FtsZ Exhibits Rapid Movement and Oscillation Waves in Helix-like Patterns in *Escherichia coli*

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Supplemental Experimental Procedures

Strains

All strains were derivatives of *E. coli* K12 and are listed in Table S1. For construction of the Δ*mreB::cat* strain, we performed PCR with *mreB*-homologous primers mreBKO1 (AGATGATTTTTGCGCCTTG TCGCTGCTGCGCAGGAGCTAAGGAAG) and mreBKO2 (CCAGAA TAAGGCGAATCTGTAGCGACGGGCCTTACGCCCCGCCCT) to amplify the chloramphenicol acetyltransferase (CAT) gene, and we electroporated the PCR product into WM1826 that had been transiently induced at 42°C. This induces phage λ recombination functions, allowing for high-efficiency chromosomal gene replacement [S1]. Introducing the pET28-FtsA* plasmid [S2] carrying *ftsA* with the R286W substitution into DY329 (WM1826) promoted cytokinesis in round cells, which are thought to be deficient in the activity of *ftsQAZ*. The resulting Δ*mreB*::*cat* strain (WM1933) had a complete replacement of the *mreB* gene with *cat*; it was verified by PCR and by the fact that it was possible to cotransduce Cm^R and spherical morphology to a new strain background. Because of technical difficulties in transducing the chromosomal *ftsZ-gfp* construct into the Δ*mreB::cat* strain WM1933 from EC448, we instead introduced pWM711, a plasmid that synthesizes a low level of FtsZ-GFP constitutively (WM2002). Similar Z rings and mobile helices were observed with pWM711 in *mreB*+ cells (data not shown), indicating that the FtsZ-GFP expression level was similar to that in EC448. To express GFP-MinD in Δ*mreB::cat* cells, we transformed WM1933 with pWM1255 [S3] to make WM1928. WM1135 is an FtsZ depletion strain (WM746) that contains an *ftsZ* null allele on the chromosome and an *ftsZ*-containing plasmid with a thermosensitive origin of replication and into which the *ftsZ-gfp* from EC448 was introduced by P1 phage transduction.

Plasmids

All plasmids are shown in Table S1. To make plasmid pWM711, we cloned the *ftsZ-gfp* fusion from pZG [S4] as a EcI136II-ApaI fragment into pcDNA 3.1 (Invitrogen) cleaved with EcoRV and ApaI. The *ftsZ-gfp* fusion is expressed constitutively from a vector promoter. To construct plasmid pWM2024, expressing GFP-MreB, we amplified the chromosomal *mreBC* region with primers mreB9 (TGAGCTCAT GGTTGGTAAAGTA) and mreCnostop (TTTCTAGATTGCCCTCCCG GCGCAC) and cloned the product between the SacI and XbaI sites of pDSW207 [S5]. To construct plasmid pWM1736 expressing SulA, we amplified the chromosomal *sulA* gene with primers SulA5 (AAG AGCTCACAGGGGCTGGATTG) and SulA4 (GGTCTAGAATGATACA AATTAGAGTG) and cloned the product between the SacI and XbaI sites of pBAD33 [S6].

Growth Conditions and Microscopy

All strains containing FtsZ-GFP were grown in LB medium at the temperatures indicated in the figure legends. Unless otherwise stated, FtsZ-GFP expression was induced with 40 μM IPTG for 2–3 hr. Because the basal expression levels of GFP-MinD from pWM1255 and FtsZ-GFP from pWM711 were sufficient for detection, no IPTG was necessary in those experiments. Induction of SulA expression with 0.1% arabinose in WM2012 completely inhibited cell division, whereas addition of no arabinose resulted in only slightly elongated cells. When cells were ready for microscopic examination, they were immobilized in LB containing a final concentration of 1.5% low-melting-point agarose under glass coverslips, and GFP fluorescence time courses were performed essentially as described previously [S3]. Great care was taken to keep the specimen in the focal plane throughout the time courses; this was aided by zooming in on a part of the field.

Immunofluorescence microscopy was performed as described previously, by methanol fixation followed by probing with affinity-purified anti-FtsZ, Alexa-488-conjugated goat anti-rabbit secondary antibody and fluorescence microscopy [S7]. Live cells expressing GFP fusions were immobilized and examined by time-lapse fluorescence microscopy as described previously [S3].

Table S1. Strains Used in this Study

Strain or Plasmid	Genotype	Source/Reference
TOP10	F- *mcrA* Δ*(mrr-hsdRMS-mcrBC)* ø80*lacZ*Δ*M15* Δ*lacX74 deoR recA1 araD139* Δ*(araA-leu)7697 galU galK rpsL endA1 nupG*	Invitrogen
DY329	W3110 Δ*lacU169 nadA*::Tn*10 gal490* λcI857 Δ(*cro-bioA*)	[S1]
TX3772	MG1655 (wild-type) with Δ*lacU169*	Lab collection
EC448	MC4100 Δ(λ*attL-lom*)::*bla lacI*^{q}Ptrc$_{208}$ *-ftsZ-gfp*	[S5]
EC488	EC448 *ftsZ84*(ts)	[S5]
WM746	WX7 (*ftsZ* null)/*ftsZ* on thermosensitive plasmid (pCX41)	[S8]
WM947	MG1655 Δ*minCDE*::*aph*	[S7]
WM1135	WM746 with Δ(λ*attL-lom*)::*bla lacI*^{q}Ptrc$_{208}$ *-ftsZ-gfp*	EC448 P1 → WM746
WM1826	pET28-*ftsA* (R286W)* in DY329	This study
WM1928	Ptrc$_{207}$ *-gfp-minD, minE* in Δ*mreB::cat*/pET28-*ftsA*(R286W)*	pWM1255 → WM1933
WM1933	DY329 Δ*mreB*::*cat* pET28-*ftsA*(R286W)*	Δ*mreB*::*cat* in WM1826
WM1993	EC488 Δ*minCDE::aph*	WM947 → EC488
WM1994	EC448 Δ*minCDE*::*aph*	WM947 → EC448
WM2002	Δ*mreB*::*cat* with constitutive *ftsZ-GFP*	pWM711 → WM1933
WM2012	MC4100 Δ(λ*attL-lom*)::*bla lacI*^{q}Ptrc$_{208}$ *-ftsZ-gfp* P$_{ara}$-*sulA*	pWM1736 → EC448
WM2024	Ptrc$_{207}$ *-gfp-mreB, mreC*	pWM2024 → TOP10
pcDNA3.1	Mammalian expression vector	Invitrogen
pZG	P$_{lac}$-*ftsZ-gfp* (pZG +*lacI*^{q})	[S4]
pWM711	Constitutive expression of *ftsZ-gfp* in pCDNA3.1	This study
pWM1255	Ptrc$_{207}$ *-gfp-minD, minE*	[S3]
pWM1736	P$_{ara}$-*sulA* in pBAD33	This study
pWM2024	Ptrc$_{207}$ *-gfp-mreB, mreC*	This study

Analysis of Fluorescence Dynamics

To determine whether the movement of fluorescence in cells expressing FtsZ-GFP was oscillatory and to quantitate the oscillation period, we opened the time-lapse images of a given cell in ImageJ 1.31 freeware to make a stack. We then used the time-lapse images to make a borderless montage similar to that shown in Figure 1B and rotated the montage such that the time lapse read from left to right. Transects were then made across the two cellular regions to be compared, usually the top half and bottom half (or one end and the middle of a filament, for the cell in Figure 3B), and the grayscale intensity profile was plotted over the entire time course for each transect. Numerical values of the peaks from the resulting plots for each time point were paired, and the differences between values (e.g., left half of the cell minus the right half) were obtained in Microsoft Excel. The results were imported into Kaleidagraph, and these differences were then plotted against time to reveal when the fluorescence was the same in each cell half (value = zero) or when fluorescence in one half was greater than that in the other half, which would give either a positive or negative numerical value depending on which half of the cell had the most fluorescence intensity. The advantages of this subtractive calculation method are that periodic oscillations can be readily visualized in a plot, and the frequent decrease of fluorescence over time due to photobleaching is automatically normalized.

Supplemental References

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