Cell, Vol. 81, 1075–1084, June 30, 1995, Copyright © 1995 by Cell Press

SRP Samples Nascent Chains for the Presence of Signal Sequences by Interacting with Ribosomes at a Discrete Step during Translation Elongation

Stephen C. Ogg and Peter Walter
Department of Biochemistry and Biophysics
Medical School
University of California, San Francisco
San Francisco, California 94143-0448

Summary

The signal recognition particle (SRP) binds to ribosomes that synthesize nascent chains bearing signal sequences and catalyzes their targeting to the endoplasmic reticulum membrane. In S. cerevisiae, a temperature-sensitive mutation in the *SEC65* gene, encoding an SRP subunit, results in lowered levels of SRP. Growth and protein translocation defects induced by this mutation can be suppressed specifically by sublethal doses of cycloheximide but not anisomycin, each inhibitors of different steps of translation elongation. Cycloheximide also suppresses protein translocation defects caused by depletion of a different SRP subunit. We propose that reduced elongation rates in the presence of cycloheximide allow otherwise insufficient SRP to interact efficiently with ribosomes. These results are consistent with a sampling model in which SRP cycles on and off of translating ribosomes at specific steps during the elongation cycle to inspect all nascent chains for the presence of signal sequences.

Introduction

The signal recognition particle (SRP) is a cytosolic ribonucleoprotein that selects ribosomes synthesizing membrane and secretory proteins and targets these ribosome–nascent chain complexes to the endoplasmic reticulum (ER) membrane (for review, see Walter and Johnson, 1994). Selection involves the binding of SRP to signal sequences as they emerge as part of the nascent chain from the ribosome. As a consequence of this interaction, SRP binds more tightly to the ribosome–nascent chain complex, and the elongation of the nascent protein chain pauses (elongation arrest), suggesting that SRP interacts directly with the ribosome (Wolin and Walter, 1989). Upon targeting of the SRP–ribosome–nascent chain complex to the ER membrane, the interaction of the SRP with the SRP receptor, an ER membrane protein, displaces SRP in a GTP-dependent reaction from both the signal sequence and the ribosome (Connolly et al., 1991; Miller et al., 1993). Released from SRP, the ribosome engages with the protein translocation apparatus in the ER membrane, translation resumes, and, concomitant with its synthesis, the nascent protein chain is translocated across or, in the case of an integral membrane protein, integrated into the ER membrane. Thus, SRP can be viewed as an adapter between the protein translation machinery in the cytosol and protein translocation machinery in the ER membrane that ensures that selection and targeting of ribosomes synthesizing signal sequence–bearing nascent chains occurs in a cotranslational manner.

Much of this model describing SRP function was derived from biochemical studies of mammalian SRP. Recently, structurally similar SRP and SRP receptor homologs from the yeast Saccharomyces cerevisiae have been identified and shown to function in protein translocation in vivo (Brown et al., 1994; Hann et al., 1989; Ogg et al., 1992; Stirling and Hewitt, 1992). Deletion of the genes encoding any SRP or SRP receptor component leads to identical phenotypes: slow cell growth and inefficient protein translocation across the ER membrane. In the absence of SRP, the efficiency with which proteins are translocated across the ER differs among individual proteins: the translocation of some proteins is severely impaired, while some others are not affected at all (Hann and Walter, 1991). Because of this observation and because yeast cells can grow, albeit slowly, in the absence of SRP, there must be an alternative, SRP-independent route by which proteins can be translocated across the ER membrane in cells lacking SRP. In vitro studies have shown that protein translocation across ER-derived membranes can occur posttranslationally (Hansen et al., 1986; Rothblatt and Meyer, 1986; Waters and Blobel, 1986), and hence it is plausible that in yeast, SRP-dependent cotranslational and SRP-independent posttranslational pathways coexist that are, at least in part, functionally redundant.

SRP homologs have been identified in all cells analyzed to date, including bacteria, archae, and eukaryotes. In Escherichia coli, the SRP consists of a single protein subunit, Ffh, that is homologous to the eukaryotic Srp54 protein, the SRP subunit that binds to signal sequences, and a small RNA, 4.5S RNA, that shares a structurally similar domain with eukaryotic SRP RNA. There is increasing evidence that these structurally related bacterial RNPs function like their eukaryotic counterparts in signal sequence recognition and protein targeting (Wolin, 1994). As in S. cerevisiae, an SRP-independent targeting pathway can operate in E. coli; but in contrast with yeast, this pathway cannot sustain life in the absence of the SRP.

The evolutionary conservation of SRP suggests that its function must be important. One explanation is that the cotranslational nature of the signal recognition event by SRP guarantees that nascent chains are targeted to the appropriate membrane early during their synthesis, i.e., before too much of the protein has been synthesized. This may assure targeting of short nascent chains that can be efficiently translocated before they, after further elongation, may have had the opportunity to misfold or aggregate into a translocation-incompetent state in the cytosol. Here, we present evidence that the function of SRP early in the synthesis of a protein is assured through a mechanism where the ribosome plays a direct role in the signal recognition event. In particular, we show that the interaction of SRP with the ribosome is obligatory for SRP function in vivo. Furthermore, our data suggest that the SRP–ribo-

some interaction is restricted to a defined state in the elongation cycle of the ribosome. Taken together, our results point at a much more intimate and exquisitely regulated interaction between SRP and ribosomes than previously appreciated.

Results

SEC65 encodes the yeast homolog of mammalian SRP19, an essential component of SRP (Stirling and Hewitt, 1992). The gene was identified genetically and cloned by complementation of the recessive *sec65-1* mutation, which renders yeast cells temperature sensitive for growth (Stirling et al., 1992). We mapped the mutation responsible for the temperature-sensitive phenotype to a C to T transition that results in a change of Pro-152 (CCA) to leucine (CTA) (see Experimental Procedures). As previously shown, this mutation causes Sec65p to be unstable at the nonpermissive temperature, causing SRP in these cells to be partially disrupted (Hann et al., 1992). Surprisingly, yeast cells bearing the *sec65-1* mutation not only show growth arrest, but also die when incubated at the nonpermissive temperature. In contrast, cells bearing a complete disruption of *SEC65* are viable, although, like cells in which genes encoding other SRP components are disrupted, they have growth and protein translocation defects. Thus, it seems a paradox that *sec65-1* cells are conditionally lethal, yet cells bearing the *SEC65* gene disruption are viable.

To test whether the temperature-sensitive phenotype of *sec65-1* cells is related to SRP function or is due to some indirect interference of mutant Sec65p with some essential cellular process, we constructed strain SOY288 (see Table 1), bearing both a disruption of the gene encoding SRP RNA (*scr1Δ*) and the *sec65-1* mutation (see Experimental Procedures). Growth analyses showed that SOY288 cells were not temperature sensitive: cells grew at the rate characteristic for SRP-depleted cells at both 24°C and 37°C, the permissive and nonpermissive temperatures for *sec65-1* (Figure 1). Transformation with a plasmid bearing the wild-type *SCR1* gene complemented the growth defects and restored the temperature-sensitive phenotype. We infer from these data that the temperature-sensitive phenotype of the *sec65-1* mutation is manifested only in cells that contain SRP; i.e., cells that lack SRP are immune to the detrimental effects of the *sec65-1* mutation at the nonpermissive temperature. This result suggests that the temperature-induced disruption of the mutant SRP directly causes cell death.

A possible explanation for the paradox described above is that yeast cells are sensitive to the kinetics with which SRP is lost: as SRP levels drop suddenly when *sec65-1* cells are shifted to the nonpermissive temperature, cells might not have enough time to switch over to an SRP-independent mode of protein targeting. This failure to adapt would result in cell death. This model suggests that it might be possible to suppress the temperature-sensitive phenotype of the *sec65-1* mutation first of all if SRP-independent targeting were enhanced, second, if SRP levels at the nonpermissive temperature were stabilized, or third, if an insufficient SRP concentration present in *sec65-1* cells at the nonpermissive temperature could be made to suffice.

To explore these possibilities, we pursued the observation, first made by D. Feldheim and R. Schekman, that sublethal concentrations of cycloheximide, an inhibitor of translation elongation, can suppress the temperature-sensitive growth defect of *sec65-1* cells. To demonstrate suppression, a lawn of *sec65-1* cells was plated on yeast extract–peptone–dextrose (YEPD, or yeast complete media) plates and filter disks soaked either in water (Figure 2A, minus CHX) or in a cycloheximide solution (Figure 2A, plus CHX) were placed in the center of the plates. When plates were incubated at 37°C, a distinct ring of cell growth was observed surrounding the filter disk that contained cycloheximide (Figure 2A, bottom right), whereas no cells grew on the plate containing the disk soaked in water (Figure 2A, bottom left). The diameter of this ring was dependent on the amount of cycloheximide added to the filter disk: higher amounts produced a ring with a larger diameter, whereas lower amounts produced a ring with a smaller diameter (data not shown). The most plausible interpretation of these results is that, during the incubation time, cycloheximide diffuses from the disk and forms a radially symmetric concentration gradient. Within a narrow concentration range, cycloheximide suppresses the growth defect of *sec65-1* cells at the nonpermissive temperature, resulting in a ring of cell growth.

To quantitate this result, we measured the growth rate of *sec65-1* cells in liquid culture at different cycloheximide concentrations at 24°C and 37°C (Figure 2B). In agree-

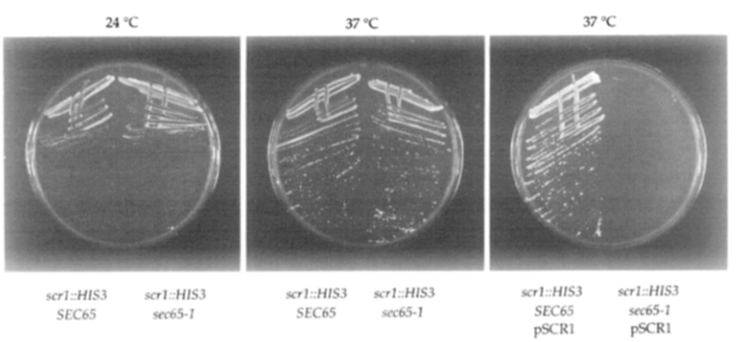

Figure 1. Intact SRP Is Required for *sec65-1* Cells to Manifest the Temperature-Sensitive Defect

Cells with the indicated relevant genotype (SOY288 (*scr1::HIS3*, *sec65-1*) and BHY132 (*scr1::HIS3*, *SEC65*) were streaked onto YEPD plates and incubated at the indicated temperatures (left and middle plates). Both strains were transformed with pSCR1, a plasmid containing the wild-type *SCR1* sequence, streaked onto a YEPD plate, and incubated at 37°C (right plate).

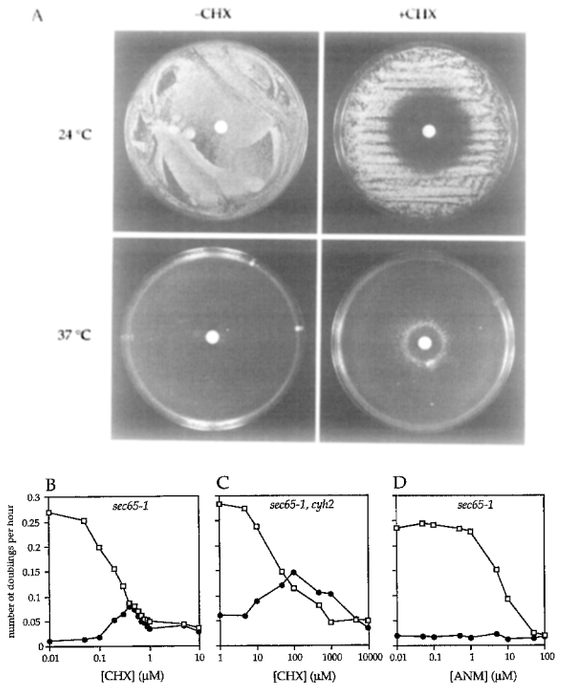

Figure 2. Cycloheximide, but Not Anisomycin, Suppresses the Temperature-Sensitive Defect of *sec65-1* Cells

(A) Cells (RSY457, *sec65-1*) were plated on YEPD plates. Filter disks containing 6 nmol of cycloheximide (in water) were placed in the center of each plate (plus CHX). Filter disks containing only water serve as the control (minus CHX). Plates were incubated at the indicated temperatures for three days.

(B and C) Cells were grown in liquid YEPD medium containing the indicated amount of cycloheximide at 24°C (open squares) or 37°C (closed circles). Growth rates were determined by measuring the OD_{600} of the cultures. Each data point represents the average of two experiments. (B) shows the growth rates for strain RSY457 (*sec65-1*, *CYH2*), and (C), the growth rates for strain SOY158 (*sec65-1*, *cyh2*).

(D) RSY457 (*sec65-1*) cells were grown in the presence of anisomycin at 24°C (open squares) or 37°C (closed circles).

ment with the plate assay, addition of low concentrations of cycloheximide resulted in cell growth at the nonpermissive temperature. The growth rate was maximal at 0.4 μM cycloheximide and, at this concentration, was identical at the permissive and nonpermissive temperature. Cycloheximide concentrations in excess of 0.4 μM inhibited the growth rate equally at either temperature, presumably because the rate of translation became limiting for cell growth.

sec65-1 cells show marked protein translocation defects upon shift to the nonpermissive temperature (Stirling and Hewitt, 1992; Stirling et al., 1992). To ask whether cycloheximide suppresses the protein translocation defects in addition to the growth defect, we pulse labeled *sec65-1* cells with [^{35}S]methionine, either at 24°C or 37°C, and in the presence or absence of 0.4 μM cycloheximide. Using antibodies that recognize Kar2p or DPAP-B, two proteins that use the SRP-dependent protein-targeting pathway to the ER membrane, we performed immunoprecipitation reactions from extracts of the radiolabeled cells. Accumulated precursor forms of both Kar2p and DPAP-B were significantly increased when *sec65-1* cells were shifted to the nonpermissive temperature (see Figure 3; compare lane 3 with lane 1). Translocation defects in Kar2p are indicated by the accumulation of a more slowly migrating band, pre-Kar2p, containing an uncleaved signal sequence, whereas translocation defects of DPAP-B result in the accumulation of a faster-migrating band, pre-DPAP-B, lacking N-linked glycosylation. The addition of 0.4 μM cycloheximide completely abolished precursor accumulation of both Kar2p and DPAP-B at the nonpermissive temperature. Thus, both cell growth and protein translocation defects manifested in *sec65-1* cells at the nonpermissive temperature are suppressed by cycloheximide.

Cycloheximide is a well-characterized inhibitor of eukaryotic protein translation elongation (Gale et al., 1981). We therefore sought to ascertain that suppression resulted from direct effects of cycloheximide on protein synthesis, rather than from pleiotropic effects of the drug on an unrelated target. To this end, we isolated mutations in ribosomal protein L29, encoded by *CYH2*, which render cells resistant to higher levels of cycloheximide (Stöcklein and Piepersberg, 1980). Such mutants arise spontaneously and can be readily selected by growing cells on plates containing cycloheximide. This allowed us to isolate *cyh2* mutant cells in the same strain background bearing the *sec65-1* mutation. Cycloheximide-resistant colonies were isolated from strain RSY457 (*sec65-1*). One of these colonies was designated as strain SOY158 and chosen for further study. To confirm that the cycloheximide resistance of these cells was due to a mutation in *CYH2*, cells from strain SOY158 were crossed to strain 5114-2 bearing the *cyh2* mutation. The resulting diploid strain was resistant to cycloheximide, and, after sporulation, the cycloheximide resistance phenotype segregated 4:0 in 20 tetrads analyzed, indicating that the cycloheximide resistance phenotype was due to a mutation in *CYH2*. Like the parent strain, SOY158 cells were temperature sensitive for growth. Growth of RSY457 (*sec65-1*, *CYH2*) cells at the permissive temperature was inhibited by 50% at 0.2 μM cycloheximide, whereas growth of SOY158 (*sec65-1*, *cyh2*) cells was inhibited by 50% at 50 μM cycloheximide (compare Figures 2B and 2C). We therefore conclude that SOY158 cells bear both the *sec65-1* and the *cyh2* mutations.

To test whether cycloheximide would suppress the temperature-sensitive growth defect of SOY158 cells, we grew the cells in liquid culture at either the permissive or nonpermissive temperature in the presence of increasing concentrations of cycloheximide. As shown in Figure 2C, cycloheximide suppressed their growth defect at the nonpermissive temperature; however, suppression required levels of cycloheximide 250-fold higher (100 μM) than that required to suppress the growth defect of *sec65-1* cells (Figure 2B). This difference correlated directly with the resistance of SOY158 cells to a 250-fold higher level of cycloheximide when grown at the permissive temperature.

These results strongly suggest that the effects of cyclo-

heximide on translating ribosomes are directly responsible for the suppression of the defects caused by the *sec65-1* mutation. Furthermore, the defects caused by the *sec65-1* mutation must result from a loss of SRP, because they are only manifested in cells that initially harbor intact SRP. We can explain these results with the following model: in the presence of limiting concentrations of cycloheximide, nascent polypeptide chains emerge more slowly from the ribosome. This results in a longer time window during which the signal peptide of a nascent polypeptide chain can be recognized by SRP cotranslationally. Given this model, the cycloheximide-induced reduction of the elongation rate would allow a smaller, and otherwise insufficient amount of SRP, to perform its normal function.

We examined the fate of Sec65p in *sec65-1* cells to ask directly whether a small amount of Sec65p still remained after incubation at the nonpermissive temperature. To this end, we probed Western blots of lysates identical to those used for Figure 3 with antibodies specific for Sec65p. When shifted to 37°C, Sec65p levels fall to roughly 10% of those seen in cells grown at 24°C in the absence of cycloheximide (Figure 4A; compare lane 3 with lane 1). Growth in the presence of cycloheximide at 37°C further reduced the level of Sec65p, to roughly 5% of that seen in cells grown in the absence of cycloheximide (Figure 4A; compare lanes 4 and 6 with lane 1. Lane 6 contains ten times the amount of cell extract as lane 4). This reduced level of Sec65p in cycloheximide-treated cells grown at 37°C was the same whether the cells were tested at 30 min or 1.5 hr after a shift to 37°C (data not shown). These results show that a small amount of Sec65p remains in cells, even after prolonged incubation at the nonpermissive temperature, and thus are consistent with the model presented above. The data also rule out the alternative possibility that Sec65p is somehow being stabilized by cycloheximide at the nonpermissive temperature.

According to another possible mechanism of suppression, cycloheximide treatment might cause detrimental precursor proteins to be degraded or would induce an SRP-independent, posttranslational translocation pathway. To test these possibilities, we accumulated radiolabeled precursor proteins by shifting *sec65-1* cells to 37°C followed by pulse labeling with [^{35}S]methionine. After the addition of a large excess of unlabeled methionine, the incubation was continued at 37°C in either the presence or the absence of cycloheximide, and the fate of labeled pre-Kar2p was monitored. Aliquots of the cell culture were removed at the intervals indicated in Figure 4B and immunoprecipited with antibodies to Kar2p. As demonstrated by the results shown in Figure 4B, pre-Kar2p was stable throughout the duration of a 32 min chase period, regardless of the presence or absence of cycloheximide. Because the time needed for cycloheximide to exert its effect is no longer than 10 min (conditions under which the experiment in Figure 3 was performed), we know that the temperature-induced protein translocation defect can be suppressed by cycloheximide during the first third of the chase period shown in Figure 4B. Thus, we can conclude that the suppression of the translocation defect shown in Figure 3 was not simply due to precursor degradation, nor was it caused by the precursors being translocated in a posttranslational manner.

To test the validity of our model, we used an independent approach, not involving the *sec65-1* mutation, to reduce

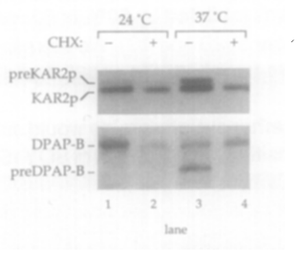

Figure 3. Cycloheximide Suppresses the Protein Translocation Defect of *sec65-1* Cells

RSY457 (*sec65-1*) cells growing at the indicated temperatures in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of 0.4 μM cycloheximide were pulse labeled with [^{35}S]methionine for 7 min. Cell extracts were immunoprecipitated with antibodies against either Kar2p or DPAP-B. The precursor and mature forms of each of the immunoprecipitated proteins are indicated. Immunoprecipitated proteins were visualized by autoradiography following SDS–PAGE.

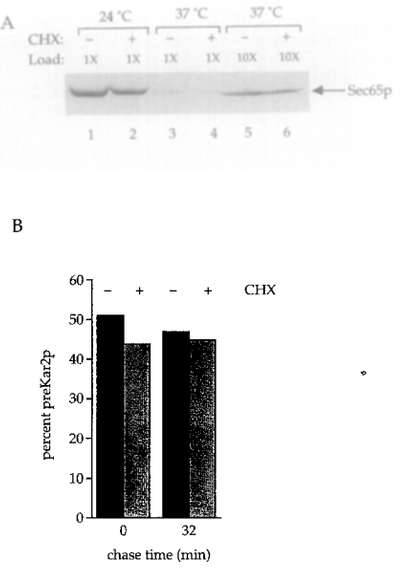

Figure 4. Cycloheximide Does Not Stabilize Sec65p or Increase the Efficiency of Protein Translocation into the ER

(A) Lysates made from cells grown as in Figure 3 were subjected to Western blot analysis. Lanes 1–4 contain 0.5 OD_{600} cell equivalents of lysate. Lanes 5 and 6 are duplicates of lanes 3 and 4, except that 5.0 OD_{600} cell equivalents of lysate were loaded. The position of Sec65p is indicated with an arrow.

(B) RSY457 (*sec65-1*) cells were shifted from 24°C to 37°C for 30 min prior to pulse labeling for 1 min with [^{35}S]methionine. Concomitant with the addition of nonradiolabeled methionine in a 1000-fold molar excess, cycloheximide was added to cells as indicated. Cells were incubated for 0 or 32 min and then processed for immunoprecipitation using anti-Kar2p. Kar2p and pre-Kar2p were separated by SDS–PAGE followed by quantitation on a PhosphorImager.

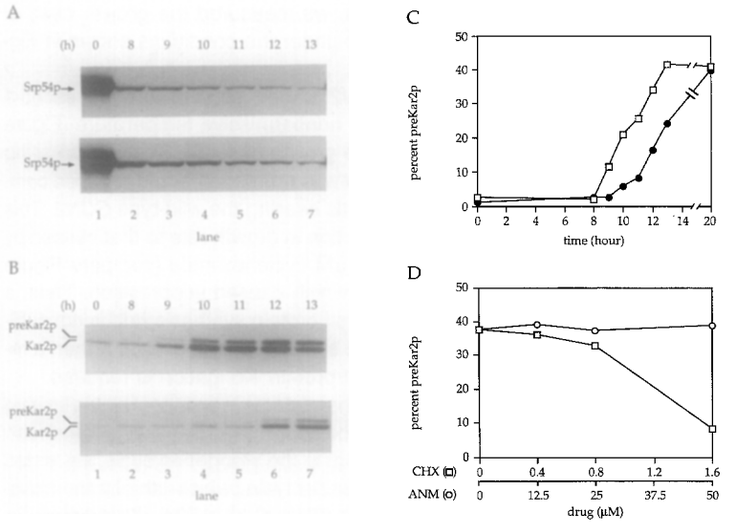

Figure 5. Protein Translocation Defects Caused by SRP Depletion Are Suppressed by Cycloheximide

BHY104 cells containing *SRP54* under the control of the *GAL1* promoter were shifted from galactose to glucose to repress the expression of Srp54p. At the indicated time points after repression of Srp54p, samples were incubated in the presence (bottom in [A] and [B]) or absence (top in [A] and [B]) of cycloheximide for 5 min. Cells were pulse labeled with Trans[^{35}S]-label for 15 min, followed by a 2 min chase period to complete processing of all Kar2p chains. Kar2p and pre-Kar2p were visualized as in Figure 3. Higher levels of Kar2p at later time points are due to the induction of the heat shock response upon accumulation of cytoplasmic precursors (Arnold and Wittrup, 1994). (A) Portions of the radiolabeled extracts were subjected to Western blot analysis with anti-Srp54p antibodies. Each lane contains 0.5 OD_{600} cell equivalents of lysate and contained equivalent amounts of protein as judged by Ponceau S staining.

(B) Immunoprecipitations were performed with anti-Kar2p antibodies.

(C) Quantification of the data in (B) was performed on a PhosphorImager. Squares, minus CHX; circles, plus CHX.

(D) In a similar but separate experiment, cycloheximide (squares) and anisomycin (circles) were titrated after cells were grown for 11 hr in glucose medium to deplete Srp54p. After incubation of the cells for 5 min with the indicated amount of each drug, cells were processed as above, and the ratio of pre-Kar2p to Kar2p was determined. The concentration range of each drug was chosen on the basis of the data in Figure 2. In the absence of the drug, 40% pre-Kar2p accumulated, compared with only 25% at the corresponding time point in (C). This is due to variation among separate experiments.

the amount of functional SRP in cells and then monitored the effects of cycloheximide on the efficiency of protein translocation. Strain BHY104 contains *SRP54* under the control of the *GAL1* promoter, and SRP can be depleted from these cells by switching the carbon source from galactose- to glucose-containing media (Figure 5A). As shown previously, precursors of normally translocated proteins accumulate as the concentration of SRP becomes limiting in these cells (Hann and Walter, 1991). Thus, monitoring the efficiency of protein translocation at different times after repression of Srp54p synthesis, i.e., in cells containing decreasing concentrations of SRP, allowed us to test directly whether a reduction of the elongation rate by cycloheximide would allow a limiting concentration of SRP to work more efficiently. To perform this experiment, aliquots were taken from a culture of BHY104 cells at different times after repression of *SRP54* and incubated both in the presence and absence of cycloheximide for 5 min. [^{35}S]Methionine was then added to pulse label newly synthesized proteins. We examined the accumulation of pre-Kar2p in cells labeled in the absence or presence of cycloheximide after immunoprecipitation.

Pulse labeling in the absence of cycloheximide gave the expected results (Figure 5B, top, and Figure 5C, squares). Pre-Kar2p started to accumulate at 9 hr after *SRP54* expression was repressed and reached a plateau at 13 hr at about 40% of the total immunoprecipitated Kar2p species. In contrast, in the presence of cycloheximide, the accumulation of pre-Kar2p was significantly delayed (Figure 5B, bottom, and 5C, circles), indicating that cycloheximide allowed the limiting concentration of SRP in these cells to work more efficiently. As shown in the Western blot analysis in Figure 5A, the delay of pre-Kar2p accumulation was not due to differences in Srp54p levels in the untreated and cycloheximide-treated samples. While the pre-Kar2p accumulation in the presence of cycloheximide was significantly reduced at the 9–13 hr time points, it eventually reached the same plateau as in the absence of cycloheximide. This result is expected, because cells must switch from an SRP-dependent mode of protein translocation to an SRP-independent mode when SRP is depleted. From the data shown in Figure 5C, we surmise that between 9 and 11 hr after repression of Srp54p synthesis, the levels of functional SRP become limiting but are still high enough to afford efficient protein translocation, if the rate of protein elongation is reduced by cycloheximide. At the later time points, however, an increasing fraction of Kar2p becomes targeted through the SRP-independent pathway and, because the level of functional SRP becomes too low, efficient cotranslational SRP-dependent protein translocation can no longer be reinstated, even if the elongation rate is reduced by the addition of cycloheximide. Consistent with this interpretation, the growth and protein translocation defects in strains deleted for *SRP54* or *SEC65* cannot be abrogated by the addition of cycloheximide (data not shown).

To determine the concentration of cycloheximide that would give the greatest difference of precursor accumulation, we performed a titration experiment with cells taken from a fixed time point (11 hr after shift to glucose) in the SRP depletion experiment. The results show that increasing amounts of cycloheximide progressively improved

translocation efficiency (Figure 5D, squares), indicating that the more protein elongation is slowed, the more efficiently protein translocation occurs in the presence of a fixed concentration of SRP. The optimum cycloheximide concentration was found to be 1.6 μM. The difference between this concentration and the one required to suppress the growth defect of *sec65-1* cells (0.4 μM) may be due to differences in the strain background between RSY457 (*sec65-1*) and BHY104 (pGALSRP54) cells. Alternatively, the optimal concentration of cycloheximide that allows efficient Kar2p translocation in a 5 min pulse (conditions in Figure 5) may not allow sustained growth of yeast in liquid culture (conditions in Figure 2).

According to our model, the nascent protein chain has to emerge from the ribosome more slowly to allow a reduced concentration of SRP to target the nascent chains cotranslationally. If this is the case, then inhibiting protein synthesis initiation should not suppress the *sec65-1*-induced growth defects, because, while reducing the flux of newly synthesized protein, this would not reduce the rate at which they emerge from the ribosome. To reduce translation initiation, we expressed dominant, constitutively active alleles of *GCN2* in either *sec65-1* or wild-type cells. Gcn2p is a protein kinase that inactivates initiation factor eIF-2α by phosphorylation, and cells expressing dominant alleles of *GCN2* grow at reduced rates because of limiting concentrations of active eIF-2α. The severity of the growth defect correlates with the degree to which the mutations in *GCN2* activate its kinase activity (Dever et al., 1992). We expressed the wild-type and three constitutively active alleles of *GCN2* (in increasing strengths, *GCN2*^{c}-516, *GCN2*^{c}-518, and *GCN2*^{c}-515) in either *sec65-1* or wild-type cells. Figure 6A depicts plates on which either *sec65-1* or wild-type cells were grown at 24°C or 37°C. The particular allele of *GCN2* that was present in the cells in each quadrant of the plates is indicated below. To quantitate the severity of the growth defect produced by each of the different *GCN2*^{c} alleles, we measured the growth rates of cells in liquid culture under the conditions shown in Figure 6A.

None of the *GCN2* alleles suppressed the *sec65-1* growth defect at the nonpermissive temperature (Figure 6A), even though the growth of *sec65-1* cells expressing the *GCN2*^{c}-515 allele was more than 4-fold reduced compared with that of cells bearing the wild-type *GCN2*. This is a similar fold reduction in growth rate to that caused by the presence of 0.4 μM cycloheximide (compare Figure 6B with Figure 2B), which caused suppression. Thus, a reduction of the overall protein synthesis rate is not sufficient to suppress the *sec65-1* growth defect; rather, a reduction in the rate of protein elongation is required.

To determine whether defects in cells containing limiting amounts of SRP can be suppressed by slowing translation elongation at any point in the elongation cycle, we tested an additional drug, anisomycin, which inhibits the transpeptidylation reaction (Gale et al., 1981). Surprisingly, in contrast with cycloheximide, anisomycin was unable to suppress the growth defect of *sec65-1* cells at 37°C (see Figure 2D, closed circles). Similarly, anisomycin was unable to suppress the translocation defect at early times after Srp54p depletion (see Figure 5D, circles). Anisomycin was able, however, to enter yeast cells efficiently and inhibit growth of *sec65-1* cells at the permissive temperature (see Figure 2D, squares).

These results raised the possibility that the different effects of cycloheximide, anisomycin, and the *GCN2*^{c} mutation could, in principle, result from differential effects of these three inhibitors on the translation of individual mRNAs. Translation of a subset of proteins could, for example, be irreversibly blocked in one case but not another, and the different effects leading to the suppression of the translocation defect under conditions of limiting SRP might then result from the different spectrum of proteins synthesized. To test for this possibility directly, we deter-

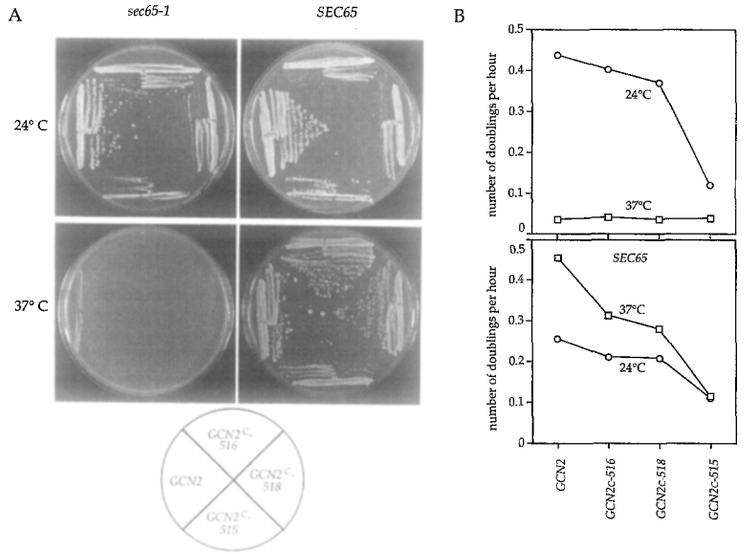

Figure 6. Inhibition of Translation Initiation Does Not Suppress the Growth Defect of *sec65-1* Cells

(A) RSY457 or W303-1A cells (either *sec65-1* or *SEC65*) were transformed with plasmids containing the indicated dominant allele of *GCN2*. These cells were grown on media selecting for the plasmid at either 24°C or 37°C. The diagram illustrates which quadrant of each plate contains cells harboring the indicated allele of *GCN2*.

(B) The growth rates of the strains used in (A) were determined. The average of two separate experiments is shown.

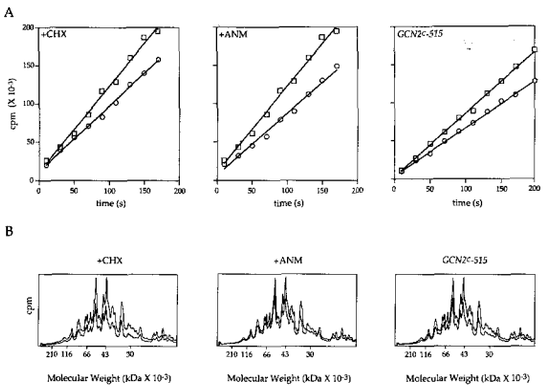

Figure 7. Translation Elongation and Initiation Inhibitors Affect the Same Spectrum of Proteins

(A) Incorporation of [^{35}S]methionine at 24°C is plotted as a function of time. *sec65-1* cells were pulse labeled in the absence (open squares) or presence (open circles) of either cycloheximide (plus CHX) or anisomycin (plus ANM). At the indicated times after [^{35}S]methionine addition, hot TCA-precipitable counts in aliquots containing equal OD_{600} cell equivalents were measured by scintillation counting. W303 cells containing a plasmid bearing wild-type *GCN2* (open squares) or the dominant allele *GCN2^{c}*-515 (open circles) were treated identically (*GCN2^{c}*-515).

(B) Aliquots (0.1 OD_{600} cell equivalents per lane) from lysates prepared at the 130 s time point from the labeling reaction shown in (A) were subjected to SDS–PAGE, followed by scanning of the lanes with a PhosphorImager. The positions of molecular mass markers are indicated below each panel. In each panel, the top trace is that obtained in the absence of inhibitor, while the bottom trace is obtained from cell lysate labeled in the presence of either cycloheximide (plus CHX), anisomycin (plus ANM), or the dominant negative allele of *GCN2* (*GCN2^{c}*-515).

mined the effects of cycloheximide, anisomycin, and the *GCN2^{c}*-515 mutation on the overall pattern of proteins synthesized. To this end, we monitored the rate of incorporation of [^{35}S]methionine into protein in the absence or in the presence of the three inhibitors under conditions that were shown in Figures 2 and 6 to slow cell growth to approximately similar extents (Figure 7A). Incorporation of [^{35}S]methionine was linear over the course of the 3 min experiment, and its rate was decreased by about 25% in the presence of the inhibitors. We chose a late time point (130 s) from this labeling reaction to analyze the pattern of newly synthesized proteins in the presence of the three inhibitors. Total labeled cell protein was fractionated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), and the lanes were scanned along their lengths with a PhosphorImager (Figure 7B). In each case, the upper trace represents the profile of proteins synthesized in the absence of inhibitor, while the lower trace represents the profile of proteins synthesized in the presence of inhibitor. These results show that all three inhibitors diminish uniformly the incorporation of [^{35}S]methionine across the entire spectrum of the labeled protein species. From these data, we conclude that the observed differences between the inhibitors most likely result because they affect different steps of protein translation and not because they have differential effects on the translation of individual mRNAs.

Discussion

We have shown that growth and protein translocation defects induced in yeast cells containing limiting concentrations of SRP can be suppressed by slowing protein elongation with low concentrations of cycloheximide. Two independent means of lowering SRP levels were used: shifting *sec65-1* cells to the nonpermissive temperature and depleting Srp54p after transcriptional repression. Cycloheximide ameliorates growth and translocation defects in both cases, but not after prolonged Srp54p depletion or in cells bearing complete SRP gene disruptions, indicating that some residual SRP must be present in cells for cycloheximide to exert this effect. Furthermore, cycloheximide-resistant cells harboring a mutation in ribosomal protein L29 (encoded by *CYH2*) require significantly higher concentrations of cycloheximide to affect suppression, thereby demonstrating that suppression is mediated directly through the effects of cycloheximide on the ribosome.

These observations strongly support a model in which cycloheximide causes nascent polypeptide chains to emerge more slowly from the ribosome, creating a longer time window during which the signal peptide of a nascent polypeptide chain can be recognized by SRP and targeted to the ER membrane cotranslationally. Thus, the cycloheximide-induced reduction of the elongation rate allows a limiting amount of SRP to perform its normal function.

Consistent with this model, we found that inhibition of translation initiation (experimentally induced by the introduction of the *GCN2^{c}* mutations, Figure 6) does not cause suppression. Unexpectedly, another well-characterized inhibitor of translation elongation, anisomycin, also failed to cause suppression, indicating that slowing down the rate of protein elongation per se is not sufficient to suppress defects caused by low levels of SRP. Anisomycin blocks transpeptidylation, while cycloheximide blocks translocation, which is the eEF2-catalyzed movement of peptidyl-tRNA from the A site to the P site (Figure 8C). These data suggest that SRP can interact productively with ribosomes and signal sequence–bearing nascent chains only at a specific step(s) in the elongation cycle, which is the step at which ribosomes accumulate in the presence of cycloheximide.

The notion of an intimate, elongation step–specific interaction of SRP and the ribosome suggests a mechanism that can explain how SRP selects signal sequence–bearing ribosomes from the total ribosomal pool. Estimation of the concentration of SRP mammalian cells, yeast (B. C. Hann and P. W., unpublished data), and E. coli (Jensen and Pedersen, 1994) suggest that there is one SRP for every 10–100 translating ribosomes. SRP has to scan every nascent chain for the presence of a signal sequence, yet, on the basis of the estimated SRP levels, only a small fraction of the translating ribosomes can have an SRP associated at any one time. On the basis of the results presented here, SRP can interact with ribosomes only during a specific step in the translation cycle. This is consistent with a sampling mechanism in which SRP is constantly cycling on and off the ribosome, as opposed to a scanning mechanism in which SRP would remain ribo-

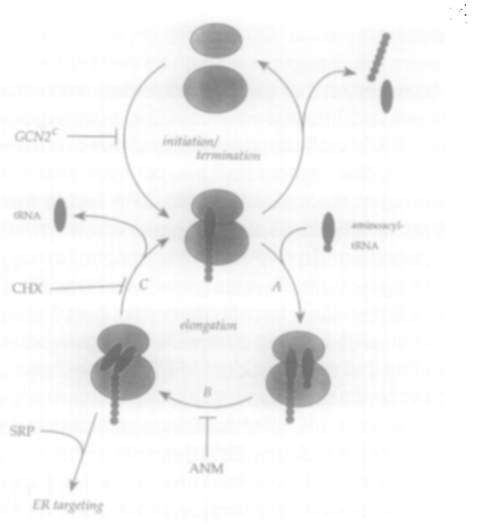

Figure 8. Model for SRP Interaction in the Translational Cycle

Translation is shown as a cycle of translational elongation preceded by initiation (blocked by *GCN2^C* alleles) and followed by termination. Translational elongation is simplified in this model 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–nascent chain complex after the anisomycin block but before the cycloheximide block. This model is adapted from the hybrid sites model of translational elongation as proposed by Moazed and Noller (1989).

some associated. We propose that SRP binds to the translating ribosome when it has just completed the transpeptidylation reaction, but before it has undergone translocation of the peptidyl-tRNA from the A site to the P site. While bound, the SRP checks whether the emerging nascent protein contains a signal sequence. If this is the case, SRP targets the ribosome to the ER membrane; if no signal sequence is found, SRP dissociates, and the ribosome continues through the elongation cycle. According to this mechanism, a substoichiometric concentration of SRP would suffice to monitor each ribosome for the presence of a signal sequence. Because SRP can recognize signal sequences on nascent chains of different lengths, we surmise that sampling need not occur during every elongation cycle (Siegel and Walter, 1988).

Concomitant with a tighter association of SRP to the ribosome, elongation pausing is observed upon binding of SRP to a signal sequence (Walter and Blobel, 1981). This suggests that SRP dissociation is obligatory for the ribosome to resume elongation. According to this model, it should be possible to isolate SRP mutants that inhibit protein synthesis because they bind inappropriately tightly to ribosomes.

The definition of the point of action of SRP during the translation elongation cycle is in agreement with results obtained in prokaryotic cells. In E. coli, the SRP RNA homolog 4.5S RNA and the Srp54p homolog Ffh are essential for cell growth (Brown and Fournier, 1984; Phillips and Silhavy, 1992). Prior to results suggesting its SRP-like role in protein secretion, 4.5S RNA was proposed to be directly involved in translation (Brown, 1987; Brown and Fournier, 1984). In particular, suppressor mutations were isolated in components of the translation apparatus that allowed cells to grow even though they contained sublethal levels of 4.5S RNA. Suppressor mutations were found in elongation factor G (EF-G, the prokaryotic homolog of eEF-2), in 23S ribosomal RNA in the region defining the EF-G-binding site, and in tRNA synthetases, resulting in higher levels of some uncharged tRNAs (Brown, 1987; Brown, 1989). Intriguingly, these mutations are predicted to impair the translation cycle at the translocation step of peptidyl-tRNA from the A to the P site. EF-G mutants and mutations in the ribosomal EF-G-binding site would impair translocation directly, whereas uncharged tRNAs might bind to the tRNA exit site (E site) on the ribosome, thereby backing up the ribosome to prevent translocation. These mutations suppressed defects caused by lowering the concentration of 4.5S RNA, but no mutations could be isolated that would allow cells to live in its absence. Brown (1989) concluded that 4.5S RNA participates in an event required for ribosomal translocation.

The results presented here and the increasing evidence pointing at an SRP-like role for the Ffh/4.5S RNP suggest a different interpretation for these data. We surmise that the lowered levels of 4.5S RNA resulted in lowered levels of functional SRP in E. coli, strictly analogous to the experimental scenarios described here in yeast. Mutations that slow the translocation step would therefore suppress the defects caused by reduced SRP levels, because of an increase in the time window in which SRP can productively recognize a signal sequence. Our data are in agreement with evidence suggesting that the E. coli Ffh/4.5S RNP indeed functions as a prokaryotic SRP.

Experimental Procedures

Strains, Plasmids, Antibodies, and General Methods

Yeast strains used in this study are listed in Table 1. Anti-DPAP-B serum was provided by T. Stevens, University of Oregon; anti-Sec65p is described by Brown et al. (1994); anti-Srp54p is described by Hann and Walter (1991); anti-Kar2p was produced in our laboratory from protein overexpressed in E. coli from a clone provided by J. Vogel and M. Rose (Princeton University). SDS–PAGE was performed on 10%–15% gradient gels, and Western blots were visualized by enhanced chemiluminescence (ECL; Amersham Corporation, Arlington Heights, IL) according to the instructions of the manufacturer. Plasmid pSCR1 was constructed by ligating the *SCR1* containing KpnI to HindIII fragment from pSR6.21 into the KpnI–HindIII sites of pRS316 (Felici et al., 1989).

Strain SOY288, containing both the *sec65-1* and the *scr1-Δ2* mutations, was constructed by mating strain RSY457 to strain BHY133. Sporulation of the resulting diploid followed by tetrad dissection allowed the identification of tetrads that contained colonies resulting from two of the four spores with the *SEC65*, *SCR1* genotype. These colonies were identified, because they were not temperature sensitive and grew at wild-type rates. We therefore reasoned that colonies resulting from the other two spores must contain both the *sec65-1* and *scr1-Δ2* mutations. Presence of *scr1-Δ2* was confirmed by the slow growth phenotype of the cells. Confirmation that these cells also contained the *sec65-1* mutation was achieved by transformation of the cells with a plasmid (pSCR1) bearing wild-type *SCR1*, resulting in the recovery of the temperature-sensitive phenotype as shown in Figure 1.

Strain SOY158, containing both *sec65-1* and *cyh2* mutations, was

Table 1. Yeast Strains

Strain	Genotype	Source
RSY457	MATa *sec65-1, ura3-52, ade2-1, trp1-Δ1, leu2-3,112, his3*	Stirling et al. (1992)
W303-1A	MATa *ura3-1, ade2-1, trp1-1, leu2-3,112, his3-11, can1-100*	R. Rothstein, Columbia University
CSY186	MATα *trp1, lys2, his3, ura3, ade2, sec65::HIS3*	Stirling and Hewitt (1992)
BHY104	MATα *trp1, lys2, his3, ura3, ade2, srp54-Δ1,* (pGALSRP54)	Hann and Walter (1991)
BHY133	MATα *trp1, lys2, his3, ura3, ade2, scr1::HIS3* [rho$^-$]	Brown et al. (1994)
SOY158	MATa *sec65-1, cyh2, ura3-52, ade2-1, trp1-Δ1, leu2-3,112, his3*	This study
SOY288	MATα *sec65-1, scr1-Δ2, his3, ura3, trp1*	This study
5114-2	MATα *leu2-3,112, ura3-52, trp1-289, his7, hom3, can1, chy2, gal1*	N. Hollingsworth, State University of New York at Stonybrook

constructed in the following manner. RSY457 cells were plated onto YEPD plates containing 10 mg/ml of cycloheximide. From approximately 10^8 cells, we recovered three cycloheximide-resistant colonies. One of these colonies was designated SOY158. That the cycloheximide-resistant phenotype resulted from a mutation at the *CYH2* locus was confirmed by crossing strains SOY158 and 5114-2. The resulting diploid was able to grow on cycloheximide plates, indicating that whatever mutation had occurred did not complement the *cyh2* mutation of 5114-2. Colonies resulting from the sporulation and subsequent tetrad dissection (20 tetrads analyzed) of the diploid strain SOY158 × 5114-2 were all resistant to high concentrations of cycloheximide.

Sequencing of the *sec65-1* Allele

Plasmid pJDY32 was constructed by polymerase chain reaction (PCR) using oligonucleotides complementary to bases −336 to −354 on the N-terminal end of the *SEC65* gene and +1051 to +1068 on the C-terminal side, and pCS52 as template DNA (Stirling and Hewitt, 1992). The PCR product was ligated into the SmaI site of pRS314 (Sikorski and Heiter, 1989). We recovered the *sec65-1* allele from RSY457 by gap repair (Orr-Weaver et al., 1988). Toward this end, pJDY32, containing the entire coding sequence of *SEC65*, as well as *TRP1*, *CEN*, and *ARS* sequences, was digested with the restriction enzymes NheI and NdeI, which cut at two unique sites to remove a portion of the *SEC65* gene. The gap begins 63 nt upstream of the A of the initiating codon and continues through the last nucleotide of codon 207, removing a fragment that encodes 207 of the 273 amino acids of the *SEC65* gene. The resulting vector fragment was transformed into strain RSY457 (*sec65-1*). Eight Trp$^+$ colonies failed to grow at 37°C, indicating that they were not able to complement the *sec65-1* temperature-sensitive growth defect. Plasmids were isolated from two of these colonies, amplified in bacteria, and shown to contain the entire *SEC65* gene. The plasmids were transformed into strain CSY186 (*sec65::HIS3*). Trp$^+$ transformants were tested for their ability to complement the growth defect of the deletion allele. Deletion of any of the components of the SRP pathway results in a 3- to 5-fold reduction in the growth rate of the cells containing the deletion. Two plasmids, pSO438-1 and pSO438-2, restored temperature-sensitive growth to cells containing the *sec65* deletion (CSY186) but failed to complement the temperature-sensitive growth of the original *sec65-1* mutation (RSY457). Thus, we concluded that the plasmids contain the mutation that causes the *sec65-1* temperature-sensitive growth. Because the *sec65-1* allele was repaired to a gapped plasmid, the mutation must occur within the boundaries of the gap. The DNA sequences of pSO438-1, pSO438-2, and pJDY32 were determined between these two restriction sites on both strands. Both pSO438-1 and pSO438-2 contained the same single base change when compared with the sequence of pJDY32. This difference was a C to T transition at position 795 of the coding strand. This occurs in the second position of codon 152 and is predicted to change a proline codon (CCA) in the wild-type allele of *SEC65* to a leucine codon (CTA) in the *sec65-1* mutant allele. Ethyl methanesulfonate, the mutagen used in the selection that yielded *sec65-1*, is known to produce this type of mutation.

Cell Labeling and Immunoprecipitations

Radiolabeling and immunoprecipitations were performed as described by Ogg et al. (1992). The pulse–chase experiment shown in Figure 4 was performed by pulse labeling cells for 2 min at 30°C with 60 μCi/OD_{600} Tran[^{35}S]-label (ICN Biomedicals, Costa Mesa, CA) followed by the addition of a 1000-fold molar excess of nonradiolabeled methionine, and cycloheximide at 0.4 μM to one of two aliquots. The tubes were incubated at 30°C for the duration of the 32 min chase period. Aliquots containing 5 OD_{600} cell equivalents were processed for immunoprecipitation at both the 0 and 32 min time points. Following immunoprecipitation with anti-Kar2p antibodies, immunoprecipitated proteins were visualized and quantified by SDS–PAGE followed by analysis with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Quantitation of the rate of [^{35}S]methionine incorporation was performed by labeling *sec65-1* cells for 200 s at 24°C with 30 μCi/OD_{600} Tran[^{35}S]-label, in the presence of a 50-fold excess of nonradioactive methionine to prevent depletion of methionine during the experiment. Analysis of the media after removal of the cells confirmed that during the time course of this experiment, the external pool of [^{35}S]methionine remained unchanged. An exponentially growing culture of *sec65-1* cells was split into three portions; ANM (10 μM) and CHX (0.4 μM) were each added to a separate portion, while the third served as a control. These cultures were incubated at 24°C for 10 min prior to initiating the labeling experiment. In addition, cells containing either wild-type *GCN2* or *GCN2^C*-515 on a plasmid were subjected to the same analysis. At different time points, aliquots of cells were plunged into ice-cold 10% trichloroacetic acid (TCA) and lysed immediately in the presence of a two-thirds volume of zirconium beads (Biospec Products, Bartelseville, OK) by use of a benchtop vortex. The lysates were incubated at 100°C for 10 min in 10% TCA to discharge any methionyl-tRNAs containing [^{35}S]methionine. After TCA precipitation, proteins were solubilized in SDS sample buffer and subjected to scintillation counting or SDS–PAGE analysis.

Growth of Cells on Cycloheximide and Anisomycin

Sterile filter disks containing 6 nmol of cycloheximide in distilled water were placed on YEPD plates. In liquid culture, YEPD was supplemented with the indicated concentration of cycloheximide or anisomycin. Cycloheximide was prepared as a 100 mM stock solution in distilled water and sterilized by passage through a 0.2 μm filter. Anisomycin was prepared in a similar manner as a 7.5 mM stock solution in 70% ethanol.

Acknowledgments

We thank the members of the laboratory for critical reading of the manuscript, and Jeff Cox and Jeremy Brown for kindly providing the plasmids pSCR1 and pJDY32, respectively. We thank Tom Stevens, Joe Vogel, Mark Rose, and Nancy Hollingsworth for strains and antibodies, and Allan Hinnebusch for *GCN2^C* plasmids. We also thank David Feldheim and Randy Schekman for communicating the initial observation on which this work is based. This work was supported by a Howard Hughes Medical Institute predoctoral fellowship to S. C. O. and grants from the National Institutes of Health to P. W.

Received January 20, 1995; revised May 4, 1995.

References

Arnold, C. E., and Wittrup, K. D. (1994). The stress response to loss

of signal recognition particle function in *S. cerevisiae*. J. Biol. Chem. *269*, 30412–30418.

Brown, J. D., Hann, B. C., Medzihradszky, K. F., Niwa, M., Burlingame, A. L., and Walter, P. (1994). Subunits of the *Saccharomyces cerevisiae* signal recognition particle required for its functional expression. EMBO J. *13*, 4390–4400.

Brown, S. (1987). Mutations in the gene for EF-G reduce the requirement for 4.5S RNA in the growth of E. coli. Cell *49*, 825–833.

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

Brown, S., and Fournier, M. J. (1984). The 4.5S RNA gene of *Escherichia coli* is essential for cell growth. J. Mol. Biol. *178*, 533–550.

Connolly, T., Rapiejko, P. J., and Gilmore, R. (1991). Requirement of GTP hydrolysis for dissociation of the signal recognition particle from its receptor. Science *252*, 1171–1173.

Dever, T. E., Feng, L., Wek, R. C., Cigan, A. M., Donahue, T. F., and Hinnebusch, A. G. (1992). Phosphorylation of initiation factor 2α by protein kinase *GCN2* mediates gene-specific translational control of *GCN4* in yeast. Cell *68*, 585–596.

Felici, F., Cesareni, G., and Hughes, J. M. X. (1989). The most abundant small cytoplasmic RNA of *Saccharomyces cerevisiae* has an important function required for normal cell growth. Mol. Cell. Biol. *9*, 3260–3268.

Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., and Waring, M. J. (1981). The Molecular Basis of Antibiotic Action, Second Edition (London: J. Wiley and Sons).

Hann, B. C., and Walter, P. (1991). The signal recognition particle in S. cerevisiae. Cell *67*, 131–144.

Hann, B. C., Poritz, M. A., and 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*, 3223–3230.

Hann, B., Stirling, C. J., and Walter, P. (1992). *SEC65* gene product is a subunit of the yeast signal recognition particle required for its integrity. Nature *356*, 532–533.

Hansen, W., Garcia, P. D., and Walter, P. (1986). In vitro protein translocation across the yeast endoplasmic reticulum: ATP-dependent post-translational translocation of the prepro-α factor. Cell *45*, 397–406.

Jensen, C. G., and Pedersen, S. (1994). Concentrations of 4.5S RNA and Ffh protein in *Escherchia coli*: the stability of Ffh protein is dependent on the concentraion of 4.5S RNA. J. Bacteriol. *176*, 7148–7154.

Moazed, D., and Noller, H. F. (1989). Intermediate states in the movement of transfer RNA in the ribosome. Nature *342*, 142–148.

Miller, J. D., Wilhelm, H., Gierasch, L., Gilmore, R., and Walter, P. (1993). GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation. Nature *366*, 351–354.

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

Orr-Weaver, T. L., Nicolas, A., and Szostak, J. W. (1988). Gene conversion adjacent to regions of double-strand break repair. Mol. Cell. Biol. *8*, 5292–5298.

Phillips, G. J., and Silhavy, T. J. (1992). The E. coli *ffh* gene is necessary for viability and efficient protein export. Nature *359*, 744–746.

Rothblatt, J. A., and Meyer, D. I. (1986). Secretion in yeast: translocation and glycosylation of prepro-α factor in vitro can occur via ATP-dependent post-translational mechanism. EMBO J. *5*, 1031–1036.

Siegel, V., and Walter, P. (1988). The affinity of signal recognition particle for presecretory proteins is dependent on nascent chain length. EMBO J. *7*, 1769–1775.

Sikorski, R. S., and Heiter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Stirling, C. J., and Hewitt, E. W. (1992). The *Saccharomyces cerevisiae SEC65* gene encodes a component of the yeast signal recognition particle with homology to human SRP19. Nature *356*, 534–537.

Stirling, C. J., Rothblatt, J., Hosobuchi, M., Deshaies, R., and Schekman, R. (1992). Protein translocation mutants defective in the insertion of integral membrane proteins into the endoplasmic reticulum. Mol. Biol. Cell *3*, 129–142.

Stöcklein, W., and Piepersberg, W. (1980). Altered ribosomal protein L29 in a cycloheximide-resistant strain of *Saccharomyces cerevisiae*. Curr. Genet. *1*, 177–183.

Walter, P., and 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*, 557–561.

Walter, P., and Johnson, A. E. (1994). Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. Annu. Rev. Cell Biol. *10*, 87–119.

Waters, G., and Blobel, G. (1986). Secretory protein translocation in a yeast cell free system can occur post-translationally and requires ATP hydrolysis. J. Cell Biol. *102*, 1543–1550.

Wolin, S. L. (1994). From the elephant to E. coli: SRP-dependent protein targeting. Cell *77*, 787–790.

Wolin, S. L., and Walter, P. (1989). Signal recognition particle mediates a transient elongation arrest of preprolactin in reticulocyte lysate. J. Cell Biol. *109*, 2617–2622.