Translation arrest of SecM is essential for the basal and regulated expression of SecA

Akiko Murakami, Hitoshi Nakatogawa*, and Koreaki Ito†

Institute for Virus Research and Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Kyoto University, Kyoto 606-8507, Japan

Communicated by William T. Wickner, Dartmouth Medical School, Hanover, NH, July 8, 2004 (received for review March 28, 2004)

The SecM protein of *Escherichia coli* contains an arrest sequence (F^{150}XXXXWIXXXXGIRAGP^{166}), which interacts with the ribosomal exit tunnel to halt translation elongation beyond Pro-166. This inhibition is reversed by active export of the nascent SecM chain. Here, we studied the physiological roles of SecM. Arrest-alleviating mutations in the arrest sequence reduced the expression of *secA*, a downstream gene on the same mRNA. Among such mutations, the arrest-abolishing P166A substitution mutation on the chromosomal *secM* gene proved lethal unless the mutant cells are complemented with excess SecA. Whereas secretion defect due either to azide addition, a *secY* mutation, or low temperature leads to up-regulated SecA biosynthesis, this regulation was lost by a *secM* mutation, which synergistically retarded growth of cells with lowered secretion activity. Finally, an arrest-alleviating rRNA mutation affecting the constricted part of the exit tunnel lowered the basal level of SecA as well as its secretion defect-induced up-regulation. Thus, the arrest sequence of SecM has at least two roles in SecA translation. First, the transient elongation arrest in normal cells is required for the synthesis of SecA at levels sufficient to support cell growth. Second, the prolonged SecM elongation arrest under conditions of unfavorable protein secretion is required for the enhanced expression of SecA to cope with such conditions.

In the Sec pathway of protein translocation from the cytosol to the extracytoplasmic locations of the *Escherichia coli* cell (1, 2), the SecYEG complex serves as a protein-conducting channel, and the SecA ATPase drives preprotein movement into and across the channel. Whereas recent progress in the structural biology of Sec translocase is remarkable (3, 4), we still lack full understanding of the dynamic and coordinated actions of the Sec components (5, 6). Also, our knowledge about their regulation at the transcription/translation level is limited.

The *secA* gene is known to be up-regulated under the conditions of lowered functionality of the Sec translocation machinery (7). This regulation is at the translation level, in which *secM* (secretion monitor) located upstream of *secA* in the *secM-secA-mutT* polycistronic messenger RNA (8) plays a crucial role (9). The product of *secM* is a peculiar secretory protein (10, 11), which undergoes translation elongation-arrest at the Pro-166 position of the 170-codon-long *secM* ORF (12). Thus, ribosome-tethered $SecM_{1–166}$-tRNA accumulates transiently in normal cells and more stably in secretion-defective cells (11, 12). We identified amino acid sequence F^{150}XXXXWIXXXXGIRAGP^{166}, including the arrest point Pro-166, as the SecM element that is required and sufficient for the elongation stall (ref. 12; the residues denoted X are changeable functionally). This arrest sequence can halt translation even when it is attached to a polypeptide of unrelated amino acid sequence.

We isolated arrest-alleviating mutations, which all proved to affect specific residues of either the 23S rRNA or the L22 r-protein that are located in positions facing the constricted part of the exit tunnel (12, 13). Thus, the arrest sequence of SecM seems to interact with the exit tunnel and thereby retards its elongation beyond the Pro-166 residue.

In normal cells, in which the N-terminal part of the nascent SecM is pulled by the Sec translocase, the elongation arrest is soon released, whereas the arrest is strikingly prolonged under conditions in which the translocation machinery or the SecM signal sequence is functionally defective (11, 14, 15). Although it is believed that the stalled ribosome will disrupt the secondary structure of the *secM-secA* mRNA to allow the easier entry of new ribosomes that translate *secA*, the importance of SecM elongation arrest in basal and regulated *secA* expression has not been established. The present study was aimed at clarifying these issues and at establishing the SecM system as a regulatory mechanism, in which macromolecular interactions within the ribosomal exit tunnel have been exploited.

Materials and Methods

Media. L-medium contained 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl, and 1.7 mM NaOH; for plating, 1.2% agar was added. M9 (16) was used as a minimal salt medium; for pulse labeling, 18 amino acids (20 μg/ml, except Met and Cys) and thiamine (2 μg/ml) were added. For growing plasmid-bearing cells and for selection of transformants and transductants, ampicillin (50 μg/ml), chloramphenicol (20 μg/ml), tetracycline (25 μg/ml), kanamycin (25 μg/ml), or spectinomycin (40 μg/ml) was included.

Plasmid Constructions. To construct a plasmid carrying the *secM-secA* gene complex, a *secM-secA* fragment was PCR-amplified from the MC4100 (see below) chromosome with primers 5′-GAGAGCTCCACCGACTTGAATCCACCGG-3′ and 5′-ATAATACCTACCGCAATTTGCAGC-3′. The product (≈2 kbp) was digested with *Sph*I and ligated with a ≈7-kbp *Sma*I–*Sph*I fragment of pKY173, a pBR322-derived *secA* plasmid (17). The resulting plasmid, pAN1, carried a 5′ truncated *lpxC* fragment followed by the intact *secM* and *secA* genes. Site-directed *secM* mutations were introduced into pAN1 by the procedures published by Sawano and Miyawaki (18). The mutations were designed to have a GCT (an Ala codon) substitution for the codons for Phe-150, Pro-153, Trp-155, Arg-163, and Pro-166. These mutations are called *secM150*, *secM153*, *secM155*, *secM163*, and *secM166*, respectively, in this paper. Plasmid pAN31 carried *secA* under the P_{araBAD} promoter; the *secA*-coding region was amplified from pAN1 by using primers 5′-AAA**GAGCTC**CGCAACGCGGGGCGTTTGAG-3′ and 5′-AAACCCGGGTTATTGCAGGCGG**CCATGG**C-3′ (the *Sac*I and the *Xma*I recognition sequences are shown in bold) and cloned into pBAD33 (19) after *Sac*I–*Xma*I digestions. Plasmid pAN37 carried a dimer form of *secA* (ref. 20; also see below). For its construction, pAN5, a derivative of pSTV28 (a pACYC184-based *lac* promoter vector; ref. 21) carrying the same *secA* insert as pAN31, was subjected to site-directed introduction of CATATG (His-Met dicodons recognizable by *Nde*I) in place of either the Met-854–Gln-855 codons or Val-126–His-127 codons

Freely available online through the PNAS open access option.

*Present address: Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan.

†To whom correspondence should be addressed. E-mail: kito@virus.kyoto-u.ac.jp.

of *secA*, resulting in two plasmids, pAN35 and pAN36, respectively, which were then digested with *Sac*I and *Nde*I, followed by ligation of the respective ≈2.5- and ≈6-kbp fragments. The resulting plasmid (pAN37) complemented the temperature-sensitive $secA_{amb}$ mutant (7).

***E. coli* Strains.** MC4100 [F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 ptsF25 deoC1 thi*; ref. 16] and HPT264 (MC4100, *ara714 leu*::Tn*10*; ref. 22) were used as wild-type strains. HM1246 (MC4100, ara^+; H. Mori, personal communication) and CU141 (MC4100, F′$lacI^q$; ref. 23) were used as hosts to express genes under the control of the *araBAD* and the *lac* promoters, respectively. CK4706 (20) carried a tandemly duplicated and fused *secA* dimer gene, which encodes a functional variant of SecA with residues Met-1–Gln-853 and Val-128–Gln-901 linked covalently.

Chromosomal *secM* mutants were constructed as follows. Strain AN3 was a derivative of a *polA* strain, CU101 (24), into which *leu-82*::Tn*10* (17) was introduced by P1 transduction. It was then transformed with the *secM150* or the *secM155* plasmid to select ampicillin-resistant plasmid cointegrates generated by a single crossing-over within the *secM-secA* homology region. Transformants obtained were screened for those having the mutant *secM* allele in the segment flanked by the Tn*10* and the integrated plasmid by PCR amplification of the segment and sequencing of *secM*. They were then used to propagate P1*vir* phages (25), which were allowed to infect MC4100 recipient cells and to select tetracycline-resistant transductants, which were subsequently scored for unselected ampicillin marker. Ampicillin-sensitive transductants with confirmed *secM150* and *secM155* alleles were established as strains AN26 and AN27, respectively. AN52 was a corresponding $secM^+$ strain. AN210 and AN211 were similar to AN52 and AN26 except that they carried *ompT*::*kan*. The *secM166* mutation was introduced into the chromosome of strain HM1246 that carried pAN31 (P_{araBAD}-*secA*) by transduction in the presence of 0.2% arabinose. The resulting strain was named AN145 (*secM166*/pAN31) and its isogenic $secM^+$ strain as AN144 ($secM^+$/pAN31).

Strains carrying a chromosomal *secM* mutation in combination with the *secA* dimer on plasmid (pAN37) were constructed similarly by using CU141/pAN37 as a recipient; they were AN169 ($secM^+$), AN170 (*secM150*), AN171 (*secM155*), and AN173 (*secM166*). The *secM150 secY39* double mutant (AN205) was constructed by P1 transduction by using AN26 and GN31 (*secY39*; ref. 17) as a donor and a recipient, respectively.

Strain carrying the *rrlB2058* mutation was constructed as follows. TA542 (26), having the chromosomal 7 copies of the ribosomal RNA operons all inactivated and rRNA provided from a kanamycin-resistant plasmid pHK-*rrnC*, was transformed further with an ampicillin-resistant plasmid, either pNK+ encoding the wild-type *rrnB* operon (27) or its A2058G mutant, pNH152 (12). Transformants, selected on L-agar containing ampicillin and spectinomycin at 37°C, were then screened for kanamycin-sensitive colonies to complete plasmid shuffling and to establish strains NH592 (Δ*7rrn*/pNK+) and NH593 (Δ*7rrn*/pNH152).

Pulse-Labeling Analysis of SecA Biosynthesis and OmpA Export. Procedures for pulse-labeling and immunoprecipitation experiments were described in ref. 17. To measure SecA biosynthesis, cells were labeled with [^{35}S]methionine for 2 min and chased with unlabeled methionine for 3 min. Samples of a fixed total acid-insoluble radioactivity were subjected to anti-SecA immunoprecipitation, SDS/PAGE (10% acrylamide), and visualization/quantification by a phosphor imager (BAS1800, Fuji). The relative band intensities represent the relative differential synthesis rates of SecA (SecA synthesis rate normalized by total protein synthesis). To assess export of OmpA, cells were pulse-labeled for 30 s and processed immediately for anti-OmpA immunoprecipitation. The band intensities of the precursor (containing 6 Met residues) and the mature (5 Met residues) forms of OmpA were used to calculate the proportion of the latter.

Immunoblotting Analysis of SecA Abundance. Immunoblotting experiments were described in ref. 21. Samples containing proteins from a fixed cellular mass (≈1×10^7 cells) were used, and final protein images were quantified by a lumino-imager (LAS1000, Fuji).

Results

An Arrest-Defective *secM166* Mutation Is Lethal by Lowering SecA Abundance and Protein Export Activity. A segment of SecM from Phe-150 to Pro-166 (arrest sequence) halts translation elongation at the position of Pro-166 (12). Among Ala substitution mutations introduced into this segment of *secM* on plasmid, the one for Pro-166 (*secM166*) abolished the elongation arresting property of SecM almost completely (12). To examine physiological consequences of this mutation, we attempted to introduce it into the chromosome. In contrast to the less defective *secM150* and *secM155* mutations (12), construction of the chromosomal *secM166* mutant was only possible in the presence of a SecA-overproducing plasmid (see *Materials and Methods*).

When the complementing *secA* was arabinose-controlled, the *secM166* mutant exhibited clear dependence on arabinose for growth (Fig. 1*a*). Thus, the Ala substitution for Pro-166, an arrest-essential residue in SecM, is lethal unless SecA is provided in excess. When the arabinose promoter-directed synthesis of SecA from the plasmid was shut off by removal of arabinose from the medium, cell growth gradually declined after ≈7 h. Cellular abundance of SecA and export efficiency of an outer membrane protein, OmpA, were determined for the arabinose-deprived cells by immunoblotting and pulse labeling/immunoprecipitation, respectively. Whereas the SecA level in the control $secM^+$/P_{araBAD}-*secA* strain reached the steady-state level that was equivalent to the SecA abundance observed for plasmid-free wild-type cells (Fig. 1*b*, lane 4), SecA abundance in the *secM166* mutant cells was only ≈20% of the normal abundance (Fig. 1*b*, lane 2). Protein export was severely defective for the latter strain, in which newly synthesized OmpA was observed almost exclusively as the unprocessed precursor form (Fig. 1*b*, lane 2). These results indicate that the elongation-arresting property of SecM is essential for the cell to express SecA at the level sufficient to support cell growth.

SecM Mutational Effects on Expression of Cis-Located *secA* Gene. Because the *secM166* mutational effect was observed without involving any specific conditions known to induce a secretion-defect response, SecM elongation arrest seems to be required for the basal level synthesis of SecA, even though the arrest is only transient in sec^+ cells. To investigate the relationships between the arrest ability of SecM and rates of SecA synthesis from the cis-located *secA* gene, we carried out pulse labeling and immunoprecipitation experiments by using normally growing cells. For this purpose, we used a plasmid encoding a dimeric SecA (ref. 20; see also *Materials and Methods*) to complement the growth defect of the *secM166* mutant. This functional SecA variant enabled us to distinguish between the complementing SecA dimer and the chromosomal SecA monomer after their electrophoretic separation. The differential synthesis rate of SecA monomer was revealed to be only ≈30% of that in the $secM^+$ control strain (Fig. 1*c*, lanes 3 and 4). Another control strain expressing the dimeric SecA both from the plasmid and from the chromosome did not produce any significant amount of SecA band at the protomer position (Fig. 1*c*, lane 2).

We then examined physiological consequences of the *secM150*

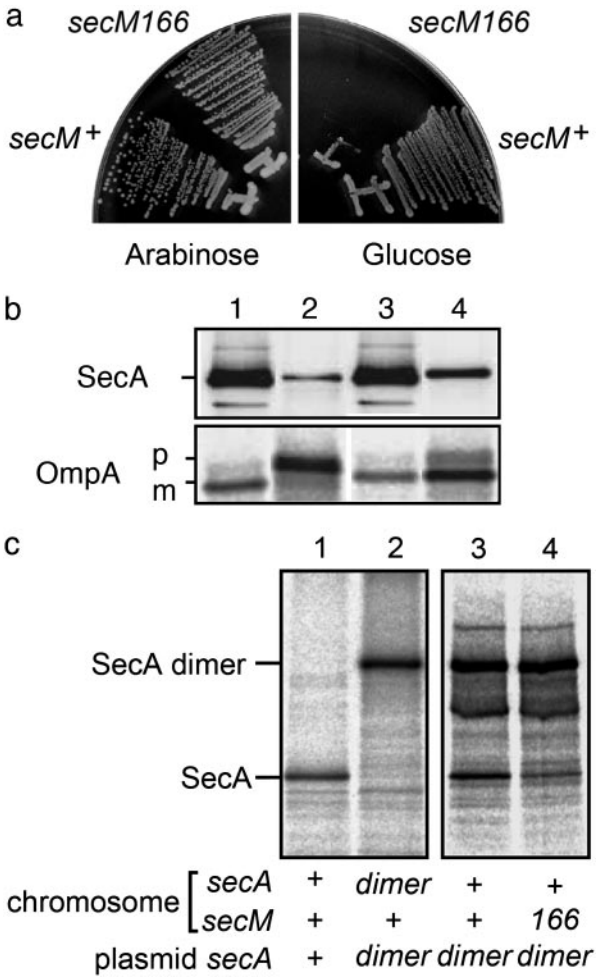

Fig. 1. Effects of the arrest-abolishing *secM166* mutation on cell viability, protein export, and SecA expression. (*a*) The *secM166* mutation is lethal unless excess SecA is provided from plasmid. Strains AN144 (*secM*$^+$/pAN31) and AN145 (*secM166*/pAN31) were grown on L-agar plates containing chloramphenicol and either 0.2% arabinose or 0.4% glucose at 37°C overnight. (*b*) Reduced SecA level and OmpA export in the *secM166* mutant after shutting off the complementing SecA. The above pair of *secM166* (lanes 1 and 2) and *secM*$^+$ (lanes 3 and 4) strains were grown at 37°C in M9-amino acids-arabinose (0.2%) medium. Cells were then washed three times with M9-salts by centrifugations and resuspensions and grown further in the medium containing 0.4% glucose instead of arabinose with appropriate dilutions. Samples were withdrawn after one doubling (lanes 1 and 3) and after 600-min growth (lanes 2 and 4). SecA abundance (*Upper*) and OmpA export (*Lower*) were examined by immunoblotting and pulse labeling/immunoprecipitation, respectively. Quantifications revealed that the eventual SecA abundance in *secM166* (lane 2) was only ≈20% of the steady-state wild-type (MC4100) level, whereas that in *secM*$^+$ (lane 4) was essentially identical with the wild-type abundance. p, precursor form; m, mature form. (*c*) Cis-specific *secM166* effect on SecA biosynthesis in growing states. Cells of CU141 (*secA*$^+$) carrying pAN5 (*secA*$^+$) (lane 1), CK4706 (*secA*-dimer) carrying pAN37 (lane 2, *secA*-dimer), AN169 (lane 3, *secM*$^+$/pAN37), and AN173 (lane 4, *secM166*/pAN37) were grown at 37°C to an early exponential phase in the presence of 1 mM isopropyl β-D-thiogalactoside and subjected to pulse labeling and SecA immunoprecipitation. Comparison of the radioactivities for the chromosomal SecA monomer in lanes 3 and 4 indicates a 3-fold reduction in the latter.

and *secM155* mutations, which only moderately affected the elongation arrest (12). These leaky mutations were successfully introduced into the chromosome without any complementing plasmid. Straightforward experiments using the chromosomal *secM150* and the *secM155* mutants under growing conditions showed that they synthesized SecA at levels of ≈80% and ≈60%

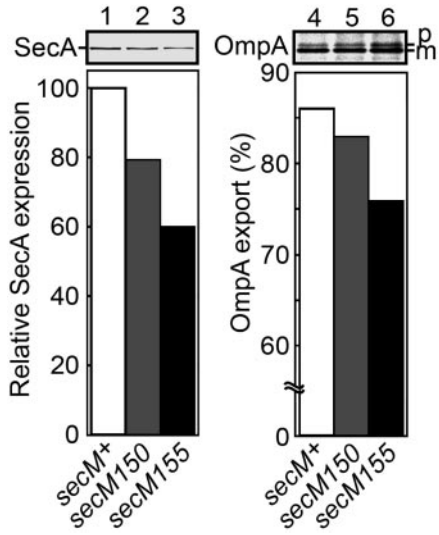

Fig. 2. SecA synthesis and protein export in viable *secM* mutants. Strains AN52 (lanes 1 and 4, *secM*$^+$), AN26 (lanes 2 and 5, *secM150*), and AN27 (lanes 3 and 6, *secM155*) were grown at 37°C in the presence of 0.4% glucose and subjected to pulse-labeling analyses of SecA biosynthesis (lanes 1–3) and OmpA export (lanes 4–6). The SDS/PAGE images of SecA and quantifications of the SecA intensities (relative to the wild-type value) are shown below lanes 1–3. The OmpA labeling patterns and the proportions of the mature (exported) species are shown below lanes 4–6. p, precursor form; m, mature form.

of the wild-type level, respectively (Fig. 2, lanes 1–3). Weak but significant retardation was observed for export of OmpA in these mutants (Fig. 2, lanes 4–6). These results, taken together, indicate that the normal *secM* is required in the cis-position of *secA* for its normal level expression and for normal protein export activity of the cell. The severity of the mutational effect on elongation arrest correlated with the extent of reduction in SecA level, consistent with a notion that duration of the arrest determines the level of *secA* translation (see *Discussion*).

Importance of the SecM Translation Arrest Under Sec Translocase-Compromised Conditions. When cells carrying chromosomal *secA* dimer and *secM-secA* on plasmid were exposed to NaN_3, an inhibitor of SecA (28), for 30 min, cellular abundance of both of these SecA species was up-regulated (Fig. 3*a*, compare lanes 1 and 7; Fig. 3*b*). Effects of different *secM* mutations on the SecA levels were then studied. Whereas the chromosomal *secA* dimer expression responded to azide addition in all of the mutant strains (Fig. 3*a*, compare lanes 1–6 and 7–12 for the SecA dimer), plasmid-encoded SecA monomer responded to the inhibitor to different extents depending on the *secM* alleles (Fig. 3 *a* and *b*). Whereas the level of SecA encoded by the wild-type *secM-secA* gene complex on the plasmid increased ≈3-fold in the presence of azide, such induction of SecA monomer was not observed at all when the upstream *secM* had either of the severest arrest-abolishing mutations, *secM166* and *secM163* (ref. 12; Fig. 3*b*). The leakier *secM155* and *secM150* mutations also reduced the azide response significantly, the effect of the former being more pronounced than the latter (Fig. 3*b*). In contrast, substitution for an unimportant residue, Pro-153 (12), gave nearly normal azide response (Fig. 3*b*). Thus, the azide response of *secA* expression correlated with the elongation arresting abilities of cis-located *secM*.

We found that the *secM155* chromosomal mutant was sensitive to a low concentration (0.5 mM) of NaN_3 (Fig. 3*c*). Thus, SecM has a positive role in the survival of the *E. coli* cell under conditions of reduced SecA function. We also studied whether SecM has a role of circumventing a SecY defect. Thus, we

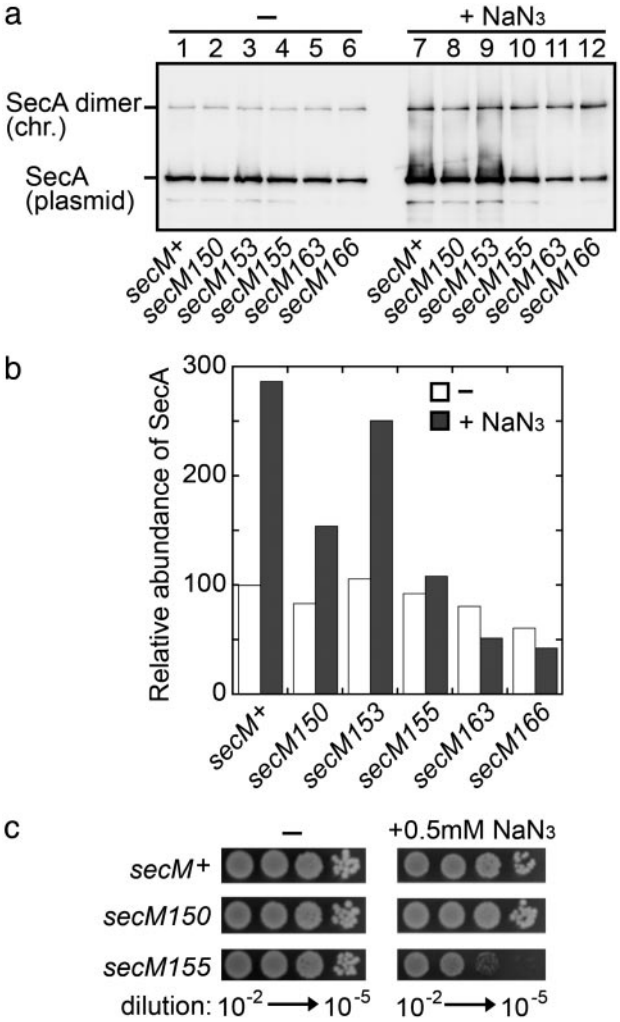

Fig. 3. Effects of *secM* mutations on azide induction of SecA. (*a* and *b*) Azide response of SecA abundance. (*a*) CK4706 (*secA* dimer) was transformed with *secM-secA* plasmids with the indicated *secM* alleles. Cells were grown at 37°C in L–ampicillin medium throughout (lanes 1–6) or in the presence of 0.2% NaN_3 for the last 30 min (lanes 7–12). SecA abundance was examined by anti-SecA immunoblotting. (*b*) Abundance of the SecA monomer relative to that encoded by the *secM*$^+$-*secA* plasmid in the absence of NaN_3 (lane 1 in *a*) is depicted graphically for each strain grown in the absence (open column) or presence (filled column) of NaN_3. (*c*) Cell growth in the presence of a low concentration of azide. AN52 (*secM*$^+$), AN26 (*secM150*), and AN27 (*secM155*) were fully grown in L-medium and diluted 10^2-, 10^3-, 10^4-, and 10^5-fold, as indicated, with 0.9% NaCl. Three microliter portions were then spotted onto L-agar plates that contained 0 (*Left*) or 0.5 mM (*Right*) NaN_3, followed by incubation at 37°C overnight.

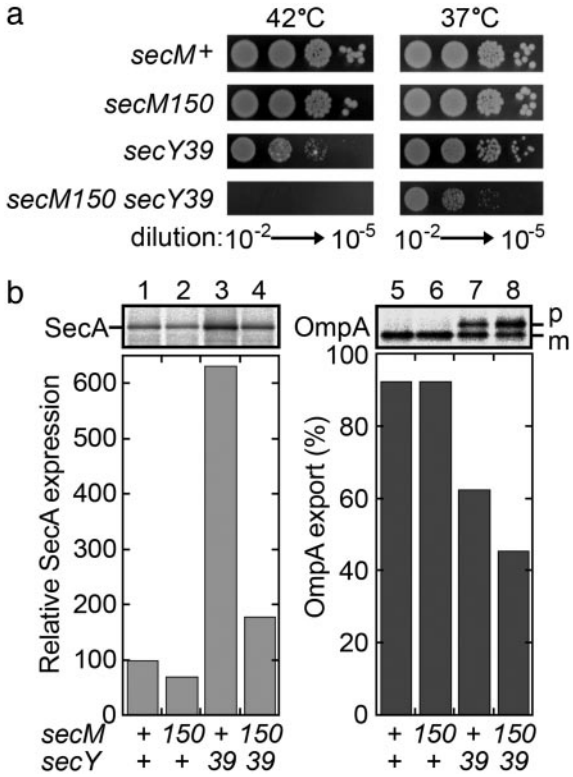

Fig. 4. The *secM150* mutation exacerbates the defect of the *secY39* mutant. (*a*) Cell growth at 37°C and 42°C. Growth of AN210 (wild type), AN211 (*secM150*), AN205 (*secY39*), and AN206 (*secM150 secY39*) were examined on L-agar plates at 37°C or at 42°C, as described in the legend to Fig. 3*c*. (*b*) SecA biosynthesis and OmpA export in the mutants. SecA expression (lanes 1–4) and OmpA export (lanes 5–8) were examined for the four strains used in *a* by pulse labeling and immunoprecipitation. Temperature was shifted to 42°C 20 min before the pulse labeling. p, precursor form; m, mature form.

attempted to combine either the *secM155* or the *secM150* mutation with *secY39*, a cold-sensitive mutation with different extents of protein export defects at different temperatures (29, 30). However, we were unable to construct a *secM155-secY39* double mutant, due presumably to a synthetic lethal consequence of this combination. In contrast, the less defective *secM150* mutation could be combined with *secY39* at 37°C, resulting in a slow-growing double mutant (Fig. 4*a*). This double mutant was found to be unable to grow at 42°C (Fig. 4*a*). The *secY39* single mutant exhibited a moderate defect in export of OmpA at 42°C, consistent with a previous note that this mutation itself showed weak temperature sensitivity in some strain backgrounds (30). We now found that the *secY39* export defect at 42°C was exaggerated in the *secM150-secY39* double mutant (Fig. 4*b*, lane 8). Moreover, the up-regulation of SecA by the *secY39* mutation (Fig. 4*b*, lane 3) was greatly suppressed in the double mutant (Fig. 4*b*, lane 4) despite the severer export defect. These results indicate that SecM has a role in better survival of cells under the conditions of lowered activities of Sec translocase.

SecA Is Cold-Inducible. It is questionable whether *E. coli* encounters azide and whether it must prepare against a forthcoming *sec* mutation in natural settings. Pogliano and Beckwith (31) showed that protein secretion in *E. coli* is intrinsically cold-sensitive. We examined whether SecA expression is up-regulated at low temperature. Pulse-labeling and SecA immunoprecipitation experiments indeed revealed that the differential synthesis rate of SecA in a *secM*$^+$ strain HPT264 was ≈3-fold higher at 20°C than at 37°C (data not shown). Higher SecA accumulation at 20°C is also illustrated in Fig. 5*b* for strain NH592.

Growth of the *secM155* mutant was abnormal at 20°C in that it formed mucoid colonies. Furthermore, we found that the *secM98* mutant described by Tian and Beckwith (22) was cold-sensitive and defective in SecA induction at 20°C. The protein export defects seen in *secM155* (Fig. 2) and in *secM98* (22) were exacerbated significantly at the low temperature (data not shown). These observations suggest that SecM function is required for the cold induction of SecA.

Proper SecM–Ribosome Interaction Is Required for the Normal SecA Expression and Its Regulation. We described a mutation in the 23S ribosomal RNA, *rrlB2058*, in its domain V, affecting the constricted part of the exit tunnel and partially alleviating the SecM

MICROBIOLOGY

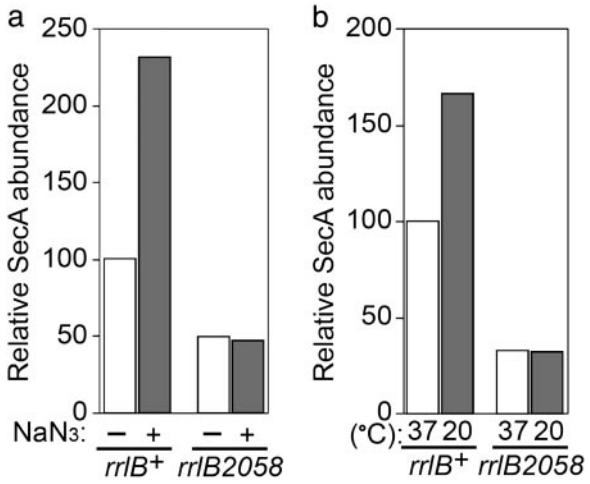

Fig. 5. The *rrlB2058* tunnel mutation affects SecA regulation. Strains NH592 (Δ*7rrn*/*rrlB*$^+$) and NH593 (Δ*7rrn*/*rrlB2058*) were cultured at 37°C in L-medium containing ampicillin and spectinomycin, and portions were treated with 0.02% NaN_3 for 1 h (*a*) or temperature-shifted to 20°C for 2 h (*b*). SecA abundance was determined by anti-SecA immunoblotting. Values relative to that of the untreated *rrlB*$^+$ strain are shown.

elongation arrest (12). We constructed an *E. coli* strain having *rrlB2058* as a sole rRNA allele (see *Materials and Methods*). Although it was viable at 37°C, its SecA abundance was only ≈50% that of the corresponding *rrlB*$^+$ strain (Fig. 5*a*, open columns). Furthermore, the SecA abundance in the *rrlB2058* mutant did not elevate either in response to azide addition (Fig. 5*a*) or in response to a temperature shift to 20°C (Fig. 5*b*). We found that growth of the mutant cells was much more sensitive to azide than that of the *rrlB*$^+$ cells (data not shown). Although the *rrlB2058* mutant continued to grow at the low temperature for the time scale of the Fig. 5*b* experiment, its growth on agar plates was very poor at 20°C (data not shown). These results show that not only the SecM sequence but also the normal ribosomal components are required for cells to have a normal level of SecA and to up-regulate the SecA synthesis in response to secretion-inhibiting environmental conditions.

Discussion

The *secM-secA* intergenic region of the messenger RNA can form stem-loop secondary structures, one of which occludes the ribosome-binding SD sequence of *secA* within a stem and is responsible for the secretion-defect response (32). This feature of mRNA is well corroborated by the properties of SecM, which monitors its own secretion status to affect effectiveness of the elongation arrest at the C-terminal region. This event will generate the stalled ribosome, which disrupts the mRNA secondary structure and thereby facilitates entry of new ribosomes for *secA* translation (9, 11, 12, 15). Although the ribosome stall might also activate a recently described ribonucleolytic quality control mechanism (33–35), its significance in the regulation is unknown.

Although the elongation arrest of SecM is released soon in normal cells, presumably by the pulling force generated by the nascent chain translocation across the membrane (11, 12, 14, 36), our results now demonstrated that even such transient arrest is important for *E. coli* to express SecA at sufficient levels. Indeed, mutational alterations of the arrest-important amino acid residues in SecM reduced the expression of the cis-located *secA* gene. The extent of this reduction was ≈70%, 40%, and 20% for the *secM166*, *secM155*, and *secM150* mutations, respectively. These values correlated with the extents of the arrest suppression by the mutations (12), rendering a support to a notion that the frequency of *secA* translation initiation is determined by the duration of the elongation arrest; the arrest will provide a time window, in which the *secA* SD sequence is accessible to ribosomes. Without this mechanism, the *E. coli* cell does not acquire enough amount of SecA required for growth. This notion is now supported strongly by our finding that the arrest-defective chromosomal *secM166* mutation is lethal in a manner it is complemented by overproduction of SecA.

It must be stressed that our results indicate that the elongation arresting property of SecM is important not only for the secretion-defect response but also for the basal level expression of *secA*. It is conceivable further that variations in the translocase activities within a physiological range could result in the fine-tuning of the level of SecA translation. In addition, our recent results indicate that SecM has a positive role in the better functioning of SecA presumably through its ability to target the mRNA to the membrane (H.N., A.M., and K.I., unpublished results).

Another conclusion of this study is that SecM elongation arrest is important for cells to cope with conditions of lowered Sec translocase. First, addition of azide up-regulated the SecA synthesis/abundance in combination with cis-located *secM*$^+$ or *secM153* (a silent allele), but not in combination with the arrest-compromising alleles of *secM*. The azide response was also lost by the *rrlB2058* ribosomal mutation affecting the exit tunnel and partially canceling the SecM elongation arrest. The *secM155* and the *rrlB2058* mutants proved to be hypersensitive to azide.

Second, the *secM150* mutation suppressed the SecA elevation caused by the *secY39* secretion-defective mutation. Moreover, synthetic growth defect was observed when this *secY* mutation was combined either with *secM150* or *secM155*. The *secY39* alteration in SecY impairs the SecY's ability to activate the SecA ATPase (37, 38) and thereby to facilitate the initial steps of preprotein translocation (39). The defect can be suppressed by *secA* mutations that up-regulate the ATPase activity (39). These properties of SecY39 are consistent with its increased dependence on the SecM's function to up-regulate the SecA expression.

Finally, we found that the SecA abundance was increased at 20°C, in accordance with the notion that the *E. coli* protein export processes include some intrinsically cold-sensitive elements (31). This cold-induction of SecA was seen neither in the *secM98* nor the *rrlB2058* mutant. These mutants also grew poorly at low temperature. The cold-sensitivity of the ribosomal mutant could be ascribed to the lack of SecA induction, some translation defect, or both. At any rate, SecM elongation arrest that involves normal interaction between the arrest sequence and the ribosome is clearly required for the cold-induction of SecA. Undoubtedly, low temperature is a major environmental condition that *E. coli* cells encounter in nature and in which the secretion-defect response takes place.

In this study, we have established the physiological importance of SecA regulation that involves SecM. In the evolution of this system, the features of interaction between the SecM arrest sequence and the ribosomal exit tunnel must have been exploited to fit the regulatory purpose. SecM represents a new class of proteins, whose regulatory functions are expressed in their ribosome-tethered nascent states by virtue of its interactions with the ribosomal interior components.

We thank Hiroyuki Mori and Yoshinori Akiyama for materials and useful discussion; Catherine L. Squires and Jon Beckwith for bacterial strains; and Kiyoko Mochizuki, Michiyo Sano, and Kunihito Yoshikaie for their technical support. This work was supported by Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, and by grants from Japan Society for the Promotion of Science and the National Project on Protein Structural and Functional Analyses of the Ministry of Education, Culture, Sports, Science, and Technology (Japan). H.N. was supported by a Japan Society for the Promotion of Science fellowship for Japanese Junior Scientists.

1. Mori, H. & Ito, K. (2001) *Trends Microbiol.* **9,** 494–500.
2. Driessen, A. J., Manting, E. H. & van der Does, C. (2001) *Nat. Struct. Biol.* **8,** 492–498.
3. van den Berg, B., Clemons, W. M., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C. & Rapoport, T. A. (2004) *Nature* **427,** 36–44.
4. Hunt, J. F., Weinkauf, S., Henry, L., Fak, J. J., McNicholas, P., Oliver, D. B. & Deisenhofer, J. (2002) *Science* **297,** 2018–2026.
5. Or, E., Navon, A. & Rapoport, T. (2002) *EMBO J.* **21,** 4470–4479.
6. Ding, H., Mukerji, I. & Oliver, D. (2003) *Biochemistry* **42,** 13468–13475.
7. Oliver, D. B. & Beckwith, J. (1982) *Cell* **30,** 311–319.
8. Schmidt, M. G., Rollo, E. E., Grodberg, J. & Oliver, D. B. (1988) *J. Bacteriol.* **170,** 3404–3414.
9. Oliver, D., Norman, J. & Sarker, S. (1998) *J. Bacteriol.* **180,** 5240–5242.
10. Rajapandi, T., Dolan, K. M. & Oliver, D. B. (1991) *J. Bacteriol.* **173,** 7092–7097.
11. Nakatogawa, H. & Ito, K. (2001) *Mol. Cell* **7,** 185–192.
12. Nakatogawa, H. & Ito, K. (2002) *Cell* **108,** 629–636.
13. Jenni, S. & Ban, N. (2003) *Curr. Opin. Struct. Biol.* **13,** 212–219.
14. Butkus, M. E., Prundeanu, L. B. & Oliver, D. B. (2003) *J. Bacteriol.* **185,** 6719–6722.
15. Sarker, S. & Oliver, D. (2002) *J. Bacteriol.* **184,** 2360–2369.
16. Silhavy, T. J., Berman, M. L. & Enquist, L. W. (1984) *Experiments with Gene Fusions* (Cold Spring Harbor Lab. Press, Plainview, NY).
17. Matsumoto, G., Yoshihisa, T. & Ito, K. (1997) *EMBO J.* **16,** 6384–6393.
18. Sawano, A. & Miyawaki, A. (2000) *Nucleic Acids Res.* **28,** E78.
19. Guzman, L. M., Belin, D., Carson, M. J. & Beckwith, J. (1995) *J. Bacteriol.* **177,** 4121–4130.
20. McFarland, L., Francetic, O. & Kumamoto, C. A. (1993) *J. Bacteriol.* **175,** 2255–2262.
21. Shimoike, T., Taura, T., Kihara, A., Yoshihisa, T., Akiyama, Y., Cannon, K. & Ito, K. (1995) *J. Biol. Chem.* **270,** 5519–5526.
22. Tian, H. & Beckwith, J. (2002) *J. Bacteriol.* **184,** 111–118.
23. Akiyama, Y., Ogura, T. & Ito, K. (1994) *J. Biol. Chem.* **269,** 5218–5224.
24. Ueguchi, C., Wittekind, M., Nomura, M., Akiyama, Y. & Ito, K. (1989) *Mol. Gen. Genet.* **217,** 1–5.
25. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY)
26. Asai, T., Zaporojets, D., Squires, C. & Squires, C. L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 1971–1976.
27. Xiong, L., Kloss, P., Douthwaite, S., Andersen, N. M., Swaney, S., Shinabarger, D. L. & Mankin, A. S. (2000) *J. Bacteriol.* **182,** 5325–5331.
28. Oliver, D. B., Cabelli, R. J., Dolan, K. M. & Jarosik, G. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8227–8231.
29. Baba, T., Jacq, A., Brickman, E., Beckwith, J., Taura, T., Ueguchi, C., Akiyama, Y. & Ito, K. (1990) *J. Bacteriol.* **172,** 7005–7010.
30. Taura, T., Akiyama, Y. & Ito, K. (1994) *Mol. Gen. Genet.* **243,** 261–269.
31. Pogliano, K. J. & Beckwith, J. (1993) *Genetics* **133,** 763–773.
32. McNicholas, P., Salavati, R. & Oliver, D. (1997) *J. Mol. Biol.* **265,** 128–141.
33. Hayes, C. S. & Sauer, R. T. (2003) *Mol. Cell* **12,** 903–911.
34. Sunohara, T., Jojima, K., Yamamoto, Y., Inada, T. & Aiba, H. (2004) *RNA* **10,** 378–386.
35. Sunohara, T., Jojima, K., Yamamoto, Y., Inada, T. & Aiba, H. (2004) *J. Biol. Chem.* **279,** 15368–15375.
36. Nakatogawa, H. & Ito, K. (2004) *Chembiochem* **5,** 48–51.
37. Taura, T., Yoshihisa, T. & Ito, K. (1997) *Biochimie* **79,** 517–521.
38. Mori, H. & Ito, K. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 5128–5133.
39. Mori, H. & Ito, K. (2003) *J. Bacteriol.* **185,** 405–412.