Requirements for the Translocation of Elongation-arrested, Ribosome-associated OmpA across the Plasma Membrane of Escherichia coli*  

(Received for publication, October 6, 1997, and in revised form, February 4, 1998)

Mathias Behrmann, Hans-Georg Koch‡, Thomas Hengelage, Beate Wieseler, Hedda K. Hoffschulte, and Matthias Müller§§

From the Adolf Butenandt Institut für Physikalische Biochemie, Ludwig-Maximilians-Universität München, D-80336 München, Germany

An oligodeoxynucleotide-dependent method to generate nascent polypeptide chains was adopted for use in a cell-free translation system prepared from Escherichia coli. In this way, NH2-terminal pOmpA fragments of distinct sizes were synthesized. Because most of these pOmpA fragments could be covalently linked to puromycin, precipitated with cetyltrimethylammonium bromide, and were enriched by sedimentation, they represent a population of elongation-arrested, ribosome-associated nascent chains. Translocation of these nascent pOmpA chains into inside-out membrane vesicles of E. coli required SecA and (depending on size) SecB. Whereas their translocation was strictly dependent on the H+-motive force of the vesicles, no indication for the involvement of the bacterial signal recognition particle was obtained. SecA and SecB, although required for translocation, did not mediate binding of the ribosome-associated pOmpA to membrane vesicles. However, SecA and SecB cotranslationally associated with nascent pOmpA, since they could be co-isolated with the ribosome-associated nascent chains and as such catalyzed translocation subsequent to the release of the ribosome. These results indicate that in E. coli, SecA also functionally interacts with preproteins before they are targeted to the translocase of the plasma membrane.

Protein export across the plasma (cytoplasmic, inner) membrane of Escherichia coli is achieved by the concerted action of a distinct set of Sec proteins (summarized in Ref. 1). Most of them are integral membrane proteins of the plasma membrane. SecY and SecE most likely are core constituents of the translocation pore in the membrane; SecG appears to change its membrane topography during polypeptide translocation (2); the exact roles of SecD, SecF, and YajC are yet to be established. SecA on the other hand, is a peripheral membrane protein that binds to SecYE (3) probably serving as a membrane receptor for a prepeptide-SecB complex. SecB functions as a chaperone (4) and as a targeting factor (5). Due to its ATPase activity, SecA inserts in, and deinserts from, the membrane in a cyclic manner (6, 7), which leads to the stepwise translocation of the precursor across the membrane bilayer (8, 9). In addition to ATP, the H+-motive force (ΔμH+) is utilized as an energy source for translocation.

This model does not ascribe a function to the soluble form of SecA, which in E. coli partitions roughly equally between cytosol/ribosomes and the plasma membrane (10, 11). The occurrence of cytosolic complexes between precursor proteins and SecA (12, 13) suggests that SecA actually might interact with its protein substrate well before it has been targeted to the membrane. Besides the Sec proteins, E. coli possesses a signal recognition particle (SRP)7/SRP receptor system whose eukaryotic equivalents mediate the cotranslational targeting of nascent secretory and membrane proteins to the endoplasmic reticulum (14). Whereas more recent results (15–18) indicate that the bacterial SRP has a specialized role in the integration of hydrophobic membrane proteins, several reports had suggested a role of the bacterial SRP in the export of signal sequence-containing proteins (19–21).

To elucidate early events during translocation of bacterial secretory proteins in general, and the function of soluble SecA in particular, the translocation of ribosome-associated, nascent chains of OmpA was analyzed. The outer membrane protein OmpA was chosen because it is one of the most widely studied model precursors of E. coli. In vitro synthesis of ribosome-associated, nascent polypeptide chains requires a truncated mRNA lacking a stop codon so that ribosomes carrying nascent chains stall at the end of this mRNA fragment. Truncation of mRNA is most commonly achieved by the use of linearized DNA. This method has successfully been used with both eukaryotic and prokaryotic mRNAs translated in a wheat germ extract to demonstrate direct molecular interaction of the E. coli SRP subunit Ffh (P48) with signal sequences (22, 23). In our hands, however, linearized DNA turned out to be extremely unstable in cell-free transcription/translation systems made from E. coli, giving rise to a large population of undefined peptides. The use of a homologous translation system from E. coli, however, appeared to be imperative for the analysis of early translational steps, since recent studies show that results obtained with wheat germ ribosomes might not necessarily reflect the authentic events occurring in E. coli (24).

EXPERIMENTAL PROCEDURES

Subcloning and Transcription of ompA—An EcorI–PstI fragment of plasmid pRD87 (25) containing the E. coli ompA gene was subcloned into vector pGEM-3Z (Promega) to yield plasmid pDMB in which ompA

* This work was supported by a grant from the Sonderforschungsbereich 184 and the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Institut für Biochemie und Molekularbiologie, Universität Freiburg, Hermann-Herderstr. 7, D-79104 Freiburg, Germany.

§ To whom correspondence should be addressed. Tel.: 761-203-5265; Fax: 761-203-5274; E-mail: numatthi@ruf.uni-freiburg.de.

1 The abbreviations used are: SRP, signal recognition particle; RCS, reconstituted system; BANC, ribosome-associated, nascent chain; INV, inside-out, plasma membrane vesicle(s); AMP-PNP, 5'-adenyl γ,γ-imidodiphosphate; GMP-PNP, 5'-guanylyl β,γ-imidodiphosphate; GDP-βS, guanosine 5'-O-2-(thio)ridiphosphate.
is under the control of the T7 phage promoter. For in vitro transcription, pDMB was linearized with PstI. 20 mg of linearized DNA was transcribed in 100-μl reactions containing 40 μM Tris/HCl, pH 7.5, 10 mM NaCl, 6 mM MgCl₂, 2 mM spermidine; 10 mM dithiothreitol; a 0.5 M concentration each of ATP, GTP, CTP, and UTP; 0.1 mg/ml acetylated bovine serum albumin; 400 units/ml placental RNase inhibitor; and 600 units/ml T7 RNA polymerase. Incubation was for 60 min at 37 °C. The mRNA was precipitated with ethanol in the presence of 200 μM NH₄CH₃COO and redissolved in 50 μl of H₂O.

Cell-free Protein Synthesis—A nuclease-treated wheat germ extract was prepared, and the translation system was operated as described (26) with the following modifications. The final concentrations of K⁺, Mg²⁺, spermidine, and S-adenosylmethionine were 80 mM, 2.5 mM, 0.08 mM, and 5 μM, respectively; 25-μl reactions contained 4 μl of wheat germ extract, 12 units of placental RNase inhibitor, 2 μl of transcribed ompA RNA, and 10 μCi of [³⁵S]methionine (1000 Ci/mmol). Incubation was for 2 h at 25 °C.

The components of the reconstituted system (RCS), salt-washed ribosomes, initiation factors, and the undefined fraction of soluble translation factors were prepared as described (27). In vitro protein synthesis by use of the RCS was performed in 25-μl aliquots of translation buffer (40 mM triethanolamine/CH₃COO, pH 7.5, 70 mM KCH₃COO, 10 mM Mg(CH₃COO)₂, 0.8 mM spermidine) containing 3.2% polyethylene glycol 6000; 2.5 mM ATP; a 0.5 mM concentration each of GTP, CTP, and UTP; 0.01 mM KOH; 12 mM phosphoenolpyruvate; 8 mM creatine phosphate; 40 μg/ml creatine phosphokinase; 2 mM dithiothreitol; a 40 μM concentration each of 19 amino acids; 10 μCi of [³⁵S]methionine (1000 Ci/mmol); incubation was for 30 min at 37 °C.

Isolation of Ribosome-associated, Nascent Chains (RANCs)—RANCs of pOmpA were synthesized by the RCS in the presence of the oligodeoxynucleotides β2 (5'-AACGCAATACGGTGAAC-3'), β5 (5'-TAACGTTGGATATTGCTG-3'), and β8 (5'-GGAGCTGCCTCGCCCT-3') (amounts are given in the legend to Fig. 1). Aliquots of 25–100 μl were centrifuged through a cushion of 30% sucrose at 80 °C for further use.

Membrane Vesicles—Gradient-purified inner membrane vesicles were obtained as described (28) and extracted with 6 M urea (29). For extraction with 1 M KCH₃COO, 50-μl aliquots of inside-out, plasmatic membrane vesicles (INV) were incubated with 25 μl of 3 M KCH₃COO for 15 min on ice and centrifuged through a cushion of 30% sucrose prepared in 50 mM triethanolamine/CH₃COO, pH 7.5, 1 mM dithiothreitol for 10 min at 30,000 x g in a Beckman Airfuge. Low salt washing of INV (wINV) was performed as described (30).

Purification of Components—The purification of SecA (29), SecB (31), and F₁-ATPase (up to the gel filtration step) was performed according to Lurink et al. (21) except that the first Mono Q column was replaced by Q-Sepharose.

RESULTS

Synthesis of Discrete Nascent Chains of pOmpA by Wheat Germ and E. coli Ribosomes—In order to synthesize nascent chains of pOmpA in vitro, we adopted a method described previously (31) to create elongation-arrested chains by use of oligodeoxynucleotides complementary to discrete sections of the mRNA under investigation. The DNA-RNA hybrids thus formed in vitro are cleaved by endogenous RNase H, leading to 5'-fragments of mRNA. Three oligodeoxynucleotides were designed so that the resulting nascent chains would be caused by translational stops after the second, the fifth, and the eighth β-strand of pOmpA (32); pOmpA-β₂, 65 amino acids; pOmpA-β₅, 125 amino acids; pOmpA-β₈, 191 amino acids.

The method was tested both in a wheat germ translation system programmed with ompA-mRNA and in a subfractionated version of an E. coli transcription/translation system (RCS) programmed with plasmid pDMB. These translation systems were used because they are essentially free of SecA and SecB activities (13, 33). When present, the three oligodeoxynucleotides gave rise to a few peptides each, the more prominent of which had the expected sizes (Fig. 1A, lanes 1–4, arrows). By varying the concentration of the oligodeoxynucleotide, different degrees of co-synthesis of full-length pOmpA and the nascent peptide was achieved as depicted in Fig. 1A (lanes 5–9) for pOmpA-β₅. The peptide corresponding in size to pOmpA-β₅ was processed by pure signal (leader) peptidase (Fig. 1A, lanes 10 and 11), suggesting that it contained a correct NH₂ terminus, including the signal sequence. Ribosome association of the NH₂-terminal fragments of OmpA was demonstrated in three different ways (illustrated in Fig. 1B for pOmpA-β₅). First, the fragments were precipitated by cetyltrimethylammonium bromide (lanes 1–3), which does so only if proteins are bound to tRNA. Second, puromycin caused a slight increase in molecular mass of the fragments due to covalent binding, which requires the peptidyl-tRNA be bound to the P-site of the ribosome (lanes 4 and 5). These two criteria were found fulfilled also for a fragment 3 kDa larger than pOmpA-β₅, which was expressed only by the wheat germ system and whose nature remained obscure (cf. legend to Fig. 1). Finally, when synthesized in the E. coli cell-free system, the bona fide nascent pOmpA-β₅ pelleted indeed in a time-dependent manner (lanes 6–12), indicating attachment to the ribosomes. Thus, this method in fact proved suitable in producing ribosome-associated, nascent pOmpA chains whose elongation was arrested at sites determined by the oligodeoxynucleotide used.

The Translocation of Nascent pOmpA Chains Is Mediated by SecA and SecB—Next, the translocation of RANCs of pOmpA into INV of E. coli was examined (translocation was assayed via resistance to proteinase K as illustrated in Fig. 2D, lanes 5–7, and Fig. 5, lanes 18–20). Routinely, INV had been extracted with 1 M potassium acetate (K-INV), which removes a considerable amount of the INV-bound SecA (13). Only background levels of translocation into K-INV were observed when pOmpA-β₅ was synthesized by wheat germ ribosomes (Fig. 2A, compare lanes 8 and 10). Translocation was completely suppressed by removing additional SecA from the INV by extraction with 6 M urea (U-INV, Fig. 2A, lane 4). Translocation into both types of membranes, however, was clearly stimulated by the addition of pure SecA and SecB (Fig. 2A, lanes 1–4 and 5–8).

A striking difference was observed when RANCs of pOmpA-β₅ were produced by the E. coli cell-free system and collected by centrifugation before membrane vesicles were added. In this case, translocation into U-INV could not be restored by substituting both Sec proteins (Fig. 2C, lanes 1–4). This was also observed when a total translation mix instead of isolated RANCs was used (not shown). As expected, Sec-dependent transport into INV required the release of the elongation-arrested pOmpA from both types of ribosomes by puromycin (Fig. 2, B and D, compare lanes 1 and 2 to lanes 3 and 4).

The Bacterial SRP Is Not Required for Translocation of Nascent Chains of pOmpA—The finding that RANCs of pOmpA could not be translocated into U-INV by SecAB alone when synthesized in the E. coli cell-free system prompted the question of whether an additional necessary component had been removed from INV by urea treatment. We have recently found
that urea treatment of INV also interferes with the integration of membrane proteins, a property that is partially restored by the addition of the purified components of the bacterial SRP/SRP-receptor system but not by SecA and SecB. In contrast, the failure of SecA/B to translocate pOmpA-β5 into U-INV (Fig. 3, lanes 1–4) was not overcome by Ffh (P48), FtsY, and 4.5 S RNA when added as purified components (lanes 5 and 6) at concentrations that had restored integration of a membrane protein to U-INV. There was also no translocation of pOmpA-β5 and full-length pOmpA when the SRP components

![Figure 1](image-url)
were added without SecA/B (lanes 7 and 8). However, translocation of pOmpA-β5 into U-INV occurred if, in addition to SecA/B, purified F1-ATPase was provided to restore the DmH1 (not shown). In order to examine a potential participation of SRP by another approach, the nucleotide dependence of the translocation of nascent pOmpA-β5 into membrane vesicles was analyzed. RANCs of pOmpA-β5 were isolated by centrifugation and incubated with K-INV, SecA/B, and the nucleotides indicated in Fig. 4. Following dissociation of the nascent chain-ribosome complexes by puromycin, processing and translocation was monitored as before. As described previously for full-length pOmpA (8), translocation of pOmpA-β5 required the presence of hydrolyzable ATP, since it was completely inhibited by the nonhydrolyzable analog AMP-PNP (lane 4). In complete agreement with a previous report (8), processing was not affected under these conditions (lane 3) because binding of AMP-PNP to SecA initiates translocation up to the signal sequence cleavage site. The omission of GTP (compare lanes 1 and 2 with lanes 5 and 6) and the addition of nonhydrolyzable analogs of GTP (lanes 7 and 8) and GDP (lanes 9 and 10) did not reveal any significant inhibition of pOmpA-β5 translocation. These results render an involvement of the bacterial SRP in the export of pOmpA-β5 very unlikely.

Cotranslational Binding of SecA/B to Nascent pOmpA Is Not Linked to a Cotranslational Membrane Targeting—In the previous experiments, SecA and SecB had always been substituted together. The SecB-binding site of OmpA has been localized to the first 229 amino acids of the mature protein (34), which include the entire β-stranded domain. Because pOmpA-β5 contains only five of the eight β-strands, we assumed that its membrane translocation might not to the same extent depend on SecB as that of pOmpA-β8. In fact, Fig. 5 illustrates that the
SecA-mediated translocation of pOmpA-β8 into K-INV (lanes 14 and 15) was further stimulated by SecB (lanes 16 and 17), whereas that of pOmpA-β5 was not (compare lanes 5 and 6 with lanes 7 and 8).

Using immunoprecipitation of whole, 35S-labeled E. coli cells, an association of SecA (12) and SecB (35) with nascent chains of the maltose-binding protein had been reported. The experimental approach used here, in which the isolation of RANCs allowed an analysis of their biogenesis separate from the subsequent translocation process, enabled us to address the questions (i) whether SecA/B would bind also to nascent pOmpA and (ii) whether cotranslationally bound SecA/B would be functional in translocation (Fig. 6). The synthesis of pOmpA-β5 and pOmpA-β8 was first allowed to proceed in the presence or absence of SecA and SecB. RANCs were subsequently isolated by centrifugation, resuspended, and incubated with K-INV and puromycin. Compared with the Sec protein-free control (lanes 1 and 2), a clear cut increase in translocation was obtained when RANCs had been synthesized in the presence of SecA and SecB (lanes 5 and 6). The conclusion from these results is that SecA/B can interact cotranslationally with nascent pOmpA in a functional manner. Consistent with this interpretation, NaN3 (a low molecular weight inhibitor of SecA) when added during synthesis remained inhibitory to translocation after the isolation of RANCs by centrifugation (lanes 7 and 8), indicating its co-sedimentation with SecA and RANCs. The extent of translocation achieved by the co-isolated SecA/B was lower than when the Sec proteins were added posttranslationally together with the K-INV (lanes 3–6), most likely because not 100% of the nascent pOmpA-SecA-SecB complexes had remained intact during the isolation procedure.

This finding raised the question whether nascent pOmpA is targeted to INV in its ribosome-associated state by means of the bound SecA/B. In order to test this (Fig. 7), pOmpA-β5 RANCs were collected from the translation mixture by centrifugation for 60 min and then incubated with K-INV and SecA to allow for potential binding to the membrane vesicles. These were subsequently resolated by brief centrifugation (10 min) through a sucrose cushion, resuspended, and analyzed for translocation using proteinase K. When puromycin was added prior to the 10-min centrifugation step (assay shown in lanes 5–8), processed and translocated pOmpA-β5 was recovered in its majority from the membrane pellet (PEL, lanes 7 and 8), indicating that translocation into K-INV had occurred under these conditions. On the contrary, when the vesicles were treated with puromycin only after their reisolation, no processing and translocation were observed (lanes 3 and 4) with the majority of pOmpA-β5 now remaining soluble (lane 1). This distribution pattern of pOmpA-β5 was largely similar to that of the membrane-free control (assay shown in lanes 9–12). The almost complete lack of co-sedimentation of pOmpA-β5 with the membranes in the absence of puromycin indicated that...
pOmpA-β5 when still bound to ribosomes is not targeted to membranes by SecA. This situation did not change when SecB was included in an otherwise identical setup or when the longer pOmpA-β8 chains were used (data not shown).

**DISCUSSION**

**Results Obtained with RANCs Depend on the Origin of Ribosomes**—Noticeable differences between the plant and the bacterial translation system were that the former (i) was less stringently dependent on ΔpH+ for translocation and (ii) still allowed for considerable processing even if little translocation was observed in the absence of SecA/B (cf. Fig. 2A, lanes 5–8). In the E. coli system, however, both processing and translocation were abolished under these conditions (Fig. 5, compare lanes 1 and 2 to lanes 7 and 8). These differences underlie the necessity to use a homologous in vitro system in order to examine the authentic events during the biogenesis and translocation of bacterial nascent preproteins.

**No Indication for a Ribosome-involving Membrane Targeting of RANCs in E. coli**—We have obtained no evidence for a targeting of ribosome/nascent chain complexes to the E. coli membrane vesicles. Thus, isolated RANCs of pOmpA did not bind to INV in a way that would have withstood reisolation of the membranes. Such binding of RANCs to membrane vesicles is characteristic for the SRP-mediated targeting of eukaryotic nascent chains to the endoplasmic reticulum and has frequently been demonstrated with both native (cf. Refs. 36 and 37) and reconstituted (38) microsomes. On the other hand, Josefsson and Randall (39) demonstrated that in E. coli, cleavage of the signal sequence occurs with unfinished polypeptide chains and concluded that translocation can initiate late during the synthesis of bacterial precursor proteins. We assume that in this case membrane targeting of nascent chains, which have already grown up to 80% of their final size, does not involve a ribosome/membrane junction but is rather achieved by a SecA/B-dependent insertion of the NH2-terminal portion of the proteins.

Previous reports had suggested a role of the bacterial SRP in the export of signal sequence-containing proteins (19–21) including that of OmpA (40). We have recently found that the RCS used here for *in vitro* transcription/translation contains sufficient amounts of the SRP components when combined with K-INV to allow for the integration of an SRP-dependent membrane protein. Under the same experimental conditions, targeting of nascent pOmpA to K-INV could not be detected, indicating that the bacterial SRP is not involved in such a targeting step. Consistently, the use of GMP-PNP and GDP-βS, both of them interfering with the SRP cycle in eukaryotes (41), did not reveal a participation of SRP in the export of nascent pOmpA. Contradictory to our results, Powers and Walter (42) have demonstrated co-translational protein targeting mediated by the components of the E. coli SRP/SRP-receptor. However, these findings were obtained with a heterologous in vitro system employing wheat germ ribosomes and mammalian microsomal membranes, conditions that we show here are likely to bypass the authentic mechanisms of E. coli.

**A Precursor Recognition Function for SecA**—The method used here to create RANCs yielded peptides of largely distinct lengths, which is a prerequisite to show that the SecB dependence of translocation correlates with the length of the nascent pOmpA chains. Using a similar approach of producing nascent chains of the maltose-binding protein, a critical length was defined below which tight binding of SecB was not observed (43). Most of the data collected so far, including the data presented here, are consistent with the view that SecB binds within the mature part of preproteins (reviewed in Ref. 44), although involvement of the signal sequence in the binding of SecB has also been postulated (45, 46).

We demonstrate here that SecA can tightly bind to nascent pOmpA and remain functional for a proficient interaction with the translocase in the plasma membrane. We therefore propose that in addition to its widely accepted function as a membrane-located receptor for precursor proteins (1), SecA also operates as recognition factor for nascent secretory proteins. This mode of action would then be represented by the fraction of non-
membrane-associated SecA, completely compatible with the fact that a considerable amount of soluble SecA is recovered from the ribosomes (10, 47). Conceivably, this property of SecA has so far not received much attention because most of the functions of SecA were deduced from studies employing purified, denatured precursor proteins, a strategy that bypasses intermolecular contacts during the biogenesis of presecretory proteins.

Interaction of SecA with a precursor protein was reported to involve specific features of the signal sequence such as the positively charged NH₂ terminus (48). Is it also influenced by SecB? A molecular interaction between SecB and SecA was first deduced from the finding that SecB binds with high affinity to INV only in the presence of SecA (49). Later on, it was directly demonstrated to occur by the purification of an enzymatically active complex between SecA and SecB from E. coli (13). Moreover, SecA and SecB were both found attached to a completed precursor synthesized in vitro (13). Further experimentation is required to examine whether or not recognition of precursors by SecA is modulated by SecB.

REFERENCES

1. Duong, F., Eichler, J., Price, A., Rice Leonard, M., and Wickner, W. (1997) Cell 91, 567–573
2. Nishiyama, K., Suzuki, T., and Tokuda, H. (1996) Cell 85, 71–81
3. Duong, F., and Wickner, W. (1997) EMBO J. 16, 2756–2768
4. Randall, L. L., and Hardy, S. J. S. (1995) Trends Biochem. Sci. 20, 65–69
5. Ernst, F., Hoffschulte, H. K., Thome-Kromer, B., Swidersky, U. E., Werner, P. K., and Muller, M. (1994) J. Biol. Chem. 269, 12840–12845
6. Kozono, A., and Wickner, W. (1994) Cell 78, 835–845
7. Rajapandi, T., and Oliver, D. (1996) Mol. Microbiol. 20, 43–51
8. Schiebel, E., Driessen, A. J. M., Hartl, F. U., and Wickner, W. (1991) Cell 64, 927–939
9. Uchida, K., Mori, H., and Mizushima, S. (1995) J. Biol. Chem. 270, 30682–30686
10. Lünsche, H. H. (1987) J. Bacteriol. 169, 1174–1181
11. Cabelli, R. J., Delan, K. M., Qian, L., and Oliver, D. B. (1991) J. Biol. Chem. 266, 24420–24427
12. Chun, S. Y., and Randall, L. L. (1994) J. Bacteriol. 176, 4197–4203
13. Hoffschulte, H. K., Drees, B., and Muller, M. (1994) J. Biol. Chem. 269, 12833–12839
14. Raap, T. A., Jungnickel, B., and Kutay, U. (1993) Nature 365, 271–273
15. MacFarlane, J., and Muller, M. (1995) Eur. J. Biochem. 233, 766–771
16. de Gier, J. W., Mansournia, P., Valent, Q. A., Phillips, G. J., Luirink, J., and von Heijne, G. (1996) FEBS Lett. 399, 307–309
17. Seluanov, A., and Bihl, E. (1997) J. Biol. Chem. 272, 2053–2055
18. Ulbrandt, N. D., Newitt, J. A., and Bernstein, H. D. (1997) Cell 88, 187–196
19. Poritz, M. A., Bernstein, H. D., Strub, K., Zopf, D., Wilhelm, H., and Walter, P. (1990) Science 250, 1111–1117
20. Phillips, G. J., and Silhavy, T. J. (1992) Nature 359, 744–746
21. Lührink, J., ten Hagen-Jongman, C. M., van der Weijden, C. C., Oudega, B., High, S., Dobberstein, B., and Kusters, R. (1994) EMBO J. 13, 2289–2296
22. Lührink, J., High, S., Wood, H., Giner, A., Tollervey, D., and Dobberstein, B. (1995) Nature 329, 741–743
23. Valent, Q. A., Kendall, D. A., High, S., Kusters, R., Oudega, B., and Lührink, J. (1995) EMBO J. 14, 5494–5505
24. Valent, Q. A., de Gier, J. W. L., von Heijne, G., Kendall, D. A., ten Hagen-Jongman, C. M., Oudega, B., and Lührink, J. (1997) Mol. Microbiol. 23, 53–64
25. Freudl, R., Schwarz, H., Klose, M., Movva, N. R., and Henning, U. (1987) EMBO J. 4, 3593–3598
26. Krebs, H. O., Hoffschulte, H. K., and Müller, M. (1989) Eur. J. Biochem. 181, 323–329
27. Müller, M., and Blobel, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7737–7741
28. Müller, M., and Blobel, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7421–7425
29. Heide, R., Wieseler, B., Wachtler, E., Neubüser, A., Hoffschulte, H. K., Hengelage, T., Schima, K. L., Stuart, R. A., and Müller, M. (1997) J. Bacteriol. 179, 4003–4012
30. Müller, M., Fisher, R. P., Rienhöfer-Schweer, A., and Hoffschulte, H. K. (1987) EMBO J. 6, 3855–3861
31. Haegtle, M. T., Frank, R., and Dobberstein, B. (1986) Nucleic Acids Res. 14, 1427–1448
32. Klose, M., MacIntyre, S., Schwartz, H., and Henning, U. (1988) J. Biol. Chem. 263, 13297–13302
33. Watanabe, M., and Blobel, G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2728–2732
34. MacIntyre, S., Mutschler, B., and Henning, U. (1991) Mol. Gen. Genet. 227, 224–228
35. Kumamoto, C. A., and Franquet, O. (1993) J. Bacteriol. 175, 2184–2188
36. Connolly, T., and Gilmore, R. (1986) J. Cell Biol. 103, 2253–2261
37. Lauring, B., Kreibich, G., and Wiedmann, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9435–9439
38. Jungnickel, B., and Rapoport, T. A. (1995) Cell 82, 261–270
39. Josefsson, L. G., and Randall, L. L. (1981) Cell 25, 151–157
40. Patel, S., and Austin, B. M. (1996) Eur. J. Biochem. 238, 760–768
41. Rapiejko, P. J., and Gilmore, R. (1997) Cell 89, 703–713
42. Powers, T., and Walter, P. (1997) EMBO J. 16, 4880–4886
43. Randall, L. L., Topping, T. B., Hardy, S. J. S., Pavlov, M. Y., Freistroffer, D. V., and Ehrenberg, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 802–807
44. Muller, M., and MacFarlane, J. (1994) Subcell. Biochem. 22, 327–359
45. Altman, E., Ennr, S. D., and Kamamoto, C. A. (1990) J. Biol. Chem. 265, 18574–18160
46. Watanabe, M., and Blobel, G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10133–10136
47. Swidersky, U. E., Hoffschulte, H. K., and Muller, M. (1990) EMBO J. 9, 1777–1785
48. Akiya, M., Sasai, S., Matsuyama, S., and Mizushima, S. (1990) J. Biol. Chem. 265, 8164–8169
49. Hartl, F. U., Lecker, S., Schiebel, E., Hendrick, J. P., and Wickner, W. (1990) Cell 63, 269–279
50. Thome, B. M., and Müller, M. (1991) Mol. Microbiol. 5, 2815–2821; Correction (1992) Mol. Microbiol. 6, 1077
51. Gilmore, R., and Blobel, G. (1985) Cell 42, 497–505