Purified *Escherichia coli* Preprotein Translocase Catalyzes Multiple Cycles of Precursor Protein Translocation†

Martine Bassilana and William Wickner*

Molecular Biology Institute and Department of Biological Chemistry, University of California, Los Angeles, California 90024-1570

Received September 14, 1992; Revised Manuscript Received December 23, 1992

ABSTRACT: *Escherichia coli* preprotein translocase, composed of the peripheral membrane protein SecA bound at the integral membrane domain SecY/E, has been isolated and functionally reconstituted [Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M., & Wickner, W. (1990) *Cell 62*, 649–657]. It is not known whether this purified enzyme supports multiple turnover cycles and how its kinetics compare with translocase in inverted membrane vesicles. We now report a quantitative comparison of the translocation of the outer membrane protein A precursor (proOmpA) by purified preprotein translocase and by inner membrane vesicles. ProOmpA cross-linked to bovine pancreatic trypsin inhibitor was used for quantitative titration of the functional translocation sites. The rate of proOmpA translocation per active site in this purified system is 25% of that observed in inverted membrane vesicles. Each functional site can catalyze multiple cycles of precursor translocation. These results indicate that the purified preprotein translocase properly reconstitutes translocation.

Preprotein translocation across the plasma membrane of *Escherichia coli* is catalyzed by preprotein translocase, a multisubunit membrane protein which has been recently isolated and reconstituted into liposomes (Brundage et al., 1990). The subunits of this enzyme (Akimaru et al., 1991) are largely encoded by the *sec* and *prl* genes, which were isolated on the basis of effects of mutations on protein export in vivo (Schatz & Beckwith, 1990; Bieker et al., 1990). The striking concordance between genetic and enzymological approaches has established the *E. coli* translocation reaction as a model for mechanistic studies (Wickner et al., 1991).

Preprotein translocase has a peripheral membrane domain, the SecA protein, and an integral membrane domain, SecY/E. Prior to translocation, precursor proteins interact with cytosolic chaperones such as SecB (Randall et al., 1990; Lecker et al., 1989; Kumamoto, 1989). The SecA protein (Oliver & Beckwith, 1982) is the domain of translocase that binds the SecB/preprotein complex (Hartl et al., 1991). SecA is an ATPase that is activated by its association with preprotein, the SecY/E protein, and acidic phospholipids (Lill et al., 1989; Hendrick & Wickner, 1991). Both biochemical (Brundage et al., 1990; Akimaru et al., 1991) and genetic (Bieker et al., 1990; Schatz et al., 1989) studies have established that the SecY/E protein is the integral membrane protein that supports translocation. Immunoprecipitation studies (Brundage et al., 1992) and copurification have shown that SecY/E protein is a tight complex of the SecY protein (Ito, 1984), the SecE protein (Schatz et al., 1989), and an additional polypeptide, band 1. Translocation of preproteins requires ATP and is accelerated by the transmembrane electrochemical proton gradient $\Delta\mu_{H^+}$ (Date et al., 1980; Rhoads et al., 1984; Muller & Blobel, 1984) to drive a five-step catalytic cycle of translocation (Schiebel et al., 1991).

The translocation of proOmpA into proteoliposomes bearing the purified trnaslocase (Brundage et al., 1990) has the same requirements for SecA, SecY/E, ATP, and $\Delta\mu_{H^+}$ as the translocation reaction with inverted membrane vesicles (IMV). In these studies, very low concentration (i.e., 5 nM) of radioactive preprotein were used, such that translocase was present in excess to its preprotein substrate. As a result, it has been unclear whether these in vitro translocation systems (IMV and proteoliposomes) function catalytically, i.e., whether they support more than a single cycle of translocation.

We now report a quantitative analysis of proOmpA translocation, both in IMV and in proteoliposomes bearing the SecY/E protein. To determine the number of functional sites, we exploited the fact that proOmpA which is covalently cross-linked to bovine pancreatic trypsin inhibitor (proOmpA–BPTI) forms a stable translocation intermediate (Schiebel et al., 1991). The amount of this translocation intermediate, at a saturating substrate concentration, was used to determine the number of active sites. We find that only 15% of the isolated SecY/E, normalized to SecY content, can be reconstituted to form active translocation sites. However, per active site, the maximal rate of proOmpA translocation into proteoliposomes is 25% of that seen in IMV. The isolated proteoliposomes, like inverted membrane vesicles, can catalyze multiple cycles of precursor translocation.

MATERIALS AND METHODS

Bacterial Strains. E. coli strains KM9 (unc⁻:Tn10, relA1, spoT1, metB1; Klionsky et al., 1984) and D10 (rna10, relA1, spoT1, metB1) were used for the preparation of inner membrane vesicles, as described by Chang et al. (1978).

Proteins. SecA (Cunningham et al., 1989), SecB [Weiss et al., 1988, as modified by Lecker et al. (1989)], unlabeled proOmpA (Crooke et al., 1988), and ^{35}S-proOmpA (Crooke & Wickner, 1987) were isolated according to published procedures. SecY antibodies were raised against a peptide from the SecY N-terminus (Lill et al., 1989; Watanabe & Blobel, 1989) and SecE antibodies against a peptide from the cytoplasmic loop of SecE (Brundage et al., 1992). Protein concentrations were assayed according to Bradford (1976). Full-length SecY, a fragment of SecY corresponding to its

† This work was supported by a grant from the NIH. M.B. is supported by grants from CNRS, France, and NATO.

* Correspondence should be addressed to this author. Telephone: 310-825-6885. FAX: 310-206-7286.

0006-2960/93/0432-2626$04.00/0

N-terminal half, and SecE were isolated from DEAE-purified material by SDS–PAGE and electroelution. The quantitative amino acid composition was determined for each protein from a Western blot and agreed with the published sequences. ^{35}S-ProOmpA–BPTI was synthesized as described by Schiebel et al. (1991). BPTI coupled to *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP; Carlsson et al., 1978) was cross-linked to one of the two cysteines of proOmpA.

Proteoliposome Reconstitution. SecY/E proteoliposomes were reconstituted from purified SecY/E (DE52 stage) and *E. coli* phospholipids (Avanti Polar Lipids) as described (Brundage et al., 1990).

SecY/E Content. Samples of IMV and proteoliposomes were analyzed on 19.6% polyacrylamide gels containing SDS and 6 M urea (Brundage et al., 1990). Proteins were transferred onto poly(vinylidine difluoride) membranes (PVDF, Bio-Rad), 12 h, 200 mA (Towbin et al., 1979). Membranes were incubated with the appropriate sera, followed by ^{35}S-protein A (Amersham) and goat anti-rabbit horseradish peroxidase. Bands were quantified by scintillation counting. The amounts of protein were determined using the standards of purified SecY and SecE.

Quantification of ProOmpA Translocation. Translocation of ^{35}S-proOmpA into inverted membrane vesicles (IMV) and proteoliposomes was assayed by accessibility to added proteinase K as described (Cunningham & Wickner, 1989), except that digestions were for 30 min on ice in the presence of 1 mg/mL proteinase K. To confirm that the protease-resistant species were indeed translocated into the lumen, their sensitivity to proteinase K digestion was tested after vesicle disruption with 1% Triton X-100 (Schiebel et al., 1991). Fluorograms were quantified by densitometry (video densitometer, Bio-Rad Model 620).

RESULTS

Quantitative evaluation of in vitro translocation across inverted inner membrane vesicles (IMV) and proteoliposomes required determination of the SecY and SecE content of each, measurement of the number of active translocation sites in each, and assay of the respective rates of translocation at various concentrations of proOmpA substrate.

SecY and SecE Content. The amount of SecY/E was determined by immunoblot analysis, using antibodies raised against a peptide from the SecY N-terminus (Lill et al., 1989; Watanabe & Blobel, 1989) and a peptide from a cytoplasmic loop of SecE (Brundage et al., 1992). The full-length SecY polypeptide, an OmpT proteolytic fragment of SecY representing the N-terminal half of the protein, and full-length SecE were used as standards for immunoblot analysis. These proteins were purified from DEAE-purified translocase by SDS–PAGE and electroelution. Their concentration was determined by quantitative amino acid analysis. Two preparations of IMV had averages of 46 pmol of SecY and 110 pmol of SecE per milligram of membrane protein (Table I). After purification and reconstitution of SecY/E, an average of 850 pmol of SecY/mg of protein was obtained in proteoliposomes. This represents a purification of 17-fold, as previously reported by Brundage et al. (1990). From the determination of the SecE content, a SecY:SecE ratio of 1:2.4 was observed in IMV. The higher SecY:E ratio (1:1.4) seen in proteoliposomes is likely attributable to partial dissociation of SecY from the other translocase subunits upon DEAE chromatography. This dissociation phenomenon has previously been reported and studied (Brundage et al., 1992). These estimates of the ratio of SecY to SecE agree well with those reported independently by Matsuyama et al. (1992). Preprotein translocase is the complex of SecY/E protein with its bound SecA. Previous studies (Hartl et al., 1991) demonstrated that 100 pmol of SecA bound specifically per milligram of urea-treated membrane protein in IMV, i.e., approximately 70 pmol/mg of membrane protein. Taken together, these results suggest that SecY, SecE, and SecA are bound with high affinity in comparable molar ratios to form active translocase.

Table I: SecY and SecE Contents (pmol/mg of Protein) Determined from Two Different Preparations of KM9 IMV and Proteoliposomes[a]

	inner membrane vesicles		proteoliposomes	
	IMV1	IMV2	P1	P2
SecY	42 (7)	51 (8)	810 (102)	891 (257)
SecE	107 (38)	113 (16)	1110 (432)	1169 (325)
SecY/SecE	1/2.5	1/2.2	1/1.4	1/1.3

[a] Aliquots of 40 and 4 μg of IMV protein were analyzed on a 19.6% polyacrylamide SDS–PAGE for determination of SecE and SecY contents by immunoblot. A 10-fold lower amount of proteoliposomes was used. As described under Materials and Methods, proteins were transferred onto PVDF membranes and incubated with the appropriate sera followed by ^{35}S-protein A and goat horseradish peroxidase. The excised bands were quantified by scintillation counting. The amount of SecY in proteoliposomes was determined using a standard of purified SecY N-terminus, whereas the amount of SecY in IMV was determined using a standard of purified full-length SecY. Purified SecE was the standard for SecE quantitation. The values shown in this table represent an average of at least three determinations. The standard deviations are in parentheses.

Quantification of Translocase Active Sites. ProOmpA has two cysteinyl residues, at positions 290 and 302, in its mature domain. When bovine pancreatic trypsin inhibitor is covalently cross-linked to these residues (pOA–BPTI), translocation is arrested at the point where the BPTI is joined to the proOmpA. Approximately 29 kDa of proOmpA which is N-terminal to the cross-link junction was translocated into the vesicle lumen, as determined by its inaccessability to protease (Schiebel et al., 1991). This intermediate, termed I_{29} as an *I*ntermediate with 29 kDa translocated, allowed quantitation of the functional translocase. Figure 1 illustrates the specificity of intermediate formation. Translocation of pOA–BPTI into IMV (Figure 1A, lane 2) and proteoliposomes (lane 4) yielded I_{29} in the presence of ATP, but not in its absence (lanes 3 and 5). The generation of $\Delta\mu_{H^+}$, by the addition of succinate (lane 1), had no significant effect on the amount of intermediate formed, suggesting that the membrane potential does not result in the formation of additional translocation sites. To test whether this I_{29} intermediate can complete translocation, IMV and proteoliposomes containing the arrested intermediate were isolated by sedimentation through a sucrose solution to remove nonspecifically bound pOA–BPTI. As shown in Figure 1B, this intermediate (lanes 2 and 5) completed its translocation upon addition of ATP and DTT (to cleave the disulfide bond of the cross-linker to BPTI; Schiebel et al., 1991) in both IMV and proteoliposomes (lanes 3 and 6).

The number of active sites was quantified from the amount of I_{29} generated in two different preparations of IMV and proteoliposomes (Table II). The moles of functional translocation sites in IMV (approximately 120 pmol/mg of membrane protein) is 2-fold higher than the amount of SecY determined by immunoblot analysis (Table I). We presume that this discrepancy is due to the limited accuracy of the determination by these two different methods. Taking this discrepancy into account, the approximately 130 pmol of functional sites/mg of protein in proteoliposomes represents

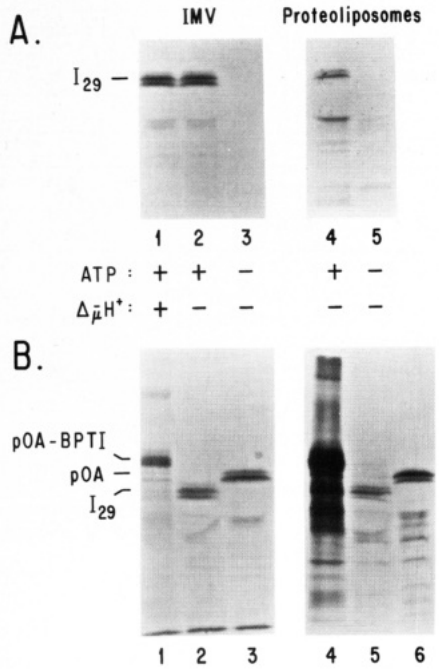

FIGURE 1: Specificity of translocation intermediate formation. (A) Translocation into IMV (60 μg of protein/mL) or proteoliposomes (30 μg of protein/mL) was performed in buffer A (50 mM Tris-HCl, pH 8.0, 50 mM KCl, and 0.5 mg/mL BSA) containing SecA (100 μg/mL), SecB (100 μg/mL), and (to preserve the disulfide linkage between proOmpA and BPTI) 0.5 mM 5,5′-dithiobis(2-nitrobenzoic acid). ATP (4 mM) and 5 mM succinate (for the generation of $\Delta\mu_{H^+}$) were added where indicated. Translocation reactions, initiated by the addition of 100 000 cpm of ^{35}S-proOmpA–BPTI, were incubated at 37 °C for 3 min (IMV) or 10 min (proteoliposomes). These incubation periods, used for the kinetic studies, were optimal for intermediate formation. Samples were then treated with proteinase K, precipitated with trichloroacetic acid, analyzed by SDS–PAGE (15% polyacrylamide), and visualized by fluorography. (B) ProOmpA–BPTI translocation intermediate was generated as in (A) in the presence of ATP. IMV and proteoliposomes containing intermediate were sedimented through a sucrose solution (200 mM sucrose, 50 mM Tris-HCl, pH 8.0, and 50 mM KCl) in an airfuge (30 min, 30 psi, 4 °C). The pellets were then resuspended in an equal volume of buffer A and incubated for 15 min either on ice (lanes 1, 2, 4, 5) without further addition or at 37 °C in the presence of 4 mM ATP and 10 mM DTT (lanes 3 and 6). The samples in lanes 2, 3, 5, and 6 were subsequently treated with proteinase K as described in (A). Samples were analyzed by SDS–PAGE and fluorography.

Table II: Quantitation (pmol/mg of Protein) of Translocase Active Sites in Two Different Preparations of IMV and Proteoliposomes[a]

	inner membrane vesicles		proteoliposomes	
	IMV1	IMV2	P1	P2
active sites	122 (38)	114 (39)	146 (46)	102 (32)

[a] Translocation intermediate was formed as described in Figure 1A using saturating substrate concentration (0.2 μM), analyzed by SDS–PAGE, and quantitated by densitometry, using proOmpA–BPTI as standard. The values are an average of three determinations for each preparation of IMV and proteoliposomes. The standard deviations are in parentheses.

15% of the total SecY. Thus, approximately 85% of the SecY protein loses its translocation activity during purification and reconstitution.

Rate of Translocation and Turnover Number. To determine whether purified preprotein translocase can act catalytically, proOmpA translocation was assayed at saturating substrate concentration. The rate of proOmpA (pOA) translocation into IMV was determined from the initial velocity of translocation at 37 °C (Figure 2, open circles). ProOmpA was translocated at a maximal rate of 1.2 (0.17 SD) pmol

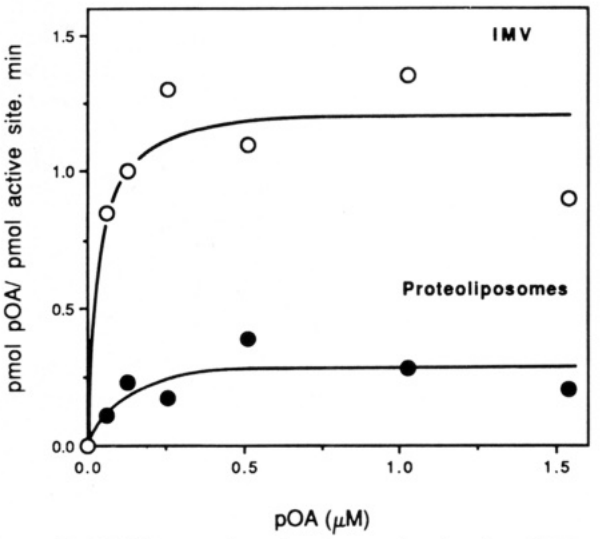

FIGURE 2: Initial rates of proOmpA translocation into IMV and proteoliposomes. ^{35}S-ProOmpA (100 000 cpm) was mixed with unlabeled proOmpA to the indicated concentration. Translocation reactions were performed with IMV (100 μg of protein/mL) or proteoliposomes (60 μg of protein/mL) in buffer A containing SecA (100 μg/mL), SecB (100 μg/mL), DTT (2 mM), and ATP (4 mM). Reactions were incubated at 37 °C for 3 min (IMV) or 10 min (proteoliposomes). Translocation reactions were stopped by chilling, treated with proteinase K as described under Materials and Methods, and then analyzed on 15% polyacrylamide SDS–PAGE, as described in Figure 1A. Fluorograms were quantified by densitometry using ^{35}S-proOmpA as standard. The number of active sites was determined as described in Table II.

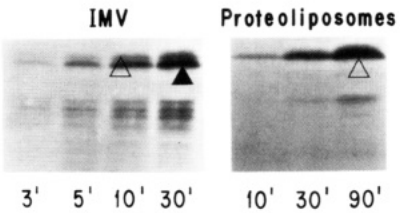

FIGURE 3: Kinetics of proOmpA translocation into IMV and proteoliposomes. Translocation reactions were performed as described in Figure 2, except that the reactions were initiated by the addition of 50 000 cpm of ^{35}S-proOmpA. The translocation reactions were stopped at the indicated times and analyzed on 15% polyacrylamide SDS–PAGE as described in Figure 1A.

min^{-1} (pmol of active site)$^{-1}$. Under the same conditions, imposition of a $\Delta\mu_{H^+}$ increased the maximal rate of proOmpA translocation by 2-fold (data not shown). ProOmpA translocation was assayed under identical conditions in proteoliposomes bearing purified SecY/E (Figure 2, filled circles). At saturating substrate concentration, the rate of proOmpA translocation was 0.32 (0.07 SD) pmol min^{-1} (pmol of active site)$^{-1}$. This rate was obtained consistently in three different preparations of proteoliposomes. From these results, we conclude that the rate of translocation per active site in proteoliposomes is approximately 4-fold lower than that observed in IMV.

We assayed whether purified preprotein translocase can catalyze multiple cycles of translocation. Translocation of proOmpA proceeded linearly for 30 min in IMV and for 90 min in proteoliposomes (Figure 3; proOmpA is indicated by open arrowheads and mature OmpA by filled arrowheads). The number of turnovers (Table III), determined at saturating substrate concentration and for these respective incubation periods, was in agreement with the number of turnovers calculated from the rate of translocation. Approximately 22 pmol of proOmpA was translocated per picomole of active site in proteoliposomes after 90-min incubation. We conclude

Table III: Turnover of Translocase in IMV and Proteoliposomes[a]

	pmol of pOA translocated/pmol of active sites	
	expected[b]	observed[c]
IMV	36 (5)	44 (10)
proteoliposomes	29 (6)	22 (7)

[a] Translocation reactions were performed at a saturating substrate concentration (2 μM) as described in Figure 2, except that the incubation period was for 30 min (IMV) or 90 min (proteoliposomes). An ATP regenerating system (1 mM creatine phosphate and 100 μg/mL creatine kinase) was added to the reaction. Samples were treated as described in Figure 2, and the fluorograms were quantified by densitometry. Each value is an average of five determinations. The standard deviations are in parentheses. The number of translocation events determined in these conditions is referred to as "observed". The number of turnovers "expected" was calculated from the initial rates (picomoles of proOmpA per picomoles of active site per minute, obtained at saturating substrate concentration) multiplied by the time of incubation. [b] Calculated from the rate, picomoles of pOA per picomoles active site per minute, obtained at saturating pOA concentration. [c] Determined at 30 min for IMV and at 90 min for proteoliposomes.

that proteoliposomes, like IMV, can catalyze multiple cycles of translocation.

DISCUSSION

To ascertain that the proteins required for an efficient translocation reaction in IMV had been reconstituted into proteoliposomes, we examined the kinetics of proOmpA translocation in both systems. We find that the rate of translocation per active site in proteoliposomes, though 4-fold lower, is of the same order of magnitude as the rate in IMV. Furthermore, isolated proteoliposomes, like IMV, can catalyze multiple cycles of precursor translocation. These results suggest that the reconstituted translocation system is not lacking a component which is vital for translocation. Nevertheless, during purification, 85% or more of SecY has lost translocation activity. This loss of activity might be due to partial dissociation of SecY and SecE during chromatography, an incorrect orientation of these proteins during reconstitution, or the loss of a stabilizing factor during purification. Studies by Bieker et al. (1990), using "sec titration" in vivo, suggested that there is a functional excess of SecE compared to SecY. An optimal SecE:SecY ratio of 5:1 has been reported in proteoliposomes reconstituted from SecE and SecY, purified separately (Nishiyama et al., 1992). Our results suggest that the ratio of SecE to SecY is higher in IMV than in proteoliposomes, partially explaining the loss of translocase activity during purification.

Despite the requirement for SecD and SecF in in vivo protein secretion (Gardel et al., 1987; Bieker et al., 1990), these proteins do not appear to be necessary for in vitro translocation (Brundage et al., 1990; Akimaru et al., 1991). Bieker-Brady and Silhavy (1992) and Gardel et al. (1992) proposed that SecD and SecF can act at a late stage of the translocation process or even after translocation. SecD and SecF may be required to release translocated protein from the translocation sites or for recycling translocase. Our data suggest that SecD and SecF may not play a major direct role in catalyzing repeated rounds of translocation when proOmpA is used as a substrate.

Assuming a cell doubling time of 35 min, 200 pmol of proOmpA must be translocated through the inner membrane per minute per milligram of membrane protein (Nikaido & Vaara, 1987). We measure an in vitro rate of translocation into IMV of 100 pmol (mg of membrane protein)$^{-1}$ min^{-1}. As another measure of the kinetic competence of the in vitro reaction, we note that individual precursor proteins are translocated in vivo in an average of 10 s (Randall, 1983). We find that, in IMV, 1 mol of translocase can translocate 1 mol of proOmpA in 50 s. Thus, the in vitro translocation rate and turnover numbers of IMV and proteoliposomes with purified SecY/E are of the same order of magnitude as those estimated in vivo. Since the kinetic properties of both in vivo and in vitro systems are comparable, we conclude that the in vitro systems used are representative of the translocation reaction occurring in vivo.

ACKNOWLEDGMENT

We thank Marilyn Leonard and Douglas Geissert for expert technical assistance. We also thank Dr. Robert Arkowitz for stimulating discussion.

REFERENCES

Akimaru, J., Matsuyama, S., Tokuda, H., & Mizushima, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 6545–6549.
Bieker, K. L., Phillips, G. J., & Silhavy, T. (1990) *J. Bioenerg. Biomembr. 22*, 291–310.
Bieker-Brady, K., & Silhavy, T. (1992) *EMBO J. 11*, 3165–3174.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Brundage, L., Hendrick, J. P., Schiebel, E., Driessen, A. J. M., & Wickner, W. (1990) *Cell 62*, 649–657.
Brundage, L., Fimmel, C., Mizushima, S., & Wickner, W. (1992) *J. Biol. Chem. 267*, 4166–4170.
Carlsson, J., Drevin, H., & Axen, R. (1978) *Biochem. J. 173*, 723–737.
Chang, C. N., Blobel, G., & Model, P. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 361–365.
Crooke, E., & Wickner, W. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 5216–5220.
Crooke, E., Guthrie, B., Lecker, S., Lill, R., & Wickner, W. (1988) *Cell 54*, 1003–1011.
Cunningham, K., & Wickner, W. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 8630–8634.
Cunningham, K., Lill, R., Crooke, E., Rice, M., Moore, K., Wickner, W., & Oliver, D. (1989) *EMBO J. 8*, 955–959.
Date, T., Goodman, J., & Wickner, W. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 4669–4673.
Gardel, C., Benson, S., Hunt, J., Michaelis, S., & Beckwith, J. (1987) *J. Bacteriol. 169*, 1286–1290.
Gardel, C., Johnson, K., Jacq, A., & Beckwith, J. (1990) *EMBO J. 9*, 3209–3216.
Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J. P., & Wickner, W. (1990) *Cell 63*, 269–279.
Hendrick, J., & Wickner, W. (1991) *J. Biol. Chem. 266*, 24596–24600.
Ito, K. (1984) *Mol. Gen. Genet.* **197**, 204–208.
Ito, K., Date, T., & Wickner, W. (1980) *J. Biol. Chem. 255*, 2123–2130.
Klionsky, D. J., Brusilow, W. A., & Simoni, R. D. (1984) *J. Bacteriol. 160*, 1055–1066.
Kumamoto, C. A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 5320–5324.
Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P. J., Jr., Kumamoto, C. A., & Wickner, W. (1989) *EMBO J. 8*, 2703–2709.
Lill, R., Cunningham, K., Brundage, L., Ito, K., Oliver, D., & Wickner, W. (1989) *EMBO J. 8*, 961–966.
Matsuyama, S., Fujita, Y., Sagara, K., & Mizushima, S. (1992) *Biochim. Biophys. Acta 1122*, 77–84.
Muller, M., & Blobel, G. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 7737–7741.
Nikaido, H., & Vaara, M. (1987) in *E. coli and S. typhimurium. Cellular and Molecular Biology* (Neidhardt, F. C., Ed.) Vol.

1, pp 7–22, American Society of Microbiology, Washington, D.C.
Nishiyama, K., Mizushima, S., & Tokuda, H. (1992) *J. Biol. Chem. 267*, 7170–7176.
Oliver, D., & Beckwith, J. (1982) *Cell 30*, 311–319.
Randall, L. L. (1983) *Cell 33*, 231–240.
Randall, L. L., Topping, T. B., & Hardy, S. J. S. (1990) *Science 248*, 860–863.
Rhoads, D. B., Tai, P. C., & Davis, B. D. (1984) *J. Bacteriol. 159*, 63–70.
Schatz, P. J., & Beckwith, J. (1990) *Annu. Rev. Genet. 24*, 215–248.
Schatz, P. J., Riggs, P. D., Annickk, J., Fath, M. J., & Beckwith, J. (1989) *Genes Dev. 3*, 1035–1044.
Schiebel, E., Driessen, A. J. M., Hartl, F.-U., & Wickner, W. (1991) *Cell 64*, 927–939.
Towbin, H., Staehelin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Watanabe, M., & Blobel, G. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 1895–1899.
Weiss, J. B., Ray, P. H., & Bassford, P. J., Jr. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 8978–8982.
Wickner, W., Driessen, A. J. M., & Hartl, F.-U. (1991) *Annu. Rev. Biochem. 60*, 101–124.