Supporting Information

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SI Materials and Methods

To make plasmid pJH110 (P_{tre}-*espP*Δ*5*), a DNA fragment encoding EspP residues 998–1300 was amplified by PCR using the oligonucleotides 5′-CACTGACAGGCTATA-ACACGGCCGCAAACA-3′ and 5′-AAAGCCGGCATTACT-GCAAGCTTTAGTC-3′ and pJH62 (1) as a template. The resulting PCR product was digested with Eag I and Hind III and cloned into the cognate sites of pHL36 (2).

1. Szabady RL, Peterson JH, Skillman KM, Bernstein HD (2005) An unusual signal peptide facilitates late steps in the biogenesis of a bacterial autotransporter. *Proc Natl Acad Sci USA* 102(1):221–226.
2. Lee HC, Bernstein HD (2002) Trigger factor retards protein export in *Escherichia coli*. *J Biol Chem* 277(45):43527–43535.

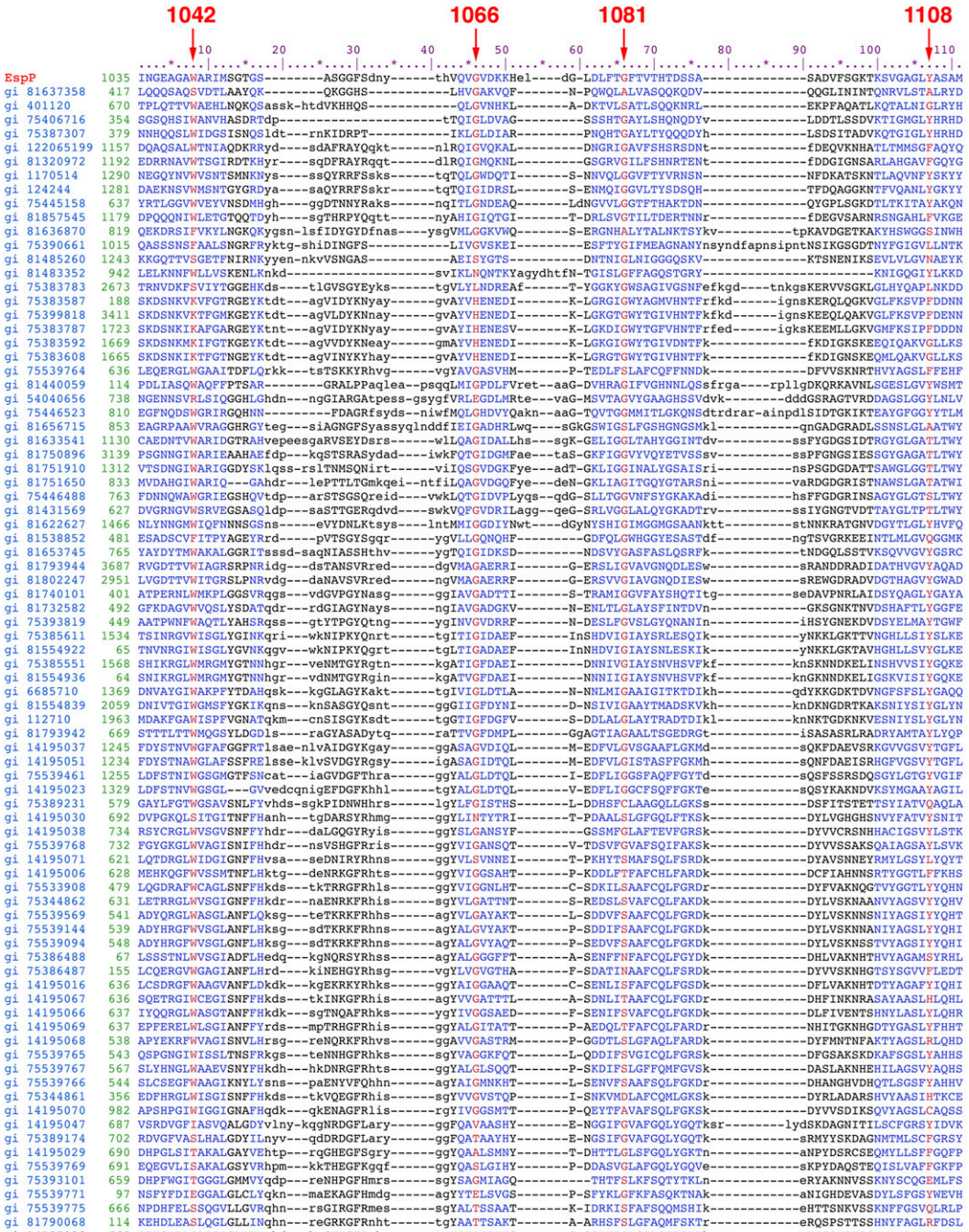

Fig. S1. Alignment of autotransporter β domain sequences. An alignment of 100 autotransporter β domain sequences was obtained by querying the Conserved Domain Database (www.ncbi.nlm.nih.gov/sites/entrez?db=cdd) with the accession no. pfam03797. The structure of the EspP β domain (accession no. 2QQM) was used as a reference to perform the alignment. The parameters were set to display the most diverse members of the pfam03797 family. The most highly conserved residues are shaded red, and less highly conserved residues are shaded blue. Uppercase residues that are unshaded were aligned but not conserved whereas lowercase residues could not be aligned. Only EspP residues 1035–1112 (*Top*) and the corresponding residues in other β domains are shown. EspP residues W1042, G1066, G1081, and Y1108 are denoted with an arrow. EspP residues Y1125, Y1144, P1170, Q1171, W1185, and Q1207 were also identified as highly conserved.

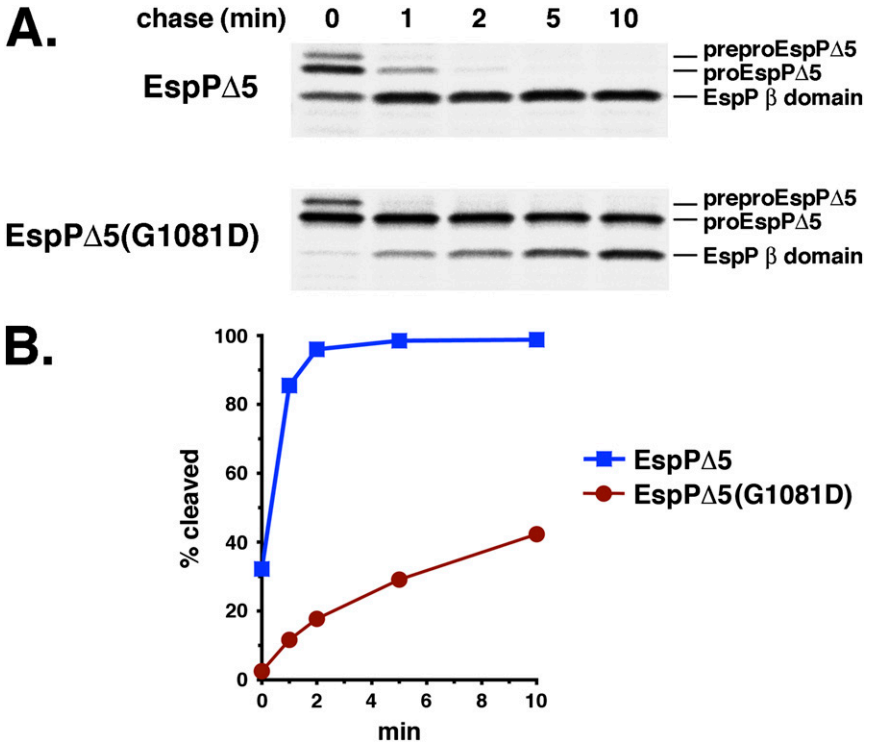

Fig. S2. Effect of the G1081D mutation on the proteolytic maturation of EspPΔ5. (*A*) AD202 transformed with pJH110 (P_{trc}-*espPΔ5*) or a pJH110 derivative encoding EspPΔ5(G1081D) were subjected to pulse-chase labeling after the addition of 10 μM isopropyl-β-D-thiogalactopyranoside (IPTG). Immunoprecipitations were performed using the C-terminal anti-EspP antiserum. (*B*) The percent of the passenger domain fragment that was cleaved at each time point in *A* is shown. A comparison of these results with those shown in Fig. 3 reveals that EspPΔ5(G1081D) undergoes proteolytic processing faster than EspP(G1081D).

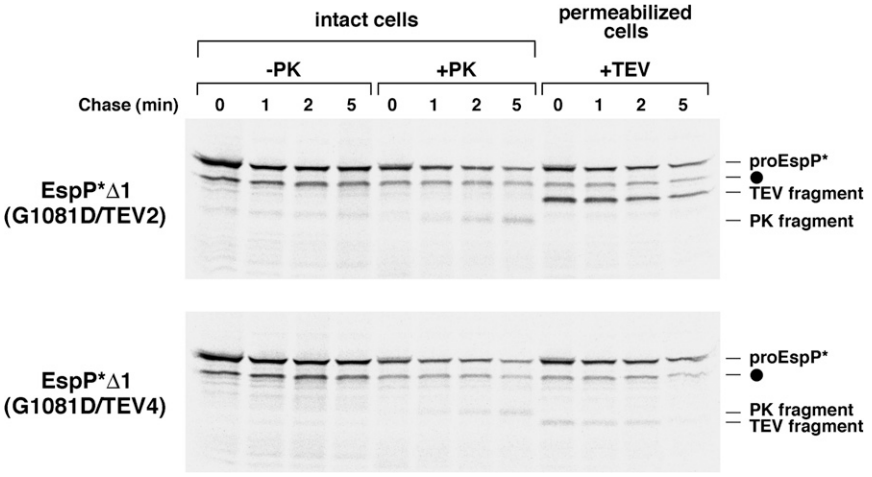

Fig. S3. The G1081D mutation does not significantly affect early steps in β domain folding. Plasmids pTEV2 and pTEV4 (1) encode a truncated version of EspP that contains a noncleavable 116-residue passenger domain fragment and a tobacco etch virus (TEV) protease recognition site at residue 974 [EspP*Δ1(TEV2)] or 1010 [EspP*Δ1(TEV4)]. Previous work has shown that EspP*Δ1(TEV2) and EspP*Δ1(TEV2/G1066A) are cleaved by TEV protease but that EspP*Δ1(TEV4) and EspP*Δ1(TEV4/G1066A) rapidly lose their susceptibility to protease digestion because of the apparent incorporation of the passenger domain–β domain junction into a partially folded β barrel structure (2). In this experiment, AD202 were transformed with a derivative of pTEV2 or pTEV4 encoding EspP*Δ1(TEV2/G1081D) or EspP*Δ1(TEV4/G1081D). Following the addition of 10 μM IPTG, cells were subjected to pulse-chase labeling and processed as previously described (2). One-third of the cells were untreated, one-third were treated with proteinase K (PK), and one-third were permeabilized and treated with TEV protease. Immunoprecipitations were then conducted using the anti-EspP C-terminal antiserum and proteins were resolved by SDS/PAGE. An unidentified background band (•) appeared in all samples. The observation that EspP*Δ1(TEV2/G1081D) is digested by TEV protease but that EspP*Δ1(TEV4/G1081D) is largely protected from digestion strongly suggests that the mutation does not interfere significantly with the overall folding of the β domain.

1. Dautin N, Barnard TJ, Anderson DE, Bernstein HD (2007) Cleavage of a bacterial autotransporter by an evolutionarily convergent autocatalytic mechanism. *EMBO J* 26(7):1942–1952.
2. Ieva R, Skillman KM, Bernstein HD (2008) Incorporation of a polypeptide segment into the β-domain pore during the assembly of a bacterial autotransporter. *Mol Microbiol* 67(1): 188–201.

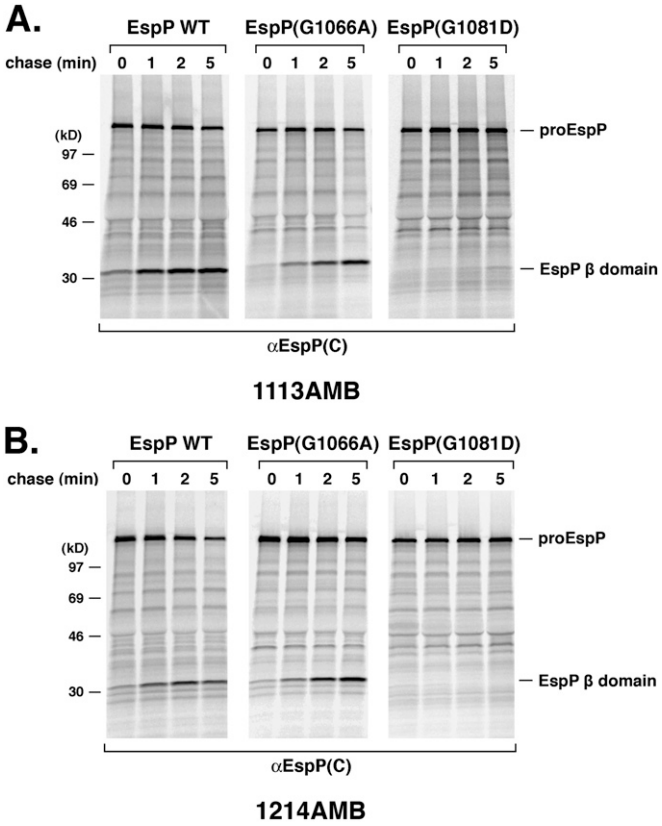

Fig. S4. Immunoprecipitation of EspP-containing polypeptides from nonirradiated samples. Samples from the experiments shown in Fig. 4*A* (*A*) and Fig. 4*B* (*B*) that were neither UV-irradiated nor treated with PK are shown.

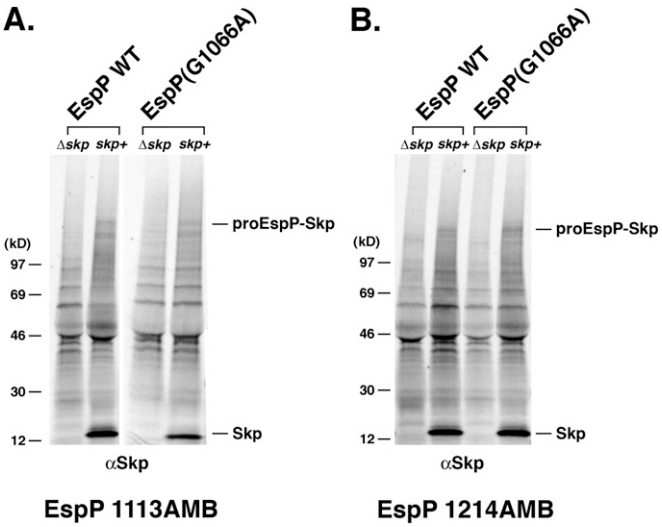

Fig. S5. An ~155-kDa crosslinking product is not observed in a Δ*skp* strain. AD202 and HDB131 (AD202 Δ*skp*) (1) were transformed with pDULEBpa and pRI22 (P_{lac}-*espP*) harboring an amber mutation at residue 1113 (*A*) or 1214 (*B*). After the addition of IPTG cells were pulse labeled and UV-irradiated. Immunoprecipitations were subsequently conducted using an anti-Skp antiserum.

1. Ieva R, Bernstein HD (2009) Interaction of an autotransporter passenger domain with BamA during its translocation across the bacterial outer membrane. *Proc Natl Acad Sci USA* 106(45):19120–19125.

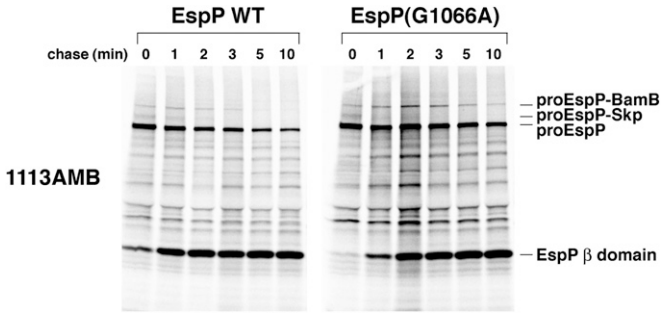

Fig. S6. The G1066A mutation does not affect the release of the EspP β domain from Skp. The experiment shown in Fig. 4*A* was repeated, except that all samples were UV-irradiated and processed without PK treatment. The results provide additional evidence that EspP(G1066A) dissociates from Skp (and then binds to the Bam complex) at the same rate as wild-type EspP.

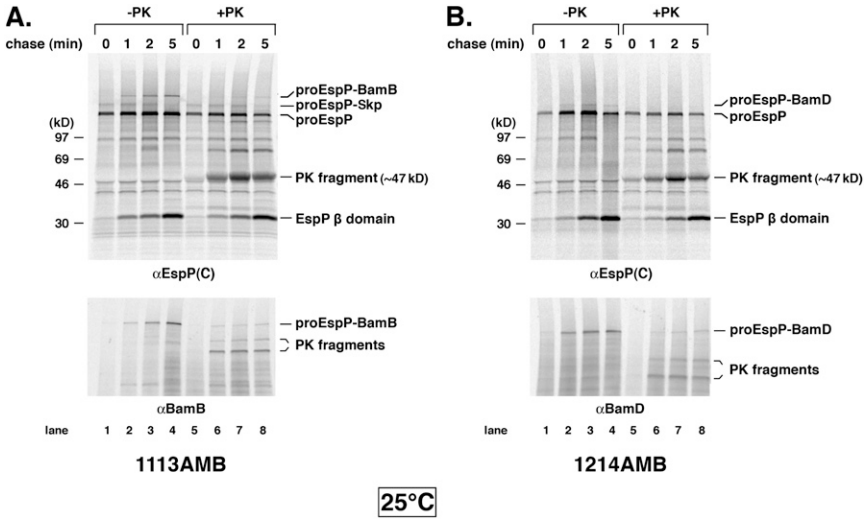

Fig. S7. The binding of the wild-type EspP β domain to the Bam complex can be uncoupled from the initiation of passenger domain translocation at low temperature. AD202 were transformed with pDULEBpa and pRI22 (P_{lac}-*espP*) harboring an amber mutation at residue 1113 (*A*) or 1214 (*B*). After the addition of IPTG cultures were shifted to 25 °C and subjected to pulse-chase labeling. Cells were UV-irradiated and PK was added to half of each sample. Immunoprecipitations were subsequently conducted using the indicated antisera.

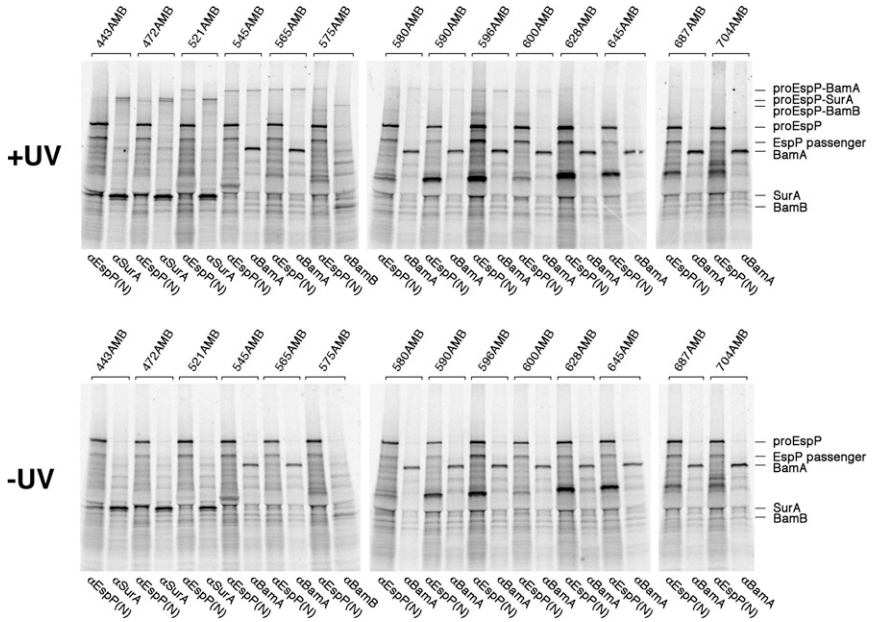

Fig. S8. A long segment of the EspP passenger domain is in proximity to BamA when translocation stalls. Previous work has shown that the translocation of the passenger domain of an EspP derivative containing a linker insertion at residue 586 [EspP(586TEV)] stalls transiently near the site of the insertion (1). In this experiment AD202 were transformed with pDULEBpa and pRI23 [P_{lac}-*espP(586TEV)*] harboring an amber codon at the indicated position. Cells were pulse labeled and subjected to a 1-min chase after the addition of 200 μM IPTG. Half of each sample was UV-irradiated, and equal portions were used for immunoprecipitations with antisera raised against an EspP N-terminal peptide (2), BamA, BamB, or SurA. Although an EspP-BamA crosslinking product was observed when Bpa was introduced into EspP within ~80 residues of the stall point (residue ~600), an EspP-SurA crosslinking product was observed only when the amino acid analog was introduced ≥80 residues from the stall point. The crosslinking of residue 575 to both BamA and BamB has been previously reported (3).

1. Ieva R, Bernstein HD (2009) Interaction of an autotransporter passenger domain with BamA during its translocation across the bacterial outer membrane. *Proc Natl Acad Sci USA* 106(45):19120–19125.
2. Szabady RL, Peterson JH, Skillman KM, Bernstein HD (2005) An unusual signal peptide facilitates late steps in the biogenesis of a bacterial autotransporter. *Proc Natl Acad Sci USA* 102(1):221–226.
3. Ieva R, Tian P, Peterson JH, Bernstein HD (2011) Sequential and spatially restricted interactions of assembly factors with an autotransporter β domain. *Proc Natl Acad Sci USA* 108(31): E383–E391.

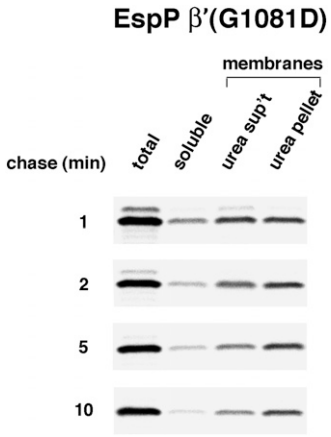

Fig. S9. The G1081D mutation impairs the membrane integration of the EspP β domain. AD202 transformed with a pJH61 derivative encoding EspP β′(G1081D) were subjected to pulse-chase labeling after the addition of IPTG. Cells were fractionated and equivalent amounts of each fraction were used for immunoprecipitations with an anti-EspP C-terminal antiserum. Although it is possible that the partial loss of EspP β′(G1081D) that occurred during the fractionation procedure resulted from proteolysis, essentially identical results were obtained when a mixture of protease inhibitors (1 mM AEBSF, 800 nM aprotinin, 50 μM bestatin, 15 μM E64, 20 μM leupeptin, 10 μM pepstatin A) was added to the cell extract.