SUPPLEMENTARY MATERIAL for:

The translational regulatory function of SecM requires the precise timing of membrane targeting

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Table S1. Oligonucleotides used in this study.

Primer set	Sequence (5'-3')	Application
P390 P008	ACCGGCAGGAAACAGACCATGGGTGG CTACTGCCGCCAGGCAAATTC	amplify *secM*
P515 P516	AAACAGACCATG**AGT**GGAATACTGA TCAGTATTCCAC**TCA**TGGTCTGTTT	construct pMY1
P257 P258	GAACACCTGGTGTTCTGAAACGGAGGCCGGGCCAG GCATGGTGTAGGCTGGAGCTGCTTC TAAAAAATCAGGCACAATTTCTTGTGCCTGATTGATA TTACATATGAATATCCTCCTTAG	construct *Δprc* allele
P400 P008	GGAATACCCATGGGCTGGCGACAG CTACTGCCGCCAGGCAAATTC	construct $\Delta5_{SP}$SecM
P401 P402	GGAATACCCATG**CGC**TGGCGACAGT ACTGTCGCCAGC**GCA**TGGGTATTCC	remove G2R mutation
P425 P426	CACAGGAAACAGACCATGGGTAAACGCTACTTCTGG CCGCAT ATGCGGCCAGAAGTAGCGTTTACCCATGGTCTGTTT CCTGTG	construct $\Delta10_{SP}$SecM
P357 P358	CAGCAACGGATCCGAACCAAACG CGTTTGGTTCGGATCCGTTGCTGAGCG	introduce BamHI site into *secM*
P381 P382	ACGAGGCGCAAACCATGGTGAAAAAGAC ACCAGGTGTTATCGGATCCAGCGGCCTGC	amplify $OmpA_{SP}$
P511 P512	AAACAGACCATGAAAAAGACAGCT AGCTGTCTTTTTCATGGTCTGTTT	remove extra valine
P509 P510	GGTTTCGCTACC**TGT**GCGCAGGCCGCT AGCGGCCTGCGC**ACA**GGTAGCGAAACC	construct $OmpA_{SP}$ (V18C)
P359 P360	CACGAGCACGGTACCAACAAGGACC ACCAGTTTACCGGATCCGATTTTGG	amplify MBP_{SP}
P394 P395	CATCCTCGCATTA**TTC**GCATTA**ATGCTG**ATGATGTTT TCCG CGGAAAACATCAT**CAGCAT**TAATGC**GAA**TAATGCGA GGATG	construct $MBP*1_{SP}$ (S13F/T16M/T17L)

P037 P458 P459 P008	CTGAAATGAGCTGTTGACAATTAATCATCCGG TTGAATTCGGCGGCGTTGCTGAGCG CGGAATTCACCCAGGAAGGCACGCC CTACTGCCGCCAGGCAAATTC	construct SecM (Δ38-127)
P037 P488 P459 P008	CTGAAATGAGCTGTTGACAATTAATCATCCGG TTGAATTCCATTGAAGGCTCGTGGTTGCGGGT CGGAATTCACCCAGGAAGGCACGCC CTACTGCCGCCAGGCAAATTC	construct SecM (Δ56-127)
P037 P488 P489 P008	CTGAAATGAGCTGTTGACAATTAATCATCCGG TTGAATTCCATTGAAGGCTCGTGGTTGCGGGT CGGAATTCGCACCGCAAACACTGCCCGTT CTACTGCCGCCAGGCAAATTC	construct SecM (Δ56-98)
P460 P461	ACTATTCCGTTGGATCCTGGCATCAACA TGTTGATGCCAGGATCCAACGGAATAGT	introduce BamHI site into *secM*
P484 P485	ATAGCATATGAAAATAAAAACAGG CGACGGCCGCGAGAGCCGAGGCGG	amplify $MBP*1_{SP}$
P361 P362	GGCAGGAAACAGCATATGAGTGGA CGCGTTTGGTTCGGCCGCGTTGCT	amplify $SecM_{SP}$
P043 P044	AGCCAGGCG**CCACCC**ATCCGTGCTGGC GCCAGCACGGAT**GGGTGG**CGCCTGGCT	introduce Q160P/ G161P mutation
P553 P554 P555	GTGGATCCAAATTCAGCCTGCAGAAACGCCTGTTTC AGAAATATGGCATTTGGCCGCCGCCGAGCATTCTGA CCTAATCTAGATA GTGGATCCAAATTCAGCCTGCA TATCTAGATTAGGTCAGAATGC	construct pMY26
P136 P134 P135	GTGGATCCCCGCACTTTTGCCTCTCTCCAAGCTACTT TCATGCGCCGATTCGCGGTAGTCCTCAACGCCTCAC CTAATCTAGATA GTGGATCCCCGCACTTTTGCCT TATCTAGATTAGGTGAGGCGTTGAG	construct pMY27

Restriction sites are underlined and codon changes are in bold.

SUPPLEMENTARY FIGURES

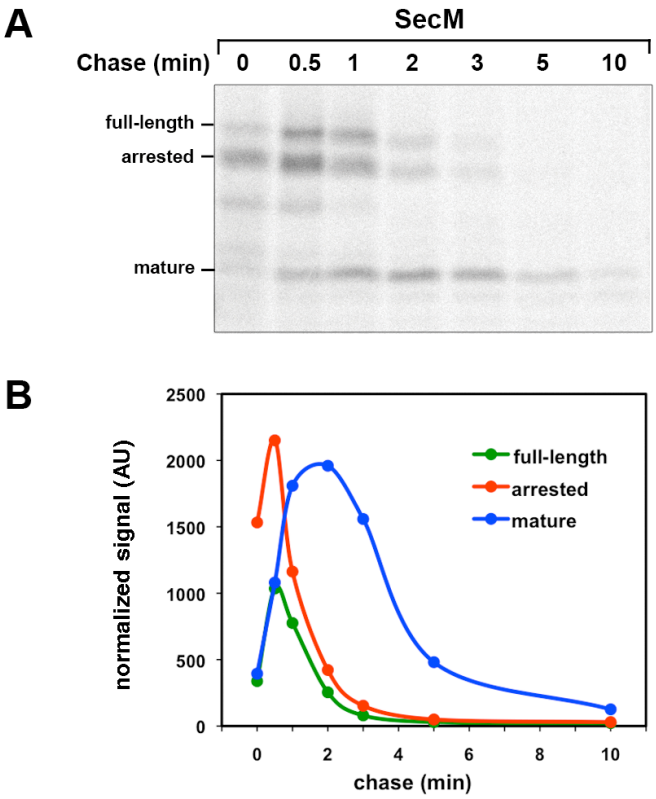

Fig. S1. Conversion of full-length SecM to the mature form. A. MNY3 transformed with a plasmid encoding wild-type SecM (pMY1) were subjected to pulse-chase labeling after the addition of IPTG to induce expression of the plasmid-borne gene and immunoprecipitations were conducted using an anti-SecM antiserum. The pulse was 30 sec. B. Quantitation of the data shown in part A. The radioactive signal generated by each band was normalized to take into account the difference in the number of methionine residues found in each form of the protein.

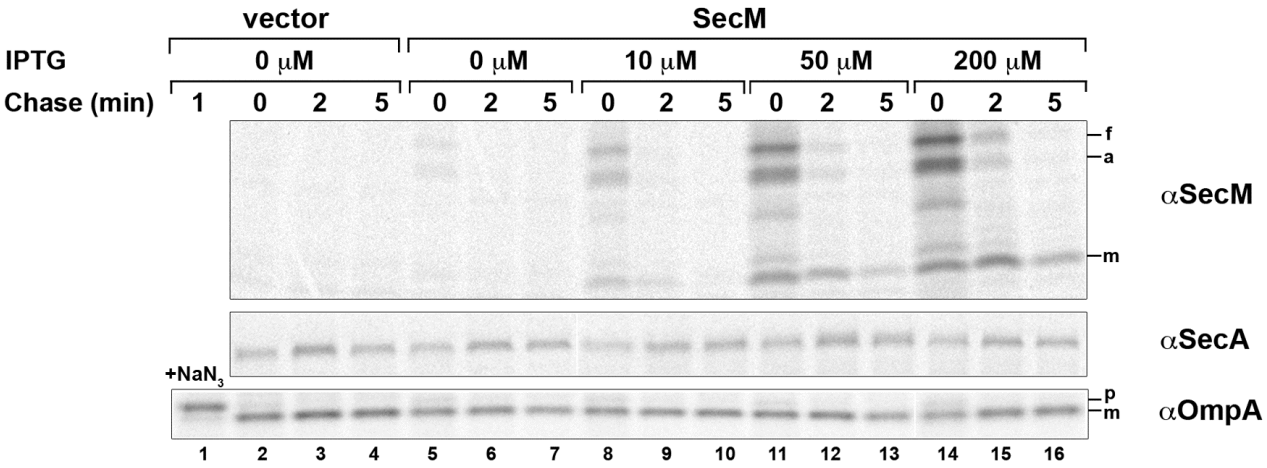

Fig. S2. Overproduction of SecM does not affect protein targeting or translocation. MNY3 transformed with the cloning vector (pTRC99a) or pMY1 were subjected to pulse-chase labeling after the addition of the indicated amount of IPTG to induce expression of the plasmid-borne gene and immunoprecipitations were conducted using anti-SecM, anti-SecA and anti-OmpA antisera. A separate aliquot of cells transformed with pTRC99a was removed and treated with 2 mM sodium azide (NaN_3) 2 min prior to labeling to show the position of the OmpA precursor.

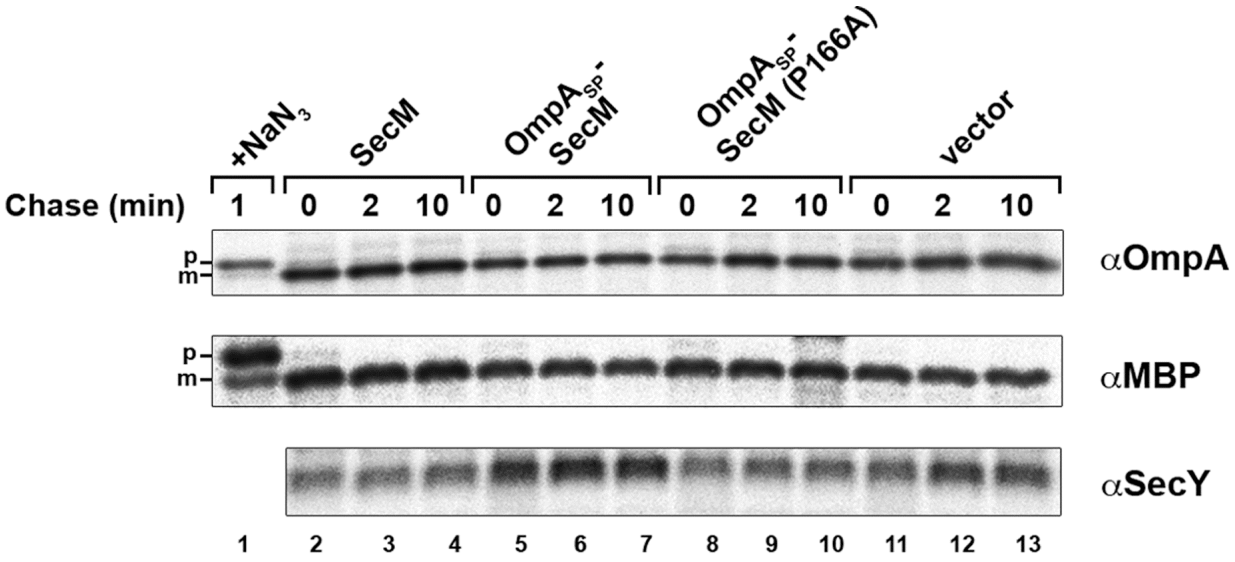

Fig. S3. Overproduction of stalled $OmpA_{SP}$-SecM RNCs does not jam or destabilize the SecYEG complex. MNY3 transformed with a plasmid that encodes the indicated SecM derivative or the cloning vector (pTRC99A) were grown in M9 and 200 μM IPTG was added to induce expression of the plasmid-borne gene. At the same time 0.2% maltose was added to induce expression of the endogenous *malE* gene. After 30 min cells were subjected to pulse-chase labeling, and immunoprecipitations were conducted with antisera generated against OmpA, MBP and SecY. To inhibit secretion and to show the position of the OmpA and MBP precursors, 2 mM sodium azide (NaN_3) was added to an aliquot of cells that produced wild-type SecM 2 min prior to labeling.

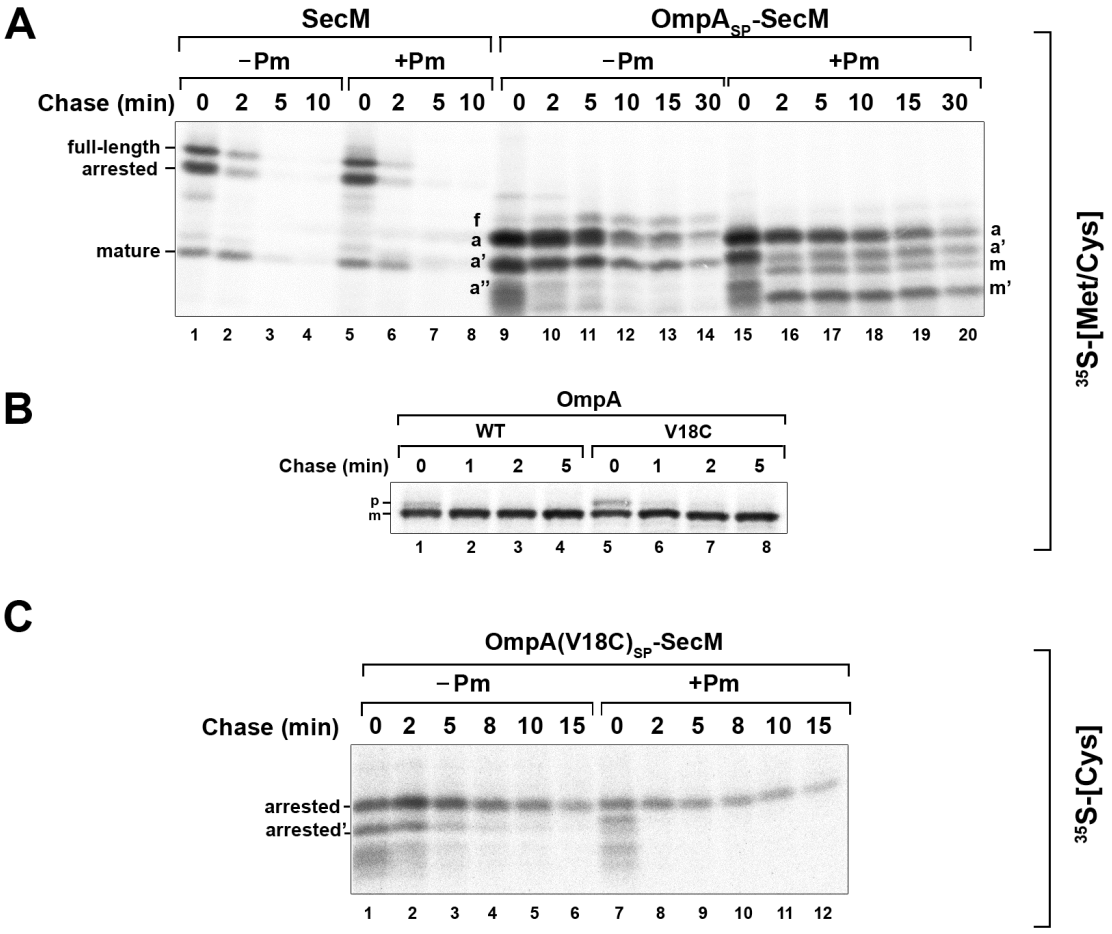

Fig. S4. Puromycin treatment promotes translocation of translation arrested OmpA$_{SP}$-SecM. A. MNY3 transformed with a plasmid encoding SecM or OmpA$_{SP}$-SecM were subjected to pulse-chase labeling with ^{35}S-[Met/Cys] after the addition of IPTG. At the time of the chase, half of the cells were treated with 5 mM puromycin. SecM-containing polypeptides were then immunoprecipitated. Consistent with previous results (Woolhead *et al.*, 2006), translation arrested SecM was resistant to puromycin treatment (lanes 1-8). In contrast, OmpA$_{SP}$-SecM nascent polypeptides that were tethered to lagging ribosomes (a' and a") were released by puromycin, and post-translational translocation of a' (which leads to the generation of m' after signal peptide cleavage) can be seen (lanes 9-20). Thus, the conversion of some of the translation arrested OmpA$_{SP}$-SecM (a) to the mature form (m) after the addition of puromycin presumably results from the clearance of trailing ribosomes and the concomitant membrane targeting and translocation of the translation arrested nascent chains. B. MC4100 transformed with a plasmid that encodes HA-tagged OmpA (pHL36) or a derivative harboring the V18C mutation were subjected to pulse-chase labeling after the addition of IPTG. The precursor (p) and mature (m) forms of the protein were then immunoprecipitated using anti-HA. The results show that the V18C mutation does not affect the targeting function of the signal peptide. C. The experiment shown in part A was repeated using MNY3 transformed with a plasmid that encodes OmpA(V18C)$_{SP}$-SecM except that cells were grown in M9 containing all of the L-amino acids except cysteine and were radiolabeled with ^{35}S-Cys. Because the only cysteine residue in the protein is in the signal peptide, only the precursor forms are radiolabeled. The results confirm that the polypeptides labeled m and m' in part A correspond to mature forms of OmpA$_{SP}$-SecM.

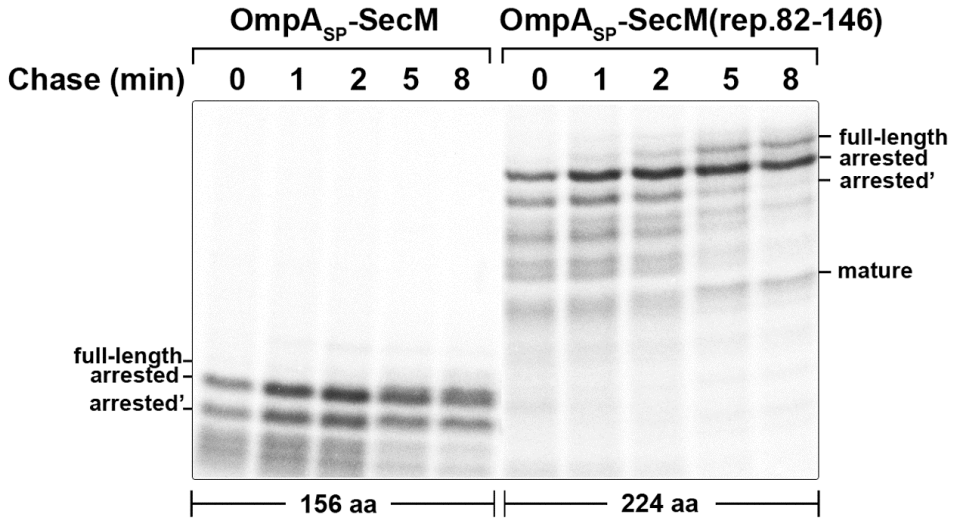

Fig. S5. Increasing the length of OmpA$_{SP}$-SecM enhances the release of translation arrest. MNY3 transformed with a plasmid encoding OmpA$_{SP}$-SecM (pMY6) or OmpA$_{SP}$-SecM (rep. 82-146) (pMY20) were subjected to pulse-chase labeling after the addition of IPTG and SecM-containing polypeptides were immunoprecipitated. When OmpA$_{SP}$-SecM was produced, only the arrested form of the protein and the nascent chain tethered to the ribosome that is situated behind the leading stalled ribosome (arrested') were observed. The appearance of the full-length and mature forms of OmpA$_{SP}$-SecM (rep. 82-146) as well as the disappearance of the arrested' polypeptide indicate that lengthening OmpA$_{SP}$-SecM augments the release of translation arrest.

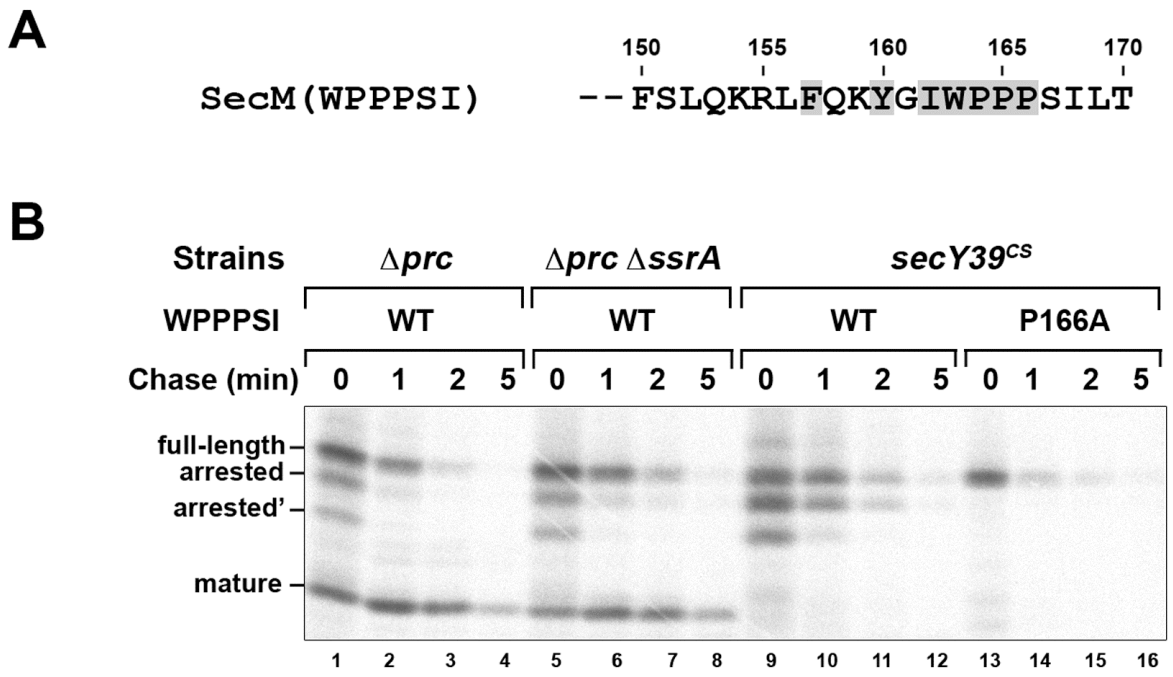

Fig. S6. An alternate translation arrest motif produces a relatively unstable arrested state. A. The C-terminal 21 residues of a SecM derivative [SecM(WPPPSI)] in which residues 151-168 were replaced with an artificial translation arrest motif that was selected from a pool of random peptide sequences (Tanner *et al.*, 2009). The residues that are required for effective translation arrest are shaded. B. MNY24 (Δ*prc*), MNY28 (Δ*prc* Δ*ssrA*) and CU164 (*secY39*^{cs}) were transformed with a plasmid (pMY26) that encodes SecM(WPPPSI) or the corresponding arrest-deficient derivative (P166A). Cells were subjected to pulse-chase labeling after the addition of IPTG and SecM-containing polypeptides were immunoprecipitated. The arrest mediated by SecM(WPPPSI) is unstable even in the absence of a force exerted by the SecYEG complex (lanes 9-12), and the WPPPSI motif is therefore incompatible with the normal regulatory function of SecM. Because proteins containing the WPPPSI motif can be tagged by the SsrA pathway and degraded in the cytoplasm following translation arrest (unlike proteins containing the *E. coli* SecM motif), we examined SecM(WPPPSI) in a Δ*prc* Δ*ssrA* strain to facilitate a comparison with other SecM derivatives.