Supporting Information

Okuda and Tokuda 10.1073/pnas.0900896106

SI Text

Construction of *E. coli* KTA2(DE3) and KT60. KTA2 (JE5506 Δ*lolA*::*kan*) harboring pKT011 (*lolA* temperature-sensitive replicon) was constructed like KT6 (JE5506 Δ*lolB*::*kan* pKT021) (1), and then lysogenized with lambda DE3, using a kit (Novagen) to construct KTA2(DE3). KT60 (JE5506 Δ*lolB*::*kan*) is a strain that was derived from KT6 by curing pKT021 and always harbored a plasmid carrying a functional LolB derivative.

1. Tanaka K, Narita S, Matsuyama S, Tokuda H (2001) Deletion of *lolB*, encoding an outer membrane lipoprotein, is lethal for *Escherichia coli* and causes accumulation of lipoprotein localization intermediates in the periplasm. *J Bacteriol* 183:6538–6542.
2. Osborn MJ, Munson R (1974) Separation of the inner (cytoplasmic) and outer membranes of gram-negative bacteria. *Methods Enzymol* 62:642–653.

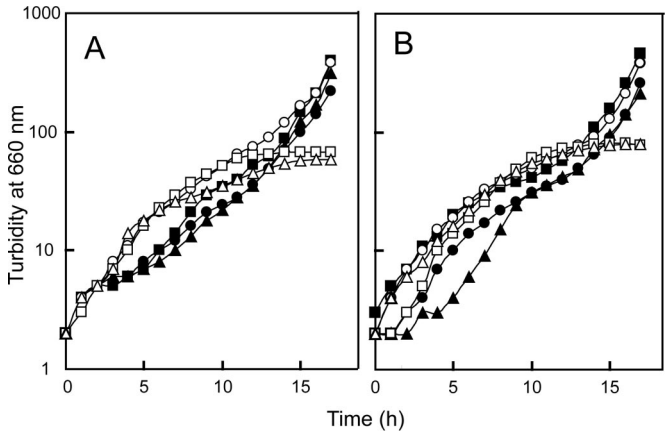

Fig. S1. LolA and mLolB derivatives containing pBPA are functional. (*A*) KTA2(DE3) cells harboring pKT011, which was constructed by inserting the *lolA* gene into pMAN997 (1) carrying a temperature-sensitive replicon, were transformed with pSS1-Xamber and pSup-BpaRS-6TRN(D286R). (*B*) KT60(DE3) cells harboring pNAS021, which was constructed by inserting the *spc* gene into *bla* of pKT021 carrying a temperature-sensitive replicon and *lolB*, were transformed with pSS24-Xamber and pSup-BpaRS-6TRN(D286R). The cells were grown on LB broth containing 10 μM IPTG with (closed symbols) or without (open symbols) 1 mM pBPA at 42 °C to delete the temperature-sensitive plasmids for the indicated times by inoculating portions of the cultures into fresh medium. The results obtained with LolA derivatives (*A*) having amber mutations at Q33 (squares) and E144 (triangles), and those with mLolB derivatives (*B*) having amber mutations at I40 (squares) and E138 (triangles) are shown. Essentially the same pBPA-dependent growth was obtained with other derivatives except LolA derivatives having amber codons at the I93 and Q180 positions. The former could not grow, and the latter, lacking only three residues, grew in the presence and absence of pBPA. As a control, growth with wild-type LolA (*A*) and mLolB (*B*) is also shown (circles).

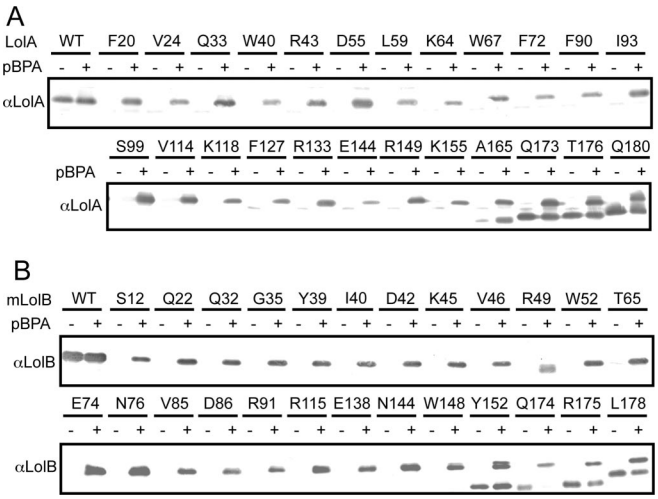

Fig. S2. Expression of amber mutants of LolA and mLolB in the presence and absence of pBPA. BL21(DE3) cells harboring pSup-BpaRS-6TRN(D286R) and pSS1-Xamber encoding a LolA derivative (*A*), which had amber mutations in place of the indicated residues, or pSS24-Xamber encoding a mLolB derivative (*B*), which had amber mutations in place of the indicated residues, were grown at 37 °C on LB broth with or without 1 mM pBPA. The expression of LolA-FLAG and mLolB-His_6 derivatives was induced by the addition of 10 μM IPTG for 2 h at the mid-log phase of growth and analyzed by SDS/PAGE and immunoblotting with anti-LolA and anti-LolB antibodies, respectively. Two bands were detected with derivatives having an amber mutation in the C-terminal region. Faster migrating bands represent truncated derivatives, whose translation was terminated at the amber codon and thereby they lacked FLAG-tag (*A*) or His_6-tag (*B*), too. Slower migrating bands represent derivatives having pBPA incorporated into the amber codon.

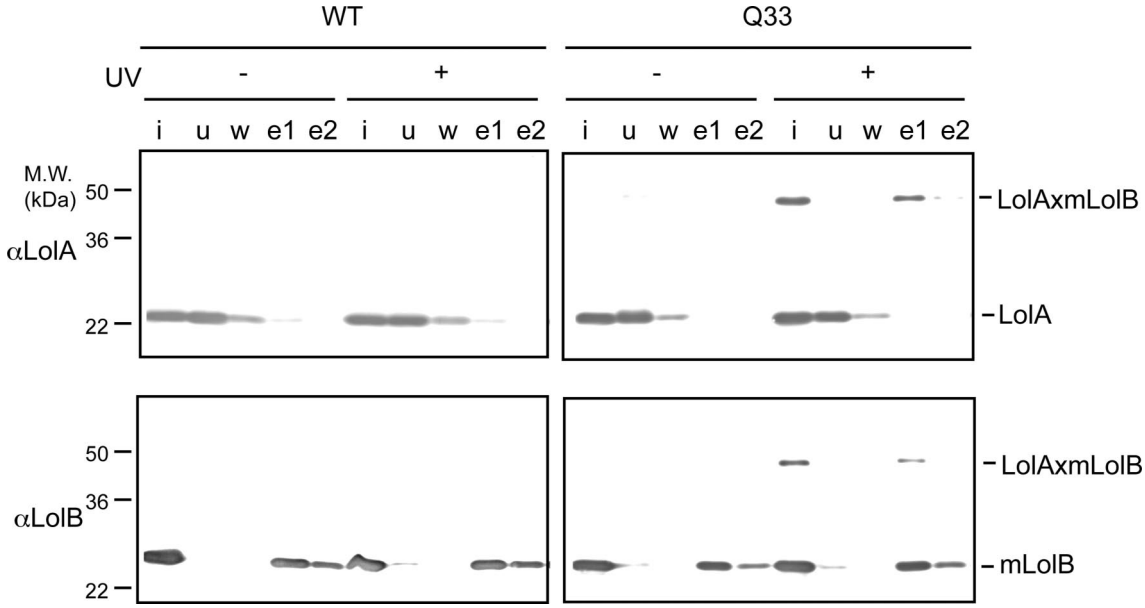

Fig. S3. Confirmation of cross-linked products generated from LolA and mLolB. Cells expressing wild-type (WT) LolA-FLAG or the LolA-FLAG derivative having pBPA in place of Q33 and mLolB-His_6 were UV-irradiated and converted into spheroplasts, as reported (2). Periplasmic fractions were obtained as spheroplast supernatants and adsorbed to a TALON affinity column. The column was then washed with 50 mM Tris-HCl (pH 7.5) containing 300 mM NaCl, followed by elution with 50 mM Tris-HCl (pH 7.5) containing 300 mM NaCl supplemented with imidazole. Each fraction was analyzed by SDS/PAGE and immunoblotting with anti-LolA and -LolB antibodies. i, input sample; u, unbound fraction; w, washing fraction; e1, eluate with 50 mM imidazole; e2, eluate with 100 mM imidzole.

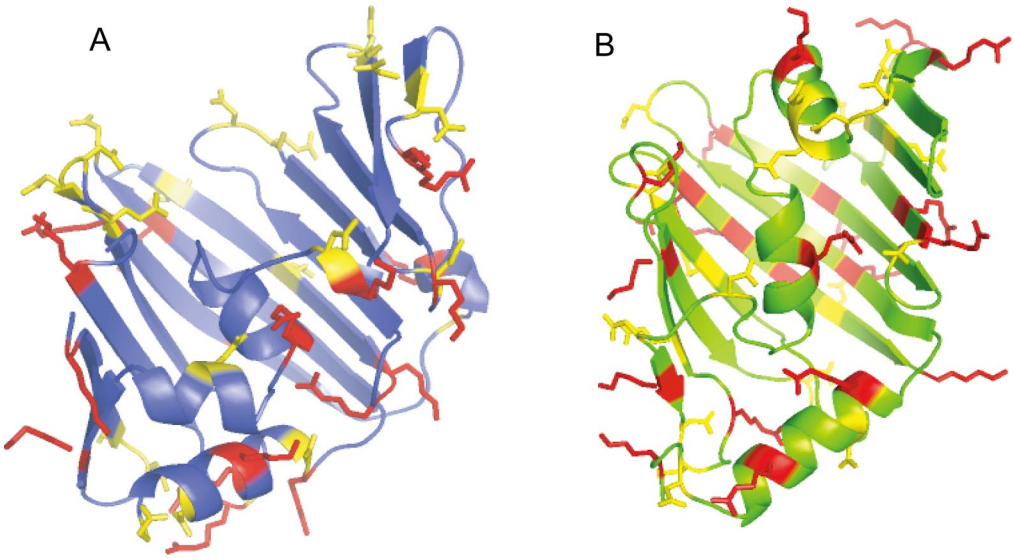

Fig. S4. Charge distribution in LolA and mLolB molecules. The LolA (*A*) and mLolB (*B*) molecules are each shown as a ribbon model. Positive and negative residues are shown as red and yellow stick models, respectively. The structural information on LolA (1IWL) and mLolB (1IWM) was obtained from the RCSB protein data bank (http://pdb.protein.osaka-u.ac.jp/pdb/), and visualized with PyMOL ver.0.98 (http://pymol.sourceforge.net/index.php).

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LolC:  ILGLMPQAIL SSEHGSLNPQ QLPETAVKLD GVNRVAPITT GDVVLQSARS VAVGVMLGID
LolB:  CSVTTPKGPG KSPDSPQWRQ HQQDVRNLNQ YQTRGAFAYI SDQQKVYAR- -FFWQQTGQD

LolC:  PAQKDPLTPY LVNVKQTDLE PGKYNVILGE QLASQLGVNR GDQIRVMVPS ASQFTPMG-
LolB:  -RYRLLLTNP L---GSTELE ---LNAQPG- --NVQLVDNK GQRYTADDAE EMIGKLTGMP

LolC:  IPSQRLFNVI GTFAANSEVD GYEMLVNIED ASRLMRYPAG NITGWRLWLD EPLKVDSLSQ
LolB:  IPLNSLRQWI ------LGLP GDATDYKLDD QYRL--SEIT YSQNGKNWKV VYGGYDTKTQ

LolC:  QKLPEGSKWQ DWRDRKGELF QAVRMEK
LolB:  PAMPANMELT DGGQR-IKLK MDNWIVK
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Fig. S5. Sequence alignment of LolB and the periplasmic region of LolC. The sequence of the I59-K264 region of LolC was compared with the sequence of LolB using GENETYX ver. 6 (GENETYX). These sequences were found to be 18.8% identical. Identical residues are indicated in red.

Table S1. Construction of pSS24 encoding mLolB-His$_6$

Primer	Sequence (5′-3′)
pSS24-F	CATG**CCATGG**CCTCCGTTACCACGCCCAAAGGTCCT
pSS24-R	CCG**CTCGAG**TTTCACTATCCAGTTATCCATTTT

Bold letters indicate restriction sites. F, forward; R, reverse.

Table S2. Amber mutants of LolA

Mutated residue	Primer	Sequence (5′-3′)
F20	F	AGCTTCCACGCCAGC**TAG**ACACAAAAAGTGACT
	R	AGTCACTTTTTGTGT**CTA**GCTGGCGTGGAAGCT
V24	F	AGCTTCACACAAAAA**TAG**ACTGACGGTAGCGGC
	R	GCCGCTACCGTCAGT**CTA**TTTTTGTGTGAAGCT
Q33	F	AGCGGCGCGGCGGTGT**TAG**GAAGGTCAGGGCGAT
	R	ATCGCCCTGACCTTC**CTA**CACCGCCGCGCCGCT
W40	F	GGTCAGGGCGATCTG**TAG**GTGAAACGTCCAAAC
	R	GTTTGGACGTTTCAC**CTA**CAGATCGCCCTGACC
R43	F	GATCTGTGGGTGAAA**TAG**CCAAACTTATTCAAC
	R	GTTGAATAAGTTTGG**CTA**TTTCACCCACAGATC
D55	F	CATATGACACAACCT**TAG**GAAAGCATTCTGGTT
	R	AACCAGAATGCTTTC**CTA**AGGTTGTGTCATATG
L59	F	CCTGATGAAAGCATT**TAG**GTTTCTGACGGTAAA
	R	TTTACCGTCAGAAAC**CTA**AATGCTTTCATCAGG
K64	F	CTGGTTTCTGACGGT**TAG**ACACTGTGGTTCTAT
	R	ATAGAACCACAGTGT**CTA**ACCGTCAGAAACCAG
W67	F	GACGGTAAAACACTG**TAG**TTCTATAACCCGTTC
	R	GAACGGGTTATAGAA**CTA**CAGTGTTTTACCGTC
F72	F	TGGTTCTATAACCCG**TAG**GTTGAGCAAGCTACG
	R	CGTAGCTTGCTCAAC**CTA**CGGGTTATAGAACCA
F90	F	ACCGGTAATACGCCG**TAG**ATGCTGATTGCCCGC
	R	GCGGGCAATCAGCAT**CTA**CGGCGTATTACCGGT
I93	F	ACGCCGTTTATGCTG**TAG**GCCCGCAACCAGTCC
	R	GGACTGGTTGCGGGC**CTA**CAGCATAAACGGCGT
S99	F	GCCCGCAACCAGTCC**TAG**GACTGGCAGCAGTAC
	R	GTACTGCTGCCAGTC**CTA**GGACTGGTTGCGGGC
V114	F	AATGGCGATGACTTT**TAG**CTGACGCCGAAAGCC
	R	GGCTTTCGGCGTCAG**CTA**AAAGTCATCGCCATT
K118	F	TTTGTCCTGACGCCG**TAG**GCCAGCAATGGCAAT
	R	ATTGCCATTGCTGGC**CTA**CGGCGTCAGGACAAA
F127	F	GGCAATCTGAAGCAG**TAG**ACCATTAACGTGGGA
	R	TCCCACGTTAATGGT**CTA**CTGCTTCAGATTGCC
R133	F	ACCATTAACGTGGGA**TAG**GATGGCACAATCCAT
	R	ATGGATTGTGCCATC**CTA**TCCCACGTTAATGGT
E144	F	CAGTTTAGCGCGGTG**TAG**CAGGACGATCAGCGC
	R	GCGCTGATCGTCCTG**CTA**CACCGCGCTAAACTG
R149	F	GAGCAGGACGATCAG**TAG**AGCAGTTATCAACTG
	R	CAGTTGATAACTGCT**CTA**CTGATCGTCCTGCTC
K155	F	AGCAGTTATCAACTG**TAG**TCCCAGCAAAATGGG
	R	CCCATTTTGCTGGGA**CTA**CAGTTGATAACTGCT
A165	F	GGGGCTGTGGATGCA**TAG**AAATTTACCTTCACC
	R	GGTGAAGGTAAATTT**CTA**TGCATCCACAGCCCC
Q173	F	ACCTTCACCCCGCCG**TAG**GGCGTCACGGTAGAT
	R	ATCTACCGTGACGCC**CTA**CGGCGGGGTGAAGGT
T176	F	CCGCCGCAAGGCGTC**TAG**GTAGATGATCAACGT
	R	ACGTTGATCATCTAC**CTA**GACGCCTTGCGGCGG
Q180	F	GTCACGGTAGATGAT**TAG**CGTAAGCTCGAGGAC
	R	GTCCTCGAGCTTACG**CTA**ATCATCTACCGTGAC

The amber codon replaced the codons in bold letters. F, forward; R, reverse.

Table S3. Amber mutants of mLolB

Mutated residue	Primer	Sequence (5′-3′)
S12	F	AAAGGTCCTGGCAAA**TAG**CCGGATTCGCCACAA
	R	TTGTGGCGAATCCGG**CTA**TTTGCCAGGACCTTT
Q22	F	CAATGGCGTCAGCAT**TAG**CAAGACGTGCGCAAT
	R	ATTGCGCACGTCTTG**CTA**ATGCTGACGCCATTG
Q32	F	AATCTTAATCAGTATT**TAG**ACTCGCGGCGCGTTC
	R	GAACGCGCCGCGAGT**CTA**ATACTGATTAAGATT
G35	F	CAGTATCAGACTCGC**TAG**GCGTTCGCTTATATT
	R	AATATAAGCGAACGC**CTA**GCGAGTCTGATACTG
Y39	F	CGCGGCGCGTTCGCT**TAG**ATTTCTGACCAACAA
	R	TTGTTGGTCAGAAAT**CTA**AGCGAACGCGCCGCG
I40	F	GGCGCGTTCGCTTATT**TAG**TCTGACCAACAAAAA
	R	TTTTTGTTGGTCAGA**CTA**ATAAGCGAACGCGCC
D42	F	TTCGCTTATATTTCT**TAG**CAACAAAAAGTGTAC
	R	GTACACTTTTTGTTG**CTA**AGAAATATAAGCGAA
K45	F	ATTTCTGACCAACAA**TAG**GTGTACGCCCGCTTT
	R	AAAGCGGGCGTACAC**CTA**TTGTTGGTCAGAAAT
V46	F	TCTGACCAACAAAAA**TAG**TACGCCCGCTTTTTC
	R	GAAAAAGCGGGCGTA**CTA**TTTTTGTTGGTCAGA
R49	F	CAAAAAGTGTACGCC**TAG**TTTTTCTGGCAGCAA
	R	TTGCTGCCAGAAAAA**CTA**GGCGTACACTTTTTG
W52	F	TACGCCCGCTTTTTC**TAG**CAGCAAACCGGCCAG
	R	CTGGCCGGTTTGCTG**CTA**GAAAAAGCGGGCGTA
T65	F	TACCGTCTGCTGCTC**TAG**AACCCATTGGGCAGC
	R	GCTGCCCAATGGGTT**CTA**GAGCAGCAGACGGTA
E74	F	GGCAGCACGGAACTG**TAG**CTGAATGCTCAACCG
	R	CGGTTGAGCATTCAG**CTA**CAGTTCCGTGCTGCC
N76	F	ACGGAACTGGAGCTG**TAG**GCTCAACCGGGTAAC
	R	GTTACCCGGTTGAGC**CTA**CAGCTCCAGTTCCGT
V85	F	GGTAACGTGCAGTTA**TAG**GACAATAAAGGTCAG
	R	CTGACCTTTATTGTC**CTA**TAACTGCACGTTACC
D86	F	AACGTGCAGTTAGTC**TAG**AATAAAGGTCAGCGT
	R	ACGCTGACCTTTATT**CTA**GACTAACTGCACGTT
R91	F	GACAATAAAGGTCAG**TAG**TATACCGCCGATGAC
	R	GTCATCGGCGGTATA**CTA**CTGACCTTTATTGTC
R115	F	CCGCTCAACAGCTTG**TAG**CAGTGGATTTTAGGT
	R	ACCTAAAATCCACTG**CTA**CAAGCTGTTGAGCGG
E138	F	CAGTACCGCCTGAGC**TAG**ATTACCTACAGCCAG
	R	CTGGCTGTAGGTAAT**CTA**GCTCAGGCGGTACTG
N144	F	ATTACCTACAGCCAG**TAG**GGCAAAAACTGGAAG
	R	CTTCCAGTTTTTGCC**CTA**CTGGCTGTAGGTAAT
W148	F	CAGAATGGCAAAAAC**TAG**AAGGTTGTTTATGGT
	R	ACCATAAACAACCTT**CTA**GTTTTTGCCATTCTG
Y152	F	AACTGGAAGGTTGTT**TAG**GGTGGTTATGACACC
	R	GGTGTCATAACCACC**CTA**AACAACCTTCCAGTT
Q174	F	CTCACCGACGGTGGT**TAG**CGCATCAAGTTAAAA
	R	TTTTAACTTGATGCG**CTA**ACCACCGTCGGTGAG
R175	F	ACCGACGGTGGTCAA**TAG**ATCAAGTTAAAAATG
	R	CATTTTTAACTTGAT**CTA**TTGACCACCGTCGGT
L178	F	GGTCAACGCATCAAG**TAG**AAAATGGATAACTGG
	R	CCAGTTATCCATTTT**CTA**CTTGATGCGTTGACC

The amber codon replaced the codons in bold letters. F, forward; R, reverse.