SecG plays a critical role in protein translocation in the absence of the proton motive force as well as at low temperature

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Abstract SecG is an integral membrane component of *E. coli* protein translocase. However, a discrepancy exists as to the importance of SecG for protein translocation at 37°C between cells and reconstituted proteoliposomes; protein translocation in Δ*secG* cells is defective at 20°C but normal at 37°C, indicating that SecG is dispensable at 37°C, whereas SecG remarkably stimulates protein translocation into reconstituted proteoliposomes at 37°C. In this study, protein translocation into membrane vesicles containing or not containing SecG was examined in the presence and absence of the proton motive force at 37°C and 20°C. We found that the absence of the proton motive force renders protein translocation strongly dependent on SecG even at 37°C. Protein translocation into proteoliposomes in the absence of the proton motive force thus required SecG whereas that in cells, which always generate the proton motive force, did not.

Key words: SecG; Protein translocation; Proton motive force; Membrane vesicle; *E. coli*

1. Introduction

The central part of *E. coli* protein translocase comprises a peripheral membrane factor, SecA, and three integral membrane factors, SecY, SecE and SecG [1]. SecA has been proposed to undergo the ATP-dependent cycle of membrane-insertion and deinsertion [2], causing the stepwise delivery of precursor proteins into the putative protein conducting channel formed by SecY and SecE. A new membrane factor, SecG, has been identified as a translocation stimulation factor [3], which forms a heterotrimeric complex together with SecY and SecE in the cytoplasmic membrane [4,5]. Furthermore, SecG was recently found to undergo a conformational change cycle, which seems to be coupled with the insertion–deinsertion cycle of SecA, and thus causes efficient protein translocation (manuscript submitted).

As to the importance of SecG for protein translocation at 37°C, however, there is a discrepancy between cells and reconstituted proteoliposomes. The activity of proteoliposomes in the presence of SecA and ATP at 37°C remarkably increases when SecG is reconstituted together with SecY and SecE [1,3]. On the other hand, the Δ*secG* strain exhibits defective protein translocation at 20°C but not at 37°C [6]. Defective SecG mutants, which suppress the toxic effect of a chimeric precursor at 37°C, were recently isolated [7]. However, the weak Sec phenotype of these mutants at 37°C does not explain the strong SecG-dependent increase in the reconstituted translocation activity at 37°C. To determine the reason for the difference in the importance of SecG at 37°C between cells and proteoliposomes, protein translocation into membrane vesicles was examined in detail. We found that the absence of the proton motive force renders the translocation strongly dependent on SecG.

2. Materials and methods

2.1. Bacterial growth and preparation of everted membrane vesicles

E. coli K003 (HfrH *pnp-13 tyr met* RNase I$^-$ Lpp$^-$ Δ*uncB-C::Tn10*) [8] and KN553 (K003 Δ*secG::kan*) were grown at 37°C as described [9]. KN553 was constructed by introducing the Δ*secG::kan* allele of KN370 [6] by P1 transduction. Everted membrane vesicles were prepared from these strains as described [9].

2.2. Determination of ΔΨ and ΔpH

The generation of ΔΨ (inside positive) and ΔpH (inside acidic) in membrane vesicles was monitored by following the fluorescence quenching of oxonol-V and quinacrine, respectively, as described [9].

2.3. In vitro protein translocation

The in vitro synthesis of proOmpA and its truncated derivative, proOmpA D26 [10], was carried out in the presence of Tran^{35}S-label (ICN), a mixture of [^{35}S]methionine and [^{35}S]cysteine, 1000 Ci/mmol. The labeled precursors were partially purified by gel filtration [11]. The translocation of [^{35}S] labeled precursors was examined at 37°C and 20°C as described [3]. The translocated protein, which was resistant to proteinase K, was detected on an SDS-polyacrylamide gel by means of fluorography. Densitometric quantification of band materials was carried out with a Shimadzu CS-930 chromatoscanner.

2.4. Other methods

SecA [12] and SecB [13] were purified from cells overproducing the respective proteins as described. NADH and carbonylcyanide *m*-chlorophenyl hydrazone (CCCP) were obtained from Sigma. SDS-PAGE was carried out for the analysis of proOmpA D26 [10] or proOmpA [11] as described. Protein was determined as described [14] using bovine serum albumin as a standard.

3. Results

3.1. Depletion of SecG does not perturb the generation of the proton motive force in membrane vesicles

It has been reported that the depletion of SecD and SecF perturbs, for an unknown reason, the generation of the proton motive force (Δ*p*) in cells and membrane vesicles [15]. Δ*p* is composed of an electrical component, ΔΨ, and a chemical component, ΔpH. To determine whether or not the depletion of SecG also affects Δ*p* generation, membrane vesicles were prepared from *secG*$^+$ and Δ*secG* cells, which lack F_0F_1-ATPase, and then examined as to the generation of ΔpH (inside acidic) and ΔΨ (inside positive), which were monitored by following the fluorescence quenching of quinacrine and oxonol-V, respectively (Fig. 1). The extent of the fluorescence quenching of both probes induced on the addition of NADH was essentially the same in normal and SecG-depleted

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membrane vesicles not only at 37°C but also at 20°C, indicating that the generation of Δp is normal in SecG-depleted membrane vesicles.

3.2. *The SecG function is critical for protein translocation in the absence of Δp or at low temperature*

The in vitro translocation of proOmpA D26, a truncated proOmpA derivative [10], into normal and SecG-depleted membrane vesicles was examined at 37°C and 20°C in the presence and absence of Δp (Fig. 2). Since protein translocation in vitro with a sub-optimal amount of SecA exhibits stronger dependence on Δp than that with a saturating amount of SecA [16], the assay was carried out in the presence of a saturating amount of SecA to minimize the Δp-dependence. When examined at 37°C in the presence of Δp, SecG depletion had little effect on the translocation (A). These results coincided with those obtained at 37°C with cells, in which Δp is usually present and SecG is dispensable [6]. In marked contrast, in the absence of Δp, SecG-depleted membrane vesicles exhibited significantly lower translocation activity than SecG-containing ones did (B). SecG was therefore required for the translocation in the absence of Δp at 37°C, as observed with proteoliposomes, which had been examined as to the translocation in the absence of Δp at 37°C [3]. On the other hand, SecG-depletion decreased the efficiency of translocation even in the presence of Δp at 20°C (C), indicating that SecG-depletion renders protein translocation sensitive to low temperature not only in vivo [6] but also in vitro. Finally, in the absence of Δp at 20°C, SecG-depleted membrane vesicles were essentially inactive whereas SecG-containing ones exhibited the translocation, albeit at a greatly reduced rate (D).

The translocation of proOmpA in Δ*secG* cells was defective at 20°C but normal at 37°C [6]. To determine whether or not the absence of Δp also renders the translocation of authentic proOmpA dependent on SecG even at 37°C, proOmpA translocation into normal and SecG-depleted membrane vesicles was examined in the absence of Δp (Fig. 3). The translocation of proOmpA was also defective in SecG-depleted membrane vesicles at 37°C, although the SecG-dependence was weaker than the translocation of proOmpA D26. These membrane vesicles translocated proOmpA in the presence of Δp as effectively as normal membrane vesicles did (data not shown). When assayed at 20°C, SecG-depleted membrane vesicles translocated little proOmpA.

These results, taken together, indicate that the SecG function is critical for protein translocation in the absence of Δp as well as at low temperature.

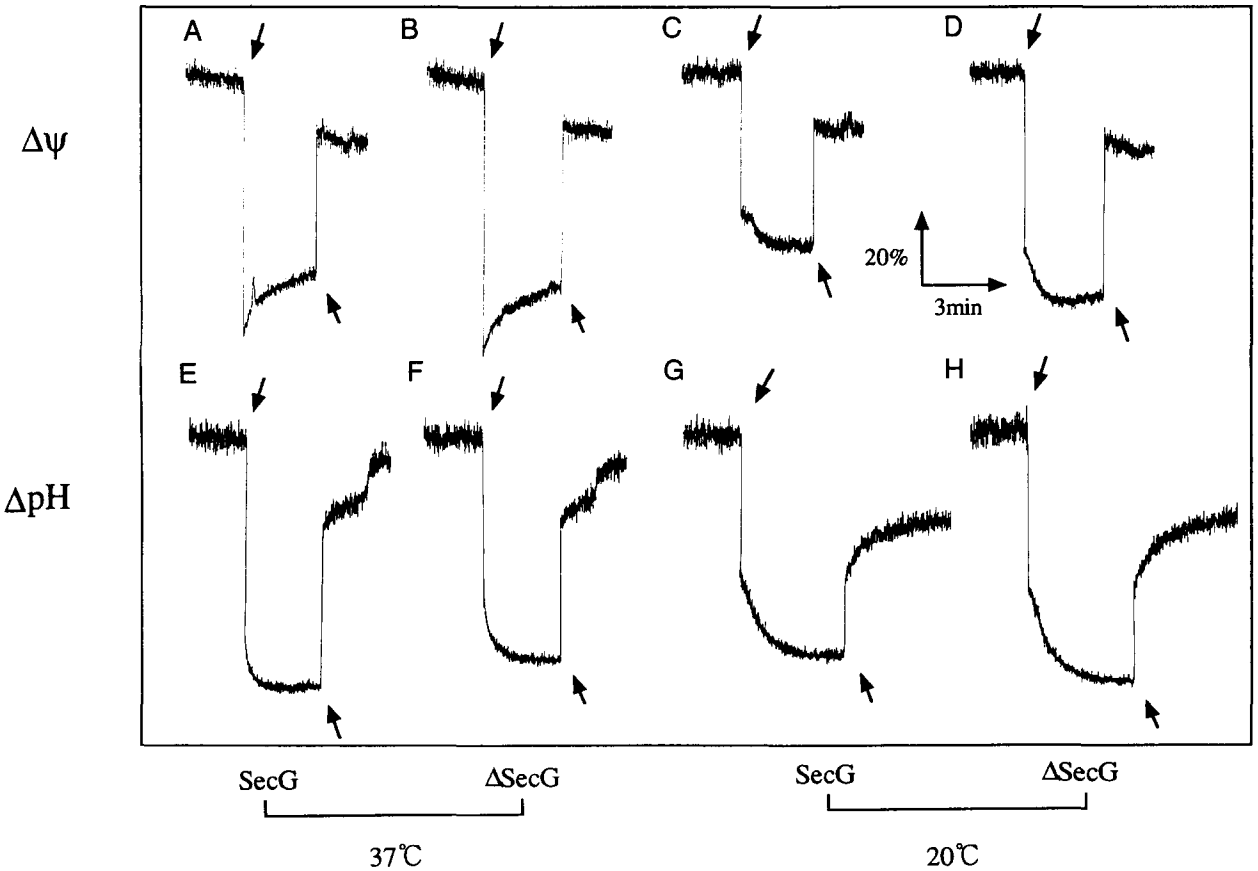

Fig. 1. Generation of ΔΨ and ΔpH in normal and SecG-depleted membrane vesicles. Everted membrane vesicles were prepared from wild type (A, C, E and G) or Δ*secG* (B, D, F and H) cells. The generation of ΔΨ (inside positive, A–D) and ΔpH (inside acidic, E–H) were monitored by following the fluorescence quenching of oxonol-V and quinacrine, respectively. The reaction mixture contained, in 2 ml, 50 mM potassium phosphate (pH 7.5), 2 mM $MgSO_4$, 1 μM oxonol-V or quinacrine, and 25 μg/ml of membrane vesicles. ΔΨ and ΔpH at 37°C (A, B, E and F) or 20°C (C, D, G and H) were generated with 1 mM NADH and then collapsed with 10 μM CCCP, which were added at the times indicated by the first and second arrows in each trace, respectively. Scales for time and fluorescence intensity (20%) are also included.

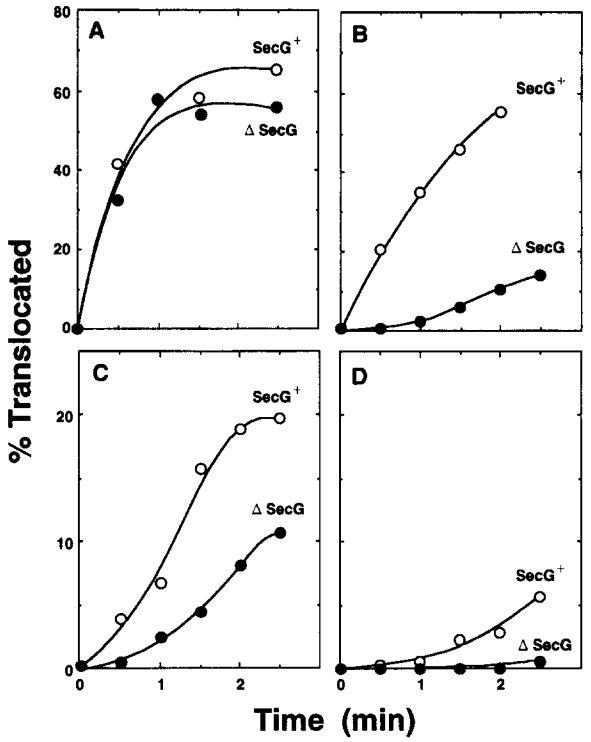

Fig. 2. Translocation of proOmpA D26 into normal and SecG-depleted membrane vesicles. The translocation of [^{35}S]proOmpA D26 into normal (○) and SecG-depleted (●) membrane vesicles was examined at 37°C (A and B) or 20°C (C and D). The reaction mixture contained 50 mM potassium phosphate (pH 7.5), 2 mM $MgSO_4$, 60 μg/ml of SecA, 2 mM ATP, an ATP-generating system consisting of creatine phosphate and creatine kinase [19], [^{35}S]proOmpA D26 (8×10^6 cpm/ml), and 0.1 mg/ml of membrane vesicles with (A and C) or without (B and D) 5 mM NADH. At the indicated times, aliquots (25 μl) of the reaction mixture were withdrawn, treated with 0.8 mg/ml of proteinase K on ice for 30 min, and then analysed by SDS-PAGE and fluorography. The amounts of translocated OmpA D26, which were quantitated as described under section 2, are expressed as percentages of the input proOmpA D26.

4. Discussion

Various lines of evidence indicate that SecG is an integral membrane component of *E. coli* protein translocase. On the other hand, the significance of the contribution of SecG to protein translocation at 37°C remained unclear since SecG is essential for neither the growth of cells nor in vivo protein translocation at 37°C [6], whereas the rate of protein translocation into proteoliposomes at 37°C increases 20- to 30-fold upon the reconstitution of SecG [3]. As shown in this paper, Δp was found to significantly affect the importance of SecG for protein translocation, indicating that the strong SecG-dependence of protein translocation observed previously with proteoliposomes is due to the absence of Δp, which also enhances the translocation efficiency but is dispensable for in vitro protein translocation, especially in the presence of a saturating level of SecA [16].

In addition to Δp and SecG, various other factors such as the level of SecA, precursor structure or temperature affect the translocation efficiency. The mechanisms whereby these factors affect the translocation efficiency are most likely not the same, however. Although the data presented in this paper appear to suggest that the roles of Δp and SecG in protein translocation are mutually compensated for, Δp is required for efficient protein translocation at 20°C irrespective of the presence or absence of SecG, indicating that Δp and SecG play distinct roles in protein translocation and independently enhance the translocation efficiency. The role of Δp in the translocation remains largely unknown, however. Efficient in vitro translocation at 20°C requires both Δp and SecG but that at 37°C requires only one of them. These results seem to support the proposal that protein translocation itself involves a cold-sensitive step [17].

SecG has been isolated as a factor which stimulates reconstituted translocation activity [3]. The *secG* gene was then identified on the basis of its partial amino acid sequence. Using a toxic chimeric precursor possessing an inefficient signal peptide, Bost and Belin [7] isolated *secG* mutants, which suppress the toxic effect of the precursor at 37°C, indicating that SecG is functioning at this temperature. The weak Sec phenotype of these mutants explains why the *secG* gene has not been identified on conventional *sec* or *prl* selection [18]. The strong SecG-dependence in the absence of Δp is an important aspect of protein translocation, which can only be revealed with in vitro assay systems.

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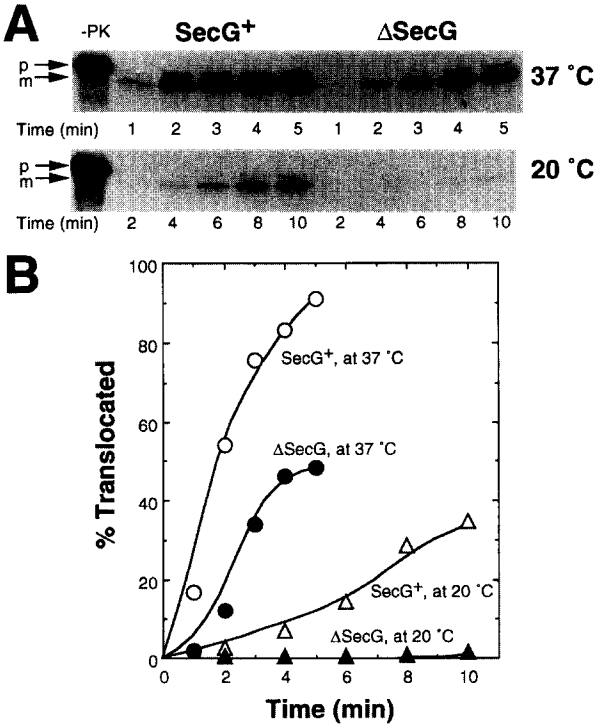

Fig. 3. Translocation of proOmpA in the absence of Δp depends on SecG. The translocation of [^{35}S]proOmpA (8×10^6 cpm/ml) into normal or SecG-depleted membrane vesicles was assayed in the absence of NADH at 37°C and 20°C as described in Fig. 2 except that the reaction mixture also contained 25 μg/ml of SecB and 10 mM dithiothreitol. (A) The translocated proteins were analysed by SDS-PAGE and fluorography. The left lane shows the amount of input proOmpA. (B) The amounts of translocated protein are expressed as percentages of the input proOmpA.

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