SUPPLEMENTARY INFORMATION

DROPPING OUT AND OTHER FATES OF TRANSMEMBRANE SEGMENTS INSERTED BY THE SECA ATPASE

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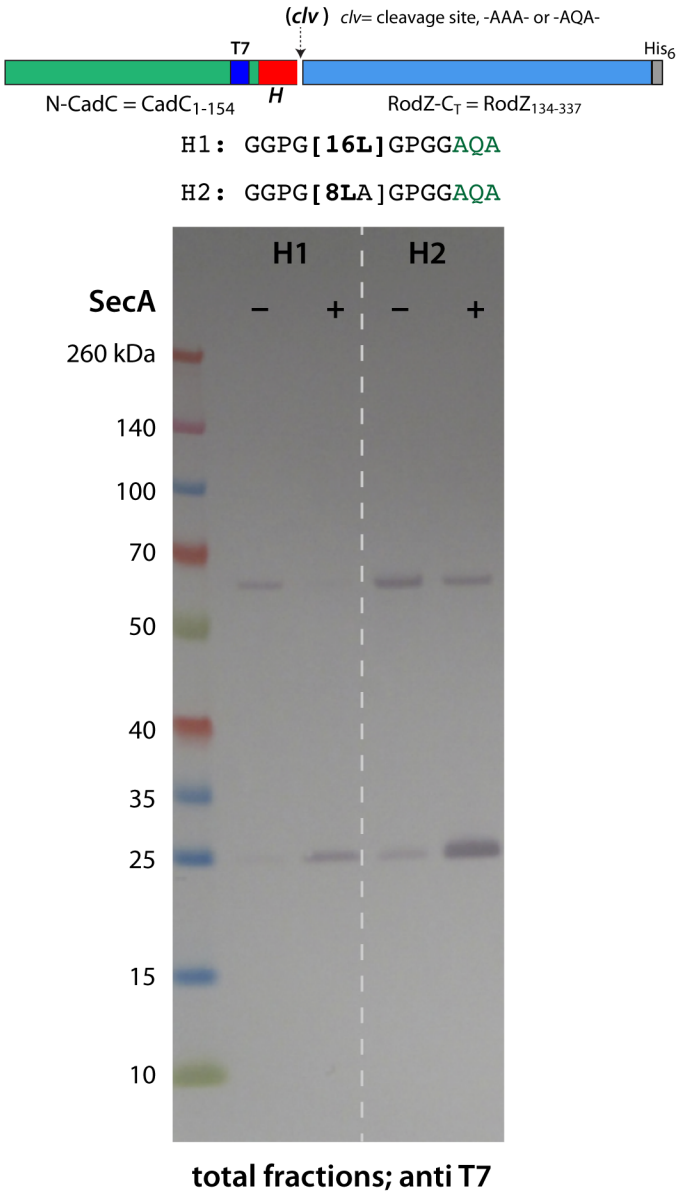

Figure S1. Dependence of *H*-segment processing on SecA. Complete processing of the C-H-R constructs requires SecA. We used *E. coli* strain EO527 in which *secA* is under the control of AraC. *C-H-R* constructs with *clv* = AQA (modified pET-vector, T7-RNA-polymerase independent system using a T5 promoter sequence which is recognized by the wt *E. coli* RNA-polymerase.) were transformed in depletion cells. Overnight cultures were grown in SOC media in the presence of 0.02 % arabinose (non-depletion condition). A 400 µl inoculum from the culture was added to 10 ml fresh SOC media with or without 0.02 % arabinose. After 2 h (OD_{600} ~ 0.6) protein expression was induced by adding 10 µM IPTG. After 0.5 h of protein expression, cells were pelleted and analyzed.

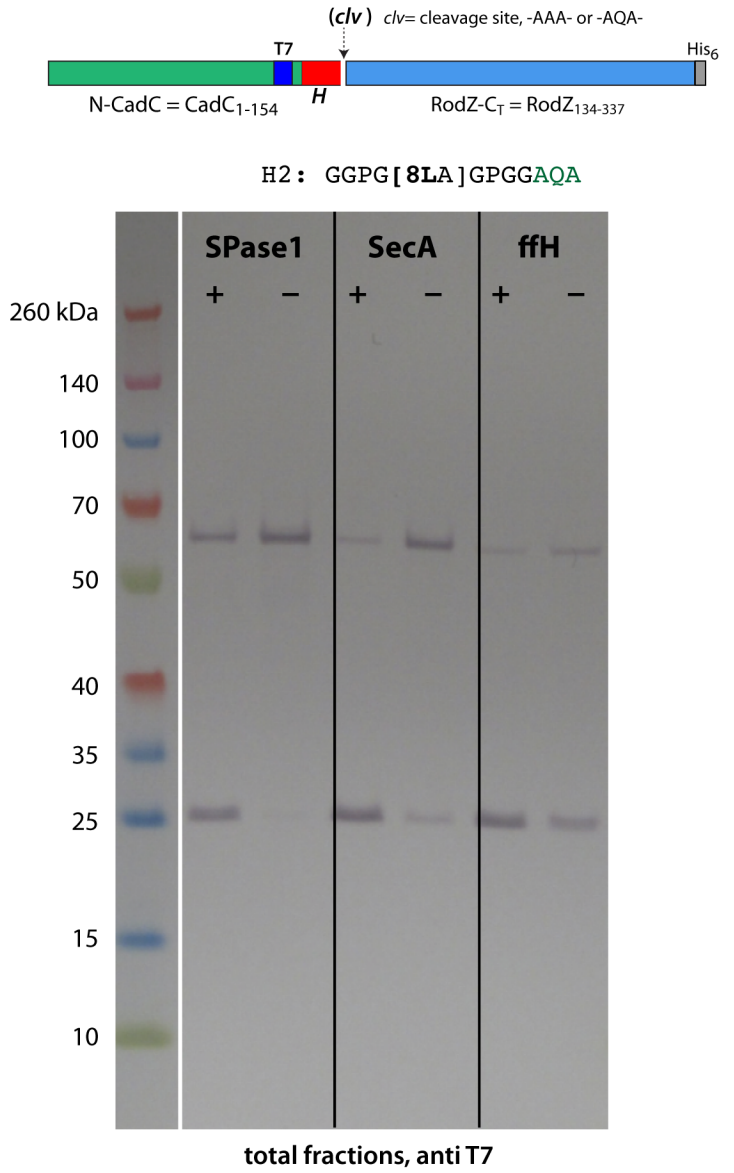

Figure S2. The dependence of SecA processing on SPase I, SecA, and ffH. While ffH has some effect, it is minor compared the requirement for SPase I and SecA. For SecA depletion studies, we used *E. coli* strain EO527 in which *secA* is under the control of AraC. For Ffh depletion studies, we used *E. coli* strain WAM121 in which *ffh* is under the control of AraC. *C-H-R* constructs with *clv* = AQA (modified pET-vector, T7-RNA-polymerase independent system using a T5 promoter sequence which is recognized by the wt *E. coli* RNA-polymerase.) were transformed in depletion cells. Overnight cultures were grown in SOC media in the presence of 0.02 % arabinose (non-depletion condition). A 400 µl inoculum from the culture was added to 10 ml fresh SOC media with or without 0.02 % arabinose. After 2 h (OD_{600} ~ 0.6) protein expression was induced by adding 10 µM IPTG. After 0.5 h of protein expression, cells were pelleted and analyzed.

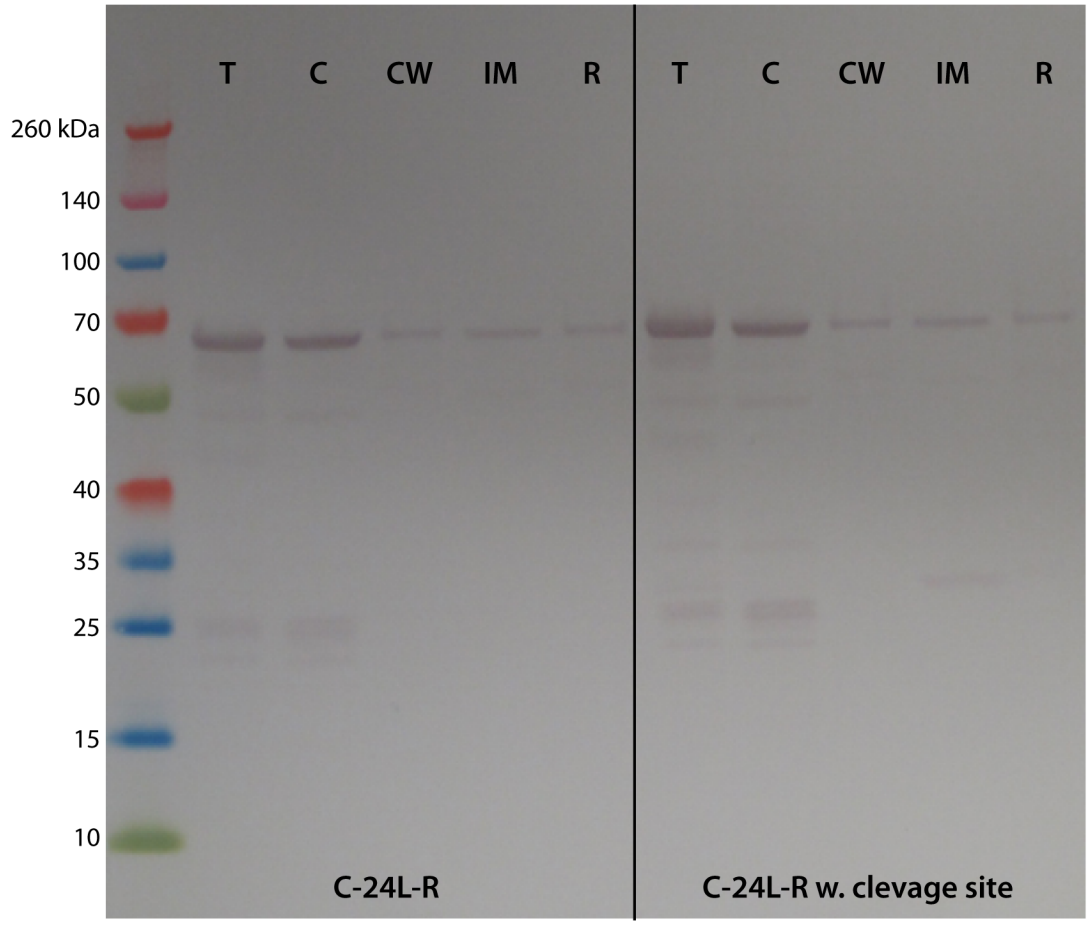

Figure S3. The cellular localization of C-24L-R constructs containing either a cleavage site or no cleavage site. The absence (left panel) or presence (right panel) of a cleavage site (AxA) C-terminal of the H segment is irrelevant. The absence of a cleavage product in the presence of a cleavage site (right panel) indicates that the C-24L-R construct is not membrane incorporated. To exclude the possibility that a cleavage site following a very long H-segment is out of the SPase I 'range', we determined the cellular localization of the construct. C-24L-R was found principally in the cytoplasm. The high intensities of the T and C bands are virtually identical while the CW, IM, and R fraction yield very weak bands. We included here an additional Na_2CO_3 washing step, because we assumed that the very hydrophobic H-segment has a high tendency to be membrane-attached. Sodium carbonate has been used successfully to remove membrane-attached proteins from subcellular organelles [1]. In addition we included the R fraction, because proteins containing very hydrophobic amino acid stretches have the strong ability to aggregate in a water environment. The R fraction contains such precipitated proteins. Interestingly, we found the C-24L-R construct in the cytoplasmic fraction. We speculate that the H-segment is a target for cytoplasmic chaperons and therefore not accessible for SecA. These experiments were carried out using BL21 cells carrying a pET21 vector. Cells were grown in SOC media at 37° C. See Materials and Methods.

[1] Fujiki Y, Hubbard AL, Fowler S, Lazarow PB. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. J Cell Biol. 1982;93:97-102.