The Tubulin Homolog FtsZ Contributes to Cell Elongation by Guiding Cell Wall Precursor Synthesis in *Caulobacter crescentus*

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Supplemental text

Construction methods for strains and plasmids.

To create pHL32, pBGST18 was cut with HindIII/NdeI releasing a 0.5kb fragment and the original pBGST18 multiple cloning site. The 3.9kb pBGST18 fragment was T4 polymerase blunted and ligated to a T4 polymerase blunted 0.8kb SapI/DraIII fragment from Bluescript pSK (Stratagene). To create pKSmegfp, enhanced *gfp* from pEGFP-N2 (BD Biosciences) was moved into Bluescript pKS (Stratagene) and mutagenized with the Promega site-directed mutagenesis kit. pKSmegfp contains enhanced *gfp* with the A206K mutation to prevent dimerization of GFP. To create pHL32TmurG-mgfp, the last 661 bases of *murG* were PCR amplified with a primer to remove the stop codon and replace it with a HindIII site. The PCR product was cloned into pKS. A digest with SpeI/HindIII was used to release the *murG* fragment. *megfp* was digested from pKSmegfp using HindIII/NotI. A triple ligation of the two fragments was performed into pHL32. To create pBGentmurG-mgfp, pHL32TmurG-mgfp was digested with BamHI/SacI to release the *murG-mgfp* insert which was cloned into pBGent. Plasmid pNJH17 (kind gift from M. Thanbichler, N. Hillson, and L. Shapiro) was transformed into CB15N to create strain CJW1550 in which *ftsZ* is tagged with *mCherry* at the *vanA* locus and is under the control of the *Pvan* promoter. To create CJW1561, pHL32TmurG-mgfp was introduced into CB15N by conjugation to integrate *murG-mgfp* on the chromosome at the *murG* locus as the only functional copy of *murG*. To create CJW1572, CJW1550 was used to create a transducing lysate which was used to transduce the *ftsZ-mCherry* fusion into CJW1561. To generate CJW1575, a transducing lysate from CJW1561 was used to move

murG::pHL32TmurG-mgfp into LS107. CJW1576 was generated by moving *vanA*::pNJH17 into CJW1575 using a transducing lysate from CJW1550. To create CJW1608, pBGentmurG-mgfp was transformed into CB15N. To create CJW1607, a transducing lysate from YB1585 was used to move *ftsZ*::pBJM1 into CJW1608. CJW1715 carrying the $mreB_{Q26P}$ mutation was isolated in a screen where an aliquot of a CB15N culture was plated on PYE plates containing 5 µg/ml A22. To generate CJW1682, pXGFP4-ClmreBQ26P was mated into CJW1715. To create CJW1997, CJW1561 which contains pHL32TmurG-mgfp was used to make a transducing lysate to move the *murG-mgfp* fusion into CJW1715. CJW1998 was generated by using a transducing lysate from CJW1550 to move pNJH17 into CJW1997.

Figure legends for supplemental figures

Figure S1: Peptidoglycan elongation occurs near midcell in *C. crescentus*.

Electron micrographs of D-Cys labeled and chased PG sacculi from cells in the stalked cell cycle stage. The central clearing of D-Cys label indicates zonal PG growth near midcell. See Figure 1B legend for details.

Figure S2: Localization pattern of MurG-mGFP.

Images of CJW1572 swarmer cells showing that the MurG-mGFP signal is not completely diffuse, but somewhat heterogeneous.

Figure S3: Localization of GFP-MreB and FtsZ-mCherry in the presence of A22.

To induce *gfp-mreB* and *ftsZ-mCherry* expression, CJW1484 cells were grown in the presence of 0.03% xylose and 0.5mM vanillic acid for 2 h. When appropriate, A22 (10 μg/ml) was added for 10 min and protein localization was visualized on M2G agarose padded slides containing A22 (10μg/ml). In the presence of A22, GFP-MreB is by and large delocalized; however, a very faint GFP-MreB accumulation near midcell can be detected in some cells (arrow).

Figure S4: Cell morphology of CB15N *mreB*$_{Q26P}$ cells.

Images of wild-type CB15N and CB15N *mreB*$_{Q26P}$ (CJW1715) cells.

Figure S5: The effect of fosfomycin on cell growth.

Two PYE cultures of CJW1572 were diluted to an optical density of about 0.05 at 660 nm in PYE containing vanillic acid (0.5 mM). After 2 h of growth at 30˚C, the two cultures were split into four and fosfomycin was added to two of them to a final concentration of 20 μg/ml. The effect on growth was then monitored by taking the optical density at 660 nm over the course of 4 h.

Figure legends for supplemental movies

Movie S1: Timelapse movie showing the relative localization of MurG and FtsZ during the cell cycle.

A CJW1572 cell culture producing MurG-mGFP and FtsZ-mCherry was treated with 0.5 mM vanillic acid for 3 hr to induce *ftsZ-mCherry* expression. Cells were then placed on an agarose-padded slide containing $M2G^{+}$ medium and 0.5 mM vanillic acid, and were examined using time-lapse microscopy at 10 min intervals throughout the cell cycle. The movie shows the localization of MurG-GFP and FtsZ-mCherry in a representative cell. MurG-GFP and FtsZ-mCherry colocalize near midcell during cell elongation (arrow) before the appearance of constriction (arrowhead).

Movie S2: Timelapse movie showing the relative localization of MreB and FtsZ during the cell cycle.

A CJW1484 cell culture producing GFP-MreB and FtsZ-mCherry was treated with 0.3% xylose and 0.5 mM vanillic acid for 3 h to induce *gfp-mreB* and *ftsZ-mCherry* expression, respectively. Cells were then placed on an agarose-padded slide containing $M2G^{+}$ medium, 0.3% xylose, and 0.5 mM vanillic acid, and were examined using time-lapse microscopy at 10-min intervals throughout the cell cycle. The movie shows the localization of GFP-MreB and FtsZ-mCherry in a representative cell.

Movie S3: Timelapse movie of MurG localization under conditions of FtsZ depletion.

CJW1607 cells producing MurG-mGFP and with *ftsZ* expression under the control of the xylose inducible promoter were synchronized. After synchronization, cells were grown in the absence of xylose and presence of glucose to shut-off *ftsZ* expression and block cell division. Cells were then placed on an agarose-padded slide and were examined using time-lapse microscopy at 10-min intervals over the course of 12 h. Under these conditions, MurG-mGFP failed to stably localize near midcell.

Movie S4: Timelapse movie of $MreB_{Q26P}$ localization in the *$mreB_{Q26P}$* mutant background.

CJW1682 cells carrying *gfp-$mreB_{Q26P}$* under xylose inducible expression were treated with 0.3% xylose for 3 h. A sample was placed on an agarose-padded slide containing $M2^{+}$ and 0.3% xylose, and cells were examined by DIC and fluorescence microscopy every 5 min to determine the localization of GFP-$MreB_{Q26P}$ during growth. GFP-$MreB_{Q26P}$ fails to form a band (ring) near midcell; instead it forms patches that move rapidly (at least every 5 min) inside the cell.

Movie S5: Timelapse movie of MurG and FtsZ localization in a *$mreB_{Q26P}$* mutant background.

CJW1998 cells were grown in $M2G^{+}$ and treated for 3 h with 0.5 mM vanillic acid to induce *ftsZ-mCherry* expression. A sample was then placed on an agarose-padded slide containing $M2G^{+}$ and 0.5mM vanillic acid, and cells were examined by DIC and

fluorescence microscopy every 10 min during growth. The localization of MurG-mGFP and FtsZ-mCherry appears mostly normal in the *mreB*$_{Q26P}$ mutant background. MurG-GFP and FtsZ-mCherry colocalize near midcell during cell elongation (arrow) before the appearance of constriction (arrowhead).

Supplemental References

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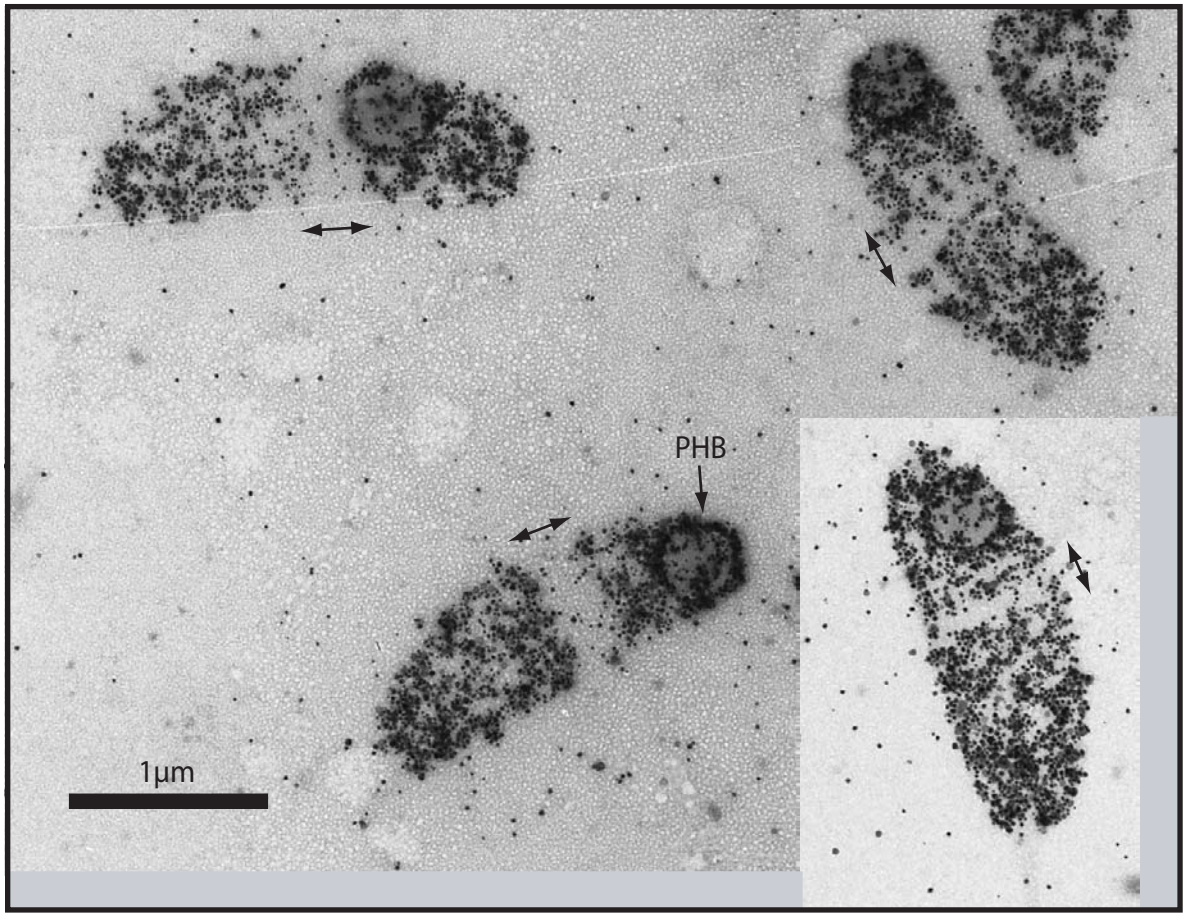
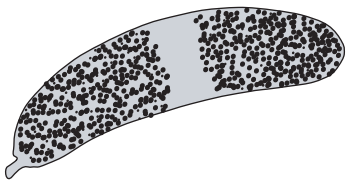
West, L., Yang, D., and Stephens, C. (2002) Use of the Caulobacter crescentus genome sequence to develop a method for systematic genetic mapping. *J Bacteriol* 184: 2155-2166.

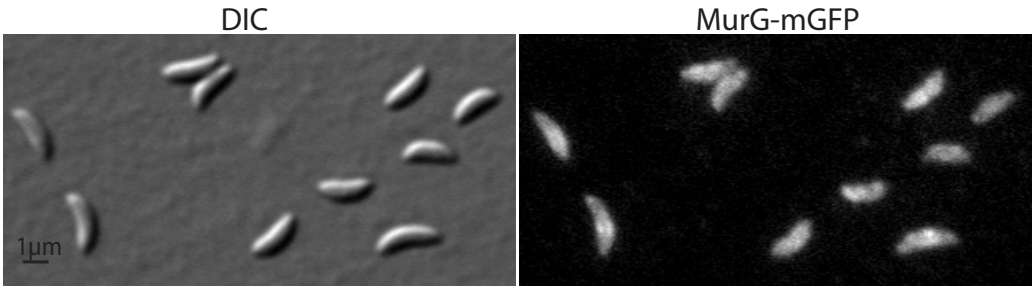
Table S1. Strains and Plasmids

Strains	**Relevant Genotype or Description**	**Reference or Source**
Caulobacter		
CJW27	CB15N (or NA1000) synchronizable variant of CB15	Evinger and Agabian, 1977
CJW1484	CB15N *xylX*::pXGFP4-ClmreB *vanA*::pNJH17	Lam et al., 2006
LS107	CB15N Δ*bla*	West et al., 2002
CJW1550	CB15N *vanA*::pNJH17	This study
CJW1561	CB15N *murG*::pHL32TmurG-mgfp	This study
CJW1572	CB15N *murG*::pHL32TmurG-mgfp *vanA*::pNJH17	This study
CJW1575	CB15N Δ*bla murG*::pHL32murGT-mgfp	This study
CJW1576	CB15N Δ*bla murG*::pHL32murGT-mgfp *vanA*::pNJH17	This study
CJW1607	CB15N *murG*::pBGentmurG-mgfp *ftsZ*::pBJM1	This study
CJW1608	CB15N *murG*::pBGentmurG-mgfp	This study
CJW1682	CB15N $mreB_{Q26P}$ *xylX*::pXGFP4-ClmreBQ26P	This study
CJW1715	CB15N $mreB_{Q26P}$	This study
CJW1997	CB15N $mreB_{Q26P}$ *murG*::pHL32TmurG-mgfp	This study
CJW1998	CB15N $mreB_{Q26P}$ *murG*::pHL32TmurG-mgfp vanA::pNJH17	This study
LS3814	CB15N *xylX*::pXGFP4-ClmreB	Gitai et al., 2004
YB1585	CB15N *ftsZ*::pBJM1	Wang et al., 2001
E. coli		
DH5α	Cloning strain	Invitrogen
S17-1	RP4-2, *Tc*::*Mu, KM*-Tn*7*, for plasmid mobilization	Simon et al., 1983
Plasmids	**Relevant Genotype or Description**	**Reference or Source**
pBGent	GentR integration vector	Matroule et al., 2004
pBGentmurG-mgfp	pBGent carrying 661bp of 3' end of *murG* fused to *mgfp*	This study
pBJM1	pBGST18T carrying the *xylX* promoter and 489bp from the 5' end of *ftsZ*	Wang et al., 2001
pBluescriptKS+	AmpR cloning vector	Stratagene
pHL32	KanR integration vector	This study
pHL32TmurG-mgfp	pHL32 integration vector carrying 658bp of 3' end of *murG* fused to *mgfp*	This study
pKSmEgfp	Cloning vector containing monomeric enhanced gfp	This study
pNJH17	AprR integration vector carrying *ftsZ-mCherry* under the vanillate-inducible promoter	M. Thanbichler, N. Hillson, and L. Shapiro
pXGFP4-ClmreB	Integration vector carrying *gfp-mreB* translational fusion under the xylose-inducible promoter	Gitai et al., 2004
pXGFP4-ClmreBQ26P	Integration vector carrying *gfp-mreBQ26P* translational fusion under the xylose-inducible promoter	This study

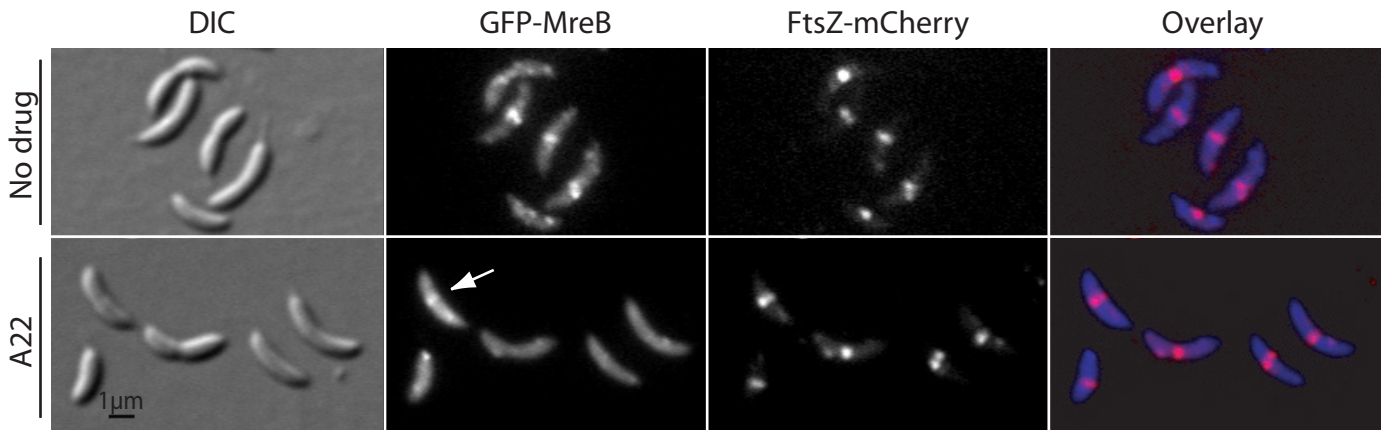




Supplemental Figure S1



Supplemental Figure S2



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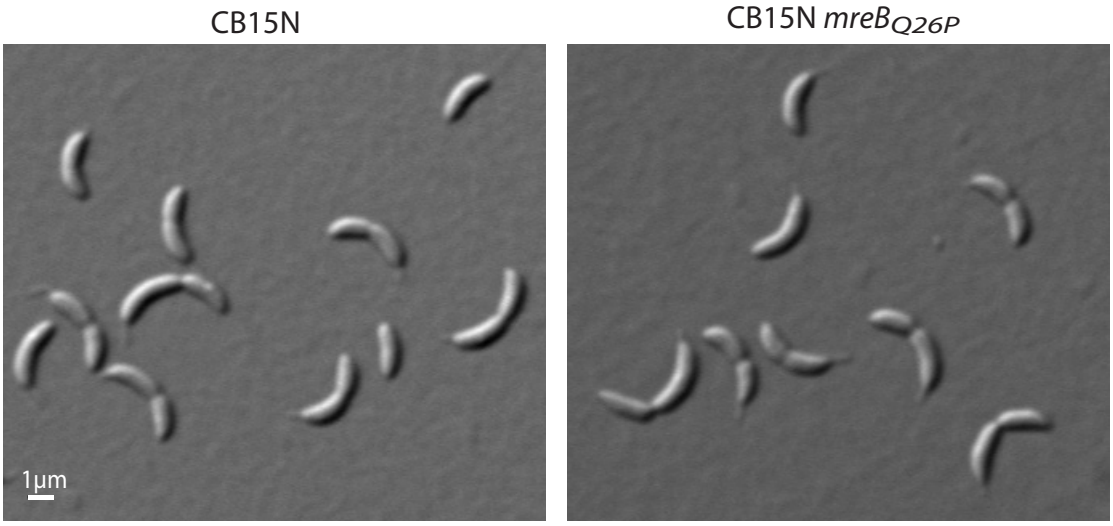
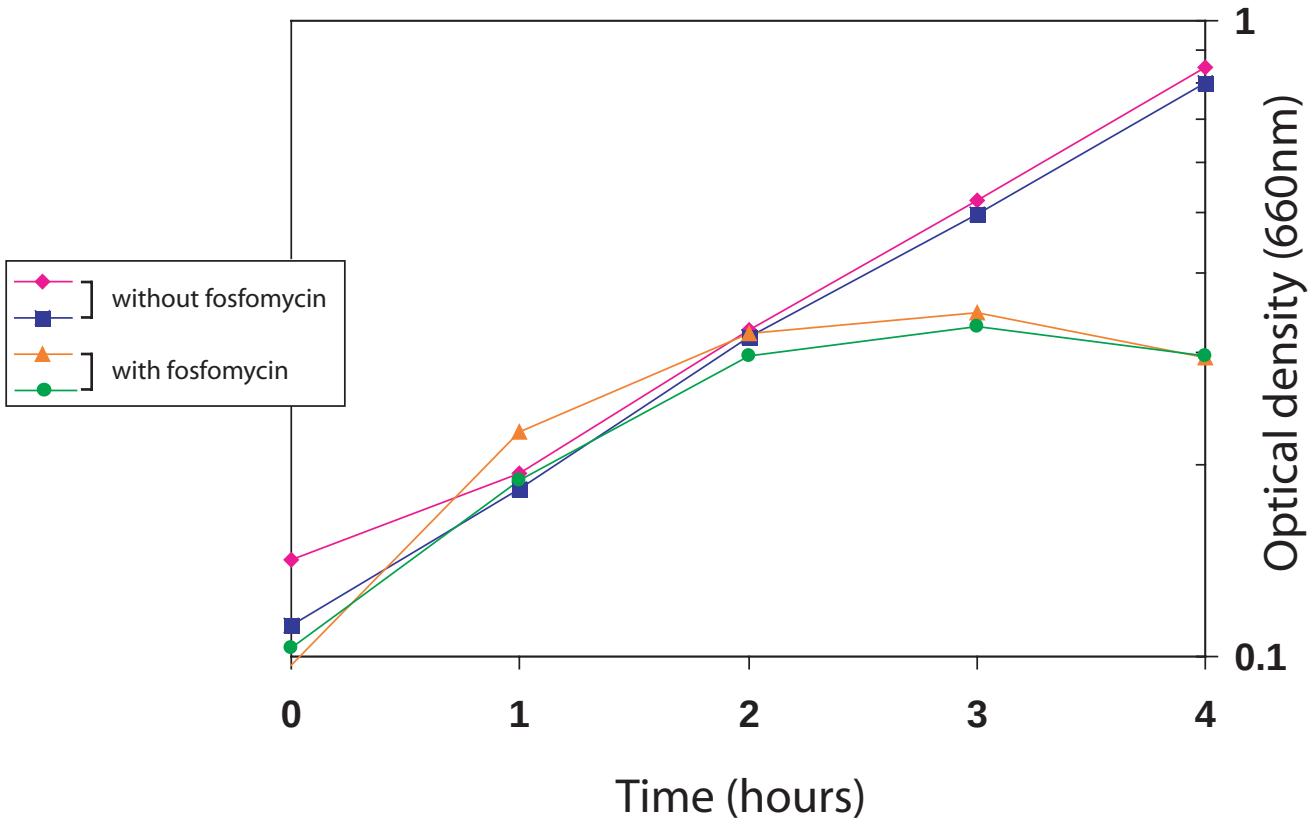


Supplemental Figure S4



Supplemental Figure S5