Roles of ATP Hydrolysis by FtsEX and Interaction with FtsA in Regulation of Septal Peptidoglycan Synthesis and Hydrolysis

Shishen Du,a Sebastien Pichoff,b Joe Lutkenhausa

aDepartment of Microbiology, College of Life Sciences, Wuhan University, Wuhan, China
bDepartment of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas, USA

ABSTRACT In Escherichia coli, FtsEX coordinates peptidoglycan (PG) synthesis and hydrolysis at the septum. It acts on FtsA in the cytoplasm to promote recruitment of septal PG synthetases and recruits EnvC, an activator of septal PG hydrolases, in the periplasm. Following recruitment, ATP hydrolysis by FtsEX is thought to regulate both PG synthesis and hydrolysis, but how it does this is not well understood. Here, we show that an ATPase mutant of FtsEX blocks septal PG synthesis similarly to cephalaxin, suggesting that ATP hydrolysis by FtsEX is required throughout septation. Using mutants that uncouple the roles of FtsEX in septal PG synthesis and hydrolysis, we find that recruitment of EnvC to the septum by FtsEX, but not ATP hydrolysis, is required to promote cell separation when the NlpD-mediated cell separation system is present. However, ATP hydrolysis by FtsEX becomes necessary for efficient cell separation when the NlpD system is inactivated, suggesting that the ATPase activity of FtsEX is required for optimal activity of EnvC. Importantly, under conditions that suppress the role of FtsEX in cell division, disruption of the FtsEX-FtsA interaction delays cell separation, highlighting the importance of this interaction in coupling the cell separation system with the septal PG synthetic complex.

IMPORTANCE Cytokinesis in Gram-negative bacteria requires coordinated invagination of the three layers of the cell envelope; otherwise, cells become sensitive to hydrophobic antibiotics and can even undergo cell lysis. In E. coli, the ABC transporter FtsEX couples the synthesis and hydrolysis of the stress-bearing peptidoglycan layer at the septum by interacting with FtsA and EnvC, respectively. ATP hydrolysis by FtsEX is critical for its function, but the reason why is not clear. Here, we find that in the absence of ATP hydrolysis, FtsEX blocks septal PG synthesis similarly to cephalaxin. However, an FtsEX ATPase mutant, under conditions where it cannot block division, rescues ftsEX phenotypes as long as a partially redundant cell separation system is present. Furthermore, we find that the FtsEX-FtsA interaction is important for efficient cell separation.

KEYWORDS amidase, EnvC, FtsA, FtsEX, peptidoglycan

For most bacteria, cell division requires the remodeling of peptidoglycan (PG) at the division site, which entails the coordinated activation of PG synthetases and hydrolases (1). These enzymes need to be highly regulated, since dysregulation could lead to a breach in the wall and to cell lysis due to turgor pressure. Spatial and temporal regulation of these enzymes is achieved by coupling their recruitment and activation to the Z ring (2). Septal peptidoglycan synthesis is carried out by FtsW, a member of the SEDS family, which was recently shown to have PG glycosyltransferase activity (3, 4), and FtsI, a transpeptidase (5, 6). The FtsWI complex is recruited to the Z ring by the FtsQLB complex and in Escherichia coli is activated by the arrival of FtsN (7). A theme that has emerged from work with E. coli and Streptococcus pneumoniae is that peptidoglycan hydrolases or their activators are recruited to the division site by FtsEX, a
member of the ABC transporter family. In *E. coli*, FtsEX recruits EnvC, which activates amidases (AmiA and AmiB) at the Z ring (8), whereas in *S. pneumoniae*, FtsEX directly recruits and regulates the PcsB hydrolase (9). Following these pioneering studies, FtsEX was also found to regulate the RipC hydrolase in *Mycobacterium tuberculosis* and *Corynebacterium glutamicum* (10, 11).

FtsEX is a member of the type VII subfamily of ABC transporters, which employ a mechanotransduction mechanism to perform work in the periplasm (12). Members of this subfamily include MacB, which expels antibiotics and virulence factors, and LolCDE, which extracts lipoproteins from the outer leaflet of the cytoplasmic membrane in Gram-negative bacteria (13). In *E. coli*, FtsEX is essential for cell division at low to moderate osmolarity and plays a role in cell separation (8, 14, 15). The complex localizes to the Z ring as it forms through an interaction between FtsE and the conserved tail (conserved C-terminal peptide [CCTP]) of FtsZ (16). In a step that does not require ATP hydrolysis, FtsEX (i) interacts with FtsA to promote the recruitment of downstream division proteins and (ii) recruits EnvC through an interaction between the large periplasmic loop of FtsX and the coiled-coil domain of EnvC (8, 17). Once the divisome is assembled, FtsEX must undergo ATP hydrolysis; otherwise, it blocks septal PG synthesis through its interaction with FtsA (17). In addition to relieving this block, ATP hydrolysis by FtsEX is thought to cause a conformational change in EnvC, which allows it to activate AmiB and AmiA at the division site to remove cross-links between the glycan strands, leading to cell separation (18).

Mutations in *ftsE* that affect ATP binding or hydrolysis have been shown to impair cell division and cell separation (8, 15, 17). However, it is not clear how ATP hydrolysis is regulated. In the case of MacB, its binding partner in the periplasm, MacA, greatly stimulates its ATPase activity (19). By analogy, EnvC would stimulate the ATPase activity of FtsEX, allowing cell division to proceed. However, an FtsEXΔlp mutant, carrying a deletion in the large periplasmic loop that eliminates interaction with EnvC, supports cell division (17), indicating that it is able to carry out ATP hydrolysis.

Despite its important roles in cell division, *ftsEX* can be deleted in *E. coli* under a variety of conditions, including increased osmolarity, overexpression of *ftsQAZ*, and mutations in *ftsA* that reduce FtsA’s self-interaction (17, 20, 21). These suppressive conditions appear to enhance the interaction between FtsA and FtsN so that the divisome is assembled and activated (22). However, EnvC is not recruited to the Z ring, and as a consequence, Δ*ftsEX* cells display a mild chaining phenotype (8). The length of these chains is limited, since cells separate due to a partially redundant pathway involving another amidase (AmiC) controlled by NlpD (18). The activities of these two systems overlap, and severe chaining is observed only when both systems are inactive, as when the activators (*ftsEX* or *envC* and *nlpD*) or all three amidases are deleted (8, 23).

In this study, we further explore how FtsEX coordinates septal PG synthesis and hydrolysis. We find that an ATPase mutant of FtsEX mimics cephalaxin in blocking division, suggesting that ATP hydrolysis by FtsEX is required throughout the septation process. We also find that the loss of *ftsEX*, like the loss of EnvC (24), results in sensitivity to hydrophobic antibiotics. However, this sensitivity is suppressed by an ATPase mutant of FtsEX, which also suppresses chaining, suggesting that EnvC can promote cell separation in the absence of ATPase hydrolysis. However, in the absence of the other cell separation system, the ATPase mutant of FtsEX only partially rescues cell separation, indicating that ATP hydrolysis by FtsEX is required for optimal activity of EnvC. In addition, we find that FtsEX must interact with FtsA for cells to separate efficiently, suggesting that tight coupling of the cell separation system with the septal PG synthetic machinery is critical for timely cell separation.

**RESULTS**

An ATPase mutant of FtsE blocks ongoing septal PG synthesis. In the current model for divisome activation, FtsN triggers septal PG synthesis by acting on FtsA and FtsQLB (25–27). Once septal PG synthesis starts, EnvC in the periplasm stimulates AmiA and AmiB to sever peptide cross-links in the newly synthesized PG, generating denuded...
peptidoglycan chains. Additional FtsN is attracted to the septum through the binding of these chains by the SPOR domain of FtsN, further enhancing septal PG synthesis (7) (Fig. 1A). Thus, FtsN activation can be divided into the triggering step and the self-enhancement stage. Previous results (17) showed that the ATPase mutant FtsED162NX (which, by analogy with MacB, would bind ATP but be defective in ATP hydrolysis [12]) acts on FtsA to generate smooth filaments, indicating that it blocks the initiation of constriction. However, it is not clear if FtsED162NX just blocks initiation or if it blocks ongoing septation as well (17).

To determine the effect of FtsED162NX on ongoing septation, we monitored the contraction of Z rings and division upon expression of ftsED162NX. We introduced a plasmid expressing ftsED162NX under the control of isopropyl-β-D-thiogalactopyranoside (IPTG) into a strain that constitutively expresses zapA-gfp from the chromosome as a proxy for Z rings. One hour after the addition of IPTG to an exponential-phase culture, a sample was spotted onto an agarose pad (with IPTG) and followed by time-lapse microscopy at room temperature for 40 min (Fig. 2; see also Fig. S1 in the supplemental material). In the control sample without IPTG, all cells with a visible constriction at time zero had completed constriction by 40 min (18/18), and this was accompanied by the disappearance of the associated Z rings and the appearance of new Z rings in the daughter cells (Fig. S1; Table S1). An example of such a cell is indicated by long arrows in Fig. 2. In other cells, a Z ring was present without an apparent constriction at time zero (short arrows), but by 40 min, the constriction was almost complete: the Z ring was reduced to a spot, and the daughter cells were nearly separated.

In the sample in which FtsED162NX was induced (with IPTG added), the cells increased in length by 1 h after IPTG addition (time zero), a finding consistent with FtsED162NX blocking division. In cells without a constriction, the Z ring persisted but did not contract during the 40 min of observation (Fig. 2 and Fig. 5). Even in cells with a
visible constriction (long arrow) at time zero, the Z ring persisted without noticeably changing in diameter (7/11). In four constricting cells, the Z ring disappeared, but in these cells the constriction was already very deep at time zero, suggesting that very late stages of cell division may not be blocked (Fig. S1 and Table S1). In rare cases (2/46), the Z ring disappeared without a constriction (Fig. 2, short arrow). These results indicate that FtsED162NX blocks ongoing constriction without disrupting the Z ring.

The block to division by FtsED162NX is phenotypically similar to the block by cephalixin, which inactivates PBP3 (FtsI) and blocks both initiation and ongoing constriction events (28). To see to what extent the block by FtsED162NX mimics the action of cephalixin, the two treatments were compared directly. An exponentially growing culture of the strain used in the experiments described above was split in two, and cephalixin was added to one half for 45 min, while IPTG was added to the other half for 60 min to induce FtsED162NX (Fig. 3A). At these time points, the cell lengths of the two cultures were comparable, indicating that cell division was inhibited to similar extents, whereas segregation of nucleoids was not affected (Fig. 3A). Both treatments resulted in almost every cell containing a single ZapA-green fluorescent protein (GFP) ring at midcell, a finding consistent with an inability of Z rings to constrict. To confirm that septal PG synthesis was inhibited, we used the fluorescent d-amino acid HADA to label newly synthesized PG (6, 29). In the control culture, a band of HADA was observed to coincide with the position of the Z ring in all cells with a constriction (Fig. 3B). In contrast, in the cephalixin-treated culture, no band of HADA was observed to overlap the Z ring (0/154) (Table S2). Similarly, in the culture with FtsED162NX induced for 45 min, bands of HADA overlapping the Z ring were infrequent (14/152) (Table S2), and they were even less frequent by 90 min (10/180) (Table S2). These results provide additional support for the observations that FtsED162NX expression phenocopies cephalixin treatment and that FtsED162NX blocks ongoing septation.

**FIG 2** Expression of FtsED162NX blocks ongoing constrictions. An overnight culture of HC261 (zapA-GFP)/pSD221-D162N (pEXT22 P_{lac}-ftsED162NX) was diluted 100-fold in LB with sucrose and antibiotics and was grown at 30°C. After 2 h, 2 μl of the culture was spotted onto a 2% agarose pad containing LB. ZapA-GFP and cell division were followed for 40 min. To follow ZapA-GFP localization in cells expressing FtsED162NX, the overnight culture of HC261 (zapA-GFP)/pSD221-D162N was diluted 100-fold in LB with sucrose and antibiotics and was grown at 30°C until the OD_{600} reached about 0.6. The culture was diluted 5 times in the same medium containing 250 μM IPTG. After induction for 1 h, 2 μl of the culture was spotted onto a 2% agarose pad containing LB with IPTG and sucrose and was monitored for 40 min. Long arrows in the left panel indicate a cell with a constriction at time zero which is completed by 20 min, with new Z rings forming in the daughter cells by 30 min. The other arrow indicates a ring that shrinks in diameter during the period of observation. The long arrow in the right panel indicates a cell with a constriction and an associated Z ring that does not change diameter during the 40 min of the experiment. The other arrow indicates a rare example of a cell with a Z ring that disappears around 20 min.
An ATPase mutant of FtsEX suppresses the sensitivity of a ΔftsEX mutant to hydrophobic antibiotics. Previous studies indicated that ATP hydrolysis by FtsEX was essential for septal PG synthesis and for the activation of amidases (AmiA and AmiB) by EnvC to cleave septal PG for cell separation (8, 17). However, in vitro studies found that EnvC activated AmiA and AmiB in the absence of FtsEX (18), raising questions about the role of ATP hydrolysis by FtsEX in EnvC-induced activation of the amidases. Thus, we decided to reassess the role of ATP hydrolysis by FtsEX in EnvC-mediated cell separation. Because ATPase mutants of FtsEX cannot support septal PG synthesis, as shown above, we took advantage of an ftsA allele (ftsA* G366D) that carries two mutations (17). One mutation (ftsA*) suppresses the growth defects of ΔftsEX cells, but the cells are sensitive to inhibition by an FtsEX ATPase mutant (17). However, the addition of a second mutation (ftsA* G366D) confers resistance to this inhibition by disrupting the FtsEX-FtsA interaction (17). Thus, the ftsA* G366D allele allows us to test if ATP hydrolysis by FtsEX is required for cell separation without worrying about inhibition of septal PG synthesis. We also took advantage of the sensitivity of chaining mutants to hydrophobic drugs to devise a complementation test.

Deletion of envC results in a mild chaining phenotype and sensitivity to hydrophobic drugs (24, 30, 31). Since deletion of ftsEX also results in a mild chaining phenotype under conditions permissive for growth, we tested the sensitivity of a ΔftsEX strain to rifampin, a hydrophobic drug. We found that the loss of ftsEX, like the loss of envC, displayed increased sensitivity to rifampin (Fig. 4). However, the ΔftsEX strain was even more sensitive than the ΔenvC strain; the ΔenvC mutant was unable to form colonies on plates with 4 μg/ml of rifampin, whereas the ΔftsEX mutant was unable to form colonies on plates with 2 μg/ml. The increased sensitivity of the ΔftsEX mutant is accompanied by a 50% increase in the average cell length (Table S3) and is consistent with FtsEX having roles beyond regulating EnvC, as previously reported (14, 17). Introduction of the ftsA* or ftsA* G366D allele into the ΔftsEX mutant partially suppressed the sensitivity to rifampin such that these mutants were comparable to the ΔenvC strain (Fig. 4). Both of these alleles also reduced the average cell length to slightly less than that of the ΔenvC strain, indicating that they behaved similarly (Table S3).
The sensitivity of the ΔftsEX ftsA∗,G366D strain to rifampin allowed us to test whether ATP hydrolysis by FtsEX was critical for the suppression of sensitivity to this drug by EnvC. For this purpose, plasmids carrying various alleles of ftsEX under the control of an IPTG-inducible promoter were introduced into this strain. As shown in Fig. 5A, ftsED162NX suppressed rifampin sensitivity as well as wild-type (WT) ftsEX. In contrast, neither ftsEX/H9004 nor ftsED162NX/H9004, which are unable to interact with EnvC due to a

The sensitivity of the ΔftsEX ftsA∗,G366D strain to rifampin allowed us to test whether ATP hydrolysis by FtsEX was critical for the suppression of sensitivity to this drug by EnvC. For this purpose, plasmids carrying various alleles of ftsEX under the control of an IPTG-inducible promoter were introduced into this strain. As shown in Fig. 5A, ftsED162NX suppressed rifampin sensitivity as well as wild-type (WT) ftsEX. In contrast, neither ftsEX/H9004 nor ftsED162NX/H9004, which are unable to interact with EnvC due to a

A  
ftsA*,G366D, ΔftsEX/P_tac::

B  
Vector
ftsEX
ftsED162NX
ftsEXΔp
ftsED162NXΔp

FIG 4  The increased sensitivity of ΔftsEX cells (relative to ΔenvC cells) to a hydrophobic drug (rifampin) is suppressed by ftsA∗ alleles. Cultures of the various strains were serially diluted 10-fold, and 3 μl of each dilution was spotted onto plates containing increasing concentrations of rifampin. The strains were derivatives of W3110 and included W3110 (leu::Tn10) (WT), SD208 (W3110 envC::kan), SD220 (W3110 leu::Tn10 ftsEX::cat), PS2343 (W3110 leu::Tn10 ftsA∗), SD221 (W3110 leu::Tn10 ftsA∗ ftsEX::cat), SD249 (W3110 leu::Tn10 ftsA∗,G366D), and SD262 (W3110 leu::Tn10 ftsA∗,G366D ΔftsEX::cat).

FIG 5  EnvC, but not ATP hydrolysis by FtsEX, is required to suppress the phenotypic defects of ΔftsEX cells. (A) Interaction with EnvC, but not ATPase hydrolysis, is required for FtsEX to suppress the sensitivity of a ΔftsEX ftsA∗,G366D strain to rifampin. Sensitivity to rifampin was assessed by streaking strains onto LB plates containing 0.2 M sucrose and 4 μg/ml of rifampin. Shown are results for SD262 (leu::Tn10 ftsA∗,G366D ΔftsEX::cat) carrying derivatives of pDSW208 expressing various alleles of ftsEX under the control of an IPTG-inducible promoter. The plasmids were pDSW208 (P204::gfp), pSEB428 (P204::ftsEX), pSEB428-D162N (P204::ftsEXΔD162N), pSD213 (P204::ftsEXΔp), and pSD213-D162N (P204::ftsEXΔD162NΔp). (B) Interaction with EnvC, but not ATPase hydrolysis, is required for FtsEX to suppress the cell separation defect of the ΔftsEX ftsA∗,G366D strain. The strains in panel A were grown to exponential phase in LB with sucrose and antibiotics, and samples were taken for photography. Note that rifampin sensitivity correlates with the chaining phenotype.
deletion in the large periplasmic loop of FtsX, was able to suppress the rifampin sensitivity. Thus, interaction of FtsEX with EnvC, but not its ATPase activity, is required for the suppression of sensitivity to hydrophobic drugs.

To see if rifampin sensitivity correlated with the chaining phenotype of the strains, they were grown in liquid medium to exponential phase and were examined by phase-contrast microscopy (Fig. 5B). Indeed, the ΔftsEX ftsA*G366D strain containing the vector produced elongated cells (average length, 21.1 ± 9.3 μM [Table S4]) due to cell chaining, whereas chaining was suppressed by induction of WT ftsEX (average length, 5.7 ± 3.8 μM [Table S4]). In agreement with the drug sensitivity test, the strain expressing ftsED162NX displayed the same cell morphology as the strain expressing wild-type ftsEX (average length, 5.8 ± 4.1 μM [Table S4]), whereas the strains expressing either ftsEXΔGip or ftsED162NXΔGip contained chains of cells similar to those of the control with the vector (average lengths, 19.4 ± 12.0 and 20.4 ± 9.5 μM, respectively [Table S4]). In stationary phase, the chaining phenotype largely disappeared, in agreement with the notion that cell chaining is due to a delay in cell separation (Fig. S2). These results suggest that under the conditions employed here, the ability of FtsEX to recruit EnvC, but not its ATPase activity, is required for cell separation.

To see if rifampin sensitivity correlated with the chaining phenotype of the strains, they were grown in liquid medium to exponential phase and were examined by phase-contrast microscopy (Fig. 5B). Indeed, the ΔftsEX ftsA*G366D strain containing the vector produced elongated cells (average length, 21.1 ± 9.3 μM [Table S4]) due to cell chaining, whereas chaining was suppressed by induction of WT ftsEX (average length, 5.7 ± 3.8 μM [Table S4]). In agreement with the drug sensitivity test, the strain expressing ftsED162NX displayed the same cell morphology as the strain expressing wild-type ftsEX (average length, 5.8 ± 4.1 μM [Table S4]), whereas the strains expressing either ftsEXΔGip or ftsED162NXΔGip contained chains of cells similar to those of the control with the vector (average lengths, 19.4 ± 12.0 and 20.4 ± 9.5 μM, respectively [Table S4]). In stationary phase, the chaining phenotype largely disappeared, in agreement with the notion that cell chaining is due to a delay in cell separation (Fig. S2). These results suggest that under the conditions employed here, the ability of FtsEX to recruit EnvC, but not its ATPase activity, is required for cell separation.

**ATPase hydrolysis by FtsEX is required for efficient cell separation in the absence of NlpD.** The results presented above are in contrast to a previous report in which the ATPase activity of FtsEX appeared essential for cell separation (8). However, in that study, nlpD, the activator of the other amidase (AmiC), was absent. In addition, increased osmolarity and extra FtsQAZ were provided to suppress the growth defects of the ΔftsEX strain. It is likely that under these conditions, the demand for the cell separation activity conferred by FtsEX is greater. To see if this was the case, we repeated the experiment from the previous report (8) with only a slight modification. We used a ΔftsEX ΔnlpD strain containing a copy of ftsEX integrated at the lambda attachment site (attλ) under the control of the arabinose promoter. In the presence of 0.2% arabinose, this strain does not have a cell separation defect, but it displays extensive chaining upon removal of arabinose. We introduced a vector (control) or plasmids harboring either ftsEX or ftsED162NX under the control of an IPTG-inducible promoter and checked cell morphology upon the removal of arabinose (basal expression from the IPTG-inducible promoter is sufficient for complementation). As shown in Fig. 6, the strain with the vector formed chains by 3 h after the removal of arabinose, and chaining was even more extensive at 6 h. The presence of the ftsEX plasmid suppressed chaining, whereas chaining was intermediate between the vector control and the ftsEX plasmid when the ftsED162NX plasmid was present (Table S5). These results are consistent with
the previous report and suggest that deletion of nlpD indeed reveals a requirement for the ATPase activity of FtsEX in cell separation.

The ΔftsEX ΔnlpD att\(^{\Delta P_{BAD}}\)/ftsEX strain used in the experiment presented above is still sensitive to the division-inhibitory activity of FtsED\(^{G366D}\)X. However, the inhibitory activity is likely suppressed by the extra FtsQAZ, which may confound the interpretation of the results. Therefore, we reexamined the requirement for the ATPase activity of FtsEX for cell separation in the absence of NlpD by using the ftsA\(^{*G366D}\) allele described above, which is resistant to the division-inhibitory activity of ftsED\(^{G366D}\)X and suppresses the division defect of the ΔftsEX mutant. We generated two derivatives of the ΔftsEX ΔnlpD att\(^{\Delta P_{BAD}}\)/ftsEX strain, differing only in their ftsA alleles (ftsA\(^*\) or ftsA\(^{G366D}\)). In the absence of arabinose, these two strains underwent extensive cell chaining, since both cell separation systems were inactive. We then introduced a vector or plasmids expressing ftsEX or ftsED\(^{G366D}\)X under the control of an IPTG-inducible promoter to examine the role of the ATPase activity.

On LB plates with 1.5% NaCl, which prevents the cell lysis that accompanies extensive cell chaining, these two strains with the vector grew regardless of the presence of arabinose (Fig. 7). However, on LB plates with 0.5% NaCl, neither strain grew in the absence of arabinose, whereas in the presence of arabinose, the strain with the ftsA\(^{G366D}\) allele grew less well than the strain with the ftsA\(^*\) allele. The growth defect of these two strains on LB plates with 0.5% NaCl allowed us to do complementation tests. As shown in Fig. 7, the presence of ftsEX on the plasmid rescued the growth of both strains, but the strain with ftsA\(^{G366D}\) required more IPTG, indicating that ftsEX was less efficient in this strain than in the ftsA\(^*\) strain. As expected, the plasmid with ftsED\(^{G366D}\)X was unable to complement the ΔftsEX ΔnlpD strain with the ftsA\(^*\) mutation, since this strain is sensitive to its inhibitory activity. However, ftsED\(^{G366D}\)X complemented the ftsA\(^{G366D}\) strain, where it cannot inhibit division, although a higher level of IPTG was required than when ftsEX was on the plasmid. These results suggest that ftsED\(^{G366D}\)X can promote cell separation but is less effective than ftsEX.

We set out to examine the effect of ftsEX or ftsED\(^{G366D}\)X on cell chaining; however, in LB with 0.5% NaCl, both strains with the vector lysed before the chaining phenotype was evident. Therefore, we examined the chaining phenotypes of these two strains in 1.5% NaCl. As shown in Fig. 8, the ΔnlpD att\(^{\Delta P_{BAD}}\)/ftsEX strain with the ftsA\(^*\) mutation and the vector displayed a normal morphology at time zero (with arabinose removed and 30 \(\mu\)M IPTG added), but cell chaining became evident after 3 h and was extensive by 6 h (Fig. 8; Table S6). Induction of ftsEX from the plasmid prevented the chaining phenotype, whereas induction of ftsED\(^{G366D}\)X from the plasmid resulted in inhibition of division and smooth filamentation, as expected. Surprisingly, cells from the strain with the vector and the ftsA\(^{G366D}\) allele were already chaining at time zero (Fig. 8; Table S6). This observation revealed that ftsEX induced from chromosomal att\(^{\Delta P_{BAD}}\)/ftsEX was not sufficient to suppress the chaining, indicating that ftsEX was less effective in the

| ΔftsEX ΔnlpD | LB (1.5% NaCl) | LB (0.5% NaCl) |
|--------------|----------------|----------------|
| att\(^{\Delta P_{BAD}}\)/ftsEX | + Ara | + Ara |
| Vec | + Ara | + Ara |
| Vec::ftsEX | 0 | 15 |
| Vec::ftsED\(^{G366D}\)X | 30 | - Ara + IPTG (\(\mu\)M) |
| Vec::ftsEX | + Ara | + Ara |
| Vec::ftsED\(^{G366D}\)X | + Ara | + Ara |

**FIG 7** Effects of the FtsA-FtsEX interaction and ATP hydrolysis by FtsEX on its ability to rescue a ΔftsEX ΔnlpD strain. A ΔftsEX ΔnlpD att\(^{\Delta P_{BAD}}\)/ftsEX strain with either ftsA\(^*\) or ftsA\(^{G366D}\) was transformed with a vector or plasmids expressing alleles of ftsEX under the control of an IPTG-inducible promoter. The transformants were spotted onto LB plates with or without arabinose and different concentrations of NaCl and IPTG. Plates were incubated overnight at 37°C and photographed. The strains were SD523 (TB28 ftsEX<->frt nlpD<->frt ftsA<->frt [P~BAD::ftsEX]) and SD524 (TB28 ftsEX<->frt nlpD<->frt ftsA\(^{G366D}\)X<->frt [P~BAD::ftsEX]) carrying plasmids expressing WT ftsEX (pEXT22 P~ext::ftsEX)), ftsED\(^{G366D}\)X (pSD221-D162N [pEXT22 P~ext::ftsED\(^{G366D}\)X]), or the vector pEXT22.
presence of \( ftsA^{G366D} \), in agreement with the complementation results (Fig. 7). Upon the removal of arabinose, the chaining of cells with the vector became more extensive, whereas induction of \( ftsEX \) from the plasmid decreased the chain length by 6 h, indicating that the higher level of \( ftsEX \) partially overcame the loss of interaction with \( ftsA \) (Fig. 8; Table S6). Induction of \( ftsED162NX \) from the plasmid also prevented the severe chaining seen with the vector, but it was not as effective as \( ftsEX \) (Fig. 8; Table S6). Taken together, these results demonstrate that the ATPase mutant of \( ftsEX \) promotes cell separation but is not sufficient to produce a WT phenotype when the partially redundant NlpD system is inactivated. Also, \( ftsEX \) is not as efficient at promoting cell separation when it cannot interact with \( ftsA \) (due to the \( ftsAG366D \) mutation).

The FtsX-FtsA interaction is important for efficient cell separation in the absence of NlpD. The results presented above (Fig. 7) suggested that in the absence of the FtsA-FtsX interaction, the efficiency of the FtsEX-mediated cell separation activity was reduced. First, a higher level of FtsEX (i.e., more IPTG) was required for the \( \Delta ftsEX \Delta nlpD \) strain to grow when the \( ftsA^{G366D} \) allele was present than when the \( ftsA^{*} \) allele was present (in the absence of arabinose on LB plates with 0.5% NaCl). Also, when \( ftsA^{G366D} \) was present, cells displayed a strong cell chaining phenotype at time zero, even though arabinose was present to induce \( ftsEX \) from its ectopic location on the chromosome. The only difference between these two strains is the \( ftsAG366D \) mutation. The fact that this mutation abolishes the FtsEX-FtsA interaction (17) suggests that this interaction is important for efficient cell separation under the conditions tested. However, in the rifampin sensitivity test (Fig. 5), there was no difference between the strains containing \( ftsA^{*} \) and \( ftsA^{G366D} \); in that case, though, \( nlpD \) was present, and \( ftsEX \) was expressed at a high level from a plasmid.

To examine the effect of the FtsA-FtsX interaction on cell division more carefully, we compared the cell length and chaining phenotype of \( \Delta ftsEX ftsA^{*} \) cells to those of \( \Delta ftsEX ftsA^{G366D} \) cells ectopically expressing \( ftsEX \) from a chromosomal copy under the control of an arabinose-inducible promoter, with or without NlpD. In the presence of arabinose (LB with 0.5% NaCl), the strain with \( ftsA^{*} \) had an average cell length of 3.8 ± 0.88 μm, whereas the strain with \( ftsA^{G366D} \) had an average cell length of 5.2 ± 1.25 μm (Table S7), indicating that loss of the interaction between FtsEX and FtsA...
delays cell division. Also, the failure to see a difference earlier (Fig. 4) was likely due to the higher level of expression of \textit{ftsEX} from a plasmid. Deletion of \textit{nlpD} had little effect on the average cell length of the Δ\textit{ftsEX} ftsA\textsuperscript{*} strain; however, the average cell length of the Δ\textit{ftsEX} ftsA\textsuperscript{*},G366D strain doubled to 10.9±5.7 µm (Fig. 9; Table S7). Increasing NaCl to 1.5% had little effect on cell separation in the Δ\textit{ftsEX} ftsA\textsuperscript{*} strain but exacerbated the defect of the Δ\textit{ftsEX} ftsA\textsuperscript{*},G366D strain, for which cell length was further increased and chaining was more pronounced. These results confirmed that loss of the FtsEX-FtsA interaction leads to a delay in cell division, which becomes more evident in the absence of \textit{nlpD} and is further exacerbated by increased salt concentrations.

DISCUSSION

In this study, we continued to explore the involvement of FtsEX in cell division, and we report several new findings that highlight its regulatory role. First, we find that an FtsEX mutant unable to hydrolyze ATP blocks cells in the process of constriction without disrupting the septal ring, implying that ATP hydrolysis by FtsEX is required throughout septation. Thus, the ATPase mutant of FtsEX mimics the action of cephalaxin, a well-characterized inhibitor of FtsI (PBP3). Second, we found that a Δ\textit{ftsEX} strain is more sensitive to a hydrophobic antibiotic than a Δ\textit{envC} strain, a finding consistent with roles of FtsEX in more than cell separation. Third, we observed that in the absence of ATP hydrolysis, FtsEX still promotes cell separation. This activity is sufficient to promote cell separation when an overlapping separation pathway (\textit{nlpD}) is present but insufficient if this pathway is missing. Last, we found that the interaction of FtsEX with FtsA is required for efficient cell division and cell separation. This effect is observed when \textit{nlpD} is present but is more pronounced when \textit{nlpD} is removed. These results argue that a physical coupling of the cell separation system to the septal PG synthesis machinery via the FtsEX-FtsA interaction is important for normal cell division and cell separation.

In our previous work (17), we found that an ATPase mutant of FtsEX blocked the start of constriction by acting on FtsA. Here, we extend those findings and show that FtsE\textsuperscript{D162NX} also blocks ongoing septation and thus phenocopies cephalaxin. This means that FtsEX must continually hydrolyze ATP throughout the constriction process. Constriction is initiated when FtsN arrives, which is thought to switch FtsA in the cytoplasm and FtsQLB in the periplasm to the “on” state, leading to activation of FtsWI.
In this model, FtsED162NX could block division by preventing FtsA from reaching the on state or communicating with downstream proteins (17). Recent evidence indicates that the septal PG synthase FtsW exists in two types of processive moving complexes at the Z ring (32). The faster complex is inactive and is driven by FtsZ treadmilling, whereas the slower-moving one likely represents the active complex synthesizing septal PG. This switch from a fast-moving to a slow-moving complex appears to correlate with the activation of the complex by FtsN. In this scenario, the ATPase mutant of FtsEX may prevent the switch, but the mechanism remains to be determined.

Cell separation in E. coli employs two distinct systems involving three amidases and two activators (18, 23). FtsEX regulates AmiA and AmiB through EnvC, whereas NlpD activates the third amidase, AmiC. Although it is generally thought that the ATPase activity of FtsEX is required for the activation of its cognate amidases, in vitro assays have raised some doubt (18). In these assays, full-length EnvC stimulated amidase activity in vitro to the same extent as the C-terminal fragment of EnvC, and FtsEX was not required (18). It is difficult, however, to tease out the role of FtsEX ATPase activity in vivo, because it is also necessary for septal PG synthesis (17). Additional confounding issues include the ability of the FtsEX ATPase mutant to inhibit division and the partially redundant pathways to activate amidases.

To circumvent these issues, we took advantage of the ftsA*G366D allele, which prevents the interaction of FtsEX with FtsA (hence rendering cells resistant to FtsED162NX) and also bypasses the requirement of FtsEX for septal PG synthesis (17). This mutant uncouples the essential role of FtsEX in septal PG synthesis from its role in septal PG hydrolysis, allowing us to assess the contribution of ATP hydrolysis by FtsEX to cell separation. FtsED162NX suppressed the increased sensitivity of ftsEX cells to the hydrophobic drug rifampin in an EnvC-dependent manner. Importantly, this rescue was dependent on EnvC and also eliminated the chaining morphology, indicating that FtsED162NX was still able to promote cell separation. However, when NlpD was deleted, the cell chaining was only partially suppressed (Fig. 6 to 8). This is consistent with the previous report which found that cell chains were somewhat shorter with the ATPase mutant than with the vector control (8). These observations suggest that EnvC can activate AmiA and AmiB to some extent when it is recruited to the septum by FtsEX; however, ATP hydrolysis by FtsEX is probably required for optimal activity of this cell separation system. It should be noted that based on the analogy to MacB (12), FtsED162NX is locked in the ATP-bound state. If this is the active form of FtsEX (for activating amidases), it may contribute to cell separation but is less efficient than the WT due to a lack of dynamics.

Using the ftsA*G366D mutation, we also uncovered an unexpected role for the FtsEX-FtsA interaction in cell separation. This role emerged from the unexpected observation that a higher level of ftsEX was required to complement the ΔftsEX ΔnlpD strain in the presence of the ftsA*K366D mutation than in the presence of ftsA* (Fig. 7). Also, ΔftsEX ΔnlpD cells with the ftsA*K366D mutation displayed a chaining phenotype even when FtsEX was provided at a level that promotes cell separation efficiently in ΔftsEX ΔnlpD cells with the ftsA* mutation (Fig. 8). These results indicate that cell separation is less effective when the FtsEX-EnvC-amidase cell separation system is uncoupled from FtsA, which is likely associated with the active FtsWI complex. This defect in cell separation is further amplified in the absence of the other cell separation system. It is likely that uncoupling the FtsEX-mediated cell separation system from the septal PG synthesis machinery delays septal PG hydrolysis, leading to cell chaining. A delay in the production of denuded PG strands by the amidases would reduce the accumulation at the septum of SPOR domain proteins (including FtsN and DedD) that bind the denuded glycan strands (33, 34). Decreased accumulation of these proteins would decrease the activation of FtsWI, leading to a decreased rate of septal PG synthesis and increased cell length.

Based on previous findings (8, 16, 17) and our observations here, we propose a model for how FtsEX coordinates septal PG synthesis and hydrolysis (Fig. 1A). FtsEX...
localizes to the Z ring via an interaction between FtsE and the conserved C-terminal peptide (CCTP) of FtsZ (16). Once at the Z ring, FtsX interacts with FtsA, promoting divisome assembly, and recruits the amidase activator EnvC through its periplasmic loop. This recruitment phase does not require ATP hydrolysis by FtsEX but acts to link the septal PG synthesis machinery (FtsA-FtsQLB-FtsWI) and the septal PG hydrolysis machinery (FtsA-FtsEX-EnvC-AmiA/AmiB) (Fig. 1A). The arrival of FtsN switches FtsA and FtsQLB to the on state, activating FtsWI to synthesize septal PG. FtsEX must hydrolyze ATP at this step, or septal PG synthesis will be blocked. Once new septal PG is synthesized, amidases are activated by EnvC to cleave the stem peptide, leading to timely cell separation (Fig. 1A). In the absence of ATP hydrolysis, FtsEX is able to recruit EnvC and activate AmiA and AmiB, sufficiently to promote cell separation when the NlpD is present. However, in its absence, this activity is insufficient, indicating a role for ATP hydrolysis. In the absence of the FtsEX-FtsA interaction (due to the ftsA^G366D mutation), FtsEX still localizes at the Z ring via an interaction with FtsZ. However, the FtsEX-EnvC-amidase cell separation system is uncoupled from the FtsA-FtsQLB-FtsWI septal PG synthesis machinery (Fig. 1B), resulting in a delay in the production of denuded peptidoglycan strands, which, in turn, delays the accumulation of SPOI domain proteins and leads to a delay in cell division. This model will be useful for further study of the role of FtsEX in regulating cell division and cell wall hydrolysis in E. coli and other bacterial species.

**MATERIALS AND METHODS**

**Media, bacterial strains, plasmids, and growth conditions.** Cells were grown in LB alone or LB plus 0.2 M sucrose medium (1% tryptone, 0.5% yeast extract, 0.5% or 1.5% NaCl, and 0.05 g/liter thymine) at 30°C or 37°C. When needed, antibiotics were used at the following concentrations: ampicillin, 100 µg/ml; spectinomycin, 25 µg/ml; kanamycin, 25 µg/ml; tetracycline, 25 µg/ml; chloramphenicol, 20 µg/ml. Rifampin was used at the concentrations indicated in the figures for the drug sensitivity test. The strains and plasmids used in this study are listed in Tables 1 and 2, respectively.

**Construction of strains.** Strains were constructed largely by P1-mediated transduction. Strain SD208 (W3110 envC::kan) was constructed by the introduction of the envC::kan allele from strain TB35 (TB28 envC::kan) into W3110. Transductants were selected on LB plates with kanamycin at 30°C. Strain SD516 [TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan] was constructed by P1-mediated transduction of the nlpD::kan cassette from strain JW2712 (BW25113 nlpD::kan) into strain TU191 [TB28 ftsEX::frt att^ (P_BAD::ftsEX)]. Transductants were selected on LB medium with 1.5% NaCl, 0.2% arabinose, and kanamycin. The kan cassette was removed from nlpD::kan strains by using plasmid pCP20 to create strain SD518 [TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan]. Strains SD523 [TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan] were constructed by P1-mediated transduction of the leuC::Tn10 ftsA* from strain PS2343 (leuC::Tn10 ftsA*) or strain SD249 (leuC::Tn10 ftsA*::Tn9) into strain SD518. ftsA* refers to the ftsA^G366D allele (35). Transductants were selected on LB medium with 1.5% NaCl, 0.2% arabinose, and tetracycline. The ftsA coding sequences from at least four transductants were PCR amplified and sequenced, and

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| JS238  | MC1061 araC Δ(ara leu) galE galK hsdS rtS rpsL ΔlacIQZ/ΔOZ/ΔPZ/ΔX74 malC::λacI857 srlC::Tn10 recA1 | Lab collection |
| JW2712 | BW25113 nlpD::kan | 37 |
| HC261  | TB28 zapa-GFP Camr | 38 |
| PS2343 | W3110 leu::Tn10 ftsA* | 39 |
| S3     | W3110 leu::Tn10 | 40 |
| SD208  | W3110 envC::kan | This study |
| SD220  | W3110 leu::Tn10 ftsEX::cat | This study |
| SD221  | W3110 leu::Tn10 ftsA* ftsEX::cat | This study |
| SD249  | W3110 leu::Tn10 ftsA*::Tn9 | This study |
| SD262  | W3110 leu::Tn10 ftsA*::Tn9 ftsEX::cat | This study |
| SD516  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan | This study |
| SD518  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan | This study |
| SD523  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan leu::Tn10 ftsA* | This study |
| SD524  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) nlpD::kan leu::Tn10 ftsA*::G366D | This study |
| SD528  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) leu::Tn10 ftsA* | This study |
| SD529  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) leu::Tn10 ftsA*::G366D | This study |
| TB35   | TB28 envC::kan | 41 |
| TU191  | TB28 ftsEX::frt att^ (P_BAD::ftsEX) | 8 |
those with the ftsA* or ftsA*Δg660 mutation were saved. Strain SD528 [TB28 ftsEX<frt att< (P_BAD::ftsEX)] leu::Tn10 ftsA* and SD529 were constructed similarly by P1-mediated transduction of leu::Tn10 ftsA* or leu::Tn10 ftsA*Δg660 into strain TU191 [TB28 ftsEX<frt att< (P_BAD::ftsEX)].

**Determination of the effect of FtsED162NX overexpression or cephalexin on cell constriction, contraction of Z rings, and septal PG synthesis.** To follow cell constriction and Z ring contraction, an overnight culture of HC261/pSD221-D162N (pEXT22 P_reg::ftsEX) was diluted 100-fold in LB with sucrose and antibiotics and was grown at 30°C. After 2 h, 2 μl of the culture was spotted onto a 2% agarose pad, and photographed. Cell length was measured using MetaMorph software.

To follow cell constriction and Z ring contractions in cells expressing FtsEΔ162NX, an overnight culture of HC261/pSD221-D162N was diluted 100-fold in LB with sucrose and antibiotics and was grown at 30°C until the optical density at 600 nm (OD600) reached about 0.6. The culture was diluted 5-fold in the same medium containing 250 μM IPTG. After incubation for 1 h, 2 μl of the culture was spotted onto a 2% agarose pad containing LB plus sucrose. 4,6-Diamidino-2-phenylindole (DAPI) was added at a final concentration of 200 ng/ml 5 min before fixation to monitor the distribution of nucleoids.

**Measurement of cell lengths.** To measure the length of ServC ΔftsEX cells with or without the ftsA* mutations (Table S3), overnight cultures were diluted 1:100 in LB with 0.2 M sucrose and were grown at 30°C. After 3 h, cells were fixed with paraformaldehyde and glutaraldehyde, immobilized on a 2% agarose pad, and photographed. Cell length was measured using MetaMorph software.

The lengths of ftsA*Δg660 ΔftsEX cells expressing different ftsEX alleles (Table S4) were measured as described above for Table S3. To measure the lengths of strain SD518 [TB28 ftsEX<frt nlpD<att< (P_SBAD::ftsEX/pBS588) (pGB2-ftsQAZ)] cells expressing different alleles of ftsEX (Table S5), overnight cultures were diluted 1:100 in LB with 1.5% NaCl and 0.2% arabinose and were grown for 3 h at 30°C. At time zero, the cells were collected by centrifugation, washed twice, and resuspended in LB with 1.5% NaCl with or without arabinose. Samples were taken for microscopy at various times after the removal of arabinose. Cells were detected and measured using the ImageJ plug-in MicrobeJ (36).

**Rifampin sensitivity test.** To determine the sensitivities of various strains to rifampin, overnight cultures were serially diluted 10-fold, and 3 μl was spotted onto LB-plus-sucrose plates with or without increasing concentrations of rifampin. The plates were incubated at 37°C overnight and photographed.

To determine whether FtsED162NX mutants can correct the sensitivity to rifampin, pSD228 (ftsEX), and their derivatives were transformed into strain SD262 (ftsEX::cat ftsA*Δg660) and transformants selected on LB-plus-sucrose plates with ampicillin. The transformants were then restreaked onto LB-plus-sucrose plates with antibiotics, with or without 4 μg/ml rifampin. The plates were incubated at 37°C overnight and photographed.

---

**TABLE 2 Plasmids used in this study**

| Plasmid           | Genotype                                      | Source or reference |
|-------------------|-----------------------------------------------|---------------------|
| pBS58             | pSC101aadAftsQAZ                              | 42                  |
| pCP20             | pSC101(Ts)aadArepA(Ts)P_BAC::flp              | 43                  |
| pDSW208           | pDSW208blaP_n::gfp                             | 44                  |
| pSD213            | pDSW208blaP_n::ftsEXcl                         | This study          |
| pSD213-D162N      | pDSW208blaP_n::ftsED162NXlp                   | This study          |
| pSD212            | pEXT22kanP_reg::ftsEX                         | 17                  |
| pSD212-D162N      | pEXT22kanP_reg::ftsED162NX                    | 17                  |
| pSEB428           | pDSW208blaP_n::ftsED162NX                     | 45                  |
| pSEB428-D162N     | pDSW208blaP_n::ftsED162NX                     | 17                  |

---

FtsEX ATPase in Peptidoglycan Metabolism
SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, PDF file, 0.3 MB.
FIG S2, PDF file, 0.2 MB.
TABLE S1, DOCX file, 0.01 MB.
TABLE S2, DOCX file, 0.01 MB.
TABLE S3, DOCX file, 0.01 MB.
TABLE S4, DOCX file, 0.01 MB.
TABLE S5, DOCX file, 0.01 MB.
TABLE S6, DOCX file, 0.01 MB.
TABLE S7, DOCX file, 0.01 MB.

ACKNOWLEDGMENTS

We thank Tom Bernhardt for strains and members of the Lutkenhaus lab for comments and advice in preparing the manuscript. The fluorescent D-amino acid HADA was a gift from Michael VanNieuwenhze.

This study was supported by NIH grant GM29746 to J.L. S.D. and J.L. designed the research; S.D. and S.P. performed the research; S.D. and J.L. analyzed data and wrote the manuscript.

REFERENCES

1. Typas A, Banzhaf M, Gross CA, Vollmer W. 2011. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nat Rev Microbiol 10:123–136. https://doi.org/10.1038/nrmicro2677.
2. Du S, Lutkenhaus J. 2017. Assembly and activation of the Escherichia coli divisome. Mol Microbiol 105:177–187. https://doi.org/10.1111/mmi.13696.
3. Taguchi A, Welsh MA, Marmont LS, Lee W, Sjodt M, Kruse AC, Kahne D, Bernhardt TG, Walker S. 2019. FtsW is a peptidoglycan polymerase that is functional only in complex with its cognate penicillin-binding protein. Nat Microbiol 4:587–594. https://doi.org/10.1038/s41564-018-0345-x.
4. Meeske AJ, Riley EP, Robins WP, Uehara T, Melkanan Jo, Kahne D, Walker S, Kruse AC, Bernhardt TG, Rudner DZ. 2016. SEDS proteins are a widespread family of bacterial cell wall polymerases. Nature 537:634–638. https://doi.org/10.1038/nature18882.
5. Picabarro AG, Prats R, Vázquez D, Rodríguez-Tébar A. 1986. Activity of penicillin-binding protein 3 from Escherichia coli. J Bacteriol 168:199–206. https://doi.org/10.1128/jb.168.1.199-206.1986.
6. Spratt BG. 1975. Distinct penicillin binding proteins involved in the division, elongation, and shape of Escherichia coli K12. Proc Natl Acad Sci U S A 72:2999–3003. https://doi.org/10.1073/pnas.72.8.2999.
7. Gerding MA, Liu B, Bendezú FO, Hale CA, Bernhardt TG, de Boer PAJ. 2009. Self-enhanced accumulation of FtsN at division sites and roles for other proteins with a SPOR domain (DamX, DedD, and RipA) in Escherichia coli cell constriction. J Bacteriol 191:7383–7401. https://doi.org/10.1128/JB.00811-09.
8. Yang DC, Peters NT, Parzych K, Uehara T, Markovski M, Bernhardt TG. 2011. An ATP-binding cassette transporter-like complex governs cell-wall hydrolysis at the bacterial cytokinetic ring. Proc Natl Acad Sci U S A 108:E1052–E1060. https://doi.org/10.1073/pnas.1007981108.
9. Sham L-T, Barendt SM, Kopecky KE, Winkler ME. 2011. Essential PcsB putative peptidoglycan hydrolase interacts with the essential FtsXSpn cell division protein in Streptococcus pneumoniae D39. Proc Natl Acad Sci USA 108:E1052–E1060. https:// doi.org/10.1073/pnas.1007981108.
10. Kaplan E, Greene NP, Crow A, Koronakis V. 2018. Insights into bacterial lipoprotein trafficking from a structure of LoLA bound to the LoLC periplasmic domain. Proc Natl Acad Sci U S A 115:E7389–E7397. https://doi.org/10.1073/pnas.1806822115.
11. Schmid KL, Peterson ND, Kustus RJ, Wissel MC, Graham B, Phillips GJ, Weiss DS. 2004. A predicted ABC transporter, FtsEX, is needed for cell division in Escherichia coli. J Bacteriol 186:785–793. https://doi.org/10.1128/JB.186.3.785-793.2004.
12. Arends SJR, Kustus RJ, Weiss DS. 2009. ATP-binding site lesions in FtsE impair cell division. J Bacteriol 191:3772–3784. https://doi.org/10.1128/JB.00179-09.
13. Du S, Henke W, Pichoff S, Lutkenhaus J. 2019. How FtsEx localizes to the Z ring and interacts with FtsA to regulate cell division. Mol Microbiol 112:881–895. https://doi.org/10.1111/mmi.14324.
14. Du S, Pichoff S, Lutkenhaus J. 2016. FtsEx acts on FtsA to regulate divisome assembly and activity. Proc Natl Acad Sci U S A 113: E5052–E5061. https://doi.org/10.1073/pnas.1606656113.
15. Uehara T, Parzych KR, Dinh T, Bernhardt TG. 2010. Daughter cell separation is controlled by cytokinetic ring-activated cell wall hydrolysis. EMBO J 29:1412–1422. https://doi.org/10.1038/emboj.2010.36.
16. Tikhonova EB, Devroy VW, Lau SY, Zgurskaya HI. 2007. Reconstitution of the Escherichia coli macrolide transporter: the periplasmic membrane fusion protein MacA stimulates the ATPase activity of MacB. Mol Microbiol 63:895–910. https://doi.org/10.1111/j.1365-2958.2006.05549.x.
17. Reddy M. 2007. Role of FtsEx in cell division of Escherichia coli: viability of ftsEx mutants is dependent on functional SufI or high osmotic strength. J Bacteriol 189:98–108. https://doi.org/10.1128/JB.01347-06.
18. Samaluru H, SaiSree L, Reddy M. 2007. Role of SufI (FtsP) in cell division of Escherichia coli: evidence for its involvement in stabilizing the assembly of the divisome. J Bacteriol 190:8044–8052. https://doi.org/10.1128/JB.00773-07.
19. Pichoff S, Du S, Lutkenhaus J. 2018. Disruption of divisome assembly rescued by FtsN-FtsA interaction in Escherichia coli. Proc Natl Acad Sci U S A 115:E6855–E6862. https://doi.org/10.1073/pnas.1806450115.
20. Heidrich C, Ursinus A, Berger J, Schwarz H, Höltje J-V. 2002. Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in Escherichia coli. J Bacteriol 184:6093–6099. https://doi.org/10.1128/JB.184.22.6093-6099.2002.
21. Tikhonova EB, Devroy VW, Zgurskaya HI. 2007. Reconstitution of the Escherichia coli macrolide transporter: the periplasmic membrane fusion protein MacA stimulates the ATPase activity of MacB. Mol Microbiol 63:895–910. https://doi.org/10.1111/j.1365-2958.2006.05549.x.
22. Pichoff S, Du S, Lutkenhaus J. 2018. Disruption of divisome assembly rescued by FtsN-FtsA interaction in Escherichia coli. Proc Natl Acad Sci U S A 115:E6855–E6862. https://doi.org/10.1073/pnas.1806450115.
23. Heidrich C, Ursinus A, Berger J, Schwarz H, Höltje J-V. 2002. Effects of multiple deletions of murein hydrolases on viability, septum cleavage, and sensitivity to large toxic molecules in Escherichia coli. J Bacteriol 184:6093–6099. https://doi.org/10.1128/JB.184.22.6093-6099.2002.
24. Starkova Z, Thomas P, Starka J. 1978. Morphological mutants of Escherichia coli: nature of the permeability barrier in mon and envC cells. Ann Microbiol (Paris) 129:265–284.
25. Liu B, Persons L, Lee L, de Boer PAJ. 2015. Roles for both FtsA and the FtsBLQ subcomplex in FtsN-stimulated cell constriction in Escherichia coli. Mol Microbiol 95:945–970. https://doi.org/10.1111/mmi.12906.
26. Tsang MJ, Bernhardt TG. 2015. A role for the FtsQLB complex in cytoxi-
netic ring activation revealed by an ftsA allele that accelerates division. Mol Microbiol 95:925–944. https://doi.org/10.1111/mmi.12905.

27. Tsang MJ, Bernhardt TG. 2015. Guiding divisome assembly and controlling its activity. Curr Opin Microbiol 24:60–65. https://doi.org/10.1016/j.mib.2015.01.002.

28. Pogliano J, Pogliano K, Weiss DS, Losick R, Beckwith J. 1997. Inactivation of FtsL inhibits constriction of the FtsZ cytokinetic ring and delays the assembly of FtsZ rings at potential division sites. Proc Natl Acad Sci U S A 94:559–564. https://doi.org/10.1073/pnas.94.2.559.

29. Kuru E, Hughes HV, Brown PJ, Hall E, Tekkam S, Cava F, de Pedro MA, Brun YV, VanNieuwenhze MS. 2012. In situ probing of newly synthesized peptidoglycan in live bacteria with fluorescent D-amino acids. Angew Chem Int Ed Engl 51:12519–12523. https://doi.org/10.1002/anie.201206749.

30. Hara H, Narita S, Karibian D, Park JT, Yamamoto Y, Nishimura Y. 2002. Identification and characterization of the Escherichia coli envC gene encoding a periplasmic coiled-coil protein with putative peptidase activity. FEMS Microbiol Lett 212:229–236. https://doi.org/10.1111/j.1574-6968.2002.tb11271.x.

31. Rodolakis A, Thomas P, Starka J. 1973. Morphological mutants of Escherichia coli. Isolation and ultrastructure of a chain-forming envC mutant. J Gen Microbiol 75:409–416. https://doi.org/10.1111/j.1365-2958-75-2-409.

32. Yang X, McQuillen R, Lyu Z, Phillips-Mason P, De La Cruz A, McCausland JW, Liang H, DeMeester KE, Grimes CL, de Boer P, Xiao J. 2019. FtsW exhibits distinct processive movements driven by either septal cell wall synthesis or FtsZ treadmilling in E. coli. bioRxiv. https://doi.org/10.1101/850073.

33. Yahashiri A, Jorgenson MA, Weiss DS. 2015. Bacterial SPOR domains are recruited to septal peptidoglycan by binding to glycan strands that lack stem peptides. Proc Natl Acad Sci U S A 112:11347–11352. https://doi.org/10.1073/pnas.1508536112.

34. Liu B, Hale CA, Persons L, Phillips-Mason PJ, de Boer PAJ. 2019. Roles of the DedD protein in Escherichia coli cell constriction. J Bacteriol 201: e00698-18. https://doi.org/10.1128/JB.00698-18.

35. Geissler B, Elraheb D, Margolin W. 2003. A gain-of-function mutation in the DedD protein in Escherichia coli cell constriction. J Bacteriol 201:16077. https://doi.org/10.1128/JB.00698-18.

36. Ducret A, Quardokus EM, Brun YV. 2016. Microbeb, a tool for high throughput bacterial cell detection and quantitative analysis. Nat Microbiol 1:16077. https://doi.org/10.1038/nmicrobiol.2016.77.

37. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol Syst Biol 2:2006.0008. https://doi.org/10.1038/msb4100050.

38. Peters NT, Dinh T, Bernhardt TG. 2011. A fail-safe mechanism in the septal ring assembly pathway generated by the sequential recruitment of cell separation amidases and their activators. J Bacteriol 193:4973–4983. https://doi.org/10.1128/JB.00316-11.

39. Pichoff S, Shen B, Sullivan B, Lutkenhaus J. 2012. FtsA mutants impaired for self-interaction bypass ZipA suggesting a model in which FtsA’s self-interaction competes with its ability to recruit downstream division proteins. Mol Microbiol 83:151–167. https://doi.org/10.1111/j.1365-2958.2011.07923.x.

40. Shen B, Lutkenhaus J. 2009. The conserved C-terminal tail of FtsZ is required for the septal localization and division inhibitory activity of MinC/MinD. Mol Microbiol 72:410–424. https://doi.org/10.1111/j.1365-2958.2009.06651.x.

41. Bernhardt TG, de Boer PA. 2004. Screening for synthetic lethal mutants in Escherichia coli and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. Mol Microbiol 52:1255–1269. https://doi.org/10.1111/j.1365-2958.2004.04063.x.

42. Bi E, Lutkenhaus J. 1990. FtsZ regulates frequency of cell division in Escherichia coli. J Bacteriol 172:2765–2768. https://doi.org/10.1128/jb.172.5.2765-2768.1990.

43. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A 97:6640–6645. https://doi.org/10.1073/pnas.120163297.

44. Weiss DS, Chen JC, Ghigo J-M, Boyd D, Beckwith J. 1999. Localization of FtsL (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. J Bacteriol 181:508–520. https://doi.org/10.1128/jb.181.2.508-520.1999.

45. Pichoff S, Du S, Lutkenhaus J. 2015. The bypass of ZipA by overexpression of FtsN motif essential for FtsA-FtsN interaction supporting a model in which FtsA monomers recruit late cell division proteins to the Z ring. Mol Microbiol 95:971–987. https://doi.org/10.1111/mmi.12907.