YidC is involved in the biogenesis of the secreted autotransporter Hbp

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Supplementary data

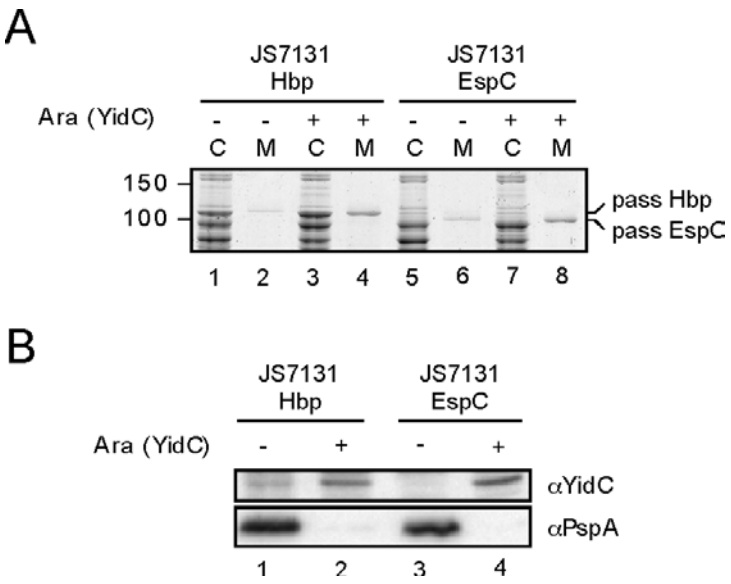

Fig. S1. Depletion of YidC affects the biogenesis of Hbp and EspC *in vivo*. (*A*) Analysis of expression and secretion of Hbp and EspC in YidC depletion strain JS7131. Cells harboring either pEH3-Hbp or pEH3-EspC were grown in the presence or absence of L-arabinose to allow expression or depletion of YidC, respectively. After 5,5h of growth, expression of Hbp or EspC was induced by addition of IPTG (1mM) and growth was continued. Samples were collected 2h after induction, separated in cells (*C*) and spent medium (*M*) and analyzed by SDS-PAGE and Coomassie staining. Cells were resuspended in SDS-sample buffer directly whereas medium samples were first TCA precipitated. Molecular mass markers (kDa) are shown at the left side of the panels. The passenger (*pass*) of Hbp and EspC is indicated at the right hand side of the panel. (*B*) Immunoblot analysis of the YidC and PspA content in 0.03 OD_{660} units of cells collected under *A*.

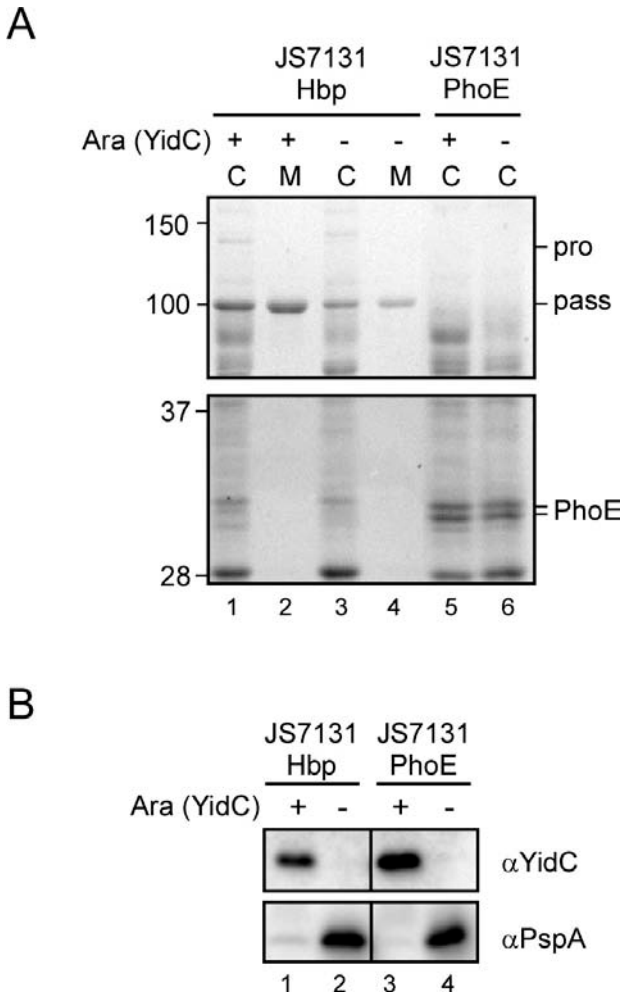

Fig. S2. Depletion of YidC does not affect biogenesis of secretory proteins in general. (*A*) Analysis of the biogenesis of Hbp and PhoE in strain JS7131. Strains JS7131(pEH3-Hbp) and JS7131(pEH3-PhoE) were grown and analyzed in parallel as described in the legend to Fig. S1. As PhoE is an integral outer membrane protein that is not secreted into the extracellular environment, medium samples of JS7131(pEH3-PhoE) were not analyzed. The relevant areas of the same gel are shown. Cells (*C*) and medium (*M*) fractions are indicated at the top of the panel. Molecular mass markers (kDa) are shown at the left side of the panels. The Hbp passenger (*pass*) and pro-form (*pro*) are indicated at the right hand side of the panels. PhoE, which runs on gel as a double band for obscure reasons, is indicated on the right hand side of the lower panel. (*B*) Immunoblot analysis of 0.03 OD_{660} of cells collected under *A* using antiserum against YidC or PspA.

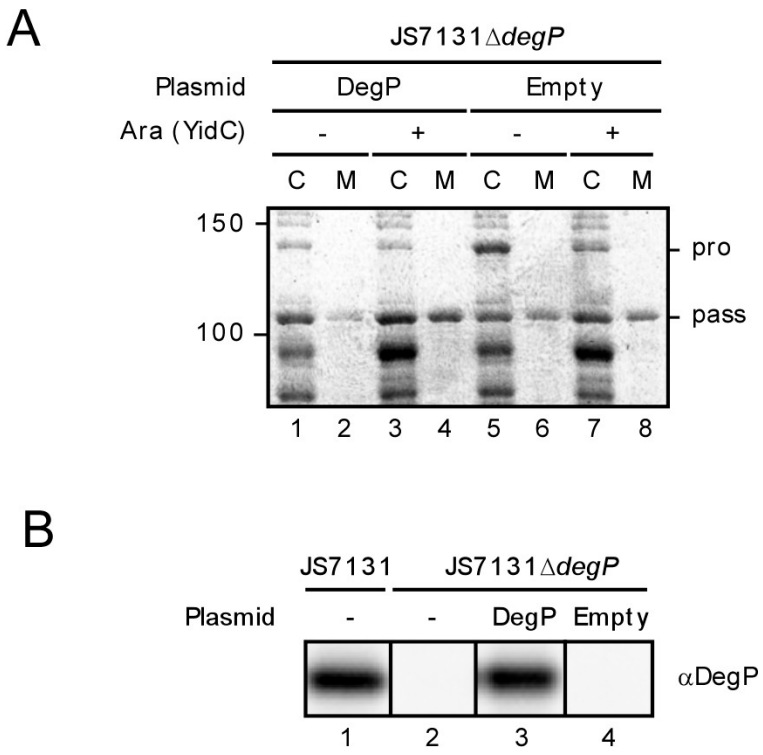

Fig. S3. Complementation of Δ*degP* with *degP in trans* restores degradation of secretion incompetent Hbp intermediates upon depletion of YidC. (*A*) Analysis of accumulation of proHbp in JS7131Δ*degP*(pEH3-Hbp) upon transformation with DegP-expression plasmid pWSK-DegP (lanes 1-4) or the empty vector pWSK-Empty (lanes 5-8) as described in the legend to Fig. S1. Cells (*C*) and medium (*M*) fractions are indicated at the top of the panel. Molecular mass markers (kDa) are shown at the left side of the panel. The Hbp passenger (*pass*) and pro-form (*pro*) are indicated at the right hand side of the panel. (*B*) DegP levels in JS7131(pEH3-Hbp) (lane 1) and JS7131Δ*degP*(pEH3-Hbp) (lanes 2-4) co-transformed with pWSK-DegP or pWSK-Empty, as indicated. Cells were grown in the absence of arabinose and collected after the addition of IPTG as described in the legend to Fig. S1. The equivalent of 0.015 OD_{660} of cell material was analyzed by immunoblotting using DegP antiserum. The image is composed of separate lanes derived from the same original chemiluminescence image.