Protein Translocation across the Yeast Microsomal Membrane Is Stimulated by a Soluble Factor

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Abstract. We have found that a soluble activity present in the postribosomal supernatant fraction of Saccharomyces cerevisiae stimulates posttranslational translocation of yeast prepro-α-factor across yeast microsomal membranes. Stimulation of translocation is not due to a nonspecific affect on ATP levels. The activity is likely to be due to protein(s) as it is destroyed by N-ethylmaleimide, protease, or heat treatment but not by incubation with RNase. Its apparent sedimentation coefficient is ~9.6 S.

Much of what is known about translocation of proteins across the endoplasmic reticulum (ER) membrane has been learned using cell-free translation systems supplemented with canine pancreatic microsomes (30). These studies have led to the discovery and isolation of three of the components involved in the process. One of the components, signal recognition particle (SRP) is a complex of six proteins and a 7 S RNA (27) that interacts with the signal sequence of a nascent secretory protein as it emerges from the ribosome (12, 26). In the case of preprolactin synthesized in an in vitro wheat germ translation system, interaction of SRP with the nascent chain–ribosome complex arrests further translation (26). Translation resumes after the arrested complex interacts with the SRP receptor (8), also termed docking protein (14), located in the ER membrane. As the nascent chain is translocated across the membrane, the signal peptide is removed by signal peptidase (3), which has recently been isolated as a complex of several polypeptide chains (5).

Until recently, translocation across the ER was thought to be strictly co-translational (29). However, it has been reported that translocation into canine pancreatic microsomes can occur posttranslationally, albeit at low efficiency, for a truncated form of the human glucose transporter (15) and for some fusion proteins (18). In addition, our laboratory (32), as well as several others (9, 20, 21), have developed in vitro translation/translocation systems with all components derived from yeast, and have shown that translocation of prepro-α-factor can occur posttranslationally and requires ATP.

In this paper we report the existence and initial characterization of a soluble activity present in the cytoplasm of S. cerevisiae that is required for efficient translocation of prepro-α-factor across the membrane of the yeast ER.

Materials and Methods

Materials

The source of most materials has been described (32). [γ-32P]ATP (28 Ci/mmol) was from New England Nuclear (Boston, MA), Norit was from Fisher Scientific Co. (Springfield, NJ), S value standards and Ribonuclease A (Type IIIA) were from Sigma Chemical Co. (St. Louis, MO), N-ethylmaleimide (NEM) was from Calbiochem (La Jolla, CA) and proteinase K was from Boehringer Mannheim (Indianapolis, IN).

Preparation of Yeast Microsomal Membranes

The microsomes were prepared as previously described (32) except that the cell lysis buffer contained 1 mM phenylmethylsulfonyl fluoride (PMSF) and the membranes were washed with 20 mM Heps-KOH, pH 7.5, 100 mM KOAc, 2 mM Mg(OAc)2, 2 mM dithiothreitol (DTT) (buffer A) instead of 50 mM triethanolamine acetate, pH 7.5, 1 mM DTT. The membranes were stored in buffer A containing 14% glycerol at a concentration of 5 eq µl−1 as previously described (25). These membranes were used for postranslational assays (Figs. 2–6). For co-translational assays (Fig. 1), the membranes were nuclease treated as described (32), and then either extracted with 500 mM KOAc, to remove peripheral proteins, or mock-extracted at 4°C as follows. One of two 400-µl aliquots of nucleate-treated membranes at 5 eq µl−1 received an equal volume of buffer A (mock extraction), whereas the other received an equal volume of 20 mM Heps-KOH, pH 7.5, 900 mM KOAc, 2 mM Mg(OAc)2, 2 mM DTT (salt extraction). After incubation on ice for 15 min, 750 µl of these preparations were overlayed onto 250-µl cushions of either buffer A containing 14% glycerol (mock extraction), or 20 mM Heps-KOH, pH 7.5, 500 mM KOAc, 2 mM Mg(OAc)2, 2 mM DTT, 14% glycerol (salt extraction) and centrifuged in a TL100 tabletop ultracentrifuge with a TL100.2 rotor (Beckman Instruments, Inc., Fullerton, CA) at 65,000 rpm (150,000 × gav) for 16 min at 4°C. The supernatant and the top half of the cushion (875 µl) were removed and discarded, 625 µl of buffer A were added to the pellet and remaining cushion in both cases, and the membranes resuspended with a tight fitting glass pestle directly in the centrifuge tube. 250 µl of either buffer A containing 14% glycerol were underlaid and the samples centrifuged as above. The supernatant and as much of the cushion as possible were removed without disturbing the flocculent pellet. The membranes were resuspended in buffer A containing 14% glycerol to a final volume of 375 µl, yielding mock-extracted or salt-extracted nucleate-treated membranes at 5 eq µl−1.

Preparation of Prepro-α-factor mRNA

In vitro transcription of a plasmid containing the complete prepro-α-factor

1. Abbreviations used in this paper: ER, endoplasmic reticulum; NEM, N-ethylmaleimide; PRS, postribosomal supernatant; SRP, signal recognition particle.
gene (a generous gift from Dr. David Julius, Columbia University, New York) using SP6 polymerase was as previously described (3, 32). Prepro-α-factor is the precursor of the yeast pheromone α-factor, which is secreted by cells of the alpha mating type (23). The precursor has a molecular mass of 18,580 D (I) and is thought to have an uncleaved signal sequence (II). Upon translocation into the ER, the protein receives three core oligosaccharide units (III).

**Yeast Translation and Co-translational Translocation**

Yeast translations were performed as previously described (32) except that Nikiol was omitted and the compensation buffer was changed to 138 mM Hepes-KOH, pH 7.5, 1.187 M KOAc, 25.2 mM Mg(OAc)₂, 0.2 mM DTT to adjust for the new membrane storage buffer. Each 25-μl reaction contained 1 μl of yeast mRNA, 2 μl of a yeast translation that was programmed with prepro-α-factor mRNA. The final conditions were 20 mM Hepes-KOH, pH 7.5, 150 mM KOAc, 3 mM Mg(OAc)₂, 3 mM DTT. The reactions were incubated at 20°C for 1 h.

**Postribosomal Supernatant (PRS)**

A yeast cytoplasmic fraction was prepared as previously described (termed S100-G25, reference 32), except that 1 mM PMSF was added to the lysis buffer. The ribosomes were removed by centrifugation in a TL100 tabletop ultracentrifuge in a TL100.2 rotor (Beckman Instruments, Inc.) at 100,000 rpm (356,000 × g,~g) at 4°C for 30 min. The supernatant was removed and is referred to as PRS. The ribosomal pellet was resuspended with a tight fitting glass pestle in buffer A to the original volume of the sample.

**Preparation of Ribosomes and Postribosomal Supernatant (PRS)**

A yeast cytoplasmic fraction was prepared as previously described (termed S100-G25, reference 32), except that 1 mM PMSF was added to the lysis buffer. The ribosomes were removed by centrifugation in a TL100 tabletop ultracentrifuge in a TL100.2 rotor (Beckman Instruments, Inc.) at 100,000 rpm (356,000 × g,~g) at 4°C for 30 min. The supernatant was removed and is referred to as PRS. The ribosomal pellet was resuspended with a tight fitting glass pestle in buffer A to the original volume of the sample.

**Protease Protection Experiments**

The protocol has been described (32).

**ATPase Assay**

First, a mock wheat germ translation was made that omitted the energy source and amino acids, creatine kinase, [35]methionine, and mRNA. Next, two mock soluble factor-stimulated posttranslational translocations were prepared: one containing a yeast PRS and the other containing buffer A. They were identical to that already described, except that the energy source and creatine kinase were replaced with 1 μl of 100 mM ATP and 0.5 μl of [γ-32P]ATP (1 μCi) per 25 μl of reaction. One of the reactions was supplemented with buffer A, whereas the other received PRS as described. The final reaction volume was 200 μl and contained 4 mM ATP.

The reactions were incubated at 20°C and at the indicated times 10-μl aliquots were transferred to 1 ml of perchloric acid, 5 mg/ml Norit (activated charcoal) and incubated at room temperature for 10 min with occasional vortexing. The samples were centrifuged for 3 min in an Eppendorf microfuge and 0.5 μl of the supernatant was used to determine cpm by Cherenkov radiation. The assay is a modification of that of Smith and Wells (22) and was done in triplicate.

**Treatment of PRS with NEM, Proteinase K, RNase, and Heat**

An aliquot of PRS (15 μl) was incubated with a final concentration of 10 mM NEM, or 10 mM NEM and 20 mM DTT, or 20 mM DTT for 15 min at 20°C. PRS (15 μl) was also incubated with a final concentration of 250 μg/ml of proteinase K, or 250 μg/ml proteinase K and 1 mM PMSF, or 1 mM PMSF for 30 min at 4°C. An aliquot of PRS (15 μl) was treated with RNase at a final concentration of 1 mg/ml for 15 min at 20°C. An aliquot of PRS was also treated at 100°C for 2 min. The final volume for each treatment was 17 μl, of which 15 μl was used to assay stimulation of posttranslational translocation of prepro-α-factor.

**Sedimentation Analysis of PRS**

An aliquot (500 μl) of PRS was placed on a 12-ml glycerol gradient (5-25%) in buffer A. The gradient was centrifuged for 20 h at 4°C at 40,000 rpm (200,000 × g,~g) using an SW40 rotor. Fractions (~0.46 ml) were collected using a fractionator (Auto Densi-Flow IIIC, Haake Buchler Instruments, Inc., Saddle Brook, NJ), diluted to 2 ml with buffer A, and then concentrated to 40 μl using filters (Centricon 10, Millipore Corp., Bedford, MA). The ability of 15 μl of concentrated fractions to stimulate posttranslational translocation of prepro-α-factor was assayed. Sedimentation coefficient standards were: cytochrome c (bovine heart), 1.7 S; albumin (human serum), 4.6 S; aldolase (rabbit muscle), 7.3 S; and catalase (bovine liver), 11.4 S.

**Determination of Percent Translocation**

It has previously been shown (9, 20, 32) that translocation of prepro-α-factor into yeast microsomes results in the appearance of higher molecular mass products. All of these products were protected from degradation by externally added protease (9, 20, 32, and Fig. 3, lane 5) indicating they are sequestered within the yeast microsome. These products have been shown to be core-glycosylated prepro-α-factor (9, 20, 32). The modification resulting in the 20-kD translocated product however, remains to be defined.

We have used a Beta Scanning System (Automated Microbiology Systems, Inc., San Diego, CA) to quantitate the radioactivity present in our dried gels. We have defined translocated products as those from 20 to 32 kD, and the untranslocated prepro-α-factor as the 19-25 kD primary translation product. Therefore, percent translocation can be calculated by dividing the cpm in the 20-32 kD region by the cpm in the 19-32 kD region.
Sample preparation and electrophoresis were as previously described (32), except that gels were autoradiographed, instead of fluorographed.

Results

To investigate whether an SRP-like component bound to yeast microsomes was required for translocation, we started with an approach similar to the one that led to the isolation of canine SRP (25). This complex was discovered and isolated because salt-washed canine pancreatic microsomes were depleted of their ability to co-translationally translocate immunoglobulin light chain (31) and preprolactin (25). Addition of the salt-wash to the treated membranes restored the translocation competence (25, 31). In an analogous manner, we salt-washed yeast microsomes and assayed them for their ability to translocate prepro-α-factor both co-translationally and posttranslationally (Fig. 1). In a co-translational translocation assay using a yeast translation system without supplemented membranes, only a small amount of prepro-α-factor was translocated because the translation system was almost completely devoid of membranes (Fig. 1A, lane 2). The small amount of translocated product is predominantly present as a 20-kD polypeptide that contains an unidentified posttranslational modification. However, protease protection and endoglycosidase H sensitivity experiments have previously shown (9, 32) that this product is sequestered in the microsome and does not contain asparagine-linked core oligosaccharides. In contrast to the results obtained without membranes, the presence of mock-extracted (control) yeast microsomes during translation resulted in efficient translocation of prepro-α-factor (96%, Fig. 1A, lane 4), as indicated by the appearance of high molecular mass glycosylated products (9, 20, 32, also see Fig. 3, lane 5). Similarly, salt-extracted microsomes efficiently translocated prepro-α-factor (91%, Fig. 1A, lane 6). In a posttranslational translocation assay, mock-extracted microsomes translocated 69% of the prepro-α-factor (Fig. 1A, lane 8), and the salt-extracted membranes translocated 67% (Fig. 1A, lane 9). The ability of the mock-extracted and salt-extracted membranes to translocate prepro-α-factor to the same extent suggested that a salt-extractable component was not required for translocation of this protein into the yeast microsomes, or that a salt-extractable component was present but present in the yeast translation system and therefore not limiting. There is a precedent for a soluble translocation factor in other systems. Cell fractionation studies (28) have shown that canine SRP exists in membrane-bound, soluble, and ribosome-bound states. Furthermore, *Escherichia coli* possess a soluble factor that stimulates translocation (16).

The possible existence of a soluble factor in the yeast translation system, which masked removal of the same factor from the membrane, was tested by using a translation system that presumably would not contain such a factor and repeating the experiment already described. We used a wheat germ translation system for this purpose. If the translation system was not supplemented with membranes, prepro-α-factor was not translocated (Fig. 1B, lane 2). The presence of mock-extracted yeast microsomal membranes resulted in ~36% translocation of prepro-α-factor (Fig. 1B, lane 4). Salt-extracted yeast membranes translocated ~48% of the prepro-α-factor (Fig. 1B, lane 6). Furthermore, a posttranslational translocation assay yielded the same low level of translocation (37%) with both mock-extracted (Fig. 1B, lane 8) and salt-extracted (Fig. 1B, lane 9) yeast membranes. Since both membrane preparations translocated prepro-α-factor to about the same extent, these data again suggested that a salt-extractable membrane component was not required to translocate this protein. In addition, the lower level of translocation obtained with the wheat germ translation system compared with the yeast translation system suggested that some component in the yeast translation system might be required for efficient translocation.

It should be noted that translocation of prepro-α-factor into yeast microsomes from a wheat germ translation (Fig. 1B) not only resulted in inefficient translocation, but also in inefficient glycosylation. This is evident from the relatively large proportion of aberrantly glycosylated products in the 21–26-kD range after translocation in the wheat germ system (Fig. 1B, lanes 4, 6, 8, 9), which were not produced when the yeast translation system was used (Fig. 1A, lanes 4, 6, 8, 9). These products were shown to be glycosylated because they were sensitive to endoglycosidase H, which removes asparagine-linked core oligosaccharides (data not shown). This finding suggested that, in addition to a soluble translocation factor, yeast may possess a soluble factor required for efficient glycosylation.

Since translocation of prepro-α-factor was more efficient in a yeast translation system than in a wheat germ translation system, we reasoned that supplementation of a wheat germ...
Figure 3. PRS affects the translocation step. 15 μl of PRS were used in 25 μl soluble factor-stimulated posttranslational translocation reactions (see legend to Fig. 2 and Materials and Methods) and then a protease protection experiment was done as previously described (31). The final concentration of trypsin was 100 μg/ml and that of Triton X-100 was 1% wt/vol.

Figure 2. Yeast PRS stimulates posttranslational translocation of prepro-α-factor. Soluble factor-stimulated posttranslational translocation assays were done as described in Materials and Methods. In summary, prepro-α-factor, previously synthesized in a wheat germ translation system, was combined with an ATP regenerating system and yeast microsomes in the presence of cycloheximide. This mixture was supplemented with the indicated fractions and incubated for 1 h. (A) Either 15 μl of buffer, a yeast cytoplasmic fraction (S100), yeast ribosomes (Ribos), a wheat germ translation system (WG), or 2, 4, 8, or 15 μl of yeast PRS were used per 25 μl reaction. (B) The experiment in A was done in triplicate and quantitated as described in Materials and Methods.

To localize the translocation-stimulating activity, we fractionated the yeast translation system into a ribosomal pellet and a PRS. We found that very little activity resided with the ribosomes (27% translocation, Fig. 2, lane 3) and that most of the activity was in the PRS (84%, Fig. 2, lane 7). The percent of prepro-α-factor that was translocated was dependent on the amount of PRS added (Fig. 2, lanes 4–7). These results are plotted in Fig. 2 B.

To test for the source of the background translocation (Fig. 2, lane 1, 20% translocation, 22.4 ± 2.4% for three experiments) we supplemented the reaction with more wheat germ extract (Fig. 2, lane 8, 22% translocation, 22.5 ± 2.3% for three experiments). We found no stimulation of translocation over background, suggesting that wheat germ does not contain a factor that can substitute for the yeast factor. This confirmed our original assumption. The amount of background translocation was dependent on the membrane concentration used, regardless of whether mock-extracted or salt-extracted membranes were used (data not shown). This suggested that either some translocation-stimulating factor is bound to the membrane in a non-salt-extractable fashion, or that a factor-independent pathway for translocation exists. If the latter is true then the factor may serve to increase the efficiency of translocation.

Since we assayed translocation by the appearance of glycosylated products (and the 20-kD translocated product, see references 9 and 32), it was possible that we had detected an activity that stimulates glycosylation without affecting translocation. This would be the case if prepro-α-factor could be translocated in the absence of PRS but the glycosylation step required PRS. A protease protection experiment was therefore done to investigate whether any translocated, unglycosylated prepro-α-factor was present in the absence of PRS. Very little prepro-α-factor was translocated in the absence of PRS (21%, Fig. 3, lane 1). Trypsin almost completely degraded the primary translation product but did not digest the background translocated products (Fig. 3, lane 2). When the membranes were solubilized with detergent before digestion with trypsin, the background translocated products were completely proteolyzed (Fig. 3, lane 3), but little or no further digestion of the primary translation product occurred. These data suggested that the unglycosylated product...
was not sequestered in the microsomes since most of it was degraded by externally added trypsin. Furthermore, the small amount of undigested material was probably aggregated because it was not degraded in the presence of detergent. These data indicated that little or no translocated, unglycosylated prepro-α-factor was present in the absence of PRS. Therefore, the addition of PRS did not affect only the glycosylation step, because there is no translocated, unglycosylated substrate for the glycosylation apparatus to act upon. These experiments indicated that the PRS contained a factor or factors that affect the translocation step. These results, however, do not rule out the possibility that PRS affected both the translocation and glycosylation steps.

A protease protection experiment of the PRS-stimulated reaction showed that the glycosylated products (Fig. 3, lane 4) were protected from degradation in the absence (Fig. 3, lane 5), but not in the presence (Fig. 3, lane 6), of detergent. This indicated that the glycosylated products are translocated. It is also evident that there are translocated products of intermediate molecular mass between the 32-kD glycosylated product and the primary translation product. We believe that these products are a population of heterogeneously glycosylated products (17, 24) because they are sensitive to endoglycosidase H (data not shown).

Since posttranslational translocation of prepro-α-factor into yeast microsomes requires ATP (9, 21, 32), the translocation-stimulating effect of PRS might be explained by a PRS-dependent change in ATP levels during the time course of the translocation. For example, if yeast microsomes contain an ATPase similar to that found in liver microsomes (19), and the PRS contains an inhibitor of the ATPase, then the ATP regenerating system in a reaction containing PRS will take longer to become depleted than in a reaction without PRS. Addition of PRS would therefore result in a longer time during which translocation could take place and the process would appear to be stimulated. We tested this by measuring the hydrolysis rate of ATP in mock translocation reactions in the absence and presence of PRS (Fig. 4). The presence of PRS in translocation reactions did not result in a statistically significant change in the rate of ATP hydrolysis compared with control reactions. Therefore, PRS does not significantly affect the time period during which translocation can take place. These data indicate that the translocation-stimulating effect of PRS is not mediated through a nonspecific effect on the endogenous ATP concentration.

To determine the nature of the component(s) in the PRS that stimulated translocation, the PRS was pretreated with NEM, protease K, RNase, or heat and then the modified PRS was assayed for activity. Treating PRS with NEM, a reagent that alkylates sulphydryl groups, reduced translocation (Fig. 5, lane 3) from the level obtained with untreated PRS (Fig. 5, lane 2) to the background level (Fig. 5, lane 1). When DTT was used to inactivate the NEM before incubation with PRS, the translocation activity was not inhibited (Fig. 5, lane 4). To further substantiate that the PRS-stimulated translocation of prepro-α-factor is due, at least in part, to a protein component, PRS was preincubated with proteinase K. Translocation was reduced to background levels when protease-treated PRS was used in the assay (Fig. 5, lane 5). Proteinase K had little effect on translocation when PMSF, an inhibitor of proteinase K, was included in the preincubation (Fig. 5, lane 6). Whether translocation is dependent on RNA was tested by treating the PRS with RNase. RNase did not appreciably reduce translocation (Fig. 5, lane 7) compared with control levels. PRS was also shown to be heat labile (Fig. 5, lane 8). To control for the possibility that the lack of appearance of glycosylated products was due to inhibition of a putative soluble glycosylation factor, instead of a translocation factor, we used protease protection experiments to test for translocated, unglycosylated prepro-α-factor. Treatment of PRS with NEM or heat did not result in accumulation of translocated, unglycosylated prepro-α-factor (data not shown), confirming that the translocation-stimulating activity was inhibited. A protease protection experiment could not be done with proteinase...
Component in the PRS required for translocation and that K-treated PRS because of the presence of the protease inhibitor PMSF. These results indicated that translocation of prepro-α-factor is stimulated by a PRS component(s), which is sensitive to NEM and heat, but resistant to RNase, and therefore most likely proteinaceous. In addition, either the translocation and/or the putative glycosylation-stimulating activity of PRS is destroyed by proteinase K.

To estimate the size of the translocation factor, an aliquot of PRS was centrifuged through a 5-25% glycerol gradient. Since the recovery of translocation activity in fractions collected from the gradient was low (∼25%) each fraction was concentrated 10-fold before assaying. The activity profile is shown in Fig. 6A and quantitated in Fig. 6B. The peak of activity is in fraction 13, which corresponds to a sedimentation value of ∼9.6 S. The fact that our activity recovery is low suggests either that the active component is being inactivated during centrifugation or that there is more than one component in the PRS required for translocation and that they are being separated on the gradient. If the latter is the case, then the activity peak at 9.6 S may represent the overlap of peaks of two or more components with S values above and below 9.6 S. It is interesting to note that fractions on either side of the activity peak that produce the same amount of translocation result in the appearance of a different population of glycosylated products (compare fractions 9 and 17). Fractions towards the top of the gradient result in more efficient glycosylation than the denser fractions do, as indicated by a higher proportion of fully glycosylated prepro-α-factor. The cause of this effect remains to be determined. However, it is possible that a soluble factor required for efficient glycosylation travels through the gradient with a sedimentation coefficient <9.6 S. This would also explain why our activity recovery is low, since our assay, the production of glycosylated prepro-α-factor, depends on both translocation and glycosylation.

Discussion

An in vitro translocation system with all components derived from yeast has recently been developed (9, 20, 32). It was shown that yeast prepro-α-factor can be posttranslationally translocated in this system and that the process requires ATP (9, 21, 32). In this paper we report the existence and initial characterization of a soluble activity present in the yeast cytoplasm that stimulates posttranslational translocation of prepro-α-factor.

The factor is not associated with yeast microsomes in a salt-extractable fashion, nor is it associated with ribosomes. Rather, the activity is present in a yeast PRS fraction. We have shown that the factor does not stimulate translocation through a nonspecific effect on ATP levels. The activity can be destroyed by NEM, protease, or heat treatment, and is therefore, at least in part, proteinaceous. RNase treatment under physiological conditions does not deplete the activity. Finally, we have found that the activity sediments at ∼9.6 S.

It is possible that the translocation-stimulating effect of PRS is due to more than one factor. It should be emphasized that, although the activity sediments at ∼9.6 S, this activity peak may be the result of peak overlap from two or more factors with sedimentation coefficients above and below 9.6 S. This may be the reason for the low activity recovery obtained with the sedimentation analysis.

It has recently been shown that posttranslational translocation and glycosylation of a truncated form of bovine opsin is also dependent on the presence of PRS (Greenburg, G., and G. Blobel, unpublished observations). Therefore we believe that the factor is not specific for only prepro-α-factor. Furthermore, we prepared our extract from diploid a/a cells that do not synthesize α-factor (23). If the translocation factor were specific for prepro-α-factor it seems unlikely that it would be synthesized in diploid cells.

It is possible that the factor acts in targeting secretory and integral membrane proteins to the ER in a manner analogous to that of canine SRP. If this is the case, the factor probably interacts with the signal sequence. Canine SRP is found about equally distributed between membrane-bound, ribosome-bound, and soluble forms (28). In contrast, we have detected the yeast factor predominantly in soluble form. By the criterion of RNase sensitivity, the soluble factor does not appear to contain RNA, which is unlike SRP (27). On the other hand, both the yeast factor and SRP are susceptible to alkalization by NEM (25), although this may be coincidental. Finally, it is noteworthy that we assay for the yeast factor by posttranslational addition of PRS. If the factor interacts with prepro-α-factor to stimulate translocation, it must be able to do so after the peptide chain is complete. This is in contrast to the co-translational interaction of SRP with the nascent chain (12, 26). It should be noted however, that Hansen et al. (9) have demonstrated that canine SRP can interact with
prepro-α-factor posttranslationally to facilitate translocation into canine microsomes, although inefficiently. Furthermore, Mueckler and Lodish (15) reported that SRP can interact posttranslationally with a truncated form of the human glucose transporter to facilitate its translocation into canine microsomes.

If the yeast translocation factor does not function in signal sequence recognition (which might instead be performed by a signal receptor [2, 7] in the membrane) it could serve some other function, perhaps in the translocation step. Because translocation of prepro-α-factor can occur posttranslationally, the peptide chain has presumably folded into a stable three-dimensional conformation. Therefore, if translocation across the membrane requires prior unfolding of the protein, an enzyme that unfolds the polypeptide (a denaturase) may be required. This unfolding may require energy in the form of ATP hydrolysis, and perhaps the factor described here serves such a function.

Much of our data support the possibility that PRS contains factors required for both translocation and glycosylation. First, we have noted that both translocation and glycosylation are inefficient when prepro-α-factor, synthesized in a wheat germ translation system, is translocated into yeast microsomes in the absence of PRS (Fig. 1 B). Furthermore, we used an E. coli PRS to stimulate posttranslational translocation and found that translocation was stimulated but, again, glycosylation was very inefficient (unpublished observations). These data suggest that the E. coli translocation factor (16) can stimulate translocation of prepro-α-factor into yeast microsomes, but as expected, this organism does not contain a factor capable of stimulating glycosylation. Finally, when the components of PRS are separated on the basis of sedimentation coefficient, we obtain a peak of translocation activity, but with a gradient of glycosylation efficiency across the peak. Fractions towards the top of the gradient, corresponding to low sedimentation coefficients, result in more efficient glycosylation than fractions towards the bottom of the gradient (Fig. 6). This suggests that a factor required for efficient glycosylation might exist and have a low sedimentation coefficient. Experiments addressing this question are in progress. It is interesting to note however, that the yeast secretory mutant sec53 (6) has been postulated to be deficient in a protein that may be required for efficient glycosylation (1). The product of the SEC53 gene is a hydrophilic, 29-kD protein that is predominantly cytosolic (1). The presence of this protein in yeast PRS, and its absence from wheat germ and E. coli, may explain the results we have obtained.

We are currently engaged in purification of this yeast translocation factor. Once obtained, a rigorous analysis of its function can be undertaken. Furthermore, this factor may serve as an entry point from which to begin a genetic analysis of protein translocation across the yeast ER membrane.

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