Asymmetric Constriction of Dividing *Escherichia coli* Cells Induced by Expression of a Fusion between Two Min Proteins

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The Min system, consisting of MinC, MinD, and MinE, plays an important role in localizing the *Escherichia coli* cell division machinery to midcell by preventing FtsZ ring (Z ring) formation at cell poles. MinC has two domains, MinCn and MinCc, which both bind to FtsZ and act synergistically to inhibit FtsZ polymerization. Binary fission of *E. coli* usually proceeds symmetrically, with daughter cells at roughly 180° to each other. In contrast, we discovered that overproduction of an artificial MinCc-MinD fusion protein in the absence of other Min proteins induced frequent and dramatic jackknife-like bending of cells at division septa, with cell constriction predominantly on the outside of the bend. Mutations in the fusion known to disrupt MinCc-FtsZ, MinCc-MinD, or MinD-membrane interactions largely suppressed bending division. Imaging of FtsZ-green fluorescent protein (GFP) showed no obvious asymmetric localization of FtsZ during MinCc-MinD overproduction, suggesting that a downstream activity of the Z ring was inhibited asymmetrically. Consistent with this, MinCc-MinD fusions localized predominantly to segments of the Z ring at the inside of developing cell bends, while FtsA (but not ZipA) tended to localize to the outside. As FtsA is required for ring constriction, we propose that this asymmetric localization pattern blocks constriction of the inside of the septal ring while permitting continued constriction of the outside portion.

Escherichia coli divides by binary fission using a machine, often called the divisome, comprised of several proteins that are recruited to midcell. In *E. coli*, two systems contribute to correct positioning of the divisome: nucleoid occlusion and the Min system (1). Both systems spatially regulate the assembly of the Z ring, which serves to organize and recruit divisome proteins (2, 3). The Z ring consists of scattered protofilaments of FtsZ in a ring-shaped structure, which is tethered to the membrane by additional divisome proteins (4, 5). ZipA and FtsA tether FtsZ to the inner membrane, and Z rings can form in the absence of one or the other, but both are required for the recruitment of downstream divisome proteins (6). In the absence of the Min system, Z rings assemble not only at midcell between the two segregated daughter nucleoids but at all nucleoid-free regions within the cell, including the cell poles (7). Because only a subset of the many Z rings formed in a Δmin mutant are competent to form a division septum at any one time, Δmin cells are a mixture of cells of normal size, filaments of various lengths because of delayed cell division, and chromosome-free minicells formed by septation near the cell poles (8).

The Min system consists of three proteins, MinC, MinD, and MinE (8). MinC binds to FtsZ and inhibits FtsZ assembly (9). By itself, MinC cannot spatially restrict Z rings to midcell; MinC binds to MinD, an ATPase that coats a large portion of the cell membrane near the cell pole when in its ATP-bound state (10, 11). MinE binds MinD and forms a ring at the edge of the MinD polar zone. MinE stimulates the ATPase activity of MinD, which removes MinD from the membrane and causes the MinD zone to collapse in front of the poleward-migrating MinE ring (12, 13). After the MinD polar zone is completely dislodged, MinD then rebinds ATP and moves toward the opposite cell pole, followed by MinE, and the pattern repeats (14–16). The oscillation of this system near the cell poles and away from midcell keeps the concentration of the MinC inhibitor lowest at midcell and highest at cell poles over time (14, 17). This has the effect of preventing FtsZ from assembling near cell poles, visualized by the oscillation of

FtsZ-green fluorescent protein (GFP)/cyan fluorescent protein (CFP) fusions from pole to pole in response (18–20).

MinC consists of an N-terminal domain and a C-terminal domain separated by a short linker (21, 22). The N terminus of MinC (MinCn) is sufficient for inhibition of FtsZ assembly, and the C terminus of MinC (MinCc) is both the binding interface for MinD and an inhibitor of FtsZ assembly (22, 23), although the inhibitory activity of MinCc requires the presence of MinD (23). MinCn binds to the conserved N-terminal domain of FtsZ, while MinCc binds to FtsZ's C-terminal core domain (24, 25). Their inhibition of FtsZ assembly is synergistic, with MinCn blocking FtsZ-FtsZ longitudinal interactions within a protofilament and MinCc inhibiting lateral interactions between FtsZ protofilaments (26). When overproduced, MinCc also acts after FtsZ assembly by competing with ZipA and FtsA for FtsZ binding, dislodging FtsA from the Z ring preferentially, and eventually dislodging ZipA (24).

As MinCc activity requires MinD, we originally sought to determine the effect on cells when MinCc and MinD are tethered together as a single fusion protein. We discovered that whereas overproduction of a MinC-MinD fusion induces cell filamentation as expected, overproduction of a MinCc-MinD fusion causes many cells to jackknife at their division septa. This prompted us to further characterize the phenotype and how the MinCc-MinD fusion induces it.

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TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Source or reference
Strains		
WM1074	MG1655 $\Delta lacU169$ (TX3772)	Laboratory collection
WM1032	WM1074 $\Delta minCDE::kan$	51
WM2026	WM1074 with $P_{lac}-ftsZ-gfp$	43
WM3486	$\Delta minCDE::kan$ with $P_{lac}-ftsZ-gfp$ (P1 WM1032 $\times$ WM2026)	This study
WM4475	$\Delta minC::kan$ (P1 WM3107 $\times$ WM1074)	This study
WM2280	$\Delta minD::kan$ in WM1074	Lab collection
WM2738	WM1032 ($\Delta minCDE$) with pWM2738	This study
WM3107	$\Delta minC::kan$ in BW25113 (Keio collection)	52
WM3997	WM1032 ($\Delta minCDE$) with pWM3563 (pBS58) + pWM2738	This study
WM4082	pWM4066 (pKG116His ₆ -minCc-minD) in WM3486	This study
XL1-Blue	Cloning strain	Stratagene
WM1659	<i>ftsA*</i> (R286W) in chromosome	53
WM4831	$\Delta minCDE::kan$ transduced into WM1659 + pWM2738	This study
Plasmids		
pKG116	Salicylate-inducible <i>nahR</i> promoter	J. S. Parkinson
pDSW210	IPTG-inducible weakened <i>trc</i> promoter	54
pWM2619	pDSW210 with His ₆ tag	23
pWM2735	pDSW210His ₆ -minC-gfp	23
pWM2736	pDSW210His ₆ -minCc-gfp	23
pWM2737	pDSW210His ₆ -minCc-minD fusion	This study
pWM2738	pDSW210His ₆ -minCc-minD fusion	This study
pWM3563	pBS58 (<i>E. coli ftsQAZ</i>)	55
pWM4005	pDSW210His ₆ -minCc-minD Δ MTS	This study
pWM4066	pKG116His ₆ -minCc-minD	This study
pWM4070	pDSW210His ₆ -minCc(R172A)-minD	This study
pWM4127	pDSW210His ₆ -minCc-ftsN	This study
pWM4580	pDSW210His ₆ -minCc(R133A)-minD	This study
pWM4581	pDSW210His ₆ -minCc-minD(G158R)	This study

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Strains and plasmids used in this study are shown in Table 1. *E. coli* strain XL1-Blue was used to clone plasmids containing fusions that were then transformed into either wild-type (WT) WM1074, WM1032 ($\Delta minCDE::kan$), or other *min* deletion strains as indicated. Bacteria were grown to mid-logarithmic phase (optical density at 600 nm [OD₆₀₀], ~0.5) at 37°C in Luria Bertani (LB) medium supplemented with 50 to 100 µg/ml ampicillin (Fisher Scientific), 25 µg/ml kanamycin (Sigma-Aldrich), or 10 to 20 µg/ml chloramphenicol (Acros Organics) as needed. Cells were induced with 0.01 to 1 mM isopropyl-β-D-galactopyranoside (IPTG) or 1 to 10 µM sodium salicylate for ~1 h prior to microscopic examination and fixation for immunofluorescence microscopy. For viability assays, cells in logarithmic phase were serially diluted in LB and spotted using a pronger onto LB plates containing appropriate antibiotics and 0, 0.1, or 1 mM IPTG and incubated overnight at 37°C.

Fusion construction. Gene fusions used in this study were constructed using primers shown in Table S1 in the supplemental material. All plasmids were introduced into the $\Delta minCDE$ strain WM1032 unless otherwise indicated. Plasmids pWM2737 and pWM2738 were made by amplifying the *minD* gene using primers 960 and 356 and inserting it as a SalI-HindIII fragment into pWM2735 (pDSW210His₆-minC-gfp) and pWM2736 (pDSW210His₆-minCc-gfp), respectively. The His₆-tagged MinCc-MinD fusion was amplified using primers 864 and 356 and inserted between the SacI and BamHI sites of pKG116 to create pWM4066. Primers 960 and 1621 were used to amplify MinD lacking a membrane targeting sequence (MTS) to make pWM4005. To create the R172A lesion in the MinCc portion of MinCc-MinD, two sets of PCRs were used, the first amplifying MinCc using primers 864 and 926 (with the R172A lesion incorporated) and MinCc(R172A)-MinD using primers 927 (with the

R172A lesion) and 356. The second reaction used primers 864 and 356 to amplify the MinCc(R172A)-MinD fusion, which was inserted between the SacI and HindIII sites of pDSW210His₆ (pWM2619) to create pWM4070. MinCc(R133A)-MinD and MinCc-MinD(G158R) were made by the same PCR method using primers 2007 and 2008 and primers 2009 and 2010, respectively. Plasmid pWM4127 was created by amplification of the *ftsN* gene using primers 1686 and 1687 and inserting it into pWM2738 digested with SalI and HindIII.

Live-cell microscopy. Microscopy of cells grown in broth cultures was performed using an Olympus BX60 microscope with a 100× oil immersion differential interference contrast (DIC) objective. Cells were spotted either on 2% LB agarose pads or directly on glass slides mixed 1:1 with molten 2% low-melting-point LB agarose before addition of a coverslip. Images were processed using Pixelmator, and ImageJ (27) was used to determine angles of bent cells. For time-lapse microscopy of WM2738, cells were induced with 1 mM IPTG for 1 h prior to image capture, spotted on an agar pad, and grown at 37°C under a microscope objective using a Tokai Hit temperature control system. Images were captured every 2 min. Time-lapse microscopy of WM4082 cells grown *in situ* was performed using an Onix Cellasic system and a B04A bacterial microfluidic plate (Millipore). Cells were diluted from overnight cultures in medium containing appropriate antibiotics and 10 µM IPTG to an OD₆₀₀ of 0.1 to 0.2 and loaded into the plate. Medium with appropriate antibiotics was made to flow through at 2 lb/in², and cultures were induced by switching to medium containing 10 µM sodium salicylate. An Olympus IX81 inverted microscope equipped with an Olympus IX2-UCB external power unit, a Lambda 10-3 filter wheel system, a 100× objective, a Lumen 200 (Prior) fluorescence illumination system, a ProScan II (Prior) motorized stage, and a high-resolution Hamamatsu C10600 camera was used for time-lapse imaging with the Cellasic system. Images were captured every 2 min.

Electron microscopy. WM2738 cells were grown with or without 1 mM IPTG induction to mid-logarithmic phase, centrifuged to pellet cells, washed in Millonig's phosphate buffer (85 mM NaCl, 165 mM Na₂HPO₄, 15 mM NaH₂PO₄, pH 7.4), and resuspended in 3% glutaraldehyde fixative. Eighty- and 120-nm thin sections were prepared and placed on copper grids, which were negatively stained for 1 min with 1% uranyl acetate and visualized using a JEM-1400 (JEOL) transmission electron microscope at 120 kV.

Immunoblotting. Pelleted cells were resuspended in 50 μ l GTE (50 mM glucose, 10 mM EDTA, 20 mM Tris pH, 7.5), and then 50 μ l of SDS buffer was added and samples were boiled for 5 min prior to separation by 12.5% SDS-PAGE. Proteins were transferred using a wet apparatus to a nitrocellulose membrane, which was stained with Swift membrane stain (G Biosciences) to evaluate the amount of protein on the blot. A monoclonal anti-His₆ antibody (Sigma-Aldrich) was used at a concentration of 1:3,000. A secondary anti-mouse antibody conjugated to horseradish peroxidase (HRP) was used at a concentration of 1:5,000. A Western Lightning ECL Pro kit (PerkinElmer) was used for HRP detection.

Immunofluorescence microscopy (IFM). Cells were fixed using glutaraldehyde and paraformaldehyde and incubated with antibodies as described previously (28). Antibodies against FtsZ, FtsA, ZipA, and His₆ were used to detect localization of FtsZ, FtsA, ZipA, and His₆-MinCc-MinD, respectively. Rabbit anti-FtsZ was used at a concentration of 1:2,000, rabbit anti-FtsA or anti-ZipA was used at a concentration of 1:500, and mouse anti-His₆ was used at 1:1,000. A secondary antibody conjugated to Alexa Fluor 488 was used at 1:200 to detect FtsZ, FtsA, or ZipA; a secondary antibody conjugated to rhodamine was used at 1:50 to detect His₆-tagged proteins. Images were overlaid and colored using Pixelmator.

Quantitation of immunofluorescence localization patterns. To quantitate the fluorescence intensities of MinCc-MinD with FtsZ, ZipA, or FtsA from dually labeled IFM images, areas of cell bending were chosen that had not deeply constricted. The fluorescent images of MinCc-MinD (red channel) and FtsZ, ZipA, or FtsA (green channel) were merged and aligned with Pixelmator. Cell bending areas were cropped from each image, boxes were drawn around the immediate area of a septal ring at each bend, and fluorescence intensity plots were generated for each color channel using ImageJ. If a fluorescent band at the bend was slanted, which would skew intensity plots, the cropped images were rotated to the same degree so that the band pattern of FtsZ would be horizontal. These plots were then merged, traced, and recolored in Pixelmator to analyze and generate the representative examples.

MinCc-MinD was scored for localization, and if either a larger peak of fluorescence or a directional shift in a fluorescence peak occurred on the same side of the cell bend, it was counted as having localization on the inside of a bend. If the peak of fluorescence was in the center of the region scored, or a directional shift was observed on the opposite side of the bend, this was scored as having localization at the center/outside of a bend. If a peak of MinCc-MinD localization colocalized with a peak of similar size of FtsZ, ZipA, or FtsA, this was counted as inclusion of localization. Conversely, if a peak of MinCc-MinD corresponded with a trough of FtsZ, ZipA, or FtsA fluorescence, this was counted as exclusion of localization.

RESULTS

Overproduction of a MinCc-MinD fusion induces frequent bending of *E. coli* cells at cell division septa. Full-length MinC or the C-terminal half of MinC (MinCc) was fused translationally to the N terminus of full-length MinD in pDSW210 containing a weakened P_{trc} promoter and His₆ tag to evaluate their effects on cell division. Constructs used in this study are shown in Fig. 1A. Cells of the WT strain (WM1074) were normal in the absence of IPTG induction of MinCc-MinD (Fig. 1D) but became extremely filamentous in the presence of inducer (Fig. 1E). This indicated that the MinCc-MinD fusion protein either is an active division

inhibitor or can activate the native Min proteins present in these cells.

We then tested the effects of these fusions in a Δ minCDE background (WM1032). Uninduced WM1032 control cells carrying the MinC-MinD fusion plasmid (Fig. 1B) displayed a mixture of cell lengths but were mostly filamentous. Uninduced WM1032 carrying the MinCc-MinD fusion plasmid (Fig. 1F) appeared as the expected mixture of filaments and minicells typical of a Δ min mutant. Upon induction of MinC-MinD production with 1 mM IPTG, cells filamented strongly (Fig. 1C). Full-length MinC inhibits cell division fairly well on its own but is a much more potent inhibitor in the presence of MinD, presumably because MinD concentrates MinC at the cytoplasmic membrane and enhances its affinity for the Z ring (17, 29, 30). The absence of MinE likely enhances the division inhibition, as there is no removal of MinD from the membrane under these conditions.

The unexpected result came from the production of MinCc-MinD after a 1-hour induction with IPTG. Instead of straight filaments that appear after overproduction of MinC-MinD or when MinCc and MinD are overproduced separately (23), the majority of cells were observed to bend dramatically at the site of division (Fig. 1G to I and Table 2). This was obvious in many cells within the culture because cell separation was often delayed, resulting in crooked cell pairs or chains. The bends occurred in either direction (Fig. 1I). As expected, minicells were sometimes observed still attached to cell poles, but unlike minicells in normal Δ min strains, minicells of MinCc-MinD overproducers were often bent at a significant angle from the mother cell (Fig. 1H). These results are consistent with a defect in the normal symmetry of division septum formation.

When overproduced at moderate levels of induction (0.1 mM IPTG), which still induced bending cell divisions in over 75% of the cells, the MinCc-MinD fusion did not appreciably affect viability of Δ minCDE cells (Fig. 2, top panels; Table 2). This indicates that a majority of cells divide despite their asymmetric constrictions for multiple generations and is consistent with the ability of cells to grow and divide in time-lapse studies (see below). Lower (10 μ M) IPTG concentrations also yielded normal viability, although the percentage of bends was a lower but still considerable 41% (Table 2). However, at the highest levels of induction (1 mM IPTG), overproduction of MinCc-MinD was lethal (Fig. 2).

Details of bending cell divisions as revealed by time-lapse and electron microscopic imaging. To observe bending at the septum as it was occurring and to trace cell lineages more closely, we cultured WM2738 (Δ minCDE) cells overproducing MinCc-MinD and spotted 3 μ l onto an agar pad to visualize growing cells by time-lapse imaging. These cells grew, bent at division sites, and often exhibited delays in septation like the cells in liquid culture (Fig. 3A; see also Movie S1 in the supplemental material). We found that most bends ultimately resulted in cell division at the site of the bend and that ~80% of all cell division events resulted in a bend (Table 2). A closer look at the region of cell separation showed that the two separating cells appeared to crack off each other. This idea is supported by electron microscopic data (see below).

As MinCc interacts directly with FtsZ, we hypothesized that the MinCc-MinD fusion somehow affected morphology and/or constriction dynamics of the Z ring. We therefore repeated the MinCc-MinD overproduction time-lapse experiment, this time in cells expressing low levels of FtsZ-GFP as a dilute label so that the

FIG 1 A MinCc-MinD fusion causes bending in $\Delta minCDE$ *E. coli* cells. (A) Fusion constructs used in this study. The black portion on the left represents the His₆ tag. The stars represent the positions of the R172A (MinC), R133A (MinC), and G158R (MinD) lesions, and ΔMTS represents the deletion of the membrane targeting sequence of MinD (MinDΔ259–270). The MinCc-MinD fusion consists of MinC122–231, a SalI restriction site, pentaglycine linker, and MinD. (B to I) Full-length MinC-MinD fusion (pWM2737) uninduced (B) and induced (C) in a $\Delta minCDE$ background (strain WM1032); MinCc-MinD fusion (pWM2738) uninduced (D) and induced (E) in an isogenic *min*⁺ background (WM1074); MinCc-MinD fusion uninduced (F) and induced (G) in a $\Delta minCDE$ background (WM1032); a bent cell from panel G (inset) is enlarged (H) and what appears to be a cell chain of the same strain as that in panel G with multiple bends is shown in panel I. All induced strains were induced with 1 mM IPTG. Bars, 4 μm.

TABLE 2 Percentage of bent division septa

Plasmid or strain ^a	Protein fusion	No. of septa	% of septa bent ^b
pWM2738	MinCc-MinD uninduced	100	7.0
	MinCc-MinD (10 μM IPTG)	84	40.5
	MinCc-MinD (0.1 mM IPTG)	93	76.3
	MinCc-MinD (1 mM IPTG)	100	82.0
pWM4005	MinCc-MinDΔMTS uninduced	91	2.2
	MinCc-MinDΔMTS (1 mM IPTG)	98	6.1
pWM4070	MinCc(R172A)-MinD uninduced	84	3.6
	MinCc(R172A)-MinD (1 mM IPTG)	100	8.0
pWM4127	MinCc-FtsN uninduced	44	9.1
	MinCc-FtsN (1 mM IPTG)	99	19.2
WM3997	MinCc-MinD uninduced + FtsQAZ	88	4.5
	MinCc-MinD (1 mM IPTG) + FtsQAZ	100	50.0
WM4831	MinCc-MinD uninduced, chromosomal <i>ftsA</i> ⁺	88	5.7
	MinCc-MinD (10 μM IPTG), chromosomal <i>ftsA</i> ⁺	76	22.4
	MinCc-MinD (0.1 mM IPTG), chromosomal <i>ftsA</i> ⁺	89	41.6
	MinCc-MinD (1 mM IPTG), chromosomal <i>ftsA</i> ⁺	98	49.0

^a All strains were derivatives of WM1032 ($\Delta minCDE::kan$).
^b If straight daughter cells are defined as having an angle of ~180°, then bent septa are defined as creating angles of <145° at the time of image capture.

localization and dynamics of the Z ring could be traced (31). This experiment was performed in a microfluidics chamber that immobilized cells and supplied nutrients for continuous growth. The growth and bending division of FtsZ-GFP-expressing cells (WM4082) were similar to those of cells not expressing FtsZ-GFP (Fig. 3). As FtsZ is required for cell division, we were not surprised to find that every bending division was associated with a fluorescent Z ring. Of cells that bent but did not show signs of septum formation during the time course, all bends were associated with Z rings. This is perhaps not surprising either, as Δmin cells form Z rings at most internucleoid spaces, although only a subset are active in septation (7). This also explains the presence of Z rings at many sites that did not divide during the time course.

Careful observation of FtsZ-GFP localization patterns at the bending septa revealed that Z rings initially appeared normal, then constricted normally as well as could be detected (Fig. 3B, arrows; see also Movie S2 in the supplemental material). Once the aberrant asymmetric septa were formed and the cell poles started to crack away from each other at one end, FtsZ rapidly formed a new ring at one or both of the new poles (Fig. 3B). This is similar to what has been observed in *B. subtilis* cells lacking the Min system and led to the idea that the Min system is important for preventing new Z ring assembly adjacent to the currently active constricting ring (32). Once a bending division was complete, the polar Z rings usually persisted in the daughter cells. We conclude that there is

FIG 2 Viability of various fusion constructs. Strains shown were grown to logarithmic phase, serially diluted at the dilutions shown, and spotted onto LB plates containing 0, 0.1, or 1 mM IPTG and grown overnight at 37°C.

no obvious abnormal localization of FtsZ prior to a bending division versus a normal division, suggesting that the root cause of asymmetric constriction is not simply a preference for FtsZ to assemble more on one side of the cell than the other.

To visualize the bending sites in greater detail, we negatively stained thin sections of WM2738 cells with MinCc-MinD fusions either uninduced or induced and viewed them by electron microscopy (Fig. 4). When the MinCc-MinD fusion was not induced, typical symmetrical cell division septa that had matched constrictions on both sides of the cell were observed (Fig. 4A). However, upon induction of MinCc-MinD, we observed a number of bending division events that showed envelope invaginations mainly on one side of the cell, on the outside of the bend (Fig. 4B to D). These observations are consistent with the idea that bending division is caused by a local disruption of septal wall growth and splitting on one side of the cell, despite the lack of any apparent asymmetry in FtsZ-GFP localization.

Localization of MinCc-MinD to cell division sites. Although FtsZ-GFP localization seemed to be normal in $\Delta minCDE$ cells overproducing the MinCc-MinD fusion, we wanted to know whether MinCc-MinD fusion proteins localized to the Z rings or any other places in the cell. We constructed a GFP-MinCc-MinD fusion, but this fusion failed to induce cell bending in the $\Delta minCDE$ background and mostly localized uniformly around the cell and not at Z rings, suggesting that the three-way fusion was inactive. We also constructed C-terminal MinCc-GFP fusions and MinCc-yellow fluorescent protein (YFP)-MinD fusions, but these also failed to localize to meaningful patterns or provoke cell bending.

To circumvent these problems, we took advantage of the N-terminal His₆ tag on our original MinCc-MinD construct in $\Delta minCDE$ cells to localize the fusion protein by immunofluorescence microscopy (IFM) using anti-His₆ tag monoclonal antibodies. The same cells were probed with polyclonal anti-FtsZ, and the signals were overlaid. A $\Delta minCDE$ strain containing an empty vector plasmid showed no localization with the anti-His₆ tag antibody (data not shown). In uninduced $\Delta minCDE$ cells with the

His₆-MinCc-MinD plasmid, weakly fluorescent bands were observed in a subset of cells, and these bands usually colocalized with Z rings (data not shown). Upon overproduction of His₆-MinCc-MinD with IPTG, fluorescent foci were usually visible instead of bands. These foci were more intense than the bands in the uninduced cells, and they were present in most cells (Fig. 5A). The foci were distributed fairly sparsely throughout the cell length, but the majority of these foci (~80%) roughly colocalized with FtsZ staining.

The bending division phenotype depends on self-interaction and membrane and septal targeting of MinCc-MinD. An R172A lesion in MinCc has been shown to disrupt its interaction with the septum, presumably FtsZ itself (33). We engineered this mutation into the MinCc-MinD fusion to test whether the likely interaction between MinCc and FtsZ contributes to the bending phenotype. As we expected, when fusions containing the R172A lesion were expressed, bending cell divisions were severely attenuated, down from ~80% to 8% of total cell division events (Table 2 and Fig. 6C and D). The R172A-containing protein fusion also failed to localize efficiently (Fig. 5D), supporting the idea that the MinCc portion of the MinCc-MinD fusion likely interacts with FtsZ in order to cause a cell to divide asymmetrically. To rule out the possibility that the fusion proteins were not being overproduced in these cells, we detected their N-terminal His₆ tags by Western blotting using anti-His₆ antibodies (Fig. 6I). The bands for MinCc-MinD were only slightly more intense than those for MinCc(R172A)-MinD, indicating that they were produced at roughly equivalent levels upon IPTG induction. Production of MinCc-MinD was reduced in a WT (WM1074) background (see Fig. S1 in the supplemental material), probably because MinCc-MinD is a potent division inhibitor in a Min⁺ strain as described above and makes cells sick. Full-length MinC-MinD was barely detectable in a $\Delta minCDE$ background (see Fig. S1), likely for similar reasons.

MinD is targeted to the membrane by an 8- to 12-residue membrane targeting sequence (MTS) on its C terminus (10, 34). We deleted the MTS in the MinD portion of the MinCc-MinD

FIG 3 Growing and dividing cells overproducing MinCc-MinD in time-lapse images. (A) DIC images of two different fields of 1 mM IPTG-induced WM2738 cells, showing bending divisions. Time (min) elapsed from the start of the experiment is shown. (B) Time-lapse images of WM4082 cells producing FtsZ-GFP with 10 μ M IPTG induction and MinCc-MinD induced with 10 μ M sodium salicylate. Top row, FtsZ-GFP fluorescence; middle row, DIC; bottom row, overlay. Time (min) elapsed from the start of the experiment is shown. Leftmost arrows highlight Z rings prior to apparent cell bending; subsequent arrows reflect localization of FtsZ-GFP to foci at the constriction site and the rims of newly formed cell poles during a bending division event. Bars, 2 μ m (A); 4 μ m (B).

fusion (MinCc-MinD Δ MTS) to test whether membrane targeting of the fusion was required for the bending division phenotype. After IPTG induction of the truncated MinCc-MinD fusion in Δ minCDE cells, we found that the percentage of cells with asymmetric septa was dramatically reduced to $\sim$ 6% (Table 2 and Fig. 6A and B), despite the overproduction of this protein to nearly the level of the normal fusion protein (Fig. 6I). Without IPTG induction, the truncated MinCc-MinD fusion weakly colocalized with FtsZ staining in IFM experiments (data not shown). Higher levels of the truncated fusion protein after IPTG induction localized to multiple foci that were not obviously colocalized with FtsZ (Fig. 5C). Although it is difficult to explain the localization results, the lack of a cell bending phenotype despite strong overproduction of the truncated fusion suggests that membrane localization is important for MinCc-MinD to induce cell bending.

To determine if MinCc-MinD fusions need to interact with other fusions to cause cells to bend, we constructed two point mutants. An R133A lesion in the MinCc portion of MinC has been shown to disrupt its interactions with MinD but not MinE (33). A G158R lesion in MinD results in loss of interaction with MinC

without affecting interactions with MinD or MinE (35). When R133A was introduced into the MinCc portion of the MinCc-MinD fusion, cell bending was suppressed (Fig. 6E and F). Likewise, when we introduced G158R into the MinD portion of the MinCc-MinD fusion, we observed that MinCc-MinD(G158R) also suppressed the bending phenotype (Fig. 6G and H). These mutant fusions were clearly overproduced as shown by Western blotting (Fig. 6I), although G158R levels were lower than R133A levels. These data suggest that interaction between the fusion proteins is required for the bending phenotype.

To test whether MinD itself is specifically required for the effect or whether targeting MinCc to the membrane by another means would be sufficient, we fused MinCc to the N terminus of FtsN, a cell division protein that contains a single transmembrane and periplasmic domain. This produced a MinCc fusion to full-length FtsN. Overproduction of MinCc-FtsN (WM4127) induced 19% of septa to have bends, more than the mutant MinCc-MinD constructs, but still much less than the normal construct (Table 2). MinCc-FtsN was stably overproduced (see Fig. S1 in the supplemental material) and should localize to the cytoplasmic mem-

FIG 4 Transmission electron microscopy of thin sections of dividing WM2738 cells producing uninduced MinCc-MinD (A) or induced MinCc-MinD (1 mM IPTG) (B to D). Bars, 100 nm (A and B), 0.2 μ m (C), and 0.1 μ m (D).

brane. These data suggest that although membrane targeting plays a role, other determinants of MinD, including residues at the MinCc-MinD fusion junction, may be important for full activity.

To investigate the activities of the MinCc and MinD portions of the fusions in cells lacking either MinC or MinD (Fig. 2 and Table 3). In cells lacking only MinC, production of the MinCc-MinD fusion caused cells to bend and filament, as in the Δ minCDE background. Producing MinCc(R172A)-MinD and MinCc-MinD Δ MTS in the Δ minC strain yielded a typical minicell phenotype indistinguishable from that of Min⁻ cells. In contrast, repeated attempts to introduce the plasmid carrying the

MinCc-MinD fusion into a Δ minD strain failed. This suggests that the native full-length MinC in the Δ minD strain can interact with the MinD portion of the MinCc-MinD fusion, even when present at low uninduced levels, to suppress assembly of FtsZ efficiently throughout the cell. It is not clear why MinCc-MinD is more toxic in a Δ minD strain than in a WT strain, although one explanation is that the Δ minD construct is polar on *minE*, which would reduce *minE* expression and increase the ability of MinC to bind to MinD at many sites on the cytoplasmic membrane. The MinCn portion of the native MinC is likely required for this inhibition. In support of this idea, production of the MinCc(R172A)-MinD fusion, which normally has little effect in a strain lacking MinC, causes

FIG 5 Localization of His₆-MinCc-MinD fusions in Δ minCDE (WM1032) cells. (A) Native FtsZ (green) and His₆-MinCc-MinD from pWM2738 (red), the latter induced with 1 mM IPTG. (B) Magnified view of red and green insets from panel A. (C) Localization of native FtsZ, along with MinCc-MinD Δ MTS induced with 1 mM IPTG. (D) Localization of native FtsZ, along with MinCc(R172A)-MinD induced with 1 mM IPTG. Arrows in panel A highlight a bend with colocalized FtsZ and MinCc-MinD. Camera exposure times were identical for all anti-His₆ or all anti-FtsZ fields. Bars, 2 μ m.

FIG 6 Membrane targeting, septal interaction, and self-interaction contribute to the effects of MinCc-MinD on cell bending. (A to H) DIC microscopy images are shown for WM1032 ($\Delta minCDE$) cells producing the MinCc-MinD Δ MTS fusion from pWM4005 uninduced (A) and induced (B) with 1 mM IPTG, MinCc(R172A)-MinD from pWM4070 uninduced (C) and induced (D) with 1 mM IPTG, MinCc(R133A)-MinD uninduced (E) and induced (F) with 1 mM IPTG, or MinCc-MinD(G158R) uninduced (G) and induced (H) with 1 mM IPTG. Bars, 2 μ m. (I) Western blot and corresponding membrane stain of uninduced (–) and induced (+) pDSW210His₆ vector control and various fusions produced from the pDSW210His₆ derivatives. The small plus sign below lane 3 represents induction of MinCc-MinD with 0.1 mM IPTG.

filamentation and ultimately cell death in the *minC*⁺ $\Delta minD$ strain as cellular levels of the fusion are increased (Fig. 2).

MinCc-MinD localizes to the inside of most developing bends and preferentially excludes FtsA. Now that we had established the need for the fusion protein to bind to FtsZ and interact with other fusion proteins, and FtsZ localization itself did not seem to be perturbed significantly by the fusion, we wanted to investigate whether any downstream cell division proteins were forced to localize asymmetrically. As we did for MinCc-MinD and FtsZ, we used IFM to examine the localization of His₆-tagged

MinCc-MinD in greater detail and whether the localization of FtsA or ZipA was perturbed by the fusion protein.

We used anti-His₆ antibody for His₆-MinCc-MinD in conjunction with either anti-FtsA or anti-ZipA antibodies in double-labeling IFM experiments on bending induced WM2738 cells or nonbending WM1032 parental cells. As before, we observed only faint background staining for His₆ in the WM1032 controls and strong His₆-MinCc-MinD foci in the induced WM2738 cells. When we examined cells at an early stage of bending division, prior to deep constriction, quantitating fluorescence intensities across isolated rings at developing cell bends, we found that 82 to 84% of the His₆-MinCc-MinD foci were found mainly at the inside of the bends (Fig. 7A and B and addition of columns A and B in Fig. 7E).

We then measured localization of FtsZ, FtsA, or ZipA in these same His₆-MinCc-MinD-overproducing cells and again quantitated fluorescence intensities across isolated rings at developing cell bends. We found that 45% of these bends exhibited FtsZ or ZipA staining that overlapped the peak His₆-MinCc-MinD staining (34% + 11% for FtsZ; 33% + 12% for ZipA), whereas 55% of the bends had FtsZ or ZipA staining that was largely distinct from the peak of His₆-MinCc-MinD staining (50% + 5% for FtsZ; 49% + 6% for ZipA). This is outlined in Fig. 7A and D versus 7B and C and in Fig. 7E, columns A and D versus columns B and C.

TABLE 3 Phenotypes of protein fusions in different Min deletion backgrounds^a

Protein	Phenotype by background:			
	Min ⁺	$\Delta minCDE$	$\Delta minC$	$\Delta minD$
Vector control	WT	Min [–]	Min [–]	Min [–]
MinCc-MinD	Filamentous	Bent	Bent	Uninduced lethal
MinCc-MinD Δ MTS	–	Min [–]	Min [–]	Min [–]
MinCc(R172A)-MinD	–	Min [–]	Min [–]	Filamentous
MinCc-MinD	–	Filamentous	–	–

^a All were overproduced with 1 mM IPTG in LB broth during logarithmic growth except as noted. –, not determined.

FIG 7 Aggregated His₆-MinCc-MinD fusion proteins preferentially displace FtsA from the Z ring. Immunofluorescent staining of WM2738 cells induced with 1 mM IPTG and stained with anti-FtsZ and anti-His₆, anti-ZipA and anti-His₆, or anti-FtsA and anti-His₆ at developing bending cell division septa was quantitated as described in Materials and Methods. Representative plots are shown above for anti-FtsA (green) and anti-His₆ (red) (A and B) and anti-FtsZ (green) and anti-His₆ (red) (C and D). (A to D) Four classes of common localization patterns are shown. (A) Representative intensity plot of His₆-MinCc-MinD localization toward the inside of a bend and displacement (or lower-intensity peak) of FtsZ, ZipA, or FtsA as represented in column A of the table in panel E. (B) Representative intensity plot of His₆-MinCc-MinD localization toward the inside of a bend and colocalization with a peak of FtsZ, ZipA, or FtsA as represented in column B of the table in panel E. (C) Representative intensity plot of His₆-MinCc-MinD localization at the center/outside of a bend with a colocalized peak of FtsZ or ZipA (none observed for FtsA), as represented in column C of the table in panel E. (D) Representative intensity plot of His₆-MinCc-MinD localization at the center/outside of a bend corresponding with displaced FtsZ, ZipA, or FtsA as represented in column D of the table in panel E. Panels A1 to D1 show representative micrographs of each example shown in panels A to D; bar, 2 μm. (E) Table of quantifications of the four observed classes of patterns. (F) Model of asymmetric constriction.

Strikingly, however, 90% of bends (74% + 16%) displayed a peak of FtsA staining intensity that was distinct from the peak of His₆-MinCc-MinD staining (Fig. 7A and D and addition of columns A and D in Fig. 7E). This suggested that FtsA localization was specifically antagonized by MinCc-MinD and thus significantly biased toward the outside of the developing cell bend, which is where most of the constriction occurs. In support of this, 74% of the bends had His₆-MinCc-MinD at the inside of the bend and FtsA localized away from His₆-MinCc-MinD toward the outside of the bend (Fig. 7A and E, column A). Although ZipA was localized more symmetrically at the bending septa and should be able to tether FtsZ to the membrane throughout the circumference of the ring, both ZipA and FtsA are required for recruitment of downstream divisome proteins (6). This supports the idea that the asymmetry of FtsA localization leads to asymmetric septation.

FtsA* or excess FtsQ, FtsA, and FtsZ partially suppress the bending phenotype. Cell division genes *ftsQ*, *ftsA*, and *ftsZ* are located adjacent to each other on the *E. coli* chromosome (36–38). Multicopy *ftsQAZ* has been shown to suppress the lethality of overproduction of MinC with MinD (39) and suppresses the filamentation of $\Delta minCDE$ strains (40). To determine if increased

expression of *ftsQAZ* could also suppress the bending division phenotype, we introduced a multicopy plasmid containing these genes under their native transcriptional controls (pBS58) (41) into our $\Delta minCDE$ strain that overproduces MinCc-MinD (WM3997). While the percentage of bending septa was lower with the *ftsQAZ* plasmid than without it (Table 2), bending was still observed at ~50% of septa, and overall viability was normal, even at the highest induction levels (see Fig. S2 and S3 in the supplemental material). As increased *ftsQAZ* stimulates cytokinesis and thus decreases cell length in $\Delta minCDE$ strains, the observed lower bending frequency could be explained by more efficient cytokinesis of these cells, which would cause us to not count cells that were bent but ultimately separated. However, the increased levels of FtsA in these cells may also help to compete with the MinCc moiety of the MinCc-MinD fusion.

This led us to ask whether a stronger FtsA-FtsZ interaction might suppress the cell bending phenotype by overcoming the effects of MinCc-MinD. Overproducing FtsA was not an option, as increasing levels of FtsA inhibit cell division (42). However, there is evidence from yeast two-hybrid assays that the gain-of-function allele of FtsA, FtsA* (R286W), interacts more strongly

with FtsZ than does WT FtsA (43, 44). To test whether FtsA* might compete more effectively with MinCc-MinD and perhaps suppress the asymmetric constriction phenotype, we overproduced MinCc-MinD in Δmin cells in which *ftsA** replaced WT *ftsA* in the chromosome. Similar to the cells expressing *ftsQAZ* in multicopy, ~50% of septa in cells with FtsA* exhibited asymmetric constrictions upon induction of MinCc-MinD with 1 mM IPTG, a significant decrease from the ~80% rate with WT FtsA (Table 2; see also Fig. S3 in the supplemental material). MinCc-MinD levels were similar in the two strains (data not shown). Moreover, these cells were completely viable at levels of MinCc-MinD (1 mM IPTG) that were toxic to *ftsA*⁺ cells (see Fig. S2). Finally, induction of MinCc-MinD at lower levels of IPTG resulted in proportionately lower bending frequencies in the *ftsA** background than in the *ftsA*⁺ background (Table 2; see also Fig. S3). These results are consistent with the idea that stronger FtsA-FtsZ binding suppresses asymmetric constrictions.

DISCUSSION

When *E. coli* and other bacilli such as *Bacillus subtilis* divide, the daughter cells generally remain in line. On solid medium, the result is that two separated *E. coli* daughter cells are roughly parallel to each other, as are their progeny. In the case of *B. subtilis*, fairly straight cell chains are produced. The symmetry of these typical cell division events also indicates that the divisome normally acts symmetrically, with septal murein synthesized evenly around the cell circumference at midcell and amidases cleaving bonds symmetrically as well. However, some rod-shaped bacteria that divide by binary fission form a sharp bend at their septum, dividing more on one side than the other. Many actinobacteria, such as mycobacteria, divide by a snapping division mechanism (45). Magnetotactic bacteria, such as *Magnetospirillum gryphiswaldense*, also divide this way and feature arc-like Z-ring structures at sites of division (46). Similar Z arcs have been observed in *E. coli* cells deficient in RodA or MreB, which grow and divide as spheres. These spherical cells also divide asymmetrically, with an invaginating septum mainly on one side of the cell corresponding to the Z arc (47–49). The difference with our cells is that we do not observe obvious asymmetric localization of the Z ring prior to or during the early stage of a bent septum. Later stages of bending division feature a V-shaped FtsZ staining pattern at the new cell poles developing on the other side of the septum, because the Min proteins are absent and cannot inhibit Z ring assembly at the new poles.

Why might MinCc-MinD, in particular, cause the bending division phenotype? When produced at high levels, the fusion protein becomes toxic, and cells producing it are unable to form colonies on plates. However, bending divisions are not lethal to the population, because lower levels of induction allow normal viability even though the majority of these cells still divide by bending (Fig. 2; Table 2). Also, Δmin cells producing MinCc-MinD fusions do not inhibit septum formation as readily as do cells producing MinC-MinD, probably because the MinCc portion has a more limited ability to disrupt FtsZ polymers than does the full-length protein containing MinCn. It is plausible that the MinD portion of the MinCc-MinD fusion is fully active, given that MinD can oscillate from cell pole to cell pole even with a large GFP protein fused to its N terminus (14). Moreover, MinCc-MinD can inhibit cell division effectively in Min⁺ cells. This is likely because of the presence of native MinC protein, as MinCc-MinD inhibits growth at

least as efficiently in cells lacking only MinD. We speculate that upon overproduction, MinCc-MinD fusions form polymers or an array. This could occur if the MinCc portion of one MinCc-MinD fusion protein interacts with the MinD portion of another fusion protein. Consistent with this idea, mutations that should prevent this interaction abolished bending cell divisions. Presumably, a subset of MinCc domains in the MinCc-MinD polymers/array remains capable of targeting the C-terminal tail of FtsZ.

As Z rings in normal cells and in cells overproducing MinCc-MinD appear similar, it is unlikely that the MinCc-MinD aggregate induces asymmetric constriction by forcing FtsZ to localize asymmetrically. Instead, we favor a model in which the Z ring assembles circumferentially, but a MinCc-MinD aggregate bound to a portion of the Z ring blocks the normal ability of those FtsZ C-terminal domains to recruit other divisome proteins. The strong tendency of FtsA in particular to avoid the region occupied by MinCc-MinD supports the idea that FtsA is specifically occluded from the Z ring by the MinCc-MinD aggregates, which would then prevent further maturation of the ring at the site of these aggregates. In support of this idea, MinCc has been reported to compete with FtsA for binding to the FtsZ C terminus (24). MinCc-MinD may displace FtsA more readily than ZipA because the latter binds to FtsZ with higher affinity.

Electron microscopic analysis of bending cells demonstrates that most of the constriction activity occurs on the outside of the developing bend, suggesting that this is the main cause of the bending phenotype. Our model predicts that MinCc-MinD should localize preferentially to the inside of a developing bend and FtsA to the outside, where additional divisome proteins such as FtsN, which has been shown to interact with FtsA directly (50), are preferentially recruited to induce constriction (Fig. 7F). In support of the model, we found that a significant majority of MinCc-MinD aggregates localize to the inside of a developing septal bend, and FtsA localizes primarily toward the outside. Replacing FtsA with FtsA* partially suppressed bending, consistent with the idea that FtsA* can compete somewhat with MinCc-MinD for binding to FtsZ and hence reduce the frequency of MinCc-MinD-induced asymmetric constrictions. Given the strong effects of the Z-ring-targeted aggregates formed by MinCc-MinD, we speculate that other proteins that target the Z ring may, if they are not fully functional, also perturb symmetrical activity of the Z ring, causing a similar bending division phenotype. Related future questions to address include how the Z ring and complete divisome maintain a mostly symmetrical circumferential shape in species such as *E. coli* and if there are advantages in dividing symmetrically.

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REFERENCES

1. Margolin W. 2000. Themes and variations in prokaryotic cell division. *FEMS Microbiol. Rev.* 24:531–548. <http://dx.doi.org/10.1111/j.1574-6976.2000.tb00554.x>.

2. Vicente M, Rico AI, Martinez-Arteaga R, Mingorance J. 2006. Septum enlightenment: assembly of bacterial division proteins. *J. Bacteriol.* 188: 19–27. <http://dx.doi.org/10.1128/JB.188.1.19-27.2006>.
3. Adams DW, Errington J. 2009. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat. Rev. Microbiol.* 7:642–653. <http://dx.doi.org/10.1038/nrmicro2198>.
4. Fu G, Huang T, Buss J, Coltharp C, Hensel Z, Xiao J. 2010. *In vivo* structure of the *E. coli* FtsZ-ring revealed by photoactivated localization microscopy (PALM). *PLoS One* 5:e12682. <http://dx.doi.org/10.1371/journal.pone.0012682>.
5. Pichoff S, Lutkenhaus J. 2005. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. *Mol. Microbiol.* 55:1722–1734. <http://dx.doi.org/10.1111/j.1365-2958.2005.04522.x>.
6. Pichoff S, Lutkenhaus J. 2002. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in *Escherichia coli*. *EMBO J.* 21:685–693. <http://dx.doi.org/10.1093/emboj/21.4.685>.
7. Yu X-C, Margolin W. 1999. FtsZ ring clusters in *min* and partition mutants: role of both the Min system and the nucleoid in regulating FtsZ ring localization. *Mol. Microbiol.* 32:315–326. <http://dx.doi.org/10.1046/j.1365-2958.1999.01351.x>.
8. de Boer PAJ, Crossley RE, Rothfield LI. 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. *Cell* 56:641–649. [http://dx.doi.org/10.1016/0092-8674\(89\)90586-2](http://dx.doi.org/10.1016/0092-8674(89)90586-2).
9. Hu Z, Mukherjee A, Pichoff S, Lutkenhaus J. 1999. The MinC component of the division site selection system in *Escherichia coli* interacts with FtsZ to prevent polymerization. *Proc. Natl. Acad. Sci. U. S. A.* 96:14819–14824. <http://dx.doi.org/10.1073/pnas.96.26.14819>.
10. Hu Z, Lutkenhaus J. 2003. A conserved sequence at the C-terminus of MinD is required for binding to the membrane and targeting MinC to the septum. *Mol. Microbiol.* 47:345–355. <http://dx.doi.org/10.1046/j.1365-2958.2003.03321.x>.
11. de Boer PAJ, Crossley RE, Hand AR, Rothfield LI. 1991. The MinD protein is a membrane ATPase required for the correct placement of the *Escherichia coli* division site. *EMBO J.* 10:4371–4380.
12. Raskin DM, de Boer PAJ. 1997. The MinE ring: an FtsZ-independent cell structure required for selection of the correct division site in *E. coli*. *Cell* 91:685–694. [http://dx.doi.org/10.1016/S0092-8674\(00\)80455-9](http://dx.doi.org/10.1016/S0092-8674(00)80455-9).
13. Hu Z, Gogol EP, Lutkenhaus J. 2002. Dynamic assembly of MinD on phospholipid vesicles regulated by ATP and MinE. *Proc. Natl. Acad. Sci. U. S. A.* 99:6761–6766. <http://dx.doi.org/10.1073/pnas.102059099>.
14. Raskin DM, de Boer PAJ. 1999. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 96:4971–4976. <http://dx.doi.org/10.1073/pnas.96.9.4971>.
15. Fu X, Shih Y-L, Zhang Y, Rothfield LI. 2001. The MinE ring required for proper placement of the division site is a mobile structure that changes its cellular location during the *Escherichia coli* division cycle. *Proc. Natl. Acad. Sci. U. S. A.* 98:980–985. <http://dx.doi.org/10.1073/pnas.98.3.980>.
16. Huang KC, Meir Y, Wingreen NS. 2003. Dynamic structures in *Escherichia coli*: spontaneous formation of MinE rings and MinD polar zones. *Proc. Natl. Acad. Sci. U. S. A.* 100:12724–12728. <http://dx.doi.org/10.1073/pnas.2135445100>.
17. Hu Z, Lutkenhaus J. 1999. Topological regulation of cell division in *Escherichia coli* involves rapid pole to pole oscillation of the division inhibitor MinC under the control of MinD and MinE. *Mol. Microbiol.* 34: 82–90. <http://dx.doi.org/10.1046/j.1365-2958.1999.01575.x>.
18. Thanedar S, Margolin W. 2004. FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. *Curr. Biol.* 14: 1167–1173. <http://dx.doi.org/10.1016/j.cub.2004.06.048>.
19. Tonthat NK, Milam SL, Cinnam N, Whitfill T, Margolin W, Schumacher MA. 2013. SlnA forms a higher-order structure on DNA that inhibits cytokinetic Z-ring formation over the nucleoid. *Proc. Natl. Acad. Sci. U. S. A.* 110:10586–10591. <http://dx.doi.org/10.1073/pnas.1221036110>.
20. Bisicchia P, Arumugam S, Schwillie P, Sherratt D. 2013. MinC, MinD, and MinE drive counter-oscillation of early-cell-division proteins prior to *Escherichia coli* septum formation. *mBio* 4(6):e00856-13. <http://dx.doi.org/10.1128/mBio.00856-13>.
21. Cordell SC, Anderson RE, Löwe J. 2001. Crystal structure of the bacterial cell division inhibitor MinC. *EMBO J.* 20:2454–2461. <http://dx.doi.org/10.1093/emboj/20.10.2454>.
22. Hu Z, Lutkenhaus J. 2000. Analysis of MinC reveals two independent domains involved in interaction with MinD and FtsZ. *J. Bacteriol.* 182: 3965–3971. <http://dx.doi.org/10.1128/JB.182.14.3965-3971.2000>.
23. Shiomi D, Margolin W. 2007. The C-terminal domain of MinC inhibits assembly of the Z ring in *Escherichia coli*. *J. Bacteriol.* 189:236–243. <http://dx.doi.org/10.1128/JB.00666-06>.
24. Shen B, Lutkenhaus J. 2009. The conserved C-terminal tail of FtsZ is required for the septal localization and division inhibitory activity of MinC^C/MinD. *Mol. Microbiol.* 72:410–424. <http://dx.doi.org/10.1111/j.1365-2958.2009.06651.x>.
25. Shen B, Lutkenhaus J. 2010. Examination of the interaction between FtsZ and MinC^N in *E. coli* suggests how MinC disrupts Z rings. *Mol. Microbiol.* 75:1285–1298. <http://dx.doi.org/10.1111/j.1365-2958.2010.07055.x>.
26. Dajkovic A, Lan G, Sun SX, Wirtz D, Lutkenhaus J. 2008. MinC spatially controls bacterial cytokinesis by antagonizing the scaffolding function of FtsZ. *Curr. Biol.* 18:235–244. <http://dx.doi.org/10.1016/j.cub.2008.01.042>.
27. Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9:671–675. <http://dx.doi.org/10.1038/nmeth.2089>.
28. Levin PA. 2002. Light microscopy techniques for bacterial cell biology, p 112–132. In Sansonetti P, Zychlinsky A (ed), *Methods in microbiology*, vol 31. Molecular cellular microbiology. Academic Press Ltd., London, United Kingdom.
29. Raskin DM, de Boer PAJ. 1999. MinDE-dependent pole-to-pole oscillation of division inhibitor MinC in *Escherichia coli*. *J. Bacteriol.* 181:6419–6424.
30. Johnson JE, Lackner LL, de Boer PAJ. 2002. Targeting of ¹⁵N-MinC/MinD and ¹⁵N-MinC/DicB complexes to septal rings in *Escherichia coli* suggests a multistep mechanism for MinC-mediated destruction of nascent FtsZ rings. *J. Bacteriol.* 184:2951–2962. <http://dx.doi.org/10.1128/JB.184.11.2951-2962.2002>.
31. Sun Q, Margolin W. 1998. FtsZ dynamics during the division cycle of live *Escherichia coli* cells. *J. Bacteriol.* 180:2050–2056.
32. Gregory JA, Becker EC, Pogliano K. 2008. *Bacillus subtilis* MinC destabilizes FtsZ-rings at new cell poles and contributes to the timing of cell division. *Genes Dev.* 22:3475–3488. <http://dx.doi.org/10.1101/gad.1732408>.
33. Zhou H, Lutkenhaus J. 2005. MinC mutants deficient in MinD- and DicB-mediated cell division inhibition due to loss of interaction with MinD, DicB, or a septal component. *J. Bacteriol.* 187:2846–2857. <http://dx.doi.org/10.1128/JB.187.8.2846-2857.2005>.
34. Szeto TH, Rowland SL, Rothfield LI, King GF. 2002. Membrane localization of MinD is mediated by a C-terminal motif that is conserved across eubacteria, archaea, and chloroplasts. *Proc. Natl. Acad. Sci. U. S. A.* 99: 15693–15698. <http://dx.doi.org/10.1073/pnas.232590599>.
35. Ma L, King GF, Rothfield L. 2004. Positioning of the MinE binding site on the MinD surface suggests a plausible mechanism for activation of the *Escherichia coli* MinD ATPase during division site selection. *Mol. Microbiol.* 54:99–108. <http://dx.doi.org/10.1111/j.1365-2958.2004.04265.x>.
36. Robinson AC, Kenan DJ, Hatfull GF, Sullivan NF, Spiegelberg R, Donachie WD. 1984. DNA sequence and transcriptional organization of essential cell division genes *ftsQ* and *ftsA* of *Escherichia coli*: evidence for overlapping transcriptional units. *J. Bacteriol.* 160:546–555.
37. Robinson AC, Kenan DJ, Sweeny J, Donachie WD. 1986. Further evidence for overlapping transcriptional units in an *Escherichia coli* cell envelope-cell division gene cluster: DNA sequence and transcriptional organization of the *ddl ftsQ* region. *J. Bacteriol.* 167:809–817.
38. Yi Q-M, Rockenbach S, Ward JE, Jr, Lutkenhaus J. 1985. Structure and expression of the cell division genes *ftsQ*, *ftsA* and *ftsZ*. *J. Mol. Biol.* 184: 399–412. [http://dx.doi.org/10.1016/0022-2836\(85\)90290-6](http://dx.doi.org/10.1016/0022-2836(85)90290-6).
39. Pichoff S, Lutkenhaus J. 2001. *Escherichia coli* division inhibitor MinCD blocks septation by preventing Z-ring formation. *J. Bacteriol.* 183:6630–6635. <http://dx.doi.org/10.1128/JB.183.22.6630-6635.2001>.
40. Begg K, Nikolaichik Y, Crossland N, Donachie WD. 1998. Roles of FtsA and FtsZ in activation of division sites. *J. Bacteriol.* 180:881–884.
41. Bi E, Lutkenhaus J. 1990. FtsZ regulates frequency of cell division in *Escherichia coli*. *J. Bacteriol.* 172:2765–2768.
42. Dai K, Lutkenhaus J. 1992. The proper ratio of FtsZ to FtsA is required for cell division to occur in *Escherichia coli*. *J. Bacteriol.* 174:6145–6151.
43. Geissler B, Shiomi D, Margolin W. 2007. The *ftsA** gain-of-function allele of *Escherichia coli* and its effects on the stability and dynamics of the Z ring. *Microbiology* 153:814–825. <http://dx.doi.org/10.1099/mic.0.2006/001834-0>.
44. Pichoff S, Shen B, Sullivan B, Lutkenhaus J. 2012. FtsA mutants im-

- paired 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. <http://dx.doi.org/10.1111/j.1365-2958.2011.07923.x>.
45. Thanky NR, Young DB, Robertson BD. 2007. Unusual features of the cell cycle in mycobacteria: polar-restricted growth and the snapping-model of cell division. *Tuberculosis* 87:231–236. <http://dx.doi.org/10.1016/j.tube.2006.10.004>.
 46. Katzmann E, Müller FD, Lang C, Messerer M, Winklhofer M, Plitzko JM, Schüler D. 2011. Magnetosome chains are recruited to cellular division sites and split by asymmetric septation. *Mol. Microbiol.* 82:1316–1329. <http://dx.doi.org/10.1111/j.1365-2958.2011.07874.x>.
 47. Addinall SG, Bi E, Lutkenhaus J. 1996. FtsZ ring formation in fts mutants. *J. Bacteriol.* 178:3877–3884.
 48. Corbin BD, Yu X-C, Margolin W. 2002. Exploring intracellular space: function of the Min system in round-shaped *Escherichia coli*. *EMBO J.* 21:1998–2008. <http://dx.doi.org/10.1093/emboj/21.8.1998>.
 49. Bendezú FO, de Boer PAJ. 2008. Conditional lethality, division defects, membrane involution, and endocytosis in *mre* and *mrd* shape mutants of *Escherichia coli*. *J. Bacteriol.* 190:1792–1811. <http://dx.doi.org/10.1128/JB.01322-07>.
 50. Busiek KK, Eraso JM, Wang Y, Margolin W. 2012. The early divisome protein FtsA interacts directly through its 1c subdomain with the cytoplasmic domain of the late divisome protein FtsN. *J. Bacteriol.* 194:1989–2000. <http://dx.doi.org/10.1128/JB.06683-11>.
 51. Sun Q, Margolin W. 2001. Influence of the nucleoid on placement of FtsZ and MinE rings in *Escherichia coli*. *J. Bacteriol.* 183:1413–1422. <http://dx.doi.org/10.1128/JB.183.4.1413-1422.2001>.
 52. 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. <http://dx.doi.org/10.1038/msb4100050>.
 53. Geissler B, Elraheb D, Margolin W. 2003. A gain-of-function mutation in *ftsA* bypasses the requirement for the essential cell division gene *zipA* in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 100:4197–4202. <http://dx.doi.org/10.1073/pnas.0635003100>.
 54. Weiss DS, Chen JC, Ghigo J-M, Boyd D, Beckwith J. 1999. Localization of FtsI (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. *J. Bacteriol.* 181:508–520.
 55. Wang X, Lutkenhaus J. 1993. The FtsZ protein of *Bacillus subtilis* is localized at the division site and has GTPase activity that is dependent upon FtsZ concentration. *Mol. Microbiol.* 9:435–442. <http://dx.doi.org/10.1111/j.1365-2958.1993.tb01705.x>.