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.

pMT12
The plasmid pMT12 [attHK022 tetA tetR lacI P_{lac}::nlpD^{S27D}] was constructed as follows. The primers 5’-GACTCTCTTCCGGGCGCTATC-3’/5’-GTGCCGGTGGATTTGAAGTGTCATCACAGCCTGC-3’ and 5’-GTTTCGCTATGGCTGGCAGGCTGTGATGACACTTC-3’/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 [attHK022 tetA tetR lacI P_{lac}::nlpD-mCherry] [5].

pMT18
The plasmid pMT18 [attHK022 bla lacI P_lac::ssdsbA-nlpD(27-379)] was constructed as follows. The primers 5'-GTCA**GGATCC**TCTGACACTTCAAATCCACCGGC-3' and 5'-GTCA**AAGCTT**CCGCCGATTATCGCTGC-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 [attHK022 bla lacI P_lac::ssdsbA-sfGFP] [5].

**pMT21**

The plasmid pMT21 [attHK022 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 [attHK022 tetA tetR lacI 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 *lacIq/P_lac* containing BglII/XbaI fragment of pHC528 [attλ cat lacI P_lac::slmA] [12] was used to replace the corresponding fragment of pDY31 [cat P ara::nativeRBS-amiB] [13]. The primers 5'-GTCA**TCTAGA**CTCTAACACTTTTGTGCATTACCGGGATG-3' and 5'-GTCA**ATCGATT**TACGGTTGAAATCGATGCGGATG-3' were used to amplify *nativeRBS_ybgC_tolQRA* from genomic DNA. The resulting PCR product was purified, digested with XbaI and Clal and ligated into the above plasmid digested with the same enzymes to create pMT94.
pMT101

The plasmid pMT101 \([att\text{HK022}} tetA tetR lacI P_{\text{lac}}::nlpD^{(1-189)}\text{-mCherry}\] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCA\textbf{CTCGAG}AACTCCTTGCTCTGCTGCGTC-3' were used to amplify \textit{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\text{HK022}} tetA tetR lacI P_{\text{lac}}::nlpD^{(1-189)}\] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCA\textbf{AAGCTT}AACTCCTTGCTCTGCTGCGTC-3' were used to amplify \textit{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\text{HK022}} tetA tetR lacI P_{\text{lac}}::nlpD^{(1-115)}\text{-mCherry}\] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCA\textbf{CTCGAG}TTTCCGGAATGTTCCCATACTGACGG-3' were used to amplify \textit{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 [attHK022 tetA tetR lacI P_{lac}::nlpD^{(1-115)}] was constructed as follows. The primers 5'-TTAGGCACCCACCAGGTATTTACAC-3' and 5'-GTCAAAAGCTTATTTCGGAATGTTCCCATACTGACGG-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.

pMT105

The plasmid pMT105 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(102-175)}] was constructed as follows. The primers 5'-GTCAAGATCCGGACGCATCGTCTATAACCGTCAGTATG-3' and 5'-GTCAAAAGCTCCGCCGATTATCGCTGC-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 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-amiB-stGFP] [5].

pMT121

The plasmid pMT121 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(27-379)}] was constructed as follows. The nlpD containing XbaI/HindIII fragment of pMT18 [attHK022 bla lacI P_{lac}::ssdsbA-nlpD^{(27-379)}] was used to replace the corresponding fragment of pNP20.

pMT147

The plasmid pMT147 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(27-379)}-mCherry] was constructed as follows. The primers 5'-TTAGGCACCCACCAGGTATTTACAC-3' and 5'-
GTCA\textbf{CTCGAG}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.

\textbf{pMT149}

The plasmid pMT149 [\textit{attHK022 tetA tetR lacI} \textit{P}_{\text{lac}}::nlpD^{(1-30)}-mCherry] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA\textbf{CTCGAG}TGAAGTGTCAGAACAGCCTGCC-3’ were used to amplify \textit{nlpD}(1-30) from pMT20. The resulting PCR product was purified, digested with XbaI and XhoI and ligated with similarly digested pNP20.

\textbf{pMT178}

The plasmid pMT178 [\textit{attHK022 tetA tetR lacI} \textit{P}_{\text{lac}}::^{ss}dsbA-nlpD^{(102-175)}-mCherry] was constructed as follows. The primers 5’-TTAGGCACCCCAGGCTTTACAC-3’ and 5’-GTCA\textbf{CTCGAG}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.

\textbf{pMT179}

The plasmid pMT179 [\textit{attHK022 tetA tetR lacI} \textit{P}_{\text{lac}}::^{ss}dsbA-nlpD^{(250-379)}] was constructed as follows. The primers 5’-GTCA\textbf{GGATCC}AGTACATCAACCAGTACGTATCTCTCC-3’ and 5’-GACGAAAGTGATTGGCGCTACC-3’ were used to amplify \textit{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 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(250-379)}-mCherry] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCACTCGAGTCGCTGCGGCAAATAACGC-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 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(189-379)}] was constructed as follows. The primers 5'-GTCACTCGAGTCGCTGCGGCAAATAACGC-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.

pMT182
The plasmid pMT182 [attHK022 tetA tetR lacI P_{lac}::ssdsbA-nlpD^{(189-379)}-mCherry] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCACTCGAGTCGCTGCGGCAAATAACGC-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) laclac::envC-LE SceI cl857 Pλ::i-sceI] is a derivative of pBL200 [aadA repA(ts) Psyn135::ftsN SceI cl857 Pλ::i-sceI] [14] in which the Psyn135::ftsN containing EcoRI/HindIII fragment was replaced with laclac::envC-LE.

pMT196

The plasmid pMT196 [attλ cat lacI P lac::yraP(1-191)] was constructed as follows. The primers 5’-GCTATCTAGATTGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3’ and 5’-GCTAAGCTTGCTGCTATTAAACGTAAACGCCG-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 pH514 [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’-GCTATCTAGATTGAAGGAGATATACATATGAAGGCATTATCGCCAATCGC-3’ and 5’-GCTACTCGAGTTAAACGTAAACGCCTAGTTACCCG-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 pH514 derivative containing mCherry.
The plasmid pMT198 [att\(\lambda\) cat lacI \(P_{lac}:: yraP^{(VA-20,21-DE)}\)] was constructed as follows. The primers 5’-

\[
\text{GCTATCTAGA} \text{TATAAGAAGGAGATATACATATGAAAGGCTATTATCGCCAATCGC-3’/5’-}
\]

\[
\text{CTACGCCAGCCTCATCACAACCTTGCAACAGCAGCG-3’ and 5’-}
\]

\[
\text{GCAAGGGTTGTGATGAGGCTGCTGCTAAGGTGTAACGC-3’/5’-}
\]

\[
\text{GCTAACGCTTGCTATTTAATAAACGTAAACGCCG-3’ were used in overlap}
\]

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 [att\(\lambda\) cat lacI \(P_{lac}::slmA\)].

The plasmid pMT199 [att\(\lambda\) cat lacI \(P_{lac}:: yraP^{(VA-20,21-DE)}-mCherry\)] was constructed as

follows. The primers 5’-

\[
\text{GCTATCTAGA} \text{TATAAGAAGGAGATATACATATGAAAGGCTATTATCGCCAATCGC-3’/5’-}
\]

\[
\text{CTACGCCAGCCTCATCACAACCTTGCAACAGCAGCG-3’ and 5’-}
\]

\[
\text{GCAAGGGTTGTGATGAGGCTGCTGCTAAGGTGTAACGC-3’/5’-}
\]

\[
\text{GCTACCTCGAG} \text{TTTAATAAACGTAAACGCCGCTAGTTACCG-3’ were used in overlap}
\]

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 \([\text{att}\lambda\ cat\ lacI\ P}_{\text{lac}}::^{ss}\text{dsbA-yraP}^{(24-191)}\] was constructed in two steps as follows. The primers 5'-GCTAGGATCCGTAGTGGGTACCGCTGCTG-3' and 5'-GCTAAAGCTTGCTGCTATTAAACGTAAACGCGC-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 \([\text{att}\ HK022\ tetA\ tetR\ lacI\ P}_{\text{lac}}::^{ss}\text{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 \([\text{att}\lambda\ cat\ lacI\ P}_{\text{lac}}::^{ss}\text{dsbA-yraP}^{(24-191)}-\text{mCherry}\] was constructed as follows. The primers 5'-TTAGGCACCCCGCTTTACAC-3' and 5'-GCTACTCGAGTTTAATAACGTAAACGCGTACGTTACCG-3' were used to amplify \(^{ss}\text{dsbA-yraP}^{(24-191)}\) 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 \([\text{bla}\\ araC\ P}_{\text{ara}}::\text{sulA}\] was constructed as follows. The \(\text{sulA}\) containing XbaI/HindIII fragment of pMT74 \([\text{tetA}\\ tetR\ lacI}\ P_{\text{lac}}::\text{sulA}\] was used to replace the corresponding fragment of pBAD24 \([\text{bla}\\ araC\ P}_{\text{ara}}::\text{empty}\] [6].
REFERENCES


