thus it was determined that dimerization of SRP9 and SRP14 is strictly required because neither protein will bind to SRP RNA in the absence of the other (Strub & Walter 1990). In contrast, SRP68 can bind weakly to SRP RNA in the absence of SRP72 (Lütcke et al 1993). SRP72 association with SRP68 significantly increases the binding affinity, but no stable binding of SRP72 alone to SRP RNA was observed. Thus SRP72 may associate with the SRP RNA via a protein-protein interaction with SRP68.

In contrast to the other SRP subunits, the sequence of SRP54 provides a wealth of information about its structure and function. As shown in the schematic alignment in Figure 4, SRP54 contains a central GTPase domain that is characterized by short sequence stretches that are conserved between most known GTPases and that are known from the X-ray structures of H-ras and EF-Tu to form juxtaposed loops on the surface of the protein that directly contact the bound nucleotide (Bourne et al 1991). The GTPase domain of SRP54 is most closely related to a GTPase domain contained in the SRP receptor subunit SRα, but is more distantly related to other known GTPases. Thus SRP54 and SRα together define a separate subfamily in the superfamily

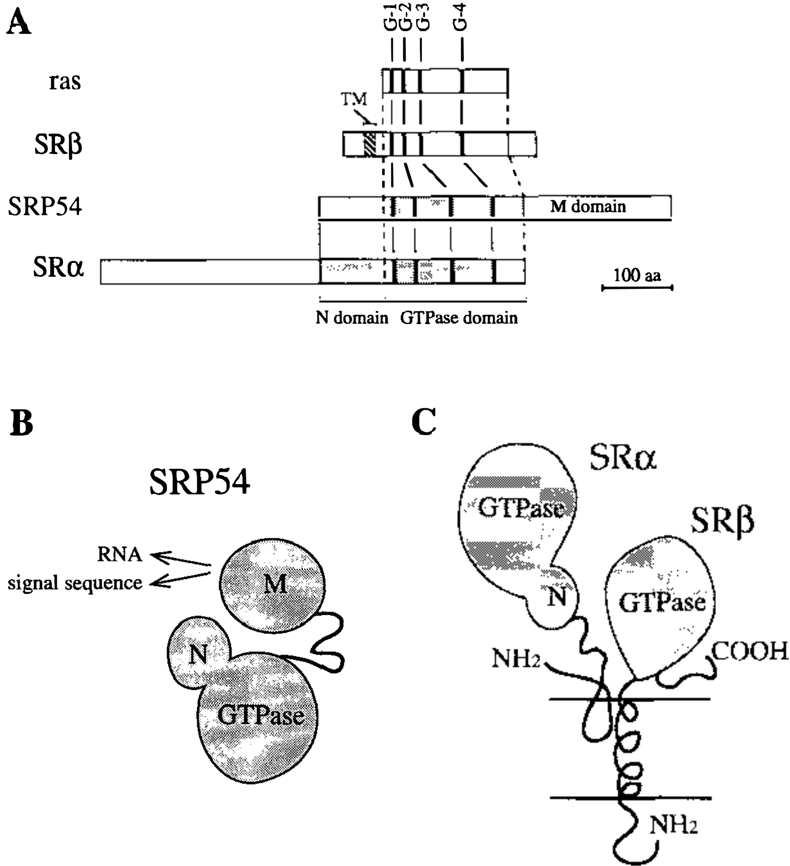


Figure 4 Domain structure of the three GTPases in SRP and SRP receptor. (*A*) The ras-like GTPase domain is characterized by four conserved sequence motifs (G-1 to G-4) as defined by Bourne et al (1991). Note that the GTPase domains of SRP54 and SRα are related to one another (***shaded box***) and that this homology extends through the N domain. The transmembrane region of SRβ is indicated (*TM*). (*B*) The M domain of SRP54 is linked to the N/G domains by a protease-sensitive hinge region. The N and the M domains are likely to be in close spatial proximity because the N and C termini of ras are in close proximity in the folded protein. (*C*) The heterodimeric SRP receptor is likely to be anchored in the membrane by the single transmembrane segment of SRβ. The N/G domains of SRα are linked to SRβ by a protease-sensitive hinge region. It is not known how the N-terminal region of SRα contacts SRβ nor whether it also contacts the hydrophobic core of the membrane.

of GTPases. The GTPase domain is flanked on its N-terminus by the N domain, and on its C-terminus by the M domain, which is characterized by an unusually high abundance of Met residues (about 12% for mammalian SRP54) (Bernstein et al 1989). The M domain is connected to the rest of

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

SRP54 by a flexible hinge region that is protease-sensitive (Figure 4B). This has been experimentally exploited to dissect SRP54 into two fragments, the M domain and a fragment containing both N and GTPase domains, here referred to as the N/G domains. Following proteolytic cleavage within the hinge region, it was shown that the M domain contains the RNA-binding site that attaches SRP54 to domain IV of SRP RNA (Römisch et al 1990; Zopf et al 1990).

The central role of SRP54 in SRP function first became apparent when the signal sequence of a nascent chain was shown to photo cross-link solely to this SRP subunit in crude translation extracts (Krieg et al 1986; Kurzchalia et al 1986). This strongly suggests that SRP54 contains the signal sequence binding site of SRP. Specific cross-linking to SRP54 has now been shown for a variety of different signal sequences including signal-anchor sequences of integral membrane proteins (High et al 1991b). Isolated SRP54 can also be cross-linked to signal sequences, which indicates that the remainder of SRP is not required for this association (Lütcke et al 1992; Zopf et al 1993). The site of signal sequence cross-linking maps to the M domain (Römisch et al 1990; Zopf et al 1990), and more specifically its C-terminal 6-kd fragment (High & Dobberstein 1991), as concluded from cross-linking combined with proteolysis.

E. coli SRP54 was discovered by data bank searches as an open reading frame encoding a protein of unknown function that is highly similar in sequence and domain structure to mammalian SRP54 (Bernstein et al 1989; Römisch et al 1989). This unanticipated discovery was the key step that allowed the characterization of SRP54 homologues from other species. Comparison of the bacterial and mammalian sequences identified highly similar regions that were used in PCR-based approaches to identify SRP54 homologues from all cells analyzed thus far. To date nine different SRP54 sequences are known including those of mammals (Bernstein et al 1989; Römisch et al 1989), yeasts (Amaya et al 1990; Hann et al 1989), prokaryotes (Bernstein et al 1989; Römisch et al 1989; Samuelsson 1992), and even chloroplasts (Franklin & Hoffman 1993). In each case, the SRP54 is part of a soluble ribonucleoprotein complex that also contains an SRP RNA with the characteristic domain IV motif (in the case of chloroplast SRP54 this is not known, however).

SRP54 is the only known protein subunit of the *E. coli* SRP. As discussed above, SRP54 binding to *E. coli* SRP RNA does not require an SRP19, and it is possible that the other SRP subunits are also dispensible in *E. coli*. Alternatively, other SRP subunits may exist, but have escaped detection because they are more loosely bound to this particle. The concept of an *E. coli* SRP was highly controversial as it emerged from the phylogenetic comparisons (Bassford et al 1991; Beckwith 1991) because neither the protein nor the RNA

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

component of *E. coli* SRP were identified genetically as secretion mutants or in biochemical assays that monitor protein translocation across the bacterial plasma membrane. By now, however, a substantial body of experimental evidence has accumulated in support of this idea. In particular, *E. coli* SRP54 can be specifically cross-linked to signal sequences in crude extracts (Luirink et al 1992), and it can replace mammalian SRP54 functionally in signal sequence recognition when it is assembled with mammalian SRP proteins and RNA into a chimeric particle (Bernstein et al 1993). Most importantly, the in vivo depletion of SRP54 from *E. coli* cells leads to translocation defects of some periplasmic proteins (Phillips & Silhavy 1992).

SRP Receptor

Mammalian SRP receptor is a heterodimeric integral membrane protein composed of SRα and SRβ (Tajima et al 1986) that is found only in the ER membrane (Meyer et al 1982b). The existence of a proteinaceous receptor was first shown when protease treatment of microsomal membranes rendered them translocation inactive, and when activity was restored by adding a cytosolic fragment back to the proteolyzed membranes (Meyer & Dobberstein 1980b; Walter et al 1979). This allowed the purification of the 52-kd cytosolic fragment (Meyer & Dobberstein 1980a), which was derived from a 70-kd protein that had been purified independently as a factor that releases the elongation arrest of presecretory proteins induced by SRP (Gilmore et al 1982a,b; Meyer et al 1982a).

From a comparison of the sequence of SRα with that of the soluble fragment released by protease treatment, it is clear that SRα is anchored to the ER membrane through an N-terminal domain (Lauffer et al 1985), presumably via interactions with SRβ (Tajima et al 1986) (Figure 4C). This N-terminal portion contains two hydrophobic regions that may contact the hydrophobic core of the lipid bilayer. However, SRα can be extracted from membranes with chaotropic agents (J Miller et al, in preparation), which indicates that it is not a bona fide integral membrane protein, and SRα synthesized in vitro can post-translationally assemble into membranes (Andrews et al 1989). After it was discovered that protein targeting is a GTP-dependent process, a reinspection of the SRα sequence revealed a GTPase domain at the C-terminus of the protein, which is released as the soluble fragment upon proteolysis (Connolly & Gilmore 1989). Consistent with this finding, SRα binds GTP. The GTPase domain of SRα is closely related to the GTPase domain of SRP54 (Figure 4A), and the sequence similarity extends through the N domain of SRP54.

FtsY, an *E. coli* protein of unknown function, was identified as an SRα homologue based on sequence similarity that extends through the N and GTPase domains of SRα and SRP54 (Bernstein et al 1989; Römisch et al

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

1989). The N-terminal portion of FtsY, however, bears no sequence similarity to the N-terminal region of SRα that anchors it to the ER membrane, and no *E. coli* SRβ subunit has been identified.

Sequence analysis of SRβ shows that it contains a standard transmembrane segment with an uninterrupted stretch of 25 hydrophobic amino acids (J Miller et al, in preparation). Surprisingly, SRβ also contains a predicted GTPase domain and experimentally binds GTP, thus bringing the number of GTPases that interact during protein targeting to three. The GTPase domain of SRβ, however, is not closely related to those of SRP54 and SRα, but is instead in its own new subfamily as a distant relative of the small GTPases SAR and ARF that are involved in vesicular trafficking.

The SRP receptor is unlikely to be an integral part of the translocon because it is present in membranes in substoichiometric amounts with respect to membrane-bound ribosomes (Tajima et al 1986). Hence it is likely that SRP receptor, like SRP, functions catalytically to promote the formation of the ribosome-translocon junction.

MECHANISM OF SRP-DEPENDENT PROTEIN TARGETING

For the purpose of this discussion, we divide the functional cycle of SRP into three distinct steps: (*a*) signal sequence recognition, which results in the recruitment into the targeting complex of those ribosomes that synthesize proteins destined for translocation across or integration into the ER membrane; (*b*) elongation arrest, which modulates the translational activity of the ribosome in the targeting complex; and (*c*) targeting, which leads to the release of SRP from the targeting complex concomitant with the formation of the ribosome-translocon junction. Upon targeting, the signal sequence of the nascent polypeptide chain has been delivered into a sealed aqueous compartment comprised of translocon components.

Selection of Signal Sequences

Current evidence suggests that signal sequences are positively selected by their ability to bind to a signal sequence-binding site on the M domain of SRP54. Historically, SRP was first shown to recognize information contained in the nascent polypeptide chain (as opposed to the mRNA encoding the protein) by experiments in which the structure of the signal sequence of a nascent protein was selectively altered by the incorporation of amino acid analogues (Walter et al 1981). Photo cross-linking experiments then showed that the signal sequence in the targeting complex is in close proximity to—and presumably bound to—the M domain of SRP54 (High & Dobberstein 1991; Krieg et al 1986; Kurzchalia et al 1986; Zopf et al 1990). The notion that SRP54 directly

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

and selectively binds signal sequences is further supported by experiments showing that synthetic functional signal peptides—but not mutant signal peptides that are inactive as signal sequences in vivo and differ from functional signal peptides only by single amino acid substitutions—inhibit GTP binding to SRP54 (Miller et al 1993). However, the results from these indirect experiments still remain to be confirmed by assays that monitor the direct binding of signal peptides to SRP54.

The characteristic feature of ER-directed signal sequences is a core comprising about 8–12 hydrophobic amino acids that presumably forms an α-helix (von Heijne 1985). Because their amino acid sequences are not conserved, such signal sequence cores must each have a different shape. The predicted structural characteristics of the M domain of SRP54 suggest a model of how signal sequences may bind to SRP despite this structural diversity. Most of the unusually abundant Met residues in SRP54M are predicted to reside on one face of a group of strongly amphipathic α helices (Bernstein et al 1989; Hann et al 1989) that have been proposed to form or contribute to a signal sequence binding groove (Bernstein et al 1989). A unique feature of Met side chains is their flexibility; the side chains of Leu and Ile, amino acids of comparable hydrophobicity, are branched and hence comparatively rigid. Thus the flexible hydrophobic Met side chains would project like bristles of a brush into such a groove and provide a hydrophobic environment with sufficient plasticity to allow signal sequence binding despite the heterogeneity in amino acid sequence. This hypothesis is supported by the remarkable phylogenetic conservation of both the unusual abundance of Met residues and their position on the predicted α helices in the M domain of SRP54 homologues from mammalian cells to bacteria. Met is typically a relatively rare amino acid and is often replaced in phylogenetic comparisions by other hydrophobic amino acids. Its conservation in SRP54 therefore indicates an importance of the Met side chains that is both structural and functional.

A paradigm for the involvement of Met side chains in the binding of heterogeneous hydrophobic surfaces is provided by calmodulin. This dumbbell-shaped molecule binds to a variety of different target proteins by clamping down on amphipathic helices exposed on the surface of the target proteins (O'Neil & DeGrado 1990). Biochemical studies, as well as NMR and crystallographic analyses, show that patches of exposed Met side chains on calmodulin provide the interaction surfaces that contact the hydrophobic parts of the amphipathic helices in the target proteins. Similar structural analyses are needed for SRP54 to validate or disprove the speculative hypothesis presented above.

SRP has a low affinity for ribosomes that are not engaged in translation, but its affinity is increased by three to four orders of magnitude when a signal sequence

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

is expressed and exposed outside the ribosome as part of a nascent chain (Walter et al 1981). It is likely that SRP normally cycles between a ribosome-bound state and a free state, thereby scanning nascent polypeptide chains for signal sequences. Regardless of their secretory activity, cells contain about one SRP for every ten ribosomes, which suggests that SRP cannot remain bound to any given ribosome waiting for a signal sequence to emerge. Moreover, in both bacteria and yeast there is evidence that SRP interacts with ribosomes at a discrete step in the elongation cycle (most likely before the translocation step catalyzed by elongation factor EF-G or eEF2, respectively) (Brown 1989; S Ogg & P Walter, unpublished). Thus free SRP may transiently bind to ribosomes during any elongation cycle. SRP then may either remain bound to the ribosome if a signal sequence has associated with SRP54, or dissociate rapidly and move to another ribosome if no signal sequence has been detected.

As nascent chains grow longer, their affinity for SRP decreases (Siegel & Walter 1988a). This could be because the signal sequence is no longer favorably positioned with respect to the ribosome-bound SRP and/or because the signal sequence is rendered less accessible through aberrant folding of the nascent chain. It is attractive to speculate that the other SRP subunits and SRP RNA help position SRP on the ribosome such that the signal sequence binding site on SRP54M and the nascent chain exit site on the large ribosomal subunit become juxtaposed. How long a nascent chain that contains a signal sequence on its amino terminus can be extended and still be recognized by SRP varies greatly between different proteins. For most proteins the affinity for SRP drops drastically after they have been elongated beyond a certain point (Siegel & Walter 1988a). Other proteins, however, can still be recognized by SRP after they are synthesized to full length, provided that translation has not terminated, i.e. that the protein remains ribosome-bound and covalently attached to tRNA (Garcia & Walter 1988). SRP will not promote post-translational translocation of signal sequence-bearing proteins that have been released from the ribosome (Garcia & Walter 1988). A reported activity (Crooke et al 1988; Sanz & Meyer 1988) of mammalian SRP to promote post-translational translocation in yeast and *E. coli* in vitro systems may not reflect a physiological pathway, but rather result from nonspecific hydrophobic interactions that retard dead-end protein folding or aggregation.

Elongation Arrest

The notion that SRP in the targeting complex interacts intimately with the ribosome is best supported by direct SRP-dependent effects on translation. When SRP is included in in vitro translation systems in the absence of ER membrane vesicles, it blocks elongation after the signal sequence has become exposed outside the ribosome (Walter & Blobel 1981). In some cases a discrete-sized protein fragment that corresponds to the elongation-arrested secre-

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

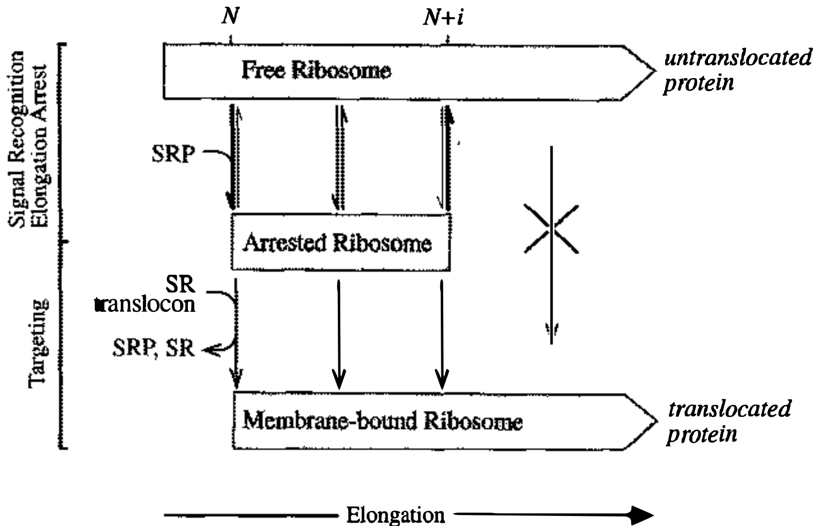


Figure 5 Kinetic model for elongation arrest and targeting (adapted from Rapoport et al 1987). SRP interacts with a ribosome and arrests translational elongation after N amino acids have been polymerized and the signal sequence is exposed outside the ribosome. The formation of the targeting complex will only occur within a window of nascent chain lengths (N to N+i). As nascent chains grow longer, their affinity for SRP decreases and drops drastically if elongation proceeds beyond this window of opportunity. In the absence of membranes, translation elongation is delayed by SRP because the ribosome spends time idling in the elongation-arrested targeting complex. In the presence of membranes on the other hand, the SRP in the targeting complex interacts with the SRP receptor, which leads to the formation of the ribosome-translocon junction, an efficient and energy-consuming reaction that leads to the synthesis of translocated protein.

tory protein can be observed by gel electrophoresis (Meyer et al 1982a; Walter & Blobel 1981); in other cases the arrested forms are more heterogeneous and difficult to detect (Anderson et al 1982; Lipp et al 1987). The positions of paused ribosomes along mRNAs confirm that SRP arrests translation just after the signal peptide emerges from the ribosome and becomes available for binding to SRP (Wolin & Walter 1988). Interestingly, the SRP enhances pausing of ribosomes at sites that are natural stutter points in the translation of the mRNA. The distribution of stutter points in the mRNA may therefore determine the spectrum of arrested nascent chain fragments. Stutter points that cause the nascent chain to pause just after a signal sequence has emerged from the ribosome may be advantageous to cells because they may increase the efficiency of signal sequence recognition by SRP.

To date, a strict block of elongation has only been observed in in vitro assays composed of heterologous components, e.g. mammalian SRP and a wheat germ translation system. In the yeast *Yarrowia lipolytica,* mutations in SRP RNA

can cause an inhibition of the translation of a secretory protein (He et al 1992; Yaver et al 1992). This phenomenon may be indicative of an elongation block, although this still remains to be shown more directly. In assays composed exclusively of mammalian components, the SRP-induced elongation arrest is transient, thus causing a kinetic delay in protein elongation (Wolin & Walter 1989). Similarly, in yeast cells in which the SRP receptor has been genetically depleted, the synthesis of presecretory proteins is not detectably reduced (Ogg et al 1992), which indicates that the lack of SRP receptor in vivo does not lead to an irreversible elongation arrest. These observations are consistent with a kinetic model (Figure 5, adapted from Rapoport et al 1987) that treats the association of SRP with the ribosome/signal sequence as an equilibrium-binding reaction. According to this model, elongation in the targeting complex is completely blocked, but elongation resumes when SRP dissociates from the targeting complex.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Molecular dissection of SRP, either by fragmentation with nuclease (Siegel & Walter 1986) or by partial reconstitution (Siegel & Walter 1985), has mapped the elongation arrest function of SRP to a discrete domain comprising the Alu-portion of SRP RNA and SRP9/14 (indicated in Figure 3A). Thus SRP can also be visualized as having one end involved in elongation arrest, while the other end is involved with the signal sequence and ER membrane components. Partial SRPs that lack the elongation arrest domain (or that contain the Alu-domain of the RNA, but without SRP9/14 or with alkylated SRP9/14 bound) still promote signal recognition and protein targeting, which indicate that elongation arrest is not a prerequisite for protein translocation (Siegel & Walter 1985, 1988c). Because most signal sequence-bearing nascent proteins lose the ability to be translocated if elongation proceeds too far, however, elongation arrest helps to maintain the translocation competence of the nascent chain by delaying its elongation. Thus one physiologically important function of elongation arrest may be to increase the fidelity of protein translocation. If the function of SRP or the SRP receptor in cells could be regulated (which presently is not known), then it is also conceivable that elongation arrest may be used as a convenient on-off switch by which cells could adapt the synthesis of secretory proteins to the secretory needs of the cell.

Because elongation arrest is selective for signal sequence-bearing proteins, it requires recognition of a signal sequence by SRP54, and SRP subparticles that lack SRP54 are inactive. An SRP subparticle, termed SRP(-54G), that lacks only the N/G domains of SRP54 because it was reconstituted with purified M domain in place of SRP54 still elicits elongation arrest activity (Zopf et al 1993). This shows that the M domain of SRP54 is sufficient for signal sequence binding and, when reconstituted with the remaining SRP subunits, is also sufficient to transmit this information to the ribosome to elicit elongation arrest. The affinity of the isolated M domain or of SRP(-54G) for

signal sequences is reduced, however, thus indicating that the presence of the N/G domains on SRP54 contributes to signal sequence binding (Zopf et al 1993). SRP(-54G) is completely inactive in promoting protein translocation across microsomal membranes presumably because it cannot interact normally with the SRP receptor. Thus the N/G domains have a dual function: they influence signal sequence recognition by promoting a tighter association between signal sequences and the M domain and, as discussed below, they play an essential role in targeting.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Targeting

Targeting to the ER membrane is mediated by multiple GTPases that comprise domains of SRP54, SRα, and SRβ. In numerous biological processes, GTPases function as molecular switches that provide unidirectionality and accuracy (Bourne et al 1990). Through GTP binding and hydrolysis, GTPases can exist in at least three discrete conformations: a nucleotide-free, a GTP-bound, and a GDP-bound conformation. Interconversion between these states in a defined sequence causes the GTPase to interact in temporal succession with its effectors, thereby regulating the biological process. In most cases, the conversion of one conformer to another is controlled by other molecules: GTP hydrolysis is often facilitated by the action of specific GTPase activating proteins, GDP release by the action of specific guanine nucleotide release factors, and GTP binding by the action of specific guanine nucleotide loading factors.

The GTPase domains of SRP54 and SRα are required to promote the progression from targeting complex to the formation of the ribosome-translocon junction. As mentioned above, SRP(-54G) does not promote targeting and, likewise, microsomal membranes that have been proteolytically depleted of the GTPase domain of SRα are inactive. The severed soluble domain of SRα can be added back to proteolyzed membranes to restore activity, thus confirming that SRα is the only membrane protein essential for this process that was destroyed by the protease treatment.

Interestingly, non-hydrolyzable GTP analogues can substitute for GTP in targeting (Connolly & Gilmore 1989; High et al 1991a). After targeting in the presence of non-hydrolyzable GTP analogues, however, SRP and SRP receptor remain locked together as a stable complex (Connolly et al 1991). Therefore, it is thought as a minimum that GTP binding to SRP54 and SRα is required for targeting, and GTP hydrolysis is required to allow the regeneration of free SRP and SRP receptor that can then engage in another round of targeting. GTP bound to SRP is hydrolyzed upon interaction with the SRP receptor, which thus functions as a GTPase-activating protein for SRP54 (Miller et al 1993).

The unprecedented direct interaction between three GTPases involved in targeting has made attempts to decipher the contributions of individual GTPases to the overall reaction challenging. Biochemical characterization of

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

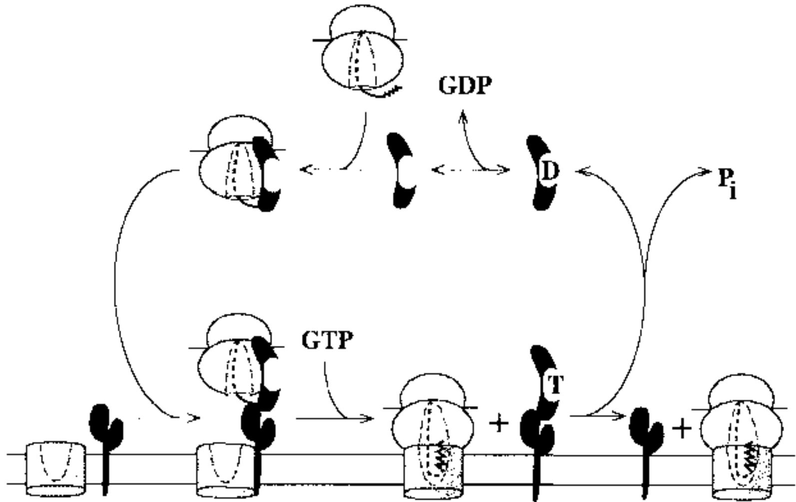


Figure 6 Model for GTP utilization by SRP54 (adapted from Miller et al 1993). As discussed in detail in the text, SRP54 is proposed to undergo a series of sequential conformational changes that drive SRP unidirectionally through cycles of protein targeting. In the experiments that led to the proposal of this model, guanine nucleotide occupancy of SRP54 was assessed by UV cross-linking (Miller et al 1993). It is possible, however, that the state depicted here as nucleotide-free does, in fact, contain a nucleotide that is bound in such a way that it fails to cross-link to SRP54 upon UV irradiation. GDP release from SRP54 may occur spontaneously or may be facilitated by binding of signal sequences and/or ribosome. T = SRP54-bound GTP; D = SRP54-bound GDP.

the interaction of purified SRP and SRP subparticles with purified SRP receptor has yielded initial insights into the mechanism by which the GTPase domain of SRP54 may regulate targeting (Figure 6, adapted from Miller et al 1993). According to these studies, the guanine nucleotide-bound state of SRP54 is influenced by at least two ligands: a signal peptide stabilizes a nucleotide-free state in SRP54 (and perhaps even stimulates the release of GDP), and interaction with the SRP receptor significantly increases the affinity of SRP54 for GTP. If these results hold true for the complete targeting complex (which needs to be confirmed experimentally), then SRP arrives at the membrane held in a nucleotide-free state by the signal sequence. The SRP receptor may then function as a guanine nucleotide-loading protein that promotes GTP binding to SRP54 and concomitantly reduces the affinity of SRP54 for the bound signal sequence. Interestingly, however, purified SRP receptor fails to stimulate GTP loading of SRP54 in the presence of signal peptides. One likely explanation for this observation is that additional components, such as translocon subunits, are required to effect signal sequence release in the purified in vitro system. This is an appealing notion because the requirement for translocon subunits

would introduce a check point: unless appropriate translocon subunits have been recruited and proper translocation is ensured, the signal sequence is not released from SRP and the reaction cannot proceed.

In this model of targeting, SRP54 emerges in a central role in which its GTPase domain is used to integrate information received from both the nascent chain and the ER membrane. Available evidence points to extensive allosteric communication between the structurally separate domains of SRP54: binding of a signal sequence to the M domain prevents nucleotide binding to the GTPase domain (Miller et al 1993) and, conversely, chemical modification of the GTPase domain prevents signal sequence binding to the M domain (Lütcke et al 1992). However, the prediction that GTP binding to the GTPase domain lowers the affinity for signal sequences has not been confirmed experimentally. SRP54 may resemble EF-Tu in which large conformational changes accompany the exchange of bound GDP for bound GTP (Berchtold et al 1993). Consistent with this observation, nucleotide occupancy of the GTPase domain could, for example, change the relative arrangement of the GTPase and M domains such that the N/G domains no longer promote tight association between signal sequences and the M domain. One can speculate that the N domain, which is likely to be closely juxtaposed to the M domain (Figure 4B), could cover the signal sequence-binding groove on the M domain and that the GTPase domain may provide a hinge module that determines whether this cover is in the open or closed position.

The GTPase domains in SRα and SRβ may also provide check points in the targeting pathway that are monitored by guanine nucleotide switches. While the importance of guanine nucleotide binding to SRβ for targeting has yet to be demonstrated, it has clearly been shown for SRα. Using proteolyzed microsomal membranes that were repopulated with a mutant form of SRα that has a lower affinity for GTP, it was shown that SRα needs to be in a GTP bound state for the targeting reaction to progress through the cycle shown in Figure 6 (Rapiejko & Gilmore 1992). Thus it is likely that only GTP-bound SRα will promote GTP binding to SRP54. As the GTPase domains of SRα and SRP54 define a unique subgroup in the superfamily of GTPases and hence may function similarly, SRα may progress through a cycle of GTP binding and hydrolysis similar to that of SRP54. It is possible that, just as SRP recruits ribosomes with nascent chains from the cytosol to the membrane, so the SRP receptor may recruit translocon components within the plane of the membrane. In this view, SRP and SRP receptor function as molecular "match makers" during the assembly of the ribosome-translocon junction.

SRP68/72 are also required for targeting in the mammalian system because SRP subparticles that lack these subunits are inactive (Siegel & Walter 1988c). Moreover, SRPs that were selectively alkylated with N-ethylmaleimide (NEM) on their SRP68/72 subunits were unable to promote targeting of the nascent

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

chains to the ER membrane, apparently because the NEM modification interfered with an interaction between the SRP68/72-modified SRP and the SRP receptor (Siegel & Walter 1988c). The challenge now is to decipher what component(s), other than SRP RNA, SRP68/72 binds during this process and how these interactions facilitate targeting.

The interactions between the *E. coli* SRP and FtsY, the bacterial homologue of SRα, closely mimic those of their mammalian counterparts (Miller et al 1994). In particular, the *E. coli* SRP binds tightly to FtsY in a GTP-dependent manner. This interaction leads to a stimulation of GTP hydrolysis, which can be inhibited by synthetic signal peptides. These results provided the first experimental evidence that FtsY has SRP receptor-like properties.

Beyond Targeting

The interaction of the targeting complex with the SRP receptor initiates the events that result in the binding of the ribosome to the membrane surface, the resumption of protein synthesis, the release of the signal sequence from SRP54, and the release of both SRP and SRP receptor from ribosome and translocon. The signal sequence is released from the SRP54 on the cytoplasmic side of the membrane during this process, but its exact location at the membrane is not well defined. In particular, it is not clear whether the signal sequence is bound to a protein in the translocon, perhaps after a direct transfer from SRP54. Such binding would have to be transitory, however, because the signal peptide is ultimately cleaved from the nascent chain by the signal peptidase on the lumenal side of the ER membrane.

Fluorescent probes that are sensitive in their emission characteristics to the hydrophobicity of their environment have been incorporated into the signal sequences of nascent chains. Measurements using such nascent chains indicate that, after completion of targeting, the signal sequence is initially in an aqueous environment, sealed off from both the cytoplasm and the lumen of the ER (Crowley et al 1994, 1993). This aqueous compartment appears to be formed by integral membrane proteins that reside in the rough ER, since photo cross-linking studies have shown that the signal sequence is positioned adjacent to at least two ER membrane proteins (High et al 1993; Krieg et al 1989; Wiedmann et al 1987) termed Sec61α and TRAM (Görlich et al 1992a; Görlich et al 1992b). Sec61α is part of a complex of three integral membrane proteins, Sec61α, Sec61β, and Sec61γ (Hartmann et al 1994). Reconstitution studies of purified components into artificial proteoliposomes have shown that the only components required to catalyze protein translocation across a lipid bilayer are the SRP receptor, the Sec61 complex and—for some proteins—TRAM (Görlich & Rapoport 1993). Thus they provide a minimal translocon for SRP-dependent protein translocation.

The endproduct of an equivalent SRP-dependent targeting reaction in *E.*

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

coli is less well defined because the existence of membrane-bound ribosomes in *E. coli* is not as well established. It is plausible, however, that signal sequence recognition and targeting lead to a ribosome/membrane junction much like that observed in mammalian cells. The *E. coli* SecY/E proteins, two known components of an *E. coli* translocon, are similar in primary structure to the α and γ subunits of the Sec61 complex, and hence could play similar roles (Görlich et al 1992b; Hartmann et al 1994). Alternatively, the co-translational features might hold true only for signal sequence recognition by the *E. coli* SRP and for FtsY-mediated targeting; the nascent chain may then be handed over to other cytoplasmic components, such as chaperonins, for subsequent post-translational delivery to a translocon.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

ALTERNATIVE TARGETING ROUTES

For the vast majority of proteins, translocation across mammalian ER membranes has a strict requirement for co-translational delivery of the nascent chain. In contrast, translocation of proteins across bacterial (reviewed in Bassford et al 1991; Randall & Hardy 1989) and yeast membranes (reviewed in Meyer 1988), and in a few cases across mammalian membranes (Schlenstedt et al 1990), can occur post-translationally, i.e. signal sequence–bearing proteins that have been released from ribosomes can be translocated from a soluble pool. SRP and SRP receptor are not involved in post-translational translocation, and it is therefore imperative to define and delineate their role (and the role of the pathway that they catalyze) in organisms in which post-translational translocation is prevalent.

SRP-independent Targeting

In yeast, protein targeting to the ER can occur by redundant pathways in vivo. One inevitably has to arrive at this conclusion because *S. cerevisiae* mutant cells lacking SRP or SRP receptor are viable, even though they grow poorly and the translocation of some proteins across the ER membrane is severely impaired (Hann & Walter 1991). Thus every protein that has to cross or become integrated into the ER membrane during its biogenesis and that is essential for cell viability must be targeted via alternate, SRP- and SRP receptor-independent pathways efficiently enough to sustain cell growth. Whereas *S. cerevisiae* cells remain viable, *S. pombe, Y. lipolytica,* and *E. coli* cells die when genes encoding SRP components are genetically disrupted, which indicates that SRP-dependent protein targeting is usually an essential pathway and that *S. cerevisiae* cells have evolved a particularly effective means of bypassing it.

The molecular details of alternative targeting pathways in the SRP-deficient *S. cerevisiae* mutant cells are presently unclear. The translocation of most soluble and membrane proteins into the lumen of the ER is impaired in

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

SRP/SRP receptor-deficient cells but, surprisingly, different proteins show translocation defects of varying severity (Hann & Walter 1991). There are two conceptually distinct explanations for this. First, SRP/SRP receptor-independent targeting could occur post-translationally: precursor proteins are released from ribosomes and are maintained in a soluble and translocation-competent state by interactions with cytosolic chaperonins (Chirico et al 1988; Deshaies et al 1988) and other putative targeting factors (Figure 7, pathway C). The folding characteristics of a particular preprotein may thus determine how efficiently it can be maintained in a translocation-competent form. This could explain why translocation defects observed in SRP and SRP receptor-deficient cells vary in magnitude for different proteins. In vitro studies corroborate this notion. The yeast pheromone prepro-α-factor, for example, which can be efficiently translocated post-translationally in vitro, shows only minor translocation defects in SRP- and SRP receptor-depleted cells in vivo.

According to a second hypothesis, SRP/SRP receptor-independent targeting could occur co-translationally: ribosomes synthesizing precursor proteins engage with the ER membrane independent of SRP and SRP receptor, but prior to termination of protein synthesis (Figure 7, pathway B). If co-translational targeting is obligate for a given precursor protein, then the kinetics of its elongation would affect the efficiency of its membrane translocation. If elongation is slow, for example, then a longer time frame would be available for the nascent precursor protein to engage with the ER membrane before it is elongated too far or is completed and released from the ribosome. Experimental results do not allow us to distinguish whether pathway B or pathway C or both operate in SRP-deficient yeast cells.

The fact that alternative pathways can be used in these mutant cells, however, does not imply that such pathways are major routes in wild-type cells. To the contrary, we consider it likely that all proteins that show translocation defects in the mutant cells are co-translationally targeted by SRP and SRP receptor to the ER membrane in wild-type cells (Figure 7, pathway A), and that they become re-routed into alternative targeting pathways only in the mutant cells lacking SRP and/or SRP receptor function or if the SRP/SRP receptor system becomes saturated. As SRP is thought to scan all nascent chains emerging from ribosomes, any protein that expresses a signal sequence that can bind to SRP with a reasonable affinity would be shunted into the co-translational pathway. Some rare proteins, however, that show no translocation defects in SRP-depleted cells may have evolved signal sequences that do not interact efficiently with SRP. Such proteins, e.g. preprocarboxypeptidase Y, may not use the SRP-dependent targeting pathway even in wild-type cells (Bird et al 1987; Hann & Walter 1991).

It is likely that a similar scenario of redundant targeting pathways also exists in *E. coli*. Because of the fast growth rates of bacterial cells, however, many

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

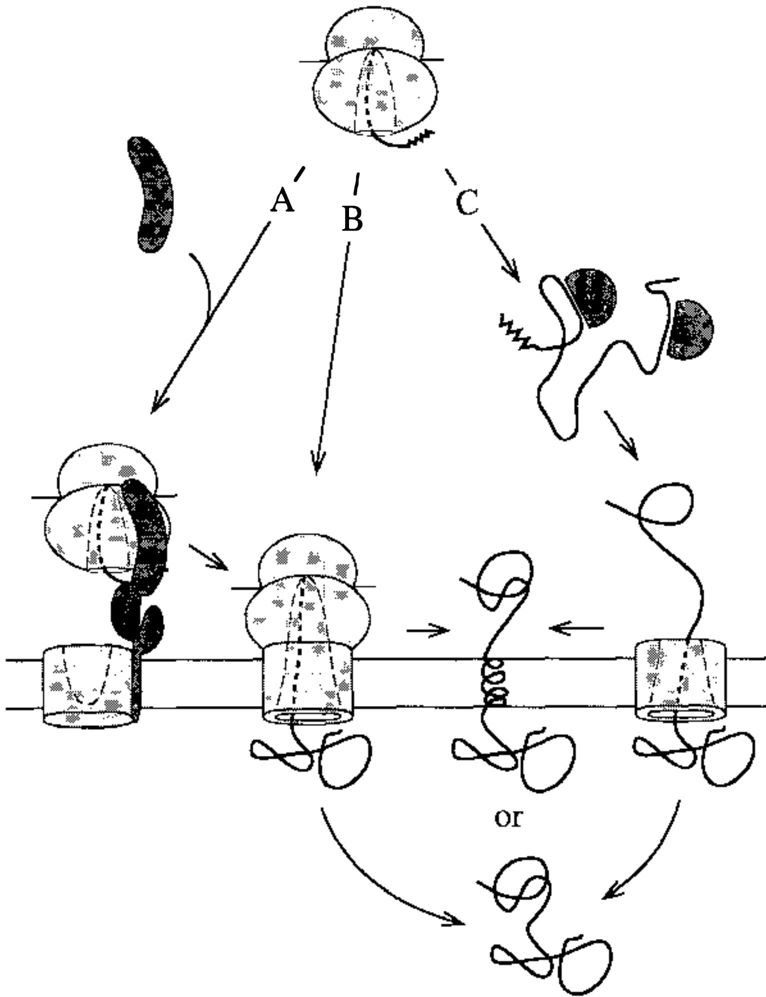


Figure 7 Three possible pathways for protein translocation in yeast (adapted from Hann & Walter 1991). SRP- and SRP receptor-dependent co-translational targeting, pathway A, can be efficiently bypassed in yeast. Pathway B shows SRP-independent co-translational targeting: SRP and SRP receptor are bypassed and the ribosome attaches to the ER membrane to yield an interaction indistinguishable from that achieved in pathway A. Attempts to identify such a pathway across mammalian ER were unsuccessful (Garcia & Walter 1988). Pathway C shows SRP-independent post-translational targeting. Prior to targeting, protein synthesis terminates and the precursor is released into a soluble pool. Translocation competence may be maintained by interactions with chaperonins, indicated by semi-circles. The preprotein interacts with a translocon, which is likely to share core components with the translocon used in pathways A and B, but which is not necessarily identical to it (see Figure 8).

abundant bacterial periplasmic proteins may have evolved such that they can use either SRP-dependent or SRP-independent pathways efficiently. *E. coli* has a particular chaperone, SecB, that seems to be dedicated to maintain preproteins competent for post-translational translocation (Kumamoto 1991). SecB-deficient cells die on rich medium, but are viable on minimal medium, which causes them to grow more slowly. The death of SecB-deficient cells on rich medium can be prevented if other chaperones are overproduced (Altman et al 1991; Wild et al 1992). This indicates that the SecB-mediated post-translational pathway is most important in fast growing cells, where an SRP-mediated pathway may be overwhelmed. The existence of at least partially redundant pathways may be the reason why genetic analyses have not identified an SRP or SRP receptor in bacteria.

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Modular Translocons?

Proteins that are delivered to the membrane as short nascent chains emerging from the ribosome have different requirements for translocation than fully synthesized proteins that are delivered post-translationally. Conceptually, co-translational translocation is the easiest mode to envision (Figure 8A). The translocation pore that is formed by the translocon underneath the tightly attached ribosome can be thought of as an extension of the tunnel in the large ribosomal subunit through which the nascent chain exits (Simon & Blobel 1991; Crowley et al 1994, 1993). The translocon and the ribosome form a tight seal and the nascent chain therefore has no alternative but to move through the pore and into the ER lumen as translation proceeds and the polypeptide grows. Co-translational translocation is therefore dictated by the topography of the ribosome-membrane junction and probably driven by passive diffusion, although some method of active transport cannot be ruled out. In evolutionary terms this is an attractive mechanism because there are no contraints on the particular sequence of the nascent chain. SRP and SRP receptor mediate the immediate membrane attachment of short nascent chains and, as the protein is never exposed to the cytosol, there is no chance for it to fold, misfold, or aggregate into conformations that might then be difficult or impossible to translocate.

A different and mechanistically more complex model emerged from in vitro studies of post-translational translocation in *E. coli* (Figure 8B). Here the translocon uses a dedicated ATPase, the SecA protein, to insert the protein into the translocation channel and then pushes the protein, presumably by ratchet-like movements, through the membrane (Wickner et al 1991). As the preprotein is fully synthesized at this point, the translocon must recognize the signal sequence, unravel the chain, and release it from chaperones such as SecB. Moreover, somehow the permeability barrier of the membrane must be

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

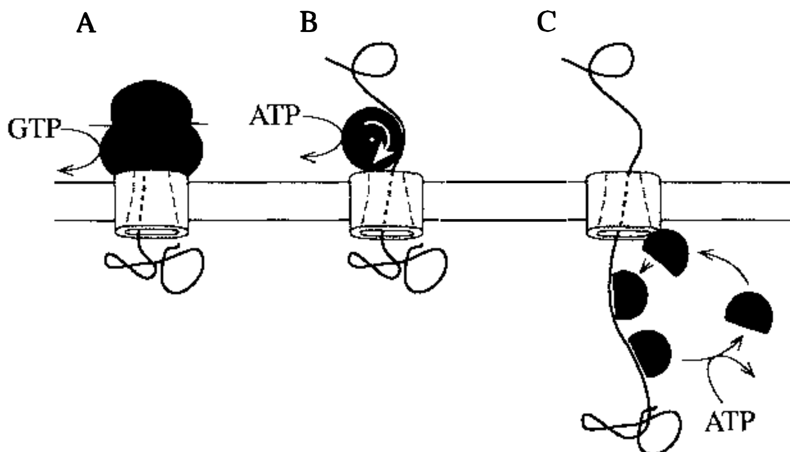


Figure 8 Three possible ways to drive protein translocation across membranes. These three modes, using chemical energy to drive protein translocation, are discussed in the text. (*A*) Translation elongation drives the nascent chain through the membrane pore (indirectly, the energy of GTP hydrolysis by elongation factors and that contributed by the charged tRNAs is utilized); (*B*) an ATP-consuming enzyme moves the polypeptide chain through the membrane pore; and (*C*) ATP-driven cycles of chaperonin binding and release pull the polypeptide chain through the membrane pore. It is possible that two, or maybe even three of these mechanisms collaborate to translocate or integrate certain proteins.

retained during translocation because in bacteria the membrane is also used to maintain a proton gradient.

A third mechanism for effecting translocation involves the sequential attachment of lumenal chaperones to pull a polypeptide across the membrane from the cytosolic side to the lumenal side (Figure 8C). There is good evidence in yeast, for example, that the ER lumenal hsp70 homologue BiP is required for protein translocation (Sanders et al 1992; Vogel et al 1990), and an analogous mechanism operates during the import of proteins into the mitochondrial matrix space (Hannavy et al 1993; Stuart et al 1994). A requirement for ER lumenal proteins was also shown for the mammalian system and may be particularly important for the completion of translocation, i.e. to pull the last section of a protein across the membrane after the ribosome has terminated its synthesis (Nicchitta & Blobel 1993).

It is likely that these three different modes of translocation all use a common "core translocon" comprising the evolutionarily conserved SecY/SecE (which contains a third uncharacterized protein called Band 1, or in eukaryotes, the Sec61 α,β,γ protein complex) that provides the basic protein-conducting channel through which the protein chain crosses the membrane. To this core translocon, auxiliary components may be attached—perhaps transiently—to

adapt the translocation process to particular needs dictated by whether the protein is delivered to the membrane co- or post-translationally or whether it is a soluble or membrane protein. For membrane protein synthesis, the core translocon has to provide not only a translocation pore, but also the means by which transmembrane segments in nascent membrane proteins are recognized. Then the translocation pore must be able to open on one side to allow the lateral exit of the membrane protein into the lipid bilayer.

Auxiliary factors might include, for example, ribosome receptors that may contribute to the seal between the membrane and ribosome: TRAM (already shown to be essential for the translocation of only some proteins; Görlich et al 1992a); the SRP receptor; the SecA protein; BiP; and the Sec62, 63, 71, and 72 proteins in yeast. The latter comprise a set of interacting membrane proteins important for protein translocation in yeast for which no homologues in mammalian cells or bacteria have been found. They may be dedicated to an SRP-independent translocation pathway and/or help the integration of membrane proteins. Because yeast cells contain much more Sec61 protein than they do Sec62 or Sec63 protein (Deshaies et al 1991), it is likely that translocons in a single cell are heterogeneous with regard to attached auxiliary components. The most challenging problem for future research is to decipher these complexities and to understand in mechanistic terms how protein translocation and membrane protein integration are catalyzed and modulated.

ACKNOWLEDGMENTS

Work in the authors' laboratories was supported by a grant from the Human Frontiers Science Program to PW and by grants from the National Institutes of Health to PW and AEJ.

Any *Annual Review* chapter, as well as any article cited in an *Annual Review* chapter, may be purchased from the Annual Reviews Preprints and Reprints service. 1-800-347-8007; 415-259-5017; email: arpr@class.org

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Literature Cited

Althoff S, Selinger D, Wise JA. 1994. Molecular evolution of SRP cycle components: functional implications. *Nucleic Acids Res.* In press

Altman E, Kumamoto CA, Emr SD. 1991. Heat-shock proteins can substitute for SecB function during protein export in *E. coli*. *EMBO J.* 10:239–45

Amaya Y, Nakano A, Ito K, Mori M. 1990. Isolation of a yeast gene, SRH1, that encodes a homologue of the 54K subunit of mammalian signal recognition particle. *J. Biochem.* 107:457–63

Anderson DJ, Walter P, Blobel G. 1982. Signal recognition protein is required for the integration of acetylcholine receptor d subunit, a transmembrane glycoprotein, into the endoplasmic reticulum membrane. *J. Cell Biol.* 93:501–6

Andreazzoli M, Gerbi SA. 1991. Changes in 7SL RNA conformation during the signal recognition particle cycle. *EMBO J.* 10:767–77

Andrews DW, Lauffer L, Walter P, Lingappa VR. 1989. Evidence for a two-step mechanism involved in assembly of functional sig-

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

nal recognition particle receptor. *J. Cell Biol.* 108:797–810

Andrews DW, Walter P, Ottensmeyer FP. 1985. Structure of the signal recognition particle by electron microscopy. *Proc. Natl. Acad. Sci. USA* 82:785–89

Andrews DW, Walter P, Ottensmeyer FP. 1987. Evidence for an extended 7SL RNA structure in the signal recognition particle. *EMBO J.* 6:3471–77

Bassford P, Beckwith J, Ito K, Kumamoto C, Mizushima S, et al. 1991. The primary pathway of protein export in E. coli. *Cell* 65:367–68

Beckwith J. 1991. "Sequence gazing?" *Science* 251:1161

Berchtold H, Reshetnikova L, Reiser CO, Schirmer NK, Sprinzl M, Hilgenfeld R. 1993. Crystal structure of active elongation factor Tu reveals major domain rearrangements. *Nature* 365:126–32

Berneabeau C, Tobin EM, Fowler A, Zabin I, Lake JA. 1983. Nascent polypeptide chains exit the ribosome in the same relative position in both eucaryotes and procaryotes. *J. Cell Biol.* 96:1471–74

Bernstein HD, Poritz MA, Strub K, Hoben PJ, Brenner S, Walter P. 1989. Model for signal sequence recognition from amino-acid sequence of 54k subunit of signal recognition particle. *Nature* 340:482–86

Bernstein HD, Zopf D, Freymann DM, Walter P. 1993. Functional substitution of the signal recognition particle 54-kDa subunit by its *Escherichia coli* homolog. *Proc. Natl. Acad. Sci. USA* 90:5229–33

Bird P, Gething MJ, Sambrook J. 1987. Translocation in yeast and mammalian cells: not all signal sequences are functionally equivalent. *J. Cell Biol.* 105:2905–14

Blobel G, Dobberstein B. 1975. Transfer of proteins across the membrane. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67:835–51

Bourne HR, Sanders DA, McCormick F. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348:125–32

Bourne HR, Sanders DA, McCormick F. 1991. The GTPase superfamily: conserved structure and molecular mechanism. *Nature* 349: 117–27

Brown S. 1989. Time of action of 4.5 S RNA in *Escherichia coli* translation. *J. Mol. Biol.* 209:79–90

Chirico WJ, Waters MG, Blobel G. 1988. 70K heat shock related proteins stimulate protein translocation into microsomes. *Nature* 332: 805–10

Connolly T, Gilmore R. 1989. The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell* 57:599–610

Connolly T, Rapiejko PJ, Gilmore R. 1991. Requirement of GTP hydrolysis for dissociation of the signal recognition particle from its receptor. *Science* 252:1171–73

Crooke E, Guthrie B, Lecker S, Lill R, Wickner W. 1988. ProOmpA is stabilized for membrane translocation by either purified E. coli trigger factor or canine signal recognition particle. *Cell* 54:1003–11

Crowley KS, Liao S, Worrell VE, Reinhart GD, Johnson AE. 1994. Secretory proteins move through the ER membrane via an aqueous, gated pore. *Cell*. Submitted

Crowley KS, Reinhart GD, Johnson AE. 1993. The signal sequence moves through a ribosomal tunnel into a noncytoplasmic aqueous environment at the ER membrane early in translocation. *Cell* 73:1101–15

Deshaies RJ, Koch BD, Werner WM, Craig EA, Schekman R. 1988. A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides. *Nature* 332:800–5

Deshaies RJ, Sanders SL, Feldheim DA, Schekman R. 1991. Assembly of yeast Sec proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex. *Nature* 349:806–8

Dobberstein B. 1994. Protein transport: On the beaten pathway. *Nature* 367:599–600

Felici F, Cesareni G, Hughes JMX. 1989. The most abundant small cytoplasmic RNA of *Saccharomyces cerevisiae* has an important function required for normal cell growth. *Mol. Cell. Biol.* 9:3260–68

Franklin AE, Hoffman NE. 1993. Characterization of a chloroplast homologue of the 54-kDa subunit of the signal recognition particle. *J. Biol. Chem.* 268:22175–80

Garcia PD, Walter P. 1988. Full-length prepro-alpha-factor can be translocated across the mammalian microsomal membrane only if translation has not terminated. *J. Cell Biol.* 106:1043–48

Gilmore R. 1993. Protein translocation across the endoplasmic reticulum: a tunnel with toll booths at entry and exit. *Cell* 75:589–92

Gilmore R, Blobel G, Walter P. 1982a. Protein translocation across the endoplasmic reticulum. I. Detection in the microsomal membrane of a receptor for the signal recognition particle. *J. Cell Biol.* 95:463–69

Gilmore R, Walter P, Blobel G. 1982b. Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J. Cell Biol.* 95:470–77

Görlich D, Hartmann E, Prehn S, Rapoport T. 1992a. A protein of the endoplasmic reticulum involved early in polypeptide translocation. *Nature* 357:47–52

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Görlich D, Prehn S, Hartmann E, Kalies K-U, Rapoport TA. 1992b. A mammalian homolog of SEC61p and SECYp is associated with ribosomes and nascent polypeptides during translocation. *Cell* 71:489–503

Görlich D, Rapoport TA. 1993. Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell* 75:615–30

Gundelfinger ED, Carlo MD, Zopf D, Melli M. 1984. Structure and evolution of the 7SL RNA component of the signal recognition particle. *EMBO J.* 3:2325–32

Hann B, Stirling CJ, Walter P. 1992. SEC65 gene product is a subunit of the yeast signal recognition particle required for its integrity. *Nature* 356:532–33

Hann BC, Poritz MA, Walter P. 1989. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* contain a homologue to the 54-kD subunit of the signal recognition particle that in *S. cerevisiae* is essential for growth. *J. Cell Biol.* 109:3223–30

Hann BC, Walter P. 1991. The signal recognition particle in S. cerevisiae. *Cell* 67:131–44

Hannavy K, Rospert S, Schatz G. 1993. Protein import into mitochondria: a paradigm for the translocation of polypeptides across membranes. *Curr. Opin. Cell Biol.* 5:694–700

Hartmann E, Sommer T, Prehn S, Görlich D, Jentsch S, Rapoport TA. 1994. Evolutionary conservation of components of the protein translocation complex. *Nature* 367:654–57

He F, Beckerich J-M, Gaillardin C. 1992. A mutant of 7SL RNA in *Yarrowia lipolytica* affecting the synthesis of a secreted protein. *J. Biol. Chem.* 267:1932–37

Herz J, Flint N, Stanley K, Frank R, Dobberstein B. 1990. The 68 kDa protein of signal recognition particle contains a glycine-rich region also found in certain RNA-binding proteins. *FEBS Lett.* 276:103–7

High S, Dobberstein B. 1991. The signal sequence interacts with the methionine-rich domain of the 54-kD protein of signal recognition particle. *J. Cell Biol.* 113:229–33

High S, Flint N, Dobberstein B. 1991a. Requirement for the membrane insertion of signal-anchor type proteins. *J. Cell Biol.* 113:25–34

High S, Görlich D, Wiedmann M, Rapoport TA, Dobberstein B. 1991b. The identification of proteins in the proximity of signal-anchor sequences during their targeting to and insertion into the membrane of the ER. *J. Cell Biol.* 113:35–44

High S, Martoglio B, Görlich D, Andersen SSL, Ashford AJ, et al. 1993. Site-specific photocrosslinking reveals that Sec61p and Tram contact different regions of a membrane inserted signal sequence. *J. Biol. Chem.* 268: 26745–51

Janiak F, Walter P, Johnson AE. 1992. Fluorescence-detected assembly of the signal recognition particle: binding of the two SRP protein heterodimers to SRP RNA is noncooperative. *Biochemistry* 31:5830–40

Krieg UC, Johnson AE, Walter P. 1989. Protein translocation across the endoplasmic reticulum membrane: identification by photocrosslinking of a 39 kDa integral membrane glycoprotein as part of a putative translocation tunnel. *J. Cell Biol.* 109:2033–43

Krieg UC, Walter P, Johnson AE. 1986. Photocrosslinking of the signal sequence of nascent preprolactin to the 54-kilodalton polypeptide of the signal recognition particle. *Proc. Natl. Acad. Sci. USA* 83:8604–8

Kumamoto CA. 1991. Molecular chaperones and protein translocation across the *E. coli* inner membrane. *Mol. Microbiol.* 5:19–22

Kurzchalia TV, Wiedmann M, Girshovich AS, Bochkareva ES, Bielka H, Rapoport TA. 1986. The signal sequence of nascent preprolactin interacts with the 54K polypeptide of the signal recognition particle. *Nature* 320: 634–36

Larsen N, Zwieb C. 1991. SRP-RNA sequence alignment and secondary structure. *Nucleic Acids Res.* 19:209–15

Larsen N, Zwieb C. 1993. The signal recognition particle database (SRPDB). *Nucleic Acids Res.* 21:3019–20

Lauffer L, Garcia PD, Harkins RN, Coussens L, Ullrich A, Walter P. 1985. Topology of the SRP receptor in the endoplasmic reticulum membrane. *Nature* 318:334–38

Liao X, Selinger D, Althoff S, Chiang A, Hamilton D, Ma M, Wise JA. 1992. Random mutagenesis of *Schizosaccharomyces pombe* SRP RNA: lethal and conditional lesions cluster in presumptive protein binding sites. *Nucleic Acids Res.* 20:1607–15

Lingelbach K, Zwieb C, Webb JR, Marshallsay C, Hoben PJ, et al. 1988. Isolation and characterization of a cDNA clone encoding the 19 kDa protein of the signal recognition particle (SRP): expression and binding to 7SL RNA. *Nucleic Acids Res.* 16:9431–42

Lipp J, Dobberstein B, Haeuptle MT. 1987. Signal recognition particle arrests elongation of nascent secretory and membrane proteins at multiple sites in a transient manner. *J. Biol. Chem.* 262:1680–84

Luirink J, High S, Wood H, Giner A, Tollervey D, Dobberstein B. 1992. Signal sequence recognition by an *Escherichia coli* ribonucleoprotein complex. *Nature* 359:741–43

Lütcke H, High S, Römisch K, Ashford A, Dobberstein B. 1992. The methionine-rich domain of the 54 kDa subunit of signal recognition particle is sufficient for the interaction with signal sequences. *EMBO J.* 11: 1543–51

Lütcke H, Prehn S, Ashford AJ, Remus M, Frank R, Dobberstein B. 1993. Assembly of

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

the 68- and 72-kD proteins of the signal recognition particle with 7S RNA. *J. Cell Biol.* 121:977–85

Meyer DI. 1988. Preprotein conformation: the year's major theme in translocation studies. *Trends Biochem. Sci.* 13:471–74

Meyer DI, Dobberstein B. 1980a. Identification and characterization of a membrane component essential for the translocation of nascent secretory proteins across the membrane of the endoplasmic reticulum. *J. Cell Biol.* 87: 503–8

Meyer DI, Dobberstein B. 1980b. A membrane component essential for vectorial translocation of nascent proteins across the endoplasmic reticulum: requirement for its extraction and reassociation with the membrane. *J. Cell Biol.* 87:498–502

Meyer DI, Krause E, Dobberstein B. 1982a. Secretory protein translocation across membranes—the role of the docking protein. *Nature* 297:647–50

Meyer DI, Louvard D, Dobberstein B. 1982b. Characterization of molecules involved in protein translocation using a specific antibody. *J. Cell Biol.* 92:579–83

Miller JD, Bernstein HD, Walter P. 1994. Interaction of *E. coli* Ffh/4.5S ribonucleoprotein and FtsY mimics that of mammalian signal recognition particle and its receptor. *Nature* 367:657–59

Miller JD, Wilhelm H, Gierasch L, Gilmore R, Walter P. 1993. GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation. *Nature* 366: 351–54

Nicchitta CV, Blobel G. 1993. Lumenal proteins of the mammalian endoplasmic reticulum are required to complete protein translocation. *Cell* 73:989–98

Nomura M, Traub P, Guthrie C, Nashimoto H. 1969. The assembly of ribosomes. *J. Cell Physiol.* 74:241–52

Nowotny V, Nierhaus KH. 1988. Assembly of the 30S subunit from *Escherichia coli* ribosomes occurs via two assembly domains which are initiated by S4 and S7. *Biochemistry* 27:7051–55

Nunnari J, Walter P. 1992. Protein targeting to and translocation across the membrane of the endoplasmic reticulum. *Curr. Opin. Cell Biol.* 4:573–80

O'Neil KT, DeGrado WF. 1990. How calmodulin binds its targets: sequence independent recognition of amphiphilic a-helices. *Trends Biochem. Sci.* 15:59–64

Ogg S, Poritz M, Walter P. 1992. The signal recognition particle receptor is important for growth and protein secretion in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 3:895–911

Palade G. 1975. Intracellular aspects of the process of protein secretion. *Science* 189:347–58

Phillips GJ, Silhavy TJ. 1992. The *E. coli ffh* gene is necessary for viability and efficient protein export. *Nature* 359:744–46

Poritz MA, Bernstein HD, Strub K, Zopf D, Wilhelm H, Walter P. 1990. An *E. coli* ribonucleoprotein containing 4.5S RNA resembles mammalian signal recognition particle. *Science* 250:111–17

Poritz MA, Strub K, Walter P. 1988. Human SRP RNA and E. coli 4.5S RNA contain a highly homologous structural domain. *Cell* 55:4–6

Randall LL, Hardy SJS. 1989. Unity in function in the absence of consensus in sequence: role of leader peptides in export. *Science* 243: 1156–59

Rapiejko PJ, Gilmore R. 1992. Protein translocation across the endoplasmic reticulum requires a functional GTP binding site in the α-subunit of the signal recognition particle receptor. *J. Cell Biol.* 117:493–503

Rapoport TA. 1992. Transport of proteins across the endoplasmic reticulum membrane. *Science* 258:931–36

Rapoport TA, Heinrich R, Walter P, Schulmeister T. 1987. Mathematical modeling of the effects of the signal recognition particle on translation and translocation of proteins across the endoplasmic reticulum membrane. *J. Mol. Biol.* 195:621–36

Römisch K, Webb J, Herz J, Prehn S, Frank R, et al. 1989. Homology of the 54K protein of signal recognition particle, docking protein, and two *E. coli* proteins with putative GTP-binding domains. *Nature* 340:478–82

Römisch K, Webb J, Lingelbach K, Gausepohl H, Dobberstein B. 1990. The 54-kD protein of signal recognition particle contains a methionine-rich RNA binding domain. *J. Cell Biol.* 111:1793–802

Samuelsson T. 1992. A mycoplasma protein homologous to mammalian SRP54 recognizes a highly conserved domain of SRP RNA. *Nucleic Acids Res.* 20:5763–70

Sanders SL, Schekman R. 1992. Polypeptide translocation across the endoplasmic reticulum membrane. *J. Biol. Chem.* 267:13791–94

Sanders SL, Whitfield KM, Vogel JP, Rose MD, W, Schekman RW. 1992. Sec61p and BiP directly facilitate polypeptide translocation into the ER. *Cell* 69:353–65

Sanz P, Meyer DI. 1988. Signal recognition particle (SRP) stabilizes the translocation-competent conformation of pre-secretory proteins. *EMBO J.* 7:3553–57

Schlenstedt G, Gudmundsson G, Bowman H, Zimmermann R. 1990. A large secretory protein translocates both cotranslationally, using signal recognition particle and ribosome, and post-translationally, without these ribonucleoparticles, when synthesized in the presence of mammalian microsomes. *J. Biol. Chem.* 265:13960–68

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.

Selinger D, Brennwald P, Liao X, Wise JA. 1993a. Identification of RNA sequences and structural elements required for assembly of fission yeast SRP54 protein with signal recognition particle RNA. *Mol. Cell. Biol.* 13: 1353–62

Selinger D, Liao X, Wise JA. 1993b. Functional interchangeability of the structurally similar tetranucleotide loops GAAA and UUCG in fission yeast signal recognition particle RNA. *Proc. Natl Acad. Sci. USA* 90:5409–13

Siegel V, Walter P. 1985. Elongation arrest is not a prerequisite for secretory protein translocation across the microsomal membrane. *J. Cell Biol.* 100:1913–21

Siegel V, Walter P. 1986. Removal of the Alu structural domain from signal recognition particle leaves its protein translocation activity intact. *Nature* 320:81–84

Siegel V, Walter P. 1988a. The affinity of signal recognition particle for presecretory proteins is dependent on nascent chain length. *EMBO J.* 7:1769–75

Siegel V, Walter P. 1988b. Binding sites of the 19-kDa and 68/72-kDa signal recognition particle (SRP) proteins on SRP RNA as determined by protein-RNA "footprinting." *Proc. Natl. Acad. Sci. USA* 85:1801–5

Siegel V, Walter P. 1988c. Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. *Cell* 52:39–49

Siegel V, Walter P. 1988d. Functional dissection of the signal recognition particle. *Trends Biochem. Sci.* 13:314–16

Simon SM, Blobel G. 1991. A protein-conducting channel in the endoplasmic reticulum. *Cell* 65:371–80

Stirling CJ, Hewitt EW. 1992. The *Saccharomyces cerevisiae SEC65* gene encodes a component of the yeast signal recognition particle with homology to human SRP19. *Nature* 356:534–37

Strub K, Moss J, Walter P. 1991. Binding sites of the 9- and 14-kilodalton heterodimeric protein subunit of the Signal Recognition Particle (SRP) are contained exclusively in the Alu domain of SRP RNA and contain a sequence motif that is conserved in evolution. *Mol. Cell. Biol.* 11:3949–59

Strub K, Walter P. 1989. Isolation of a cDNA clone of the 14-kDa subunit of the signal recognition particle by cross-hybridization of differently primed polymerase chain reactions. *Proc. Natl. Acad. Sci. USA* 86:9747–51

Strub K, Walter P. 1990. Assembly of the Alu domain of the signal recognition particle (SRP): dimerization of the two protein components is required for efficient binding of SRP RNA. *Mol. Cell. Biol.* 10:777–84

Struck J. CR, Toschka HY, Specht T, Erdmann VA. 1988. Common structural features between eukaryotic 7SL RNAs, eubacterial 4.5S RNA and scRNA and archaebacterial 7S RNA. *Nucleic Acids Res.* 16:7740

Stuart RA, Cyr DM, Craig EA, Neupert W. 1994. Mitochondrial molecular chaperones: their role in protein translocation. *Trends Biol. Sci.* 19:87–92

Tajima S, Lauffer L, Rath VL, Walter P. 1986. The signal recognition particle is a complex that contains two distinct polypeptide chains. *J. Cell Biol.* 103:1167–78

Ullu E, Murphy S, Melli M. 1982. Human 7SL RNA consists of a 140 nucleotide middle-repetitive sequence inserted in an Alu sequence. *Cell* 29:195–202

Ullu E, Tschudi C. 1984. Alu sequences are processed 7SL genes. *Nature* 312:171–72

Vogel JP, Misra LM, Rose MD. 1990. Loss of BiP/GRP78 function blocks translocation of secretory proteins in yeast. *J. Cell Biol.* 110: 1885–95

von Heijne G. 1985. Signal sequences. The limits of variation. *J. Mol. Biol.* 184:99–105

Walter P, Blobel G. 1980. Purification of membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA* 77:7112–16

Walter P, Blobel G. 1981. Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence and site specific arrest of chain elongation that is released by microsomal membranes. *J. Cell Biol.* 91:557–61

Walter P, Blobel G. 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature* 299:691–98

Walter P, Blobel G. 1983. Disassembly and reconstitution of the signal recognition particle. *Cell* 34:525–33

Walter P, Gilmore R, Blobel G. 1984. Protein translocation across the endoplasmic reticulum. *Cell* 38:5–8

Walter P, Ibrahimi I, Blobel G. 1981. Translocation of proteins across the endoplasmic reticulum I. Signal Recognition Protein (SRP) binds to in vitro assembled polysomes synthesizing secretory protein. *J. Cell Biol.* 91: 545–50

Walter P, Jackson RC, Marcus MM, Lingappa VR, Blobel G. 1979. Tryptic dissection and reconstitution of translocation activity for nascent presecretory proteins across microsomal membranes. *Proc. Natl. Acad. Sci. USA* 76:1795–99

Walter P, Lingappa VR. 1986. Mechanism of protein translocation across the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* 2:499–516

Wickner W, Driessen A. JM, Hartl F-U. 1991. The enzymology of protein translocation across the *Escherichia coli* plasma membrane. *Annu. Rev. Biochem.* 60:101–24

Wiedmann M, Kurzchalia TV, Hartmann E, Rapoport TA. 1987. A signal sequence receptor in the endoplasmic reticulum membrane. *Nature* 328:830–32

Wild J, Altman E, Yura T, Gross CA. 1992. DnaK and DnaJ heat shock proteins participate in protein export in *E. coli*. *Genes Dev.* 6:1165–72

Wolin SL, Walter P. 1988. Ribosome pausing and stacking during translation of a eukaryotic mRNA. *EMBO J.* 7:3559–69

Wolin SL, Walter P. 1989. Signal recognition particle mediates a transient elongation arrest of preprolactin in reticulocyte lysate. *J. Cell Biol.* 109:2617–22

Wood H, Luirink J, Tollervey D. 1992. Evolutionary conserved nucleotides within the *E. coli* 4.5S RNA are required for association with p48 in vitro and for optimal function in vivo. *Nucleic Acids Res.* 20:5919–25

Yaver DS, Matoba S, Ogrydziak DM. 1992. A mutation in the signal recognition particle 7S RNA of the yeast *Yarrowia lipolytica* preferentially affects synthesis of the alkaline extracellular protease: In vivo evidence for translational arrest. *J. Cell Biol.* 116:605–16

Zopf D, Bernstein HD, Johnson AE, Walter P. 1990. The methionine-rich domain of the 54 kd protein subunit of the signal recognition particle contains an RNA binding site and can be crosslinked to a signal sequence. *EMBO J.* 9:4511–17

Zopf D, Bernstein HD, Walter P. 1993. GTPase domain of the 54kD subunit of the mammalian signal recognition particle is required for protein translocation but not for signal sequence binding. *J. Cell Biol.* 120:1113–21

Zwieb C. 1991. Interaction of protein SRP19 with signal recognition particle RNA lacking individual RNA-helices. *Nucleic Acids Res.* 19:2955–60

Annu. Rev. Cell. Biol. 1994.10:87-119. Downloaded from www.annualreviews.org
Access provided by University of California - San Francisco UCSF on 04/11/16. For personal use only.