*Supplementary Information*

# SecA Drives Transmembrane Insertion of RodZ, an Unusual Single-Span Membrane Protein

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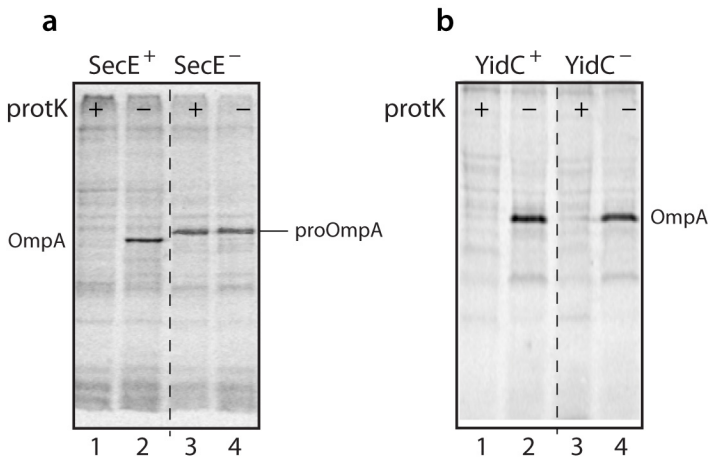
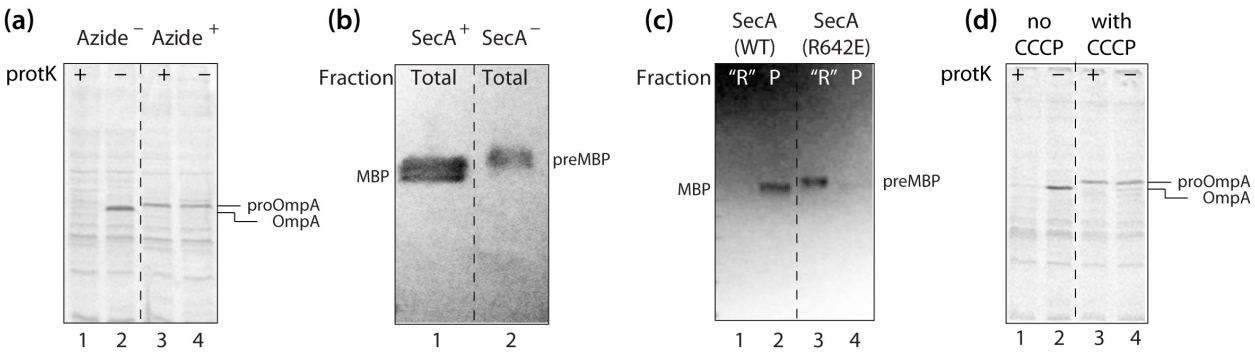
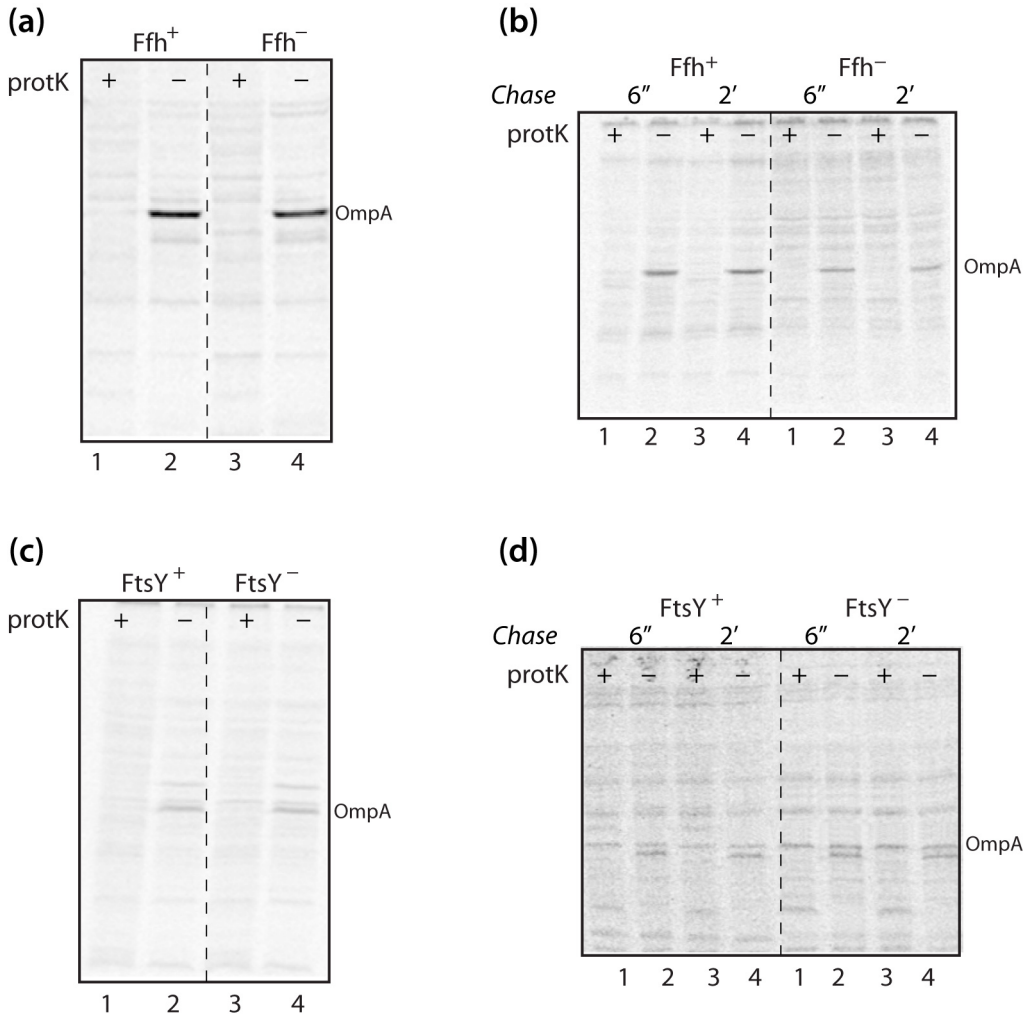



**Supplementary Figure S1:** Membrane translocation requirements for proOmpA. (**a**) The CM124 cells, expressing the OmpA protein in the $SecE^+$ and $SecE^-$ conditions, were subjected to proteinase K mapping experiment as described in the Materials and Methods. The OmpA protein was immunoprecipitated by OmpA antiserum (from rabbit) and subjected to 15% SDS-PAGE and phosphorimaging. (**b**) The YidC dependence of membrane insertion of OmpA was tested in JS7131, as described in Materials and Methods. OmpA translocation is unaffected by the absence of YidC.




**Supplementary Figure S2**: SecA is required for protein export of proOmpA and MBP. (**a**) The MC1060 cells, bearing the RodZ protein expression vector, were treated with or without sodium azide before pulse labeling as described in Materials and Methods. As a positive control, the OmpA proteins were immunoprecipitated and resolved by 15% SDS-PAGE gel. (**b**) SecA requirement was further tested in SecA depletion strain EO527. As a positive control, secretion of C-terminal-His-tagged-MBP was observed by pelleting the total cell lysate and the protein samples were subjected to SDS-PAGE gel and the MBP protein was visualized by western blot against anti-his antibody. (**c**) Secretion of his-tagged MBP was further confirmed in dominant negative SecA(R642E). Cells expressing MBP were subjected to spheroplast preparation by osmotic shock method and centrifuged at 13x000 g for 15 minutes. The supernatant was used as the periplasmic fraction "P" and the pellet fraction "R" after high-speed spin includes total minus periplasmic proteins. Both fractions were precipitated with 10% (m/V) TCA buffer and the C-terminal-His-tagged MBP protein was visualized by western blot analysis using anti-his antibody. (**d**) As a control of proteinase accessibility assay for PMF (no CCCP or with CCCP), OmpA protein was immunoprecipitated and resolved on 15% SDS-PAGE before phosphor-imaging, as described in Fig S1a.




**Supplementary Figure S3**: Ffh and FtsY proteins are not required for membrane targeting of OmpA. (**a**) and (**b**) As a negative control of Ffh dependence, the secretion of OmpA protein was tested in the WAM121 cells grown in the presence of arabinose or glucose. The OmpA protein was immunoprecipitated as described in Fig S1a and resolved by 15% SDS-PAGE as described in Materials and Methods. (**c**) and (**d**) As a negative control of FtsY dependence, protK cleavage was used to test for secretion of the OmpA. Proteins were chased and immunoprecipitated by anti-OmpA antiserum and then subjected to SDS-PAGE gel as described in Fig S1a.