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Identification of a SecM segment required for export-coupled release from elongation arrest

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ABSTRACT

SecM in *Escherichia coli* has two functionally crucial regions. The arrest motif near the C-terminus interacts with the ribosomal exit tunnel to arrest its own translational elongation. The signal sequence at the N-terminus directs the SecM nascent polypeptide to the Sec-mediated export pathway to release the arrested state of translation. Here, we addressed the importance of the central region of SecM. Characterization of internal substitution and deletion mutants revealed that a segment from residue 100 to residue109 is required for the export-coupled release of the SecM nascent chain from the elongation-arrested state. Thus, the central region of SecM is not just a geometric linker but it participates actively in the regulation of translation arrest.

1. Introduction

Protein localization to the bacterial cell envelope involves total or partial translocation of newly synthesized proteins across the cytoplasmic membrane. Signal peptides direct preproteins to the SecYEG translocon for the transmembrane movement, which is driven by the SecA translocation ATPase [1,2]. The expression level of SecA is feedback controlled by the cellular activities of protein export, such that it is upregulated in response to a defect in the Sec pathway [3]. In *Escherichia coli*, SecM plays a key role in this regulation [4–6]. It is encoded by an open reading frame of 170 codons located upstream of *secA* [7,8]. Translation of *secM* undergoes elongation arrest [5,6] in such a way that the nascent peptidyl-glycyl^{165}-tRNA resides in the P-site and Prolyl^{166}-tRNA resides in the A-site of the ribosome [9]. The nascent SecM polypeptide interacts with the components of the ribosomal exit tunnel [6], leading to transpeptidation-incompatible configurations of the ribosomal peptidyl transferase center as well as of the P-site peptidyl-tRNA and the A-site prolyl-tRNA [10,11]. Several amino acid residues of SecM, including Pro166 and Arg163, are required for the elongation arrest [6,12], although Pro166 is actually not incorporated into a polypeptide in the arrested translation complex [9]. The stalled ribosome prevents the intergenic region of the *secM–secA* mRNA from forming a stem-loop structure that sequesters the translation initiation (Shine-Dalgarno) sequence of *secA* [13], hence allowing de novo initiation of translation of *secA*. Thus, the translation level of *secA* correlates positively with the duration of the SecM translation arrest [5,14,15]. Importantly, arrested translation resumes when the SecM nascent chain engages in the Sec-mediated export process [5,6]. This "arrest release" process appears to be triggered by physical force of export that is applied to the nascent chain [16–18].

SecM represents a peculiar protein that functions in its nascent, ribosome-tethered state. The full-length SecM, which is rapidly degraded in the periplasm, is thought to be devoid of any function [5], raising a question about the role of its main body flanked by the signal and the arrest sequences. Here, we addressed this question and found that a segment of residues 100–109 plays an important role in the export-coupled arrest release.

2. Results

2.1. Stability of SecM translation arrest

The transient translation arrest of SecM in export-proficient *E. coli* cells is required for the basal level expression of SecA [15]. We assessed the rate of the arrest release in wild-type cells at 37 °C. To avoid the rapid proteolysis of the mature form of SecM by the C-terminal-tail-specific protease, Prc, [5], we attached the Flag-tag to its C-terminus (Fig. S1). SecM-Flag was induced briefly, pulse-labeled with [^{35}S]methionine for 0.5 min and isolated by

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anti-SecM immunoprecipitation. SDS–polyacrylamide gel electrophoresis revealed three radioactive bands, A, P and M (Fig. 1A). Band A represents the polypeptide moiety of the arrested SecM$^{1-165}$-tRNA. Although the material that gave this band had been linked to a tRNA in vivo [5], the ester linkage between the polypeptide and tRNA was hydrolyzed during immunoprecipitation and electrophoresis [9,19]. During a chase with unlabeled methionine, the band A intensity declined with a concomitant increase in that of band M, which represented the signal-peptide processed mature form of SecM. This conversion takes place through the full-length precursor protein (band P), in which signal peptide processing only occurs after the arrest release (H. Muto, H. Mori, Y. Akiyama and K. Ito, unpublished results). The intensity decrease of band A during chase indicated that the half life of the arrested product of SecM-Flag was ~60 s (Fig. 1A; Fig. S2, WT).

2.2. Alterations of the SecM central part affect arrest release

The mature sequence of SecM starts at Ala38 (Fig. S1) [8]. We constructed a series of substitution mutants of SecM-Flag, referred to as Sub(40–69), Sub(70–99) and Sub(100–129), in which the indicated SecM segments were replaced with an unrelated amino acid sequence derived from LacZ (Fig. S1; Table 1). Pulse-chase experiments (Fig. 1) showed that the arrest band was markedly stabilized in Sub(100–129) (Fig. 1D); the half life of the arrest product was increased at least twofold (Fig. S2). Sub(40–69) also produced a somewhat stabilized arrest product (Fig. 1B). The arrest product of Sub(70–99) appeared to be destabilized (Fig. 1C; note that this variant lacks Met97 such that its mature form is invisible). These results suggest that the central region of SecM plays some role in the regulation of the elongation arrest.

Table 1
SecM variants examined.

Abbreviated name	Amino acid alterations introduced into SecM-Flag	Plasmid name
WT	None	pKN1
Sub(40–69)	Residues 40–69 replaced with LacZ(301–330)	pKN2
Sub(70–99)	Residues 70–99 replaced with LacZ(301–330)	pKN3
Sub(100–129)	Residues 100–129 replaced with LacZ(301–330)	pKN4
Sub(90–99)	Residues 90–99 replaced with LacZ(311–320)	pKN117
Sub(100–109)	Residues 100–109 replaced with LacZ(311–320)	pKN118
Sub(110–119)	Residues 110–119 replaced with LacZ(311–320)	pKN119
Δ(90–99)	Residues 90–99 deleted	pKN10
Δ(100–109)	Residues 100–109 deleted	pKN11
Δ(110–119)	Residues 110–119 deleted	pKN12
Δ(120–129)	Residues 120–129 deleted	pKN13
Δ(130–139)	Residues 130–139 deleted	pKN14
Pro166Ala	Pro166Ala	pKN5
Pro166Ala Sub(100–129)	Pro166Ala, residues 100–129 replaced with LacZ(301–330)	pKN8
Pro166Ala Δ(100–109)	Pro166Ala, residues 100–109 deleted	pKN15

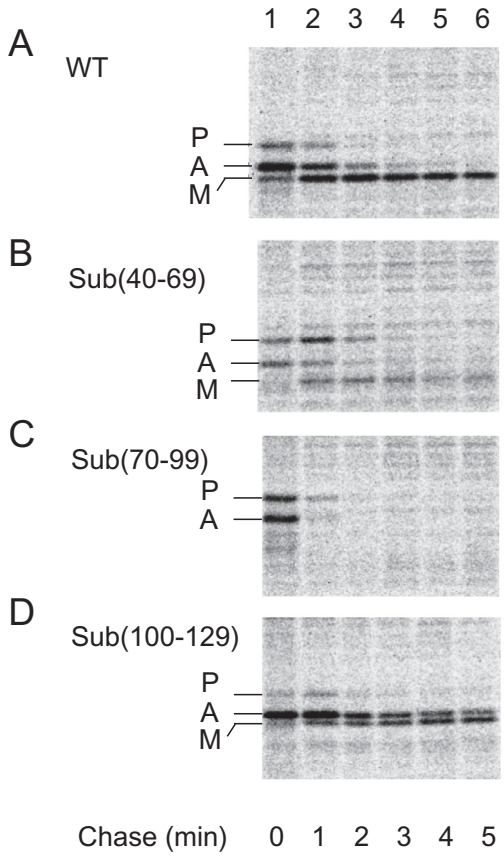

Fig. 1. Pulse-chase labeling of SecM-Flag and its variants with a 30 residues substitution in the central part. *E. coli* cells expressing SecM-Flag (A) and its Sub(40–69) (B), Sub(70–99) (C) and Sub(100–129) (D) derivatives were pulse-labeled with [^{35}S]methionine for 0.5 min at 37 °C (lane 1), followed by chase with unlabeled methionine for 1 (lane 2), 2 (lane 3), 3 (lane 4), 4 (lane 5) and 5 (lane 6) min. Radioactive SecM-Flag polypeptides were isolated by immunoprecipitation, using anti-SecM antibodies, and separated by SDS–polyacrylamide gel electrophoresis into three species; A, P and M. Band A represents the polypeptide moiety of the elongation-arrested SecM peptidyl-glycyl-tRNA. Band P represents the full-length SecM secretory precursor retaining the signal peptide. Band M represents the signal-peptide processed mature form of SecM.

To gain further insights into the role of the central region of SecM, we introduced 10 residue-deletions starting from residue 90 (Δ(90–99)), residue 100 (Δ(100–109)), residue 110 (Δ(110–119)), residue 120 (Δ(120–129)) and residue 130 (Δ(130–139)) (Fig. 2). Among the five mutant proteins tested, the arrest band of Δ(100–109) uniquely persisted up to the 5 min chase period tested (Fig. 2B, lane 6) and its half life was estimated to be ~180 s (Fig. 2; Fig. S3). The other deletion mutants were normal in stability of the arrest products. These results show that the segment 100–109 of SecM is required for the efficient arrest release. This was substantiated by constructing and analyzing SecM-Flag derivatives, in which segments 90–99, 100–109 and 110–119 were replaced with residues 301–310 of LacZ (Fig. 3). Pulse chase analysis showed that the Sub(100–109) mutation stabilized the arrest product of SecM (Fig. 3B). By contrast, neither the Sub(90–99) nor the Sub(110–119) mutation impaired the arrest release (Fig. 3A and C); the former somewhat accelerated it.

Given the evidence that the segment 100–109 is involved in the arrest release of SecM, we mutated each of the 10 residues to alanine, except for Ala105 that was mutated to Ser. None of the mutants showed a defect in the arrest release as strongly as Δ(100–109) or Sub(100–109). It is suggested that this segment as a whole contributes to the efficient arrest release of SecM in export-proficient cells in a manner that a single substitution is tolerated.

2.3. Segment 100–109 of SecM is not intrinsically required for the export competence

Engagement of the SecM nascent polypeptide in the SecA-SecYEG-mediated export across the membrane is a prerequisite for its release from the elongation-arrested state [5,6]. Therefore, the arrest release defect observed with Sub(100–129),

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