Targeting Pathways to the Endoplasmic Reticulum Membrane

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The targeting of proteins to the endoplasmic reticulum (ER) membrane is the first step in the secretory pathway through which proteins are transported to the outside of the cell, the plasma membrane, and the luminal spaces and membranes of the endomembrane system. Proteins that undergo this targeting event carry a stretch of hydrophobic amino acids, usually at the amino terminus, that constitute an ER-specific signal sequence. Several systems have been used to study the recognition, targeting, and translocation of proteins into the ER, and during the last 15 years considerable information has been gleaned from them.

The first system from which factors required for targeting to the ER were identified was an in vitro assay for the promotion of translocation of presecretory proteins into mammalian ER-derived microsomes. From this the signal recognition particle (SRP) and subsequently its ER membrane-localized receptor were identified as essential factors for the targeting of proteins to the ER (Walter and Blobel 1980; Gilmore et al. 1982; Meyer et al. 1982). SRP is a ribonucleoprotein (consisting of the 7SL RNA, or SRP RNA, and six protein subunits; SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) that acts as an adapter coupling the translation of proteins to their translocation across the ER membrane such that proteins are cotranslationally inserted. This feat is achieved by selection of ribosomes synthesizing proteins with signal sequences, mediated by direct binding of the signal sequence to the SRP54 protein. The domain of SRP54 that binds signal sequences is extremely rich in methionine residues (Römisch et al. 1990; Zopf et al. 1990), which are predicted to reside on one face of a group of amphipathic α helices forming a signal sequence binding site, or groove (Bernstein et al. 1989). This is one of several intriguing features of SRP54, another being a guanosine nucleotide-binding domain, the nucleotide occupancy of which changes during its cycle of signal sequence binding and release (Miller et al. 1993). When SRP binds to ribosomes bearing signal sequences, it causes a delay in the progression of protein synthesis (termed elongation arrest) that requires the function of the SRP9 and SRP14 subunits. Elongation recommences when the ribosome-nascent chain is released from SRP at the ER membrane, due to interaction with the SRP receptor, and transferred to the ER membrane translocation machinery, the translocon (Siegel and Walter 1988; Walter and Johnson 1994). The SRP receptor consists of two subunits, SRα and SRβ, both of which contain GTP-binding sites. Thus, there are three directly interacting GTPases that function during targeting. Although the specific roles of the individual GTP-binding sites are still incompletely understood, rapid progress in deciphering their contributions is being made (Connolly and Gilmore 1993; Miller et al. 1993, 1994; Rapiejko and Gilmore 1994; Powers and Walter 1995). It is likely that—as for other well-characterized GTPases—conformational changes that accompany GTP binding, hydrolysis, and GDP release guide the proteins through a defined series of steps and thus ultimately provide fidelity and unidirectionality to the targeting reaction.

Yeast has also been used extensively as a system in which to study targeting to and translocation across the ER membrane. In contrast to higher eukaryotic systems where study has been exclusively biochemical, most of the insight obtained from yeast started with genetic selections to identify factors required for early stages of the secretory pathway. This was through the isolation of conditional loss-of-function mutants (Deshaies and Schekman 1987; Rothblatt et al. 1989). The first factors identified from such selections were a number of ER membrane proteins, not a yeast SRP or SRP receptor. These results, along with studies that indicated that cytosolic Hsp70 proteins were required for the efficient translocation of at least some proteins into the yeast ER (Chirico et al. 1988; Deshaies et al. 1988) and that some proteins could be posttranslationally targeted to the ER in vitro (Hansen et al. 1986; Rothblatt and Meyer 1986; Hansen and Walter 1988), led to a belief that there was a fundamental difference between yeast and higher eukaryotes in the mechanism of ER targeting and translocation.

YEAST SRP AND ITS RECEPTOR

The idea that ER targeting in yeast was fundamentally different from that in higher eukaryotes was largely dispelled by the identification and characterization of a yeast SRP and SRP receptor. The yeast SRP was found by the isolation of the gene (*SRP54*) encoding the homolog of the mammalian SRP54 protein by degenerate polymerase chain reaction (PCR) (Hann et al. 1989) and serendipitous cross-hybridization of the *SRP54* gene with the bacterial *ftsY* gene (Amaya et al.

1990). Further work demonstrated that the protein encoded by this gene, Srp54p, was indeed important for secretion (Amaya and Nakano 1991; Hann and Walter 1991) and that it was part of a ribonucleoprotein containing the scR1 RNA—a major cytoplasmic RNA in yeast that had no previously ascribed function (Felici et al. 1989; Hann and Walter 1991). The gene encoding the yeast SRα was also identified through the use of degenerate PCR (Ogg et al. 1992). This result, combined with the demonstration that SRα was membrane localized, confirmed that the SRP-dependent targeting pathway is conserved in yeast.

Depletion of Srp54p or SRα (by repression of transcription from a regulated promoter) results in the accumulation of cytosolic forms of some but not all proteins normally translocated across the ER membrane. Proteins that are not affected by loss of the SRP-dependent pathway included preprocarboxypeptidase Y (ppCPY) and preproalpha factor (ppαF), the signal sequence of which was used as the ER-targeting portion of fusion proteins used in several selections for secretion mutants. With this knowledge it was perhaps not surprising that the yeast SRP had not been identified in these selections.

A surprising observation on the yeast SRP-dependent targeting pathway was that the deletion of genes encoding SRP subunits or SRα is not a lethal event—although yeast strains with such deletions grow slowly and have defects in secretion (Hann and Walter 1991). The major implication of this result is that yeast must have at least one other, SRP-independent, targeting pathway to the ER membrane. Every protein that is essential for the cells must be able to use this alternative pathway with sufficient efficacy to allow continued cell growth.

The available evidence (see below) suggests that the yeast SRP, like its mammalian homolog, interacts with the ribosome to target proteins cotranslationally to the ER membrane. SRP will, therefore, always have the "first pick"; i.e., if a signal sequence binds with sufficient affinity to Srp54p, it will be directed into a cotranslational targeting pathway. We consider it likely, then, that SRP-dependent targeting is the primary pathway taken by many proteins to the ER in wild-type cells. This notion is supported by two experimental observations. First, translocation defects observed immediately upon depletion of SRP subunits are more severe than those seen after the cells have been grown under repressing conditions for a long time or when they are deprived of SRP by germination of a spore lacking the gene encoding an SRP subunit (Hann and Walter 1991; Ogg et al. 1992). We term this phenomenon, whereby yeast switches its ER targeting into an SRP-independent mode, "adaptation." Adaptation may involve up-regulation of the alternative pathway and may rescue the cell from the otherwise lethal consequences of loss of SRP-dependent targeting.

The second indication that SRP is the primary factor directing targeting of many essential proteins to the ER membrane came when the yeast SRP19 homolog Sec65p was identified. This was through the isolation of a temperature-sensitive lethal secretion mutant *sec65-1* (Hann et al. 1992; Stirling and Hewitt 1992; Stirling et al. 1992). The lethality of the *sec65-1* mutation initially appeared as a paradox, since deletion of the *SEC65* gene, like deletion of genes encoding other subunits of SRP, is not a lethal event. Strains carrying the *sec65-1* mutation show a rapid accumulation of precursor proteins at the nonpermissive temperature, indicating that levels of functional SRP drop much more suddenly under these conditions than when SRP components are depleted or the genes encoding them are deleted. The *sec65-1* cells may not then have enough time to undergo adaptation and switch to SRP-independent protein targeting. This failure to adapt is presumably the cause of cell death. Cells carrying both the *sec65-1* mutation and a deletion of the *SCR1* gene (coding for the scR1 RNA; and therefore not containing any SRP) are not temperature-sensitive, showing that the temperature-sensitivity of *sec65-1* strains is caused by this mutation and that its effect is mediated through SRP (Ogg and Walter 1995).

SRP AND ITS RECEPTOR ARE PHYLOGENETICALLY CONSERVED

The yeast SRP has a 16S sedimentation coefficient in sucrose gradients (Hann and Walter 1991), suggesting that it contains other subunits in addition to the scR1 RNA, Srp54p, and Sec65p. Purification of the yeast SRP using immunoaffinity chromatography with antibodies against Sec65p confirmed this: Seven proteins, including Srp54p and Sec65p, were co-isolated (Brown et al. 1994). Cloning of the genes encoding four of the five new yeast SRP proteins led to the identification of three of them as homologs of mammalian SRP proteins. These are the yeast homologs of mammalian SRP14, SRP68, and SRP72 (Srp14p, Srp68p, and Srp72p). The fourth protein for which the corresponding gene has been cloned has an apparent molecular mass of 21 kD in SDS-PAGE gels and has no known homologs in the database. The fifth protein, for which the corresponding gene has yet to be isolated, has a size (7 kD) that would be consistent with its being a homolog of the mammalian SRP9 protein.

Removal of the gene encoding any one of the four novel subunits of yeast SRP results in a reduction in growth rate and translocation defects identical to those seen when genes encoding previously known SRP subunits are deleted. Thus, SRP activity in yeast requires all the subunits for which the genes have been cloned. Further study of the yeast SRP showed that yeast strains lacking any one of Srp14p, Srp21p, Srp68p, or Srp72p lack assembled SRP (Brown et al. 1994). Interestingly, mammalian SRP can target proteins to the ER in vitro in the absence of its SRP9/SRP14 proteins (although it lacks the ability to cause elongation arrest), indicating that the assembly and function of SRP

in vitro is less demanding than in vivo. Yeast cells lacking Srp54p contain a stable particle assembled from the remaining subunits, and in cells lacking Sec65p all subunits except Srp54p associate stably. These results suggest that Srp14p (and probably Srp9p) assembles onto the RNA along with Srp68p, Srp72p, and Srp21p to form a core particle onto which Sec65p and then Srp54p bind. It remains to be determined whether this is the order in which these components assemble in vivo during biosynthesis of SRP.

A yeast gene encoding a protein homologous to mammalian SRβ has recently been identified in the sequence database (Miller et al. 1995). As with all other SRP and SR genes, deletion of this gene results in slow-growing yeast strains with translocation defects. We have been able to specifically coimmunoprecipitate SRα from a detergent extract of yeast microsomes using antibodies directed against SRβ. Subcellular fractionation studies have also shown that in the absence of SRβ, SRα is no longer associated with a membrane-containing fraction (S. Ogg and P. Walter, unpubl.). Taken together, these results show that the yeast gene identified by sequence similarity to mammalian SRβ does encode the bona fide yeast SRβ and suggest that the structure of the SR is the same in these diverse eukaryotes, the β subunit anchoring the α subunit to the ER membrane.

The SRP-dependent pathway is conserved not only in eukaryotes but probably in all cellularized organisms. Homologs of some components, notably SRP54 and the SRP RNA, have been identified in all organisms examined to date. Intriguingly, in *Escherichia coli* where SRP has been characterized in detail, it consists of only the SRP54 homolog Ffh and the 4.5S RNA (Römisch et al. 1989; Poritz et al. 1990). The SRP receptor also appears to be minimal in *E. coli* where a homolog of only the SRα protein, FtsY, has been identified. The interaction between the 4.5S ribonucleoprotein and FtsY is conserved with respect to those between mammalian components (Miller et al. 1994). The particle has also been shown to bind signal sequences (Luirink et al. 1992) and to be important for secretion of a subset of proteins tested (Phillips and Silhavy 1992). These results suggest a role for the *E. coli* SRP pathway similar to that of the yeast SRP pathway, directing the secretion of some proteins. It may not have the elongation arrest function of mammalian SRP, since it does not have the domain that has been defined as necessary for this function in the mammalian particle (Siegel and Walter 1988).

INTERACTION OF SRP WITH THE RIBOSOME

As mentioned above, yeast cells bearing the *sec65-1* mutation not only undergo growth arrest, but die when incubated at the nonpermissive temperature. It has been found that the *sec65-1* mutation causes the interaction of Srp54p with the rest of the particle to be weakened, even at temperatures permissive for growth of strains carrying the mutation (Hann et al. 1992). In addition, Sec65-1p itself is unstable at nonpermissive temperatures (Stirling and Hewitt 1992). From the model postulated above (in which the lethality of the *sec65-1* mutation is caused by the defect of the mutant protein being manifested so quickly as to preclude adaptation), several possibilities can be envisaged whereby the temperature-sensitive lethal phenotype of the *sec65-1* mutation might be suppressed. These would include (1) an enhancement of SRP-independent targeting, i.e., in effect a preadaptation; (2) stabilization of SRP at the nonpermissive temperature; and (3) alteration in metabolism to allow insufficient SRP concentration present in *sec65-1* cells at the nonpermissive temperature to suffice. No evidence has yet been found to support the first of these possibilities, but data consistent with the second and third have been obtained.

Weak interactions can be stabilized by overexpression of interaction partners. It was found that overexpression of Srp54p suppresses the temperature-sensitive defects of strains carrying the *sec65-1* allele (Hann et al. 1992; Stirling and Hewitt 1992). Since Srp54p is lost from the particle and mutant Sec65-1p is destabilized at the nonpermissive temperature, the suppression of the temperature-sensitive defect is easiest to explain by the weakened interaction between Sec65-1p and Srp54p being counterbalanced by the higher concentration of Srp54p in the cells. According to the third scenario, suppression could be achieved if insufficient SRP is made to suffice. This can indeed be demonstrated in experiments in which sublethal concentrations of cycloheximide, an inhibitor of translation elongation, are shown to suppress both the temperature-sensitive growth defect and translocation defects of *sec65-1* cells (Ogg and Walter 1995). Similarly, translocation defects caused by depletion of Srp54p are also suppressed by cycloheximide, but only when the Srp54p levels are low, not after the protein has been entirely depleted. This indicates that limiting amounts of SRP, but not its total absence, can be compensated for by the presence of cycloheximide. Consistent with this observation, the growth and translocation defects of cells deleted for the *SRP54* or *SEC65* gene are not suppressed by cycloheximide. These results indicate that the suppression by cycloheximide and of SRP-dependent targeting is direct and is not via bypass of the SRP-dependent pathway.

That the effect of cycloheximide is mediated through its effect on translation is argued for by the fact that in *sec65-1* cells carrying an additional mutation causing resistance to cycloheximide (*cyh2*, encoding a ribosomal protein), a correspondingly higher level of cycloheximide is needed to cause suppression of the *sec65-1* growth defect at the nonpermissive temperature.

Surprisingly, inhibition of protein synthesis at steps other than that blocked by cycloheximide does not suppress the temperature-sensitivity and translocation

defects of *sec65-1* cells. Inhibition of the transpeptidylation reaction (with anisomycin) or initiation of protein synthesis (by down-regulating eIF-2 activity) does not suppress *sec65-1*-associated defects. This suggests that elongation is the process that is required to be slowed down and that SRP can only functionally interact with ribosomes at the cycloheximide-inhibited step, translocation of the peptidyl-tRNA from the ribosomal A-site to the P-site.

How might this suppression be mediated? The model that we favor is one of cycloheximide causing nascent polypeptide chains to emerge more slowly from the ribosome than they would normally, providing a longer time during which an otherwise insufficient amount of intact SRP can functionally interact with the signal sequences of secreted proteins. Moreover, the slowing of elongation is such that the ribosome is paused in the conformation that SRP requires to functionally interact with it. Since SRP is substoichiometric with ribosomes (one SRP for every 10–100 ribosomes), the definition of a step in the elongation cycle at which SRP can interact suggests a simple model for the way in which SRP might function in selecting nascent chains containing signal sequences (Fig. 1). SRP binds to ribosomes after the transpeptidylation reaction but before the translocation step. The affinity of SRP for the ribosome is determined by whether or not a signal sequence is present; if it is, then SRP remains bound and the elongation-arrest function of SRP decreases the likelihood of the elongation reaction's proceeding, maintaining the ribosome in a conformation compatible with docking at the ER. The complex is then targeted to the ER membrane via interaction with the SRP receptor. If a signal sequence is not found, then SRP dissociates from the ribosome, and elongation proceeds. A substoichiometric amount of SRP could monitor all nascent chains by this sampling model.

The translocation step of elongation is catalyzed by eEF-2. Mutations in EF-G, the bacterial homolog of eEF-2, have been isolated that suppress normally sublethal concentrations of the *E. coli* 4.5S RNA (Brown 1987). Because 4.5S RNA is the SRP RNA in bacteria, it is possible that the mutations in EF-G are acting to suppress the sublethal level of 4.5S RNA (and hence of the *E. coli* SRP) in a similar way to cycloheximide in *sec65-1* yeast cells by reducing the rate of translocation of the polypeptide chain.

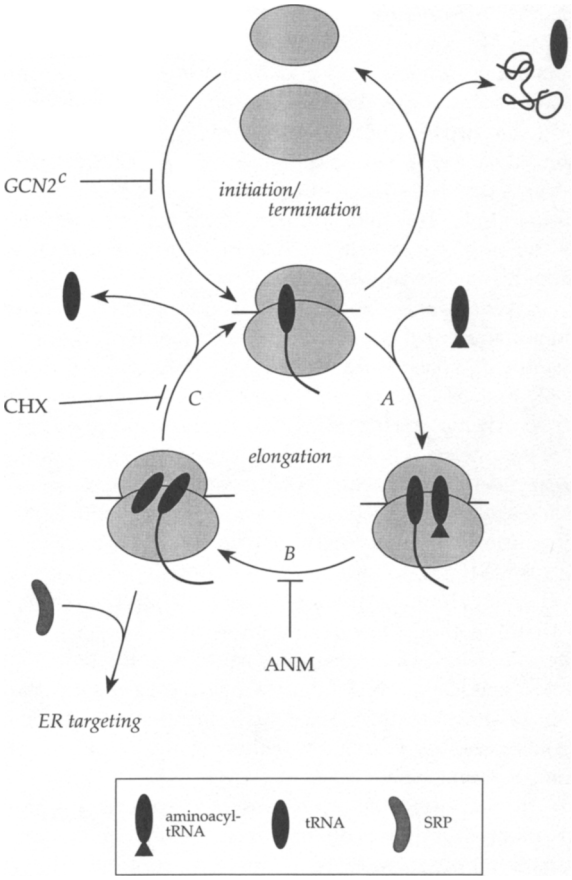

Figure 1. Model for SRP interaction with the ribosome. Translation is shown as a cycle of elongation preceded by initiation (blocked by GCN2^c alleles) and followed by termination. The elongation phase of translation is illustrated as consisting of three steps: aminoacyl-tRNA binding to the ribosomal A-site (*A*), transpeptidylation (*B*), and translocation of the peptidyl-tRNA from the A-site to the P-site (*C*). SRP interacts with the ribosome and samples the nascent chain for the presence of a signal sequence after the anisomycin (ANM) block, but before the cycloheximide (CHX) block. (Adapted, with permission, from Ogg and Walter 1995 [copyright Cell Press].)

THE SRP-INDEPENDENT TARGETING PATHWAY

As mentioned above, the survival of SRP-deficient yeast cells indicates the existence of alternative means of targeting proteins to the protein translocation apparatus in the ER membrane. To identify the components of this pathway, we used a genetic selection to isolate mutants defective in the targeting of an SRP-independent substrate. Our approach was similar to previous selections and utilized a chimeric reporter protein composed of an ER-targeting domain fused to a selectable marker enzyme that can only function in the cytosol; i.e., when transport into the ER is impeded by mutation in a targeting or translocation factor, the cells can grow. As a targeting domain, we chose the signal sequence of ppCPY because the translocation of this protein is entirely unaffected by loss of SRP function (Hann and Walter 1991). From our selection, we obtained mutants that fell into seven complementation groups, six of which correspond to known genes (*sec61*, *sec62*, *sec63*, *sec71*, *sec72*, and *kar2*), and one, which we have designated *sec73*, that appears to encode a novel factor. As expected from the design of the screen, no mutants were obtained that affected SRP pathway components. Furthermore, expression of the reporter

in SRP mutant cells did not impair translocation of the protein into the ER, again confirming the specificity of the screen. Of the proteins encoded by the previously known genes, Sec61p is an evolutionarily conserved protein found in a heterotrimeric complex that forms the pore of the translocon through which proteins are imported into the ER lumen or integrated into the ER membrane (Görlich et al. 1992; Musch et al. 1992; Sanders et al. 1992). Sec62p, Sec63p, Sec71p, and Sec72p form a complex in the ER membrane and were known previously to be important for translocation. Kar2p is the yeast ER luminal Hsp70 homolog, another factor important for translocation into the ER (Vogel et al. 1990).

When we tested the mutants for translocation of endogenous proteins, we found that alleles of *sec62* and *sec63* displayed a pattern of translocation defects inverse to that seen when SRP function is lost. As examples, pre-dipeptidyl aminopeptidase B (pDPAP-B) translocation (strongly affected by loss of SRP function) is completely unaffected, whereas ppCPY and ppαF (independent of SRP) are almost completely blocked. This pattern indicated that we had isolated mutations that blocked an SRP-independent translocation pathway that functions in parallel to the SRP-dependent pathway, rather than a salvage pathway for proteins missed by SRP. Sec62p and Sec63p are therefore two of the components important in the SRP-independent targeting pathway. The results from this mutant hunt are consistent with the results from biochemical approaches that showed by in vitro reconstitution that the Sec62p/Sec63p complex together with the Sec61p protein complex is sufficient to promote posttranslational protein translocation across membranes (Panzner et al. 1995). For efficient translocation, Kar2p is also required in this system.

Examination of the translocation defects of different proteins in cells carrying mutations in either *SEC62*, *SEC63*, or genes encoding SRP subunits (e.g., *sec65-1* cells at the nonpermissive temperature) allowed us to group substrate proteins into several classes: (1) those that are SRP-dependent (e.g., pDPAP-B, pre-alkaline phosphatase [pPho8p]); (2) those that are SRP-independent (e.g., ppCPY, ppαF, pGas1p); and (3) those that are partially affected by mutations in either pathway (e.g., pKar2p). These results demonstrate that the SRP-dependent and SRP-independent pathways are able to discriminate substrates with surprising selectivity, indicating that there are intrinsic differences between the different translocation substrates. In contrast to mutations in *SEC62*, *SEC63*, and genes encoding SRP subunits, most mutations in *SEC61* affect proteins in all three groups. We envisage, because of this pleiotropic nature of defects in *sec61*, that both pathways direct input to the same translocon, the Sec61p complex. Thus, both targeting pathways would converge on the same translocon, with the Sec62/Sec63p complex on the one hand and the SRP receptor on the other functioning to allow the translocon to receive input in either a posttranslational or cotranslational mode (Fig. 2).

SIGNAL SEQUENCES DIRECT PROTEINS INTO EITHER TARGETING PATHWAY TO THE ER

The specificity of the reporter molecule used in our screen, where the signal sequence of CPY was sufficient to direct it into the SRP-independent pathway, suggested that the signal sequences may be the discriminating feature that directs a protein into either pathway. This would add another level of complexity to their task; to date, signal sequences that direct proteins to the ER have largely been considered functionally equivalent and, in their varied composition, no obvious features or motifs have been identified that would suggest functional diversity. To investigate the possibility that signal sequences direct proteins into either targeting pathway, we asked if a normally SRP-independent protein, ppαF, could be redirected into the SRP-dependent pathway. We found that replacement of the ppαF signal sequence with the amino-terminal region of pDPAP-B, including its signal anchor region, made the protein largely dependent on SRP and, importantly, virtually independent of the blocks imposed by mutations in *SEC62* and *SEC63*. This result confirmed the suspicion that specific signal sequences determine pathway entry.

The specificity of targeting of SRP-dependent and SRP-independent proteins to the ER can be reproduced in vitro. A major advantage of this is that we can bypass the influences of adaptation by depleting SRP from a translation-competent cytosol fraction made from wild-type cells. Microsomal membranes derived from wild-type, *sec62*, or *sec63* are used in conjunction with the translation extract, and targeting is observed as signal sequence cleavage and/or glycosylation and protease protection. When wild-type extract and membranes are used, both SRP-dependent and SRP-independent proteins are efficiently translocated when they are synthesized in the extract. If, however, SRP-depleted translation extract is used, then, although proteins that are SRP-independent in vivo (e.g., ppαF) are still translocated efficiently, proteins that depend on SRP function in vivo for efficient translocation (e.g., pPho8p and the pDPAP-B-αF fusion used to test the in vivo signal-sequence dependence of targeting pathways) are poorly translocated, confirming their dependence on SRP. Wild-type extracts combined with *sec62* or *sec63* membranes show the opposite result with the translocation of ppαF and ppCPY being almost completely inhibited and SRP-dependent proteins largely unaffected. Therefore, both in vivo and in vitro, we have dissected two parallel translocation pathways to the yeast ER translocon.

As mentioned above, according to our models that suggest a strictly cotranslational signal sequence recognition function of SRP, SRP has the first choice of nascent chains as they are being synthesized and is only

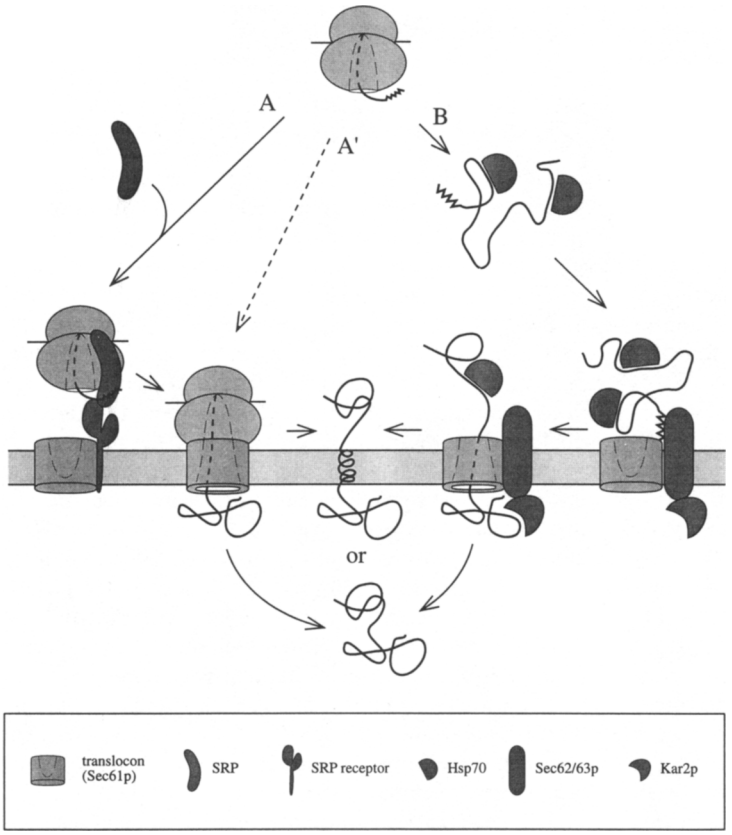

Figure 2. Targeting pathways to and translocation across, or integration into, the ER membrane. The two known targeting pathways are indicated: (*A*) SRP-dependent targeting is cotranslational, SRP examining the nascent chain as it emerges from the ribosome. Targeting to the translocon (a heterotrimeric protein consisting of Sec61p, Sss1p, and Sbh1p) is via the SRP receptor. (*B*) The SRP-independent targeting pathway is depicted as posttranslational with fully synthesized proteins, not recognized by SRP, bound by chaperones, and targeted to the translocon through interaction with the Sec62p/Sec63p complex. A third cotranslational SRP-independent pathway (*A'*) is also depicted; evidence has not been presented that supports or discounts this possibility. The Sec62p/Sec63p complex also contains Sec71p and Sec72p. It cannot be excluded, however, that this complex participates in targeting pathways *A* and/or *A'*.

capable of recognizing certain signal sequences. Since mutations in the SRP-independent pathway cannot be compensated for by SRP, but adaptation allows cells deficient in SRP to translocate most SRP-dependent proteins, the SRP-independent targeting pathway is likely responsible for the proteins that SRP does not see. These would include proteins that are less efficiently recognized by SRP, such as pKar2p, which utilizes both targeting pathways, as well as SRP-independent proteins. In the absence of SRP, the SRP-independent pathway may be up-regulated during adaptation to compensate for the loss of SRP function and possibly modified to allow it to cope with and recognize most of the normally SRP-dependent proteins.

One of the hallmarks of adaptation is the up-regulation of certain cytosolic heat shock proteins, including Hsp70 proteins that have been implicated in aiding the posttranslational import of SRP-independent proteins into the ER (Arnold and Wittrup 1994). The chaperone function of these heat shock proteins might be responsible for maintaining proteins normally targeted cotranslationally by SRP in a translationally competent state for posttranslational import into the ER in the absence of SRP.

Although translocation defects in adapted cells are largely abrogated for substrates thus far examined, SRP-deficient cells never regain wild-type levels of growth. There are several possible reasons for this phenotype. Some important yet to be identified substrates targeted by SRP might be refractory to efficient translocation in the salvage mode of the SRP-independent pathway. Their inefficient translocation would then be rate-limiting for cell growth. Alternatively, SRP may contain an additional function that is important for the growth of the cell. We favor the former, because defects in SRP receptor subunits display the identical phenotype as SRP defects, i.e., defects at multiple points in the targeting pathway lead to indistinguishable consequences for the cell.

SUMMARY AND PERSPECTIVES

Protein targeting across the membrane of the ER in the yeast *Saccharomyces cerevisiae* can occur by two partially redundant pathways. One pathway targets proteins cotranslationally to the ER membrane and is mediated by the SRP and its receptor, whereas the other pathway targets proteins posttranslationally and is SRP- and SRP-receptor-independent. We have isolated novel mutant alleles of the ER membrane proteins Sec62p and Sec63p that specifically block in vivo and in vitro the SRP-independent posttranslational translocation pathway. Mutant cells are viable, as are cells that lack the SRP or SRP receptor; the combination of mutations from both pathways, however, leads to cell death. Targeting of different proteins into either

one of the two pathways is determined by their signal sequences. We entertain a model where a common translocon, composed of the Sec61p complex, receives input from both targeting pathways, in one case via SRP and SRP receptor and in the other via the Sec62/Sec63p complex. Association with such "accessory functions" would allow the translocon to receive translocation substrates either co- or posttranslationally.

Recently, homologs of both Sec62p and Sec63p have been identified in higher eukaryotes (Noel and Cartwright 1994; Brightman et al. 1995), and there are examples of proteins that can be translocated across mammalian microsomes in a posttranslational, SRP-independent fashion (Schlenstedt et al. 1990). These results suggest that both SRP-dependent and SRP-independent pathways may coexist in all eukaryotes. Thus, why are some proteins targeted through the SRP-dependent cotranslational pathway while others bypass this route and are targeted posttranslationally? For posttranslational translocation, the substrate protein must be retained in the cytosol in a translocation-competent state, i.e., be prevented from folding up too tightly and from aggregating or precipitating. This may pose certain constraints on the proteins that can use this pathway. Cotranslational translocation is conceptually simpler: No constraints exist regarding the nature of the polypeptide chain, which is fed into the translocon as it emerges from the ribosome. Because of this mechanism, the protein does not have a chance to fold, aggregate, or precipitate in the cytosol (and potentially toxic proteins are not made in the cytosol). Thus, it seems plausible that only proteins which can use the SRP-independent translocation pathway efficiently have evolved signal sequences that allow them to bypass SRP.

Because yeast cells can live, albeit with much reduced growth rates, in the absence of SRP, the SRP-independent pathway can, at some level, handle *all* essential proteins that enter the secretory pathway. This characteristic may be unique to *S. cerevisiae*, since deletions of SRP gene homologs in *E. coli* or *Schizosaccharomyces pombe* are lethal. We consider it likely that these organisms require a greater control of the dosage of proteins crossing the bacterial plasma membrane or entering the ER and that death results from their inability to compensate for quantitative differences in the proteins using alternative targeting routes. In this light, the ability of SRP to provide feedback control of translation may be an important level of regulation. It is an attractive possibility that modification of SRP, SRP receptor, or some accessory factors might allow the synthesis of some proteins to be adjusted, such that translation and translocation are continued if and only if the cell needs them.

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