Supplemental Information:
Table S1, Plasmid and Strain Construction, Supplemental Figures, and Movie Legends

Supplemental Table

Table S1: Strains and plasmids

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### Plasmids

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<td>E. coli arabinose-inducible, Chlor&lt;sup&gt;R&lt;/sup&gt; vector</td>
<td>(Guzman et al. 1995)</td>
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<td>This study</td>
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<td>pBGENT carrying creS-tc under control of Pxy</td>
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<td>pHLS2 carrying creS-gfp under control of Pvan</td>
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<td>pXMCS7</td>
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<td>pXMCS7FtsZ</td>
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<td>This study</td>
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### Construction of plasmids

**pBAD18[Cm]creS-flag:** Cut PxyCRE-creS from pJM21PxyCRE-creS (CJW2533) with EcoRI/SpeI, blunted and ligated into pKS cut with EcoRV, and checked for orientation with 3' end toward HindIII. Cut 3' creS-flag from this plasmid with SphI/HindIII and cloned into pBAD18[Cm]creS (CJW2193) cut with these same enzymes, replacing the 3' end of creS with creS-flag.
**pBGENTP\textit{xylcreS-tc}:** pHL32P\textit{xylcreS-tc} was digested using HindIII and EcoRI enzymes. The fragment containing \textit{P\textit{xylcreS-tc}} was ligated into the pBGENT vector cut with HindIII and EcoRI.

**pBGENTP\textit{vancreS-tc}:** A HindIII fragment from pMT374P\textit{vancreS-tc} containing \textit{P\textit{vancreS-tc}} was ligated into pBGENT vector digested with HindIII.

**pHL32P\textit{vancreS-gfp}:** pKSP\textit{vancreS-gfp} was cut with HindIII and NotI to release a fragment containing \textit{P\textit{vancreS-gfp}} and ligated into the pHL32 vector cut with HindIII and NotI.

**pKSP\textit{vancreS-gfp}:** pMT374P\textit{vancreS-tc} was digested using HindIII and HincII, releasing a fragment containing \textit{P\textit{vancreS-gfp}} and part of the \textit{creS ORF}. pKS\textit{creS-gfp} was digested using NotI and HincII releasing a fragment containing part of the \textit{creS ORF} fused to \textit{gfp}. The two fragments were ligated into pKS vector cut with HindIII and NotI.

**pMT374P\textit{vancreS-tc}:** pBGENTP\textit{xylcreS-tc} was digested using NdeI and EcoRV. A fragment containing \textit{creS-tc} was ligated into pMT374 vector cut with NdeI and PmlI.

**pXGFP4-C1\textit{mreBG}_{165D}:** \textit{mreB}_{G165D} ORF was amplified by colony PCR from a CJW1789 strain (CB15N \textit{mreB}_{G165D}) using primers MREBPNIUUP (GGGGTACCATGTCTCTCCCTTTTT) and MREBXBADOWN (GCTCTAGACTAGGCCAGCGTGATT). The purified PCR product was digested with KpnI and XbaI restriction enzymes and the resulting fragment was ligated into pXGFP4-C1 vector cut with KpnI and XbaI.

**pXMCS7ftsZ:** pBJM1 was cut with HindIII and BamHI to release a fragment containing \textit{P\textit{xyl-ftsZ}} and ligated into pXMCS7 vector cut with HindIII and BamHI.
Construction of Strains

CJW1782: plasmid pBGENTPxylcreS-tc was introduced into CB15N ΔcreS (LS3812) by electroproration.

CJW1788: A φCR30 transducing phage lysate prepared from strain CB15N creS::Tn5 was used to transduce creS::Tn5 (Kan^R) into CJW1789 (CB15N mreB_G165D).

CJW1790: plasmid pXGFP4-C1mreB_G165D was conjugated into CJW1789 (CB15N mreB_G165D).

CJW1791: A φCR30 transducing phage lysate prepared from strain CB15N creS::pBGST18creS-gfp::pBGENTcreS (CJW815) was used to transduce creS::pBGST18creS-gfp::pBGENTcreS (Gent^R) into strain CJW1789 (CB15N mreB_G165D).

CJW1819: A φCR30 transducing phage lysate prepared from strain CB15N ftsZ::pBJM1 (YB1585) was used to transduce ftsZ::pBJM1 (Kan^R) into strains CB15N ΔcreS (LS3812).

CJW2046: A φCR30 transducing phage lysate prepared from strain CB15N creS::pBGST18creS-gfp::pBGENTcreS (CJW815) was used to transduce creS::pBGST18creS-gfp::pBGENTcreS (Gent^R) into strain YB1585 (CB15N ftsZ::pBJM1).

CJW2207: plasmid pBGENTPvancrcreS-tc was introduced into CJW2932 (CB15N ΔcreS Pvan::pHL32PvancrcreS-gfp) by conjugation.

CJW2209: A φCR30 transducing phage lysate prepared from strain CJW 2207 (CB15N ΔcreS Pvan::pBGENTPvancrcreS-tc::pHL32PvancrcreS-gfp) was used to transduce Pvan::pBGENTPvancrcreS-tc::pHL32PvancrcreS-gfp (Gent^R) into strain CJW1819 (CB15N ftsZ::pBJM1 ΔcreS).

CJW2210: A φCR30 transducing phage lysate prepared from strain CJW 2207 (CB15N Pvan::pBGENTPvancrcreS-tc::pHL32PvancrcreS-gfp) was used to transduce
$Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$ (Gent$^R$) into strain CJW1788 (CB15N $mreB_{G165D}$ cre$S::$Tn5)

**CJW2281:** A $\phi$CR30 transducing phage lysate prepared from strain CJW 2207 (CB15N $Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$) was used to transduce

$Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$ (Gent$^R$) into CJW1819 (CB15N $ftsZ::pBJM1$ $\Delta$cre$S$)

**CJW2283:** A $\phi$CR30 transducing phage lysate prepared from strain CJW 2207 (CB15N $Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$) was used to transduce

$Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$ (Gent$^R$) into CJW27 (CB15N $ftsZ::pXMCS7$)

**CJW2883:** plasmid pXMCS7ftsZ was introduced into CJW1788 (CB15N $mreB_{G165D}$ cre$S::$Tn5) by mating.

**CJW2884:** A $\phi$CR30 transducing phage lysate prepared from strain CJW2883 (CB15N $mreB_{G165D}$ cre$S::$Tn5 $ftsZ::pXMCS7ftsZ$) was used to transduce $ftsZ::pXMCS7ftsZ$ (Kan$^R$) into strain CJW2210 (CB15N $mreB_{G165D}$ cre$S::$Tn5 $Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp$)

**CJW3007:** plasmid pBAD18[Cm]creS-flag was electroporated into *E. coli* strain MC1000.

**CJW3035:** plasmid pMR10creS-tc was electroporated into *A. tumefaciens* strain 3101.

**Legends of supplemental figures**

**Figure S1: crescentin-TC is overproduced during de novo assembly**

Western blot of crescentin-TC levels in different strains under different conditions. Lane 1, wild-type CB15N cells; lane 2, CJW1782 cells (CB15N $\Delta$creS $Pxyl::pBGENTPxylcreS$-tc) grown in presence of glucose to repress crescentin-TC synthesis; lane 3, CJW1782 cells grown in presence
of xylose for 1h; lane 4, CJW1782 cells grown in presence of xylose for 2h; lane 5, CJW1782 cells grown in presence of xylose for 3h. Top panel shows anti-crescentin western blot and bottom panel shows anti-MreB western blot to assess sample loading. The amount of crescentin (relative to wild-type, as measured by densitometry) for each lane is given below the blots. Equivalent amounts of total protein were loaded in each lane.

**Figure S2: Crescentin-MreB interaction in *E. coli* and failure of crescentin to interact with the membrane in *A. tumefaciens.*

A) Immunoprecipitation experiment using protein extracts of FB9 cells (leftmost lane, a control strain lacking MreB) and CJW3007 cells (*E. coli* MC1000/pBAD18[Cm]creS-flag) with crescentin-FLAG production repressed or induced for 2.5 h without or with 40 µg/ml cephalexin. The reason for using cephalexin is because production of crescentin in *E. coli* results in a cell chaining phenotype and blockage of cell division with cephalexin prevents this chaining and allows the crescentin structure to continuously localize along the cell periphery in elongated cells (Cabeen et al. 2009). Extracts were immunoprecipitated with anti-FLAG antibody, eluted with FLAG peptide, resolved using SDS-PAGE, and blotted with anti-FLAG (revealing crescentin-FLAG at ~50kDa) and anti-MreB (revealing MreB at ~37kDa). The asterisk marks non-specific anti-MreB antibody cross-reactivity, as this band appears in an extract from *E. coli* cells lacking MreB (FB9; PB103 mreB<->ftr).

B) Images of ReAsH-stained *A. tumefaciens* cells (CJW3035) producing crescentin-TC from the low-copy plasmid pMR10.
Charbon_FigS1

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**A**

**B**

![Image](Charbon_FigS2)


Movie Legends

**Movie S1: Time-lapse sequence showing the crescentin structure sliding away from the site of division.**

CJW815 cells (CB15N creS::pBGST18creS-gfp::pBGENTcreS) expressing creS and creS-gfp from the native creS promoter were placed on an agarose-padded slide and imaged over time by fluorescence and DIC microscopy. Red marks show the GFP-labeled crescentin structure sliding away from the division site. Left, crescentin-GFP; middle, DIC; and right, overlay. Scale bar, 1 µm.

**Movie S2: Example of crescentin assembling at opposite cell sides.**

CJW2046 cells (CB15N creS::pBGST18creS-gfp::pBGENTcreS  
ftsZ::pBJM1) in which ftsZ is under xylose-inducible expression were first grown in absence of xylose for 2h to deplete FtsZ and cause cell filamentation. The cells were then deposited on an agarose-padded-slide containing xylose to replete FtsZ. This was immediately followed by time-lapse microscopy. This sequence shows that a new GFP-labeled crescentin structure can form at the side opposite to the existing crescentin structure, used here as a spatial reference. Left, crescentin-GFP and right, DIC overlay. Scale bar, 2 µm.

**Movie S3: Example of crescentin assembling at the same cell side.**

CJW2046 cells (CB15N creS::pBGST18creS-gfp::pBGENTcreS  
ftsZ::pBJM1) in which ftsZ is under xylose-inducible expression were first grown in absence of xylose for 2h 30min to deplete FtsZ and block division. Cells were deposited on an agarose pad containing xylose to replete FtsZ. The resulting filamentous cells were deposited on an agarose padded-slide containing
xylose to replete FtsZ. This was immediately followed by time-lapse microscopy. This sequence shows that a new GFP-labeled crescentin structure can form at the same cell side as the existing crescentin structure, used here as a spatial reference. Left, crescentin-GFP and right, overlay with DIC image. Scale bar, 2 µm.

**Movie S4: Time-lapse microscopy showing crescentin-GFP structure released from the cell side by A22 treatment.**

CJW2046 cells (CB15N creS::pBGST18creS-gfp::pBGENTcreS fisZ::pBJM1) producing a GFP-labeled crescentin structure and carrying fisZ under xylose-inducible expression were first grown in absence of xylose for 2h to deplete FtsZ and block division. The resulting filamentous cells were deposited on an agarose pad containing xylose (to replete FtsZ) and 25µM A22 (to disrupt MreB localization). This was immediately followed by time-lapse fluorescence microscopy of the GFP-labeled crescentin structure. The sequence shows that the crescentin structure detaches from the cell envelope over time and collapses into a helical structure. Scale bar, 2 µm.

**Movie S5: Time-lapse sequence showing the localization of wild-type MreB.**

CJW1225 cells (CB15N Pxyl::pXGFP4-C1mreB) producing GFP-MreB were placed on an agarose-padded slide and were imaged by time-lapse DIC and fluorescence microscopy to visualize GFP-MreB during growth. Left, GFP-MreB signal; right, DIC image. Scale bar, 2 µm.
**Movie S6: Time-lapse sequence showing the patchy localization of the MreB_{G165D} mutant.**

CJW1790 cells (CB15N mreB_{G165D} PxyI::pXGFP4-C1mreB_{G165D}) producing GFP-MreB_{G165D} (in addition to untagged MreB_{G165D}) were placed on an agarose-padded slide and were imaged by time-lapse DIC and fluorescence microscopy to visualize GFP-MreB_{G165D} during growth. The movie shows that MreB_{G165D} displays a patchy localization and, unlike wild-type MreB (Figge et al. 2004; Gitai et al. 2004), fails to form a stable ring near midcell. Left, GFP-MreB_{G165D} signal; right, DIC image. Scale bar, 2 µm.

**Movie S7: Time-lapse sequence showing a detached crescentin structure moving in a straight-shaped mreB_{G165D} mutant cell.**

CJW1791 cells (CB15N mreB_{G165D} creS::pBST18creS-gfp::pBGENTcreS) producing the MreB_{G165D} mutant were deposited on an agarose pad and immediately imaged by time-lapse DIC and fluorescence microscopy. This sequence shows an example of a straight-shaped cell in which the GFP-labeled crescentin structure is detached and moves within the cell over time. Left, crescentin-GFP signal; right, overlay with DIC image. Scale bar, 2 µm.

**Movie S8: Time-lapse sequence showing crescentin motion in Agrobacterium tumefaciens.**

*Agrobacterium tumefaciens* 3101 cells producing crescentin-TC were stained with FlAsH and deposited on an agarose pad. Crescentin-TC was imaged by time-lapse fluorescence microscopy. Scale bar, 2 µm.
Movie S9: Time-lapse sequence showing that the distortion of the crescentin structure in the \textit{mreBG165D} mutant background occurs during cell growth and not during crescentin assembly.

CJW2883 cells (CB15N \textit{mreBG165D creS::Tn5 Pvan::pBGENTPvancreS-tc::pHL32PvancreS-gfp ftsZ::pXMCS7ftsZ}) carrying the \textit{mreBG165D} mutation were grown in absence of xylose for 1h in liquid cultures to deplete FtsZ and to generate elongated cells. The cells were then placed on an agarose-padded slide containing vanillic acid (0.5mM) to induce the synthesis and co-assembly of crescentin-GFP and crescentin-TC into a hybrid structure. This was immediately followed by time-lapse microscopy. The sequence shows that as in the wild-type \textit{mreB} background, crescentin assembly occurs along a straight line parallel to the long cell axis, resulting in a straight linear structure. Distortion of the crescentin structure occurs during growth of the \textit{mreBG165D} cells, inducing cell twisting. Left, crescentin-GFP signal; right, overlay with DIC image. Scale bar, 2 µm.

Movie S10: Time-lapse sequence showing that filamentous growth on an agarose pad causes \textit{mreBG165D} mutant cells, but not \textit{mreB} wild-type cells, to twist.

CJW2882 cells (CB15N \textit{mreBG165D creS::Tn5 ftsZ::pXMCS7ftsZ}) and CJW1819 cells (CB15N \textit{ftsZ::pBJM1 ∆creS}) were placed on an agarose pad lacking xylose to deplete FtsZ and imaged by time-lapse DIC microscopy during filamentous growth. The two strains are isogenic with respect to \textit{ftsZ} being under the control of the xylose inducible promoter and the crescentin-encoding gene being inactivated, except that CJW2882 (left panel) produces the MreB\textit{G165D} mutant while CJW1819 produces wild-type MreB. The time-lapse sequences show that the \textit{mreBG165D} mutation causes the cells to twist when grown on a solid surface. Scale bar, 2 µm.
Movie S11: Cartoon model showing that crescentin structure provides an internal resistance to the torsion in mreB<sub>G165D</sub> cells.

The dots in the cartoons represent points of reference on the cell surface, and the crescentin structure is indicated in red.

Movie S12: Time-lapse sequence showing detachment and motion of the crescentin structure in an mreB<sub>G165D</sub> mutant cell in the absence of growth.

After treatment with chloramphenicol (20 µg/ml) in a liquid culture, CJW1791 cells (CB15N mreB<sub>G165D</sub> creS::pBGST18creS-gfp::pBGENTcreS) were deposited on an agarose pad containing chloramphenicol (20 µg/ml) to maintain cell growth arrest. This was followed by time-lapse microscopy. The sequence shows the GFP-labeled structure detaching from the membrane and moving within an mreB<sub>G165D</sub> mutant cell. Left, crescentin-GFP signal; right, overlay with DIC image. Scale bar, 1 µm.

References


