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SecA suppresses the temperature-sensitive SecY24 defect in protein translocation in *Escherichia coli* membrane vesicles

(protein translocation factors/*in vitro* protein export/secretion/reconstitution)

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ABSTRACT **Genetic analysis of protein secretion in *Escherichia coli* has identified *secY/prlA* and *secA* as components of the secretory apparatus. We have examined the roles of the *secY(prlA)* gene product (an integral membrane protein) and the soluble *secA* gene product in translocation of OmpA and alkaline phosphatase precursors in an *in vitro* system. The protein translocation defect of the *secY24* mutation was recently demonstrated *in vitro* as was its suppression by an S300 extract. We show here that the extract was essentially inactive in SecY24 suppression when SecA protein was removed from it by immunoaffinity chromatography. Furthermore, purified SecA protein suppressed the SecY24 defect. Preincubation of the inactivated SecY24 membrane vesicles either with S300 containing SecA or with purified SecA protein reconstituted the membranes and restored the translocation activity when assayed in the absence of additional soluble proteins. These results suggest that the SecY24 translocation defect is suppressed by SecA interacting, directly or indirectly, with SecY24 on the cytoplasmic membrane.**

In *Escherichia coli*, exported proteins are localized in the cell membrane, the periplasmic space, and the outer membrane. The mechanism by which these proteins are recognized by the translocation machinery, and their subsequent transfer into and through the cell membrane, remain to be elucidated in detail. Genetic and biochemical approaches have recently provided insights into the mechanism of protein translocation, although the components of the translocation apparatus and more specifically the interactions between components have yet to be defined (1–4).

Genetic analysis has identified components of the translocation machinery by isolation of conditional lethal mutations that are pleiotropically defective in protein translocation (5–9) and by the isolation of suppressors that allow the translocation of secretory proteins with defective signal sequences (10, 11). Extragenic suppressors of the conditional lethal mutations also have been isolated (2, 12).

The *secA* and *secY(prlA)* genes have been well-characterized genetically. The *secA* gene is essential for cell growth and encodes a 102-kDa protein (13) that is soluble and peripherally associated with the cell membrane (14). Temperature-sensitive mutations in *secA* result in the pleiotropic accumulation of the precursor forms of secreted proteins at the nonpermissive condition (5). The *secY* gene, which is identical to *prlA*, is also essential and encodes a 49-kDa integral membrane protein (6). Its conditional mutation *secY24(ts)* results in pleiotropic accumulation of the precursor forms of secretory proteins (6), and the alleles of *prlA* can suppress the translocation defects of signal sequence mutations (10, 15, 16).

Biochemical studies utilizing *in vitro* protein translocation systems have defined the energy requirements of translocation (17) and have also begun to identify protein components of the translocation machinery (18–20). Recently, the biochemical and genetic approaches were employed together to demonstrate the requirement of a genetically defined component for *in vitro* protein translocation. Membrane vesicles isolated from the *secY24* mutant grown at nonpermissive temperature were inactive for *in vitro* protein translocation (21, 22). Moreover, the temperature sensitivity of the isolated SecY24 membranes for protein translocation was demonstrated *in vitro*, indicating that the *secY24* mutation directly affected protein translocation (22). It was also shown that the SecY24 translocation defect was suppressed by the addition of an S300 extract containing soluble cytoplasmic proteins, suggesting that the *secY24* mutation may affect the interaction of SecY with a soluble factor(s) (22).

We report here that the SecY24-suppressing activity of the S300 extract requires SecA protein. We also show that purified SecA can be incorporated into membranes to suppress the SecY24 defect in protein translocation *in vitro*.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Medium. All strains are derivatives of *E. coli* K-12 except BL21(DE3), which is an *E. coli* B derivative (23). The temperature-sensitive mutant strain IQ85 (*secY24*) and the RNase⁻ strain D10 have been described (6, 24). Strain HJM114/pOmp9 (25), which overexpresses *ompA* mRNA, or strain MC1000/pHI-5 (26), which overexpresses the *phoA* mRNA and also contains stable *ompA* mRNA, were used for the isolation of mRNA. The plasmid pT7-secA, used for overproducing the SecA protein using the T7 promoter expression system (23), and the parental plasmid pET-5 are described elsewhere (27, 28). All cultures were grown with aeration in LinA medium (24) supplemented with 0.5% (wt/vol) glucose at the indicated temperature.

Preparation of Membrane Fractions and Soluble Protein Extracts. Cultures of strain IQ85 were grown at 32°C for the preparation of inverted cytoplasmic membrane vesicles as described (22). S30 extracts for *in vitro* protein synthesis were prepared from cultures of strain D10 grown at 37°C as described (17). Soluble cytoplasmic protein extracts (S300), free of ribosomes, were prepared from cultures of strains BL21(DE3)/pT7-secA and BL21(DE3)/pET-5, grown at 37°C to a cell density of 200 Klett units; this was followed by the addition of isopropyl β-D-thiogalactoside to 1 mM final concentration and an additional 30-min incubation. Cells were harvested and S300 extracts were prepared as described (29). The T7-SecA 300 contained about 40-fold more SecA protein than the parental strain BL21/pET-5, as determined

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immunologically using purified SecA as standards (28). S300 extracts were also prepared from a wild-type strain, ATCC 14948, cell paste (Grain Processing, Muscatine, IA); these extracts have been used for large-scale purification of translocation factors, and one extract was used as a standard for determining translocation activity since different S300 extracts had different maximal activity.

The purification of homogeneous SecA protein from strain BL21(DE3)/pT7-secA is described elsewhere (28). Briefly, SecA was purified from the T7-SecA S300 by ammonium sulfate fractionation, cation exchange on CM-Sephadex, hexyl-agarose hydrophobic chromatography, Sephacryl S-300 gel filtration, and Pharmacia FPLC Mono S column, and it was stored in 25 mM sodium phosphate, pH 7.5/50 mM NaCl/1 mM dithiothreitol/0.5 mM EDTA/10% glycerol (wt/vol). The purified SecA protein appeared as a single band on sodium dodecyl sulfate (SDS) gel as stained by Coomassie blue or by silver stain and appeared as a single spot on two-dimensional gel electrophoresis.

***In Vitro* Protein Synthesis and Protein Translocation.** mRNA was isolated from strain HJM114/pOmp9 or strain MC1000/pHI-5 as described (29). Precursor proteins of outer membrane protein OmpA or the periplasmic protein alkaline phosphatase were synthesized *in vitro* with [^{35}S]methionine in an mRNA-dependent system at 40°C for 15 min (29). Ribosomes were removed from some precursor mixtures by centrifugation at 90,000 rpm for 20 min in a Beckman TLA100.2 rotor.

The posttranslational protein translocation assay for the suppression of the SecY24 defect of membrane vesicles has been described (22) and essentially follows the modified method of Tian *et al.* (G. L. Tian, H. C. Wu, P. H. Ray, and P. C. Tai, unpublished data). Briefly, membrane vesicles prepared from strain IQ85 grown at 32°C were inactivated *in vitro* by incubation at 42°C for 2 hr. Membranes [0.1 A_{280} unit in 10 μl of TK buffer (10 mM Tris·HCl, pH 7.6/50 mM KCl)] were added to translocation mixtures containing soluble factors and 50 μl of the precursor mixture in a total volume of 150 μl. The translocation mixture was incubated at 30°C for 25 min, and translocation was terminated by rapid cooling on ice. Proteinase K (15 μg/ml) was added to each assay, which was incubated on ice for 15 min, and the protease digestion was stopped by addition of 700 μl of 1 mM phenylmethylsulfonyl fluoride/10 mM Tris·HCl, pH 7.6/500 mM KCl/10 mM $Mg(OAc)_2$. The mixtures were layered on 300 μl of 0.25 M sucrose in TKM buffer [10 mM Tris·HCl, pH 7.6/50 mM KCl/10 mM $Mg(OAc)_2$] and centrifuged at 90,000 rpm for 28 min in a Beckman TLA100.2 rotor. Membrane pellets were dissolved directly in 20 μl of electrophoresis sample buffer containing 2% SDS and boiled for 3 min, and the translocation products were examined by electrophoresis on SDS/10% polyacrylamide gels and fluorography as described (24).

Depletion of SecA from S300 Extracts. The preparation of affinity-purified SecA-specific antibodies and preparation of a SecA-specific immunoaffinity column using the purified IgGs are described elsewhere (28). To deplete SecA protein in an S300 extract, 0.2 ml of S300 protein (29 mg/ml) was mixed with 0.2 ml of the conjugated IgG-protein A-Sepharose, incubated at room temperature or 5°C for 20 min, and eluted with TKM buffer, and 200-μl fractions were collected. Protein concentrations of the fractions were determined by the Bio-Rad protein assay and fractions with peak protein concentrations were combined. The SecA concentrations of the S300 extracts were determined immunologically using purified SecA as standard as described (28).

Quantitation. For quantitation of protein translocation, fluorograms were scanned with a Joyce–Loebl Chromoscan 3 densitometer. Translocation activities are given relative to the activity observed for the suppression of *in vitro*-inactivated SecY24 membrane vesicles by the addition of a near-optimal amount (200 μg) of an S300 extract standard (from strain ATCC 14948), which is taken as having an activity of 1.0

Reagents. Translation-grade [^{35}S]methionine was from DuPont/New England Nuclear. Protein A-Sepharose was from Bio-Rad. All other chemicals (reagent grade) were from commercial sources.

RESULTS

Loss of the SecY24-Suppressing Activity of S300 by Depletion of SecA. The *in vitro* protein translocation defect of heat-inactivated SecY24 membranes was demonstrated and was shown to be suppressed by soluble cytoplasmic factors in an S300 extract (22). When SecA was removed from the S300 extract by passage through a SecA-specific immunoaffinity column, the SecA-depleted S300 extract lost the ability to suppress the SecY24 defect in protein translocation Fig. 1, lanes 2 and 3). In contrast, the S300 extract similarly passed through a control column, which did not affect its SecA concentration, retained SecY24-suppressing activity (lanes 4 and 5), and increased the protein translocation activity. Quantitation of fluorograms showed that the SecY24-suppressing activity of this control S300 extract was about 100-fold greater than the SecA-depleted S300 (compare lanes 2 and 4) and was similar to that of a control S300 extract that was not passed through the column (data not shown). The S300 extract routinely contained ≈180 ng of SecA per 100 μg of S300 protein as determined immunologically, whereas the S300 extract passed through the SecA-specific immunoaffinity column contained <1% of this level of SecA.

The precursor mixture used per assay contained about 173 ng of SecA and allowed a residual low level of activity of SecY24 membrane vesicles (see Fig. 1, lane 1); depletion of the SecA in this mixture by removal of ribosomes and passage through the SecA-specific immunoaffinity column greatly reduced the residual protein translocation activity (Fig. 2, lane 2).

These results suggest that the SecA-specific immunoaffinity column removed SecA from the S300 extract, and this depletion was associated with a loss of the SecY24-suppressing activity of the S300 extract. We noted that the SecA-depleted S300 was slightly inhibitory at higher protein concentrations (see Fig. 1, lanes 1–3; Fig. 2, lanes 2–4).

Suppression of the SecY24 Translocation Defect by Purified SecA Protein. The observation that removal of SecA rendered the S300 extract inactive in suppression of the SecY24 defect suggested a direct role of SecA as the SecY24-suppressing factor. We therefore tested whether purified SecA could restore the translocation activity of the SecA-depleted sys-

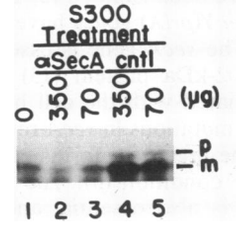

FIG. 1. Loss of SecY24-suppressing activity of S300 by depletion of SecA. Strain ATCC 14948 S300 extract was passed through a SecA-specific immunoaffinity column (αSecA) to deplete SecA or a control protein A-Sepharose column (cntl) and assayed for SecY24-suppressing activity. The translation mixtures containing OmpA precursor were used for translocation without removing ribosomes. Lane 1, background activity of the inactivated SecY24 membranes (0) without additional soluble factors. p, Precursor of OmpA; m, mature form of OmpA.

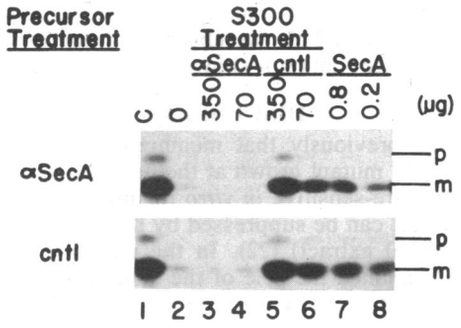

FIG. 2. SecA suppresses the SecY24 translocation defect. Post-ribosomal translation mixtures containing OmpA precursors were passed through a SecA-specific immunoaffinity column (αSecA) to remove SecA or a control protein A-Sepharose column (cntl) and assayed for protein translocation into *in vitro*-inactivated SecY24 membrane vesicles in the presence (lane 1) or absence (lane 2) of strain ATCC 14948 S300 extract (400 μg). The same S300 extract was also passed through a SecA-specific immunoaffinity column (αSecA) to deplete SecA (lanes 3 and 4) or a control protein A-Sepharose column (cntl) (lanes 5 and 6). Lanes 7 and 8, purified SecA protein. p, Precursor of OmpA; m, mature form of OmpA.

tem. Addition of purified SecA protein to the SecA-depleted system resulted in a marked increase in translocation activity; 0.8 μg of SecA stimulated the activity of heat-inactivated SecY24 membranes about 60-fold (Fig. 2, lanes 2 and 7).

To further examine the SecY24-suppressing activity of SecA we titrated a preparation of purified SecA protein and compared it with that of an S300 extract from strain BL21(DE3)/pT7-secA, which overproduces SecA ≈40-fold, and a control S300 extract from strain BL21(DE3)/pET-5, which contained wild-type levels of SecA. Fig. 3 shows that SecA stimulated the translocation activity of inactive SecY24 membrane vesicles in a concentration-dependent manner. The titration of the purified SecA showed saturation at about 1 μg of SecA, which resulted in about 1.9 units of translocation activity (Fig. 3). The maximum stimulatory activity of purified SecA was about the same as wild-type S300 extract, but the extract was 3- to 5-fold more active than purified SecA per ng of SecA.

The activity of the T7-SecA S300 per ng of SecA was intermediate between wild-type S300 (containing about 1.8 ng of SecA per μg of S300 protein) and purified SecA, but it increased to a maximum translocation activity of about 4.0 units (1.8 μg of SecA). The T7-SecA S300, which contained about 70 ng of SecA per μg of S300 protein, showed a biphasic response; it had a relatively high specific activity, close to that of purified SecA, at concentrations of total protein <5 μg, but shifted to a lower specific activity at higher concentrations of total protein (Fig. 3 *Inset*).

Kinetics of the Suppression of SecY24 Translocation Defect. We next determined the effect of the *secY24* mutation and SecA suppression on the kinetics of proOmpA translocation into active or heat-inactivated SecY24 membrane vesicles. Fig. 4 shows that the initial rate of translocation into the heat-inactivated membrane vesicles was slower by a factor of about 10 than the non-heat-treated vesicles and that the addition of 0.35 μg of SecA (contained in 5 μg of T7-SecA S300) restored the translocation rate and increased the extent of translocation. Fig. 4 also shows that the initial rate and the extent of the translocation activity of unheated active SecY24 membranes could be increased by addition of the SecA-enriched T7-SecA S300, confirming our previous observations that the *secY24* mutation renders membranes prepared from cells grown at the permissive temperature somewhat defective, and the translocation can be enhanced with additional factors (22). It is worth noting that the kinetics of translocation of unheated SecY24 membranes was much

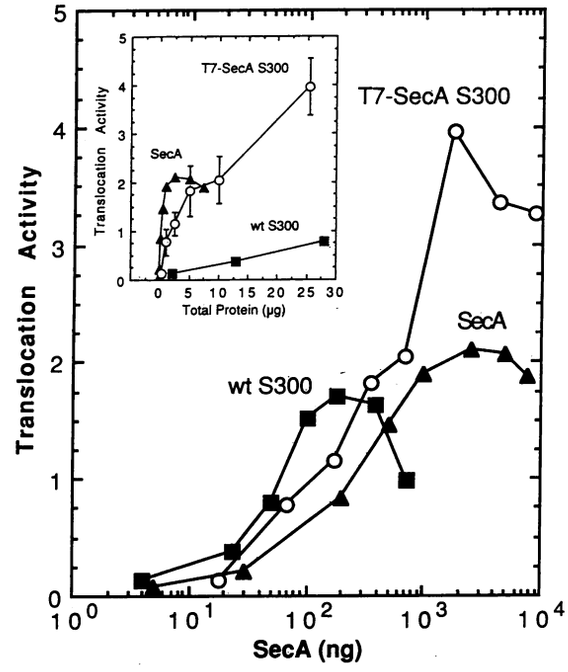

FIG. 3. Titration of SecY24-suppressing activity of SecA. Ribosomes were removed from the precursor mixture by centrifugation. The SecY24-suppressing activity of S300 from strain BL21(DE3)/pET-5, strain BL21(DE3)/pT7-secA, or purified SecA protein was determined as a function of SecA concentration. (*Inset*) Translocation activities plotted as a function of total protein; only those corresponding to <30 μg of protein were shown for clarity. Translocation activity is relative to the SecY24-suppressing activity of 200 μg of an S300 standard (ATCC 14948). Values are the average of four to seven independent experiments. Standard deviations were calculated; only that for T7-SecA S100 in the *Inset* is shown to simplify the figure. The SecA was diluted in 25 mM sodium phosphate, pH 7.5/50 mM NaCl/1 mM dithiothreitol/0.5 mM EDTA/10% glycerol/10 μg of bovine serum albumin per ml (to prevent low concentrations of SecA sticking to plastic wares). The amounts of the buffer in SecA and those of TKM buffer used in S300 have no effect on the translocation.

faster when additional SecA was provided, reaching maximal activity by 5 min.

Suppression of SecY24 Translocation Defect by SecA Associated with Membranes. SecA may suppress the SecY24 translocation defect by increasing the rate of some step in translocation prior to the precursor protein interacting with the membrane or by an effect on the membrane. To examine the latter possibility, inactive SecY24 membrane vesicles were incubated with T7-SecA S300 extract or control S300 extract in the presence of high-energy phosphates for 25 min at 30°C and then were isolated from the soluble protein factors by centrifugation through a sucrose cushion. These "reconstituted" membranes were found to possess a high level of translocation activity for OmpA and alkaline phosphatase precursors when assayed in the absence of S300 extract (Fig. 5*A*); membranes reconstituted with T7-SecA S300 extract were about 5-fold more active than membranes reconstituted with wild-type S300 extract.

To examine if purified SecA could reconstitute the inactive SecY24 membranes and to ensure that the membrane vesicles were reconstituted by the incubation and not merely contaminated with soluble translocation factors, we incubated the membranes with purified SecA and isolated the reconstituted membranes through two successive sucrose cushion sedimentations. The translocation activity of SecY24 membranes reconstituted with purified SecA protein (4 μg of SecA per 0.1 A_{280} unit of membrane) was restored to a similar level

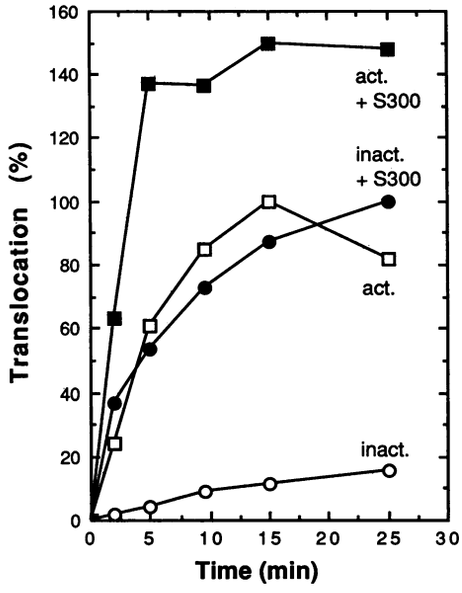

FIG. 4. Kinetics of SecA suppression on SecY24 membrane defect. The membranes were isolated from IQ85 grown at 32°C and were incubated either at 0°C (act.) or 42°C (inact.) for 2 hr. Translocation reactions were carried out with or without 5 μg of T7-SecA S300 (at which concentration the primary effect is due to SecA) and were terminated by rapid chilling on ice at the times indicated. Maximal translocation activity of unheated SecY24 membranes without additional factors is taken as 100%.

as with the T7-SecA S300 extract (10 μg of S300 per 0.1 A_{280} unit of membrane) (Fig. 5*B*, lanes 2 and 3). In fact, the translocation activity of the reconstituted membranes was similar to that of membranes assayed in the presence of purified SecA or T7-SecA S300 extract, indicating that most of the SecY24-suppressing activity of SecA was associated

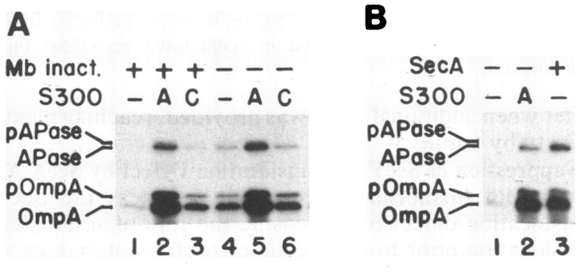

FIG. 5. Reconstitution of SecY24 membrane vesicles. (*A*) Reconstitution of SecY24 membrane vesicles by S300. *In vitro*-inactivated SecY24 membranes (Mb inact.) (lanes 1–3) or active SecY24 membranes (lanes 4–6) were incubated with S300 extract ["C" S300 from BL21(DE3)/pET-5 or "A" S300 from strain BL21(DE3)/pT7-secA] (200 μg of S300 protein per 0.1 A_{280} unit of membrane) at 30°C for 25 min in 150 μl of TK buffer containing 5 mM phospho*enol*pyruvate-Tris, 20 μM Tris-GTP, 1 mM Tris-ATP, 3 μg of pyruvate kinase, and 1 mM spermidine and then layered on 500 μl of 0.25 M sucrose in TKM buffer and centrifuged at 37,000 rpm for 90 min in an SW50.1 rotor fitted with adaptors for 4 × 51 mm tubes. The membrane pellets were resuspended in 10 μl of TK buffer and assayed for protein translocation activity. (*B*) Reconstitution of SecY24 membrane vesicles by purified SecA. *In vitro*-inactivated SecY24 membranes were incubated with "A" S300 (10 μg of T7-SecA S300 per 0.1 A_{280} unit membrane) or purified SecA protein (4 μg of SecA protein per 0.1 A_{280} unit membrane) for 25 min at 30°C as described above; membranes were sedimented twice through a sucrose cushion, resuspended, and then assayed for translocation activity in the absence of additional S300 or SecA protein. Ribosomes were removed from the translocation mixtures containing OmpA and alkaline phosphatase precursors (pOmpA and pAPase, respectively) by centrifugation.

with the membranes. The reconstitution of SecA with membranes also occurred in the absence of energy or during incubation on ice (data not shown).

DISCUSSION

We showed previously that membrane vesicles prepared from a *secY24* mutant grown at the permissive temperature are temperature-sensitive *in vitro* for protein translocation, and the defect can be suppressed by the addition of soluble proteins (S300 extract) (22). In the experiments reported here, we examined the role of the soluble SecA protein in SecY24 suppression *in vitro*. The immunochemical removal of SecA from an S300 extract resulted in the loss of SecY24-suppressing activity, whereas purified SecA protein suppressed the SecY24 defect. Furthermore, we found that SecA acted through an association with the cytoplasmic membrane that was firm enough to persist on centrifugation through a sucrose cushion.

Although purified SecA suppressed SecY24 with a maximum suppression activity similar to wild-type S300, it appeared to be less active than the same amount of SecA in the S300 extract (as determined immunologically) by a factor of 3–5. This difference in translocation activity at a given amount of SecA could be explained by the inactivation of SecA during purification and storage since SecA was found to be labile and sensitive to freeze-thawing. Alternatively, the purified SecA may require other interacting components for maximal activity.

The SecY24-suppressing activity of a T7-SecA S300 extract, which contained 40-fold more SecA than wild-type, approximated that of wild-type S300 and purified SecA below concentrations where activity of the purified SecA became saturating. However, at higher concentrations of T7-SecA S300, the suppressing activity increased at a higher differential rate to a level of activity that was >2-fold higher than the level achieved by purified SecA (Fig. 3). These results suggest that, although SecA appeared to be the major suppressing activity in S300 extracts, it may not be the only factor involved in SecY24 suppression. Indeed, we have found that SecB can also stimulate, although to a much lower degree, the translocation activity of the inactivated SecY24 membrane vesicles (J.P.F., C. Kumamoto, and P.C.T., unpublished observations). The enhanced translocation by SecB suppression may be related to the anti-folding and/or rapid membrane targeting activity of SecB with precursor molecules (30, 31). It was reported previously that more than one factor stimulated the translocation of partially purified OmpA precursors (20).

If other factors can suppress SecY24, then why was the SecA-depleted S300 not active, even at high concentrations of protein? Furthermore, if SecY24 suppression was primarily SecA-dependent, then why did the activity of the wild-type S300 not continue to increase with increasing amounts of total protein, at least until the levels of SecA in the S300 reached saturating concentrations? One possible explanation is that there exists a factor that is capable of inhibiting or modulating protein translocation at high concentrations. Indeed, we consistently observed that SecA-depleted S300 was not only inactive in SecY24 suppression, but at higher concentrations it also inhibited the residual translocation activity of the SecY24 membranes (Fig. 1, lanes 1–3; Fig. 2, lanes 2–4). Alternatively, the SecA immunoaffinity column may remove some additional SecA-interacting component.

The suppression of the SecY24 defect can be achieved either by supplementing SecA as a soluble protein in the translocation mixture or by preincubating with the defective membranes. Active SecY24 membranes and *in vitro*-inactivated SecY24 membranes possessed similar levels of SecA protein (as determined immunologically), suggesting that SecA remains on the membrane vesicles during *in vitro*

incubation at 42°C. We have found that the SecA on the heated membranes was also inactivated by incubation at 42°C. However, it was demonstrated previously that active SecY24 membrane vesicles are inactivated by incubation at 42°C for 120 min, whereas SecY$^+$ membrane vesicles remain active given the amount of SecA found normally in the translocation system (22). Moreover, unheated SecY24 membranes possessed wild-type levels of SecA (data not shown), yet they are only 10–20% as active as SecY$^+$ membranes, and additional soluble factors stimulate the activity of SecY24 membranes 5-fold but stimulate SecY$^+$ membranes <2-fold (22).

The stimulation of translocation activity, both in rate and in extent, of the unheated SecY24 membranes by SecA (Fig. 4) suggests that optimal translocation activity of SecY24 membranes requires higher levels of SecA, which is specific for the *secY24* mutation. The suppression of the SecY24 defect by the establishment of a new SecA-dependent pathway is unlikely since SecA is itself essential for protein translocation into SecY$^+$ membrane vesicles (28). Moreover, *secA* and *secY* are essential genes and are required for protein translocation *in vivo*. Accordingly, these results suggest that SecA and SecY may act in concert in the membrane during normal protein translocation.

We suggest that SecA suppresses the SecY24 defect in protein translocation either by direct interaction with SecY24 or indirectly through the involvement of an additional component of the protein translocation machinery. The nature of the interaction, direct or indirect, between SecA and SecY is not apparent, but reasonable possibilities are that the inactivation of SecY24 decreases its affinity for SecA or that an excess of SecA can reverse the conformational effect of heat on the SecY24. The possible interaction of SecA and SecY had been suggested previously based on genetic observations that the SecA levels can modulate the *prlA*-mediated suppression of signal sequence mutations (32) and that the prlA1012 allele can suppress the *secA51* protein translocation defect (33). (However, the nature of *prlA1012* mutation is not certain; see ref. 34.) Lee and Beckwith (34) have shown that slowing down protein synthesis, either by biochemical or genetic means, can compensate the translocation defect of *secA51*(ts) and *secY24 in vivo*. Conversely, it seems possible that by speeding up the rate of translocation, the SecY24 defect can also be compensated for as shown here with SecA. One prediction of this interpretation is that overproduction of SecA should suppress the *secY24* defect *in vivo*.

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