Supporting material for:

NlpD links cell wall remodeling and outer membrane invagination during cytokinesis in *Escherichia coli*

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Plasmid Construction

Plasmids used in this study are listed below. PCR was performed using KOD polymerase (Novagen) for cloning purposes and *Taq* DNA polymerase (NEB) for diagnostic purposes, both according to the manufacturer's instructions. Unless otherwise indicated, MG1655 chromosomal DNA was used as the template. Restriction sites for use in plasmid constructions are bold, italicized and underlined in the primer sequences given below. Plasmid DNA and PCR fragments were purified using the Zyppy plasmid miniprep kit (Zymo Research) or the Qiaquick PCR purification kit (Qiagen), respectively.

<u>pMT12</u>

The plasmid pMT12 [*att*HK022 *tetA tetR lacI* P_{lac}*::nlpD*$^{(S27D)}$] was constructed as follows. The primers 5'-GACTCTCTTCCGGGCGCTATC-3'/5'-GTGCCGGTGGATTTGAAGTGTCATCACAGCCTGC-3' and 5'-GTTTCGCTATGGCTGGCAGGCTGTGATGACACTTC-3'/5'-GACGAAAGTGATTGCGCCTACC-3' were used in overlap extension PCR to amplify the *nlpD* gene from pTD23 and to perform site directed mutagenesis to create the S27D mutation in the *nlpD* gene. The resulting PCR product was purified, digested with XbaI and HindIII and ligated with similarly digested pNP20 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::nlpD-mCherry*] [5].

<u>pMT18</u>

The plasmid pMT18 [*att*HK022 *bla lacI*^{q} P$_{lac}$::^{ss}*dsbA-nlpD*$^{(27\text{-}379)}$] was constructed as follows. The primers 5’-GTCA***GGATCC***TCTGACACTTCAAATCCACCGGC-3’ and 5’-GTCA***AAGCTT***CCGCCGATTTATCGCTGC-3’ were used to amplify *nlpD(27-379)* from genomic DNA. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pTB282 [*att*HK022 *bla lacI*^{q} P$_{lac}$::^{ss}*dsbA-sfGFP*] [5].

pMT21

The plasmid pMT21 [*att*HK022 *tetA tetR lacI* P$_{lac}$::*nlpD*$^{(S27D)}$*-mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCACTCGAGTCGCTGCGGCAAATAACGC-3’ were used to amplify *nlpD(S27D)* from pMT12. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP18 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$::*envC-mCherry*] [5].

pMT94

The plasmid pMT94 [*cat lacI* P$_{lac}$::*nativeRBS_ybgC_tolQRA*] was constructed as follows and involved several steps to assemble. First, the *lacI*^{q}/P$_{lac}$ containing BglII/XbaI fragment of pHC528 [*attλ cat lacI*^{q} *P*$_{lac}$::*slmA*] [12] was used to replace the corresponding fragment of pDY31 [*cat* P$_{ara}$::*nativeRBS-amiB*] [13]. The primers 5’-GTCA***TCTAGA***CTCTAACTTTTGTTGCATTACCGGGATG-3’ and 5’-GTCA***ATCGAT***TTACGGTTTGAAGTCCAATGGCG-3’ were used to amplify *nativeRBS_ybgC_tolQRA* from genomic DNA. The resulting PCR product was purified, digested with XbaI and ClaI and ligated into the above plasmid digested with the same enzymes to create pMT94.

pMT101

The plasmid pMT101 [*att*HK022 *tetA tetR lacI* P_{lac}::*nlpD*$^{(1-189)}$-*mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***CTCGAG***AACTCCTTGCTCTGCTGCGTC-3’ were used to amplify *nlpD(1-189)* from pMT20. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP18.

pMT102

The plasmid pMT102 [*att*HK022 *tetA tetR lacI* P_{lac}::*nlpD*$^{(1-189)}$] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***AAGCTT***AAACTCCTTGCTCTGCTGCGTC-3’ were used to amplify *nlpD(1-189)* from pMT20. The resulting PCR product was purified, digested with XbaI and HindIII and ligated with similarly digested pNP20.

pMT103

The plasmid pMT103 [*att*HK022 *tetA tetR lacI* P_{lac}::*nlpD*$^{(1-115)}$-*mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***CTCGAG***TTTCGGAATGTTCCCATACTGACGG-3’ were used to amplify *nlpD(1-115)* from pMT20. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP18.

pMT104

The plasmid pMT104 [*att*HK022 *tetA tetR lacI* P_{lac}::*nlpD*$^{(1-115)}$] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCA***<u>AAGCTT</u>***ATTTCGGAATGTTCCCATACTGACGG-3' were used to amplify *nlpD(1-115)* from pMT20. The resulting PCR product was purified, digested with XbaI and HindIII and ligated with similarly digested pNP20.

<u>pMT105</u>

The plasmid pMT105 [*att*HK022 *tetA tetR lacI* P_{lac}::^{ss}*dsbA-nlpD*$^{(102-175)}$] was constructed as follows. The primers 5'-GTCA***<u>GGATCC</u>***GGACGCATCGTCTATAACCGTCAGTATG-3' and 5'-GTCA***<u>AAGCTT</u>***CCGCCGATTTATCGCTGC-3' were used to amplify *nlpD(102-175)* from genomic DNA. The resulting PCR product was purified, digested with BamHI and HindIII and ligated with similarly digested pNP19 [*att*HK022 *tetA tetR lacI* P_{lac}::^{ss}*dsbA-amiB-sfGFP*] [5].

<u>pMT121</u>

The plasmid pMT121 [*att*HK022 *tetA tetR lacI* P_{lac}::^{ss}*dsbA-nlpD*$^{(27-379)}$] was constructed as follows. The *nlpD* containing XbaI/HindIII fragment of pMT18 [*att*HK022 *bla lacI*^{q} P_{lac}::^{ss}*dsbA-nlpD*$^{(27-379)}$] was used to replace the corresponding fragment of pNP20.

<u>pMT147</u>

The plasmid pMT147 [*att*HK022 *tetA tetR lacI* P_{lac}::^{ss}*dsbA-nlpD*$^{(27-379)}$*-mCherry*] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-

GTCA***<u>CTCGAG</u>***TCGCTGCGGCAAATAACGC-3’ were used to amplify *^{ss}dsbA-nlpD(27-379)* from pMT121. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

<u>pMT149</u>

The plasmid pMT149 [*att*HK022 *tetA tetR lacI* P_{lac}*::nlpD$^{(1-30)}$-mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***<u>CTCGAG</u>***TGAAGTGTCAGAACAGCCTGCC-3’ were used to amplify *nlpD(1-30)* from pMT20. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

<u>pMT178</u>

The plasmid pMT178 [*att*HK022 *tetA tetR lacI* P_{lac}*:: ^{ss}dsbA-nlpD$^{(102-175)}$-mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GCTA***<u>CTCGAG</u>***GCCAGTGATTGGCGTACCG-3’ were used to amplify *^{ss}dsbA-nlpD(102-175)* from pMT105. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

<u>pMT179</u>

The plasmid pMT179 [*att*HK022 *tetA tetR lacI* P_{lac}*:: ^{ss}dsbA-nlpD$^{(250-379)}$*] was constructed as follows. The primers 5’-GTCA***<u>GGATCC</u>***AGTACATCAACCAGTACGCCTATCTCC-3’ and 5’-GACGAAAGTGATTGCGCCTACC-3’ were used to amplify *nlpD(250-379)* from

pMT20. The resulting PCR product was purified, digested with BamHI and HindIII and ligated with similarly digested pNP19.

pMT180

The plasmid pMT180 [*att*HK022 *tetA tetR lacI* P_{lac}:: ^{ss}*dsbA-nlpD*$^{(250-379)}$*-mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***<u>CTCGAG</u>***TCGCTGCGGCAAATAACGC-3’ were used to amplify *nlpD(250-379)* from pMT179. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

pMT181

The plasmid pMT181 [*att*HK022 *tetA tetR lacI* P_{lac}:: ^{ss}*dsbA-nlpD*$^{(189-379)}$] was constructed as follows. The primers 5’-GTCA***<u>GGATCC</u>***GTTGTGATCAAGCCTGCACAAAATTCC-3’ and 5’-GACGAAAGTGATTGCGCCTACC-3’ were used to amplify *nlpD(189-379)* from pMT20. The resulting PCR product was purified, digested with BamHI and HindIII and ligated with similarly digested pNP19.

pMT182

The plasmid pMT182 [*att*HK022 *tetA tetR lacI* P_{lac}:: ^{ss}*dsbA-nlpD*$^{(189-379)}$*-mCherry*] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA***<u>CTCGAG</u>***AACTCCTTGCTCTGCTGCGTC-3’ were used to amplify *nlpD(189-379)* from pMT181. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

pMT187

This plasmid pMT187 [*aadA repA(ts)* $lacI^q$ P_{lac}*::envC-LE* SceI *cI857* $P_{\lambda R}$::i-sceI] is a derivative of pBL200 [*aadA repA(ts)* P_{syn135}*::ftsN* SceI *cI857* $P_{\lambda R}$::i-sceI] [14] in which the P_{syn135}*::ftsN* containing EcoRI/HindIII fragment was replaced with $lacI^q$ P_{lac}*::envC-LE*.

pMT196

The plasmid pMT196 [*attλ cat lacI* P_{lac}*::yraP*$^{(1-191)}$] was constructed as follows. The primers 5'-GCTA***TCTAGA***TTAAGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3' and 5'-GCTA***AAGCTT***GGCTGCTATTTAATAAACGTAAACGCCG-3' were used to amplify *yraP(1-191)* from genomic DNA. The resulting PCR product was purified, digested with XbaI and HindIII and ligated with similarly digested pHC514 [*attλ cat lacI* P_{lac}*::slmA*].

pMT197

The plasmid pMT197 [*attλ cat lacI* P_{lac}*::yraP*$^{(1-191)}$*-mCherry*] was constructed as follows. The primers 5'-GCTA***TCTAGA***TTAAGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3' and 5'-GCTA***CTCGAG***TTTAATAAACGTAAACGCCGTAGTTACCCG-3' were used to amplify *yraP(1-191)* from genomic DNA. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with a similarly digested pHC514 derivative containing mCherry.

pMT198

The plasmid pMT198 [*attλ cat lacI* P_{lac}*:: yraP*$^{(VA\text{-}20,21\text{-}DE)}$] was constructed as follows. The primers 5'-GCTA***TCTAGA***TTAAGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3'/5'-CTACGGCAGCCTCATCACAACCTTGCAACAGCAGCG-3' and 5'-GCAAGGTTGTGATGAGGCTGCCGTAGTGGGTACCGC-3'/5'-GCTA***AAGCTT***GGCTGCTATTTAATAAACGTAAACGCCG-3' were used in overlap extension PCR to amplify the *yraP* gene from genomic DNA and to perform site directed mutagenesis to create the (VA-20,21-DE) mutations in the *yraP* gene. The resulting PCR product was purified, digested with XbaI and HindIII and ligated with similarly digested pHC514 [*attλ cat lacI* P_{lac}*::slmA*].

pMT199

The plasmid pMT199 [*attλ cat lacI* P_{lac}*:: yraP*$^{(VA\text{-}20,21\text{-}DE)}$*-mCherry*] was constructed as follows. The primers 5'-GCTA***TCTAGA***TTAAGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3'/5'-CTACGGCAGCCTCATCACAACCTTGCAACAGCAGCG-3' and 5'-GCAAGGTTGTGATGAGGCTGCCGTAGTGGGTACCGC-3'/5'-GCTA***CTCGAG***TTTAATAAACGTAAACGCCGTAGTTACCCG-3' were used in overlap extension PCR to amplify the *yraP* gene from genomic DNA and to perform site directed mutagenesis to create the (VA-20,21-DE) mutations in the *yraP* gene. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with a similarly digested pHC514 derivative containing mCherry.

pMT209

The plasmid pMT209 [*attλ cat lacI* P_{lac}::^{ss}*dsbA-yraP*$^{(24-191)}$] was constructed in two steps as follows. The primers 5'-GCTA***GGATCC***GTAGTGGGTACCGCTGCTG-3' and 5'-GCTA***AAGCTT***GGCTGCTATTTAATAAACGTAAACGCCG-3' were used to amplify *yraP(24-191)* from genomic DNA. The resulting PCR product was purified, digested with BamHI and HindIII and ligated with similarly digested pNP19 to make pMT200 [*att*HK022 *tetA tetR lacI* P_{lac}::^{ss}*dsbA-yraP*$^{(24-191)}$]. The *yraP* containing XbaI/HindIII fragment of pMT200 was then used to replace the corresponding fragment of pHC514.

pMT210

The plasmid pMT210 [*attλ cat lacI* P_{lac}::^{ss}*dsbA-yraP*$^{(24-191)}$*-mCherry*] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GCTA***CTCGAG***TTTAATAAACGTAAACGCCGTAGTTACCCG-3' were used to amplify ^{ss}*dsbA-yraP(24-191)* from pMT200. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with a similarly digested pHC514 derivative containing mCherry.

pMT224

The plasmid pMT224 [*bla araC* P_{ara}::*sulA*] was constructed as follows. The *sulA* containing XbaI/HindIII fragment of pMT74 [*tetA tetR lacI*^{q} P_{lac}::*sulA*] was used to replace the corresponding fragment of pBAD24 [*bla araC* P_{ara}::*empty*] [6].

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