Supplemental Data

A Landmark Protein Essential for Establishing and Perpetuating the Polarity of a Bacterial Cell

Hubert Lam, Whitman B. Schofield, and Christine Jacobs-Wagner

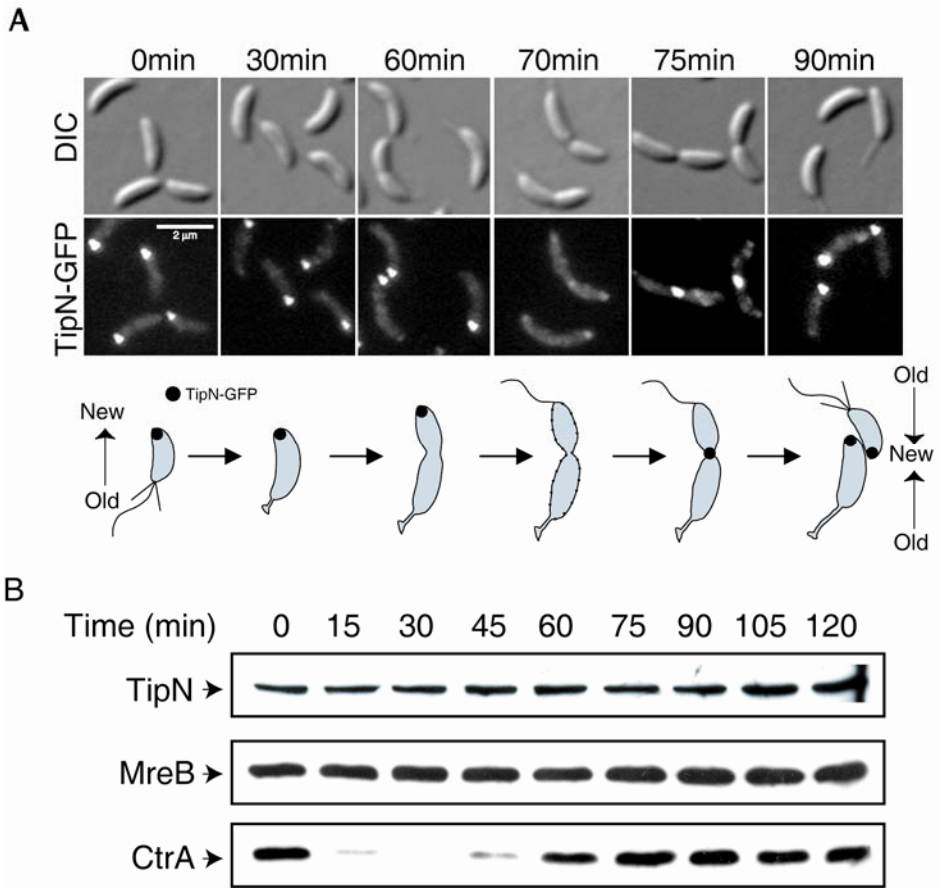

Figure S1. TipN Localization during the Cell Cycle

(A) The localization of TipN-GFP during the cell cycle was monitored by time-course light microscopy starting with a synchronized population of swarmer cells (CJW1406). A schematic representation of TipN-GFP localization is depicted below.

(B) Western blot analysis of relative TipN protein levels during the cell cycle starting with a synchronous population of wild-type CB15N swarmer cells. MreB and CtrA proteins are shown as loading control and quality control for the synchrony, respectively.

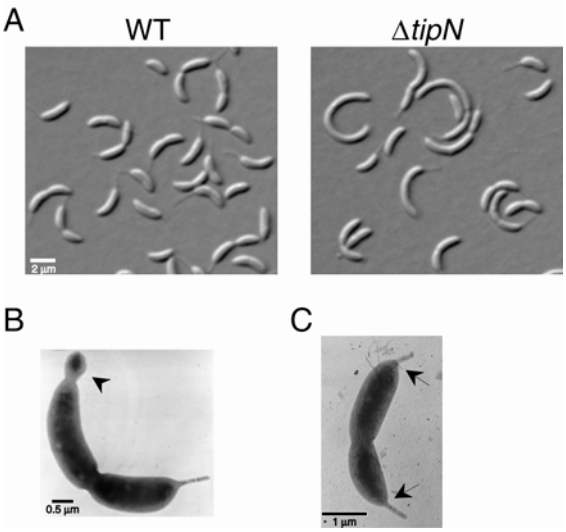

Figure S2. Aberrant Cell Morphologies in the Δ*tipN* Population

(A) DIC images showing the normal cell morphology of a population of wild-type CB15N cells and the occurrence of cell elongation in a population of Δ*tipN* cells.

(B) Electron micrograph illustrating the formation of a minicell (shown by arrowhead) in the Δ*tipN* mutant.

(C) Electron micrograph showing a Δ*tipN* cell with bipolar stalks.

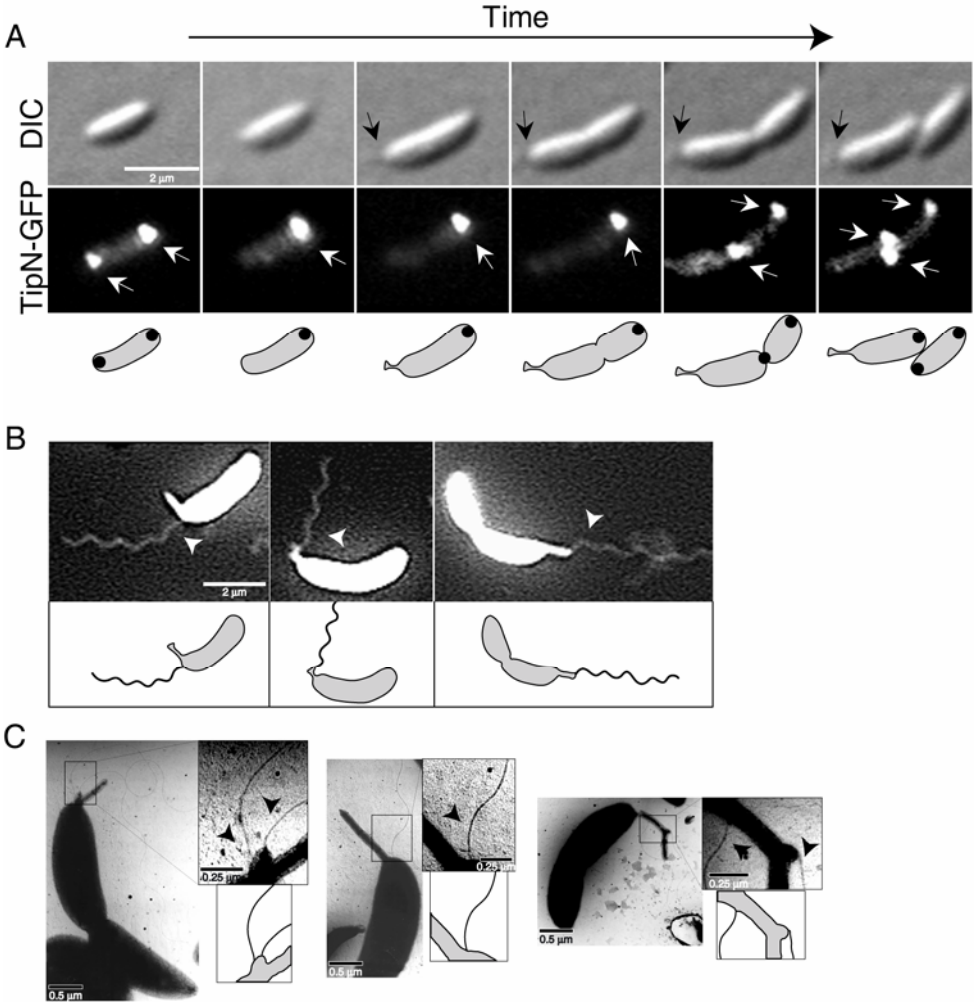

Figure S3. Overexpression of *tipN* from a Low-Copy Plasmid Perturbs the Cell Cycle-Dependent Localization of TipN and Affects the Placement of the Flagellum

(A) Time-lapse microscopy of wild-type cells expressing *tipN-gfp* from a low-copy plasmid (CJW1412) shows that plasmid-encoded TipN-GFP (white arrow) is abnormally present at both poles of a newborn swarmer cell. The aberrant TipN-GFP focus at the old pole disappears during the swarmer cell stage, restoring the normal localization pattern of TipN-GFP for most of the remainder of the cell cycle. However, in the late predivisional

cell when the TipN-GFP focus normally disappears from the pole to reappear at the division site (see Figure 2B), plasmid-encoded TipN-GFP remains at the pole while still accumulating at the division site. This results in bipolar localization of TipN-GFP in the swarmer progeny. The black arrow shows the stalk and identifies the old pole of the cell.

(B) DAPI staining shows abnormal positioning of the flagellum (arrowhead) in cells expressing *tipN* from a low-copy plasmid (CJW1411). A schematic representation is shown below. Sixty-seven percent of predivisional cells with plasmid-encoded TipN (n = 137) had a flagellum emerging from the stalked (old) pole or from the stalk itself.

(C) Electron micrographs showing flagella protruding from the stalk of cells expressing *tipN* from a low-copy plasmid (CJW1411). The boxed regions on the right are shown at a higher magnification with a schematic shown below. Flagella are indicated by arrowheads.

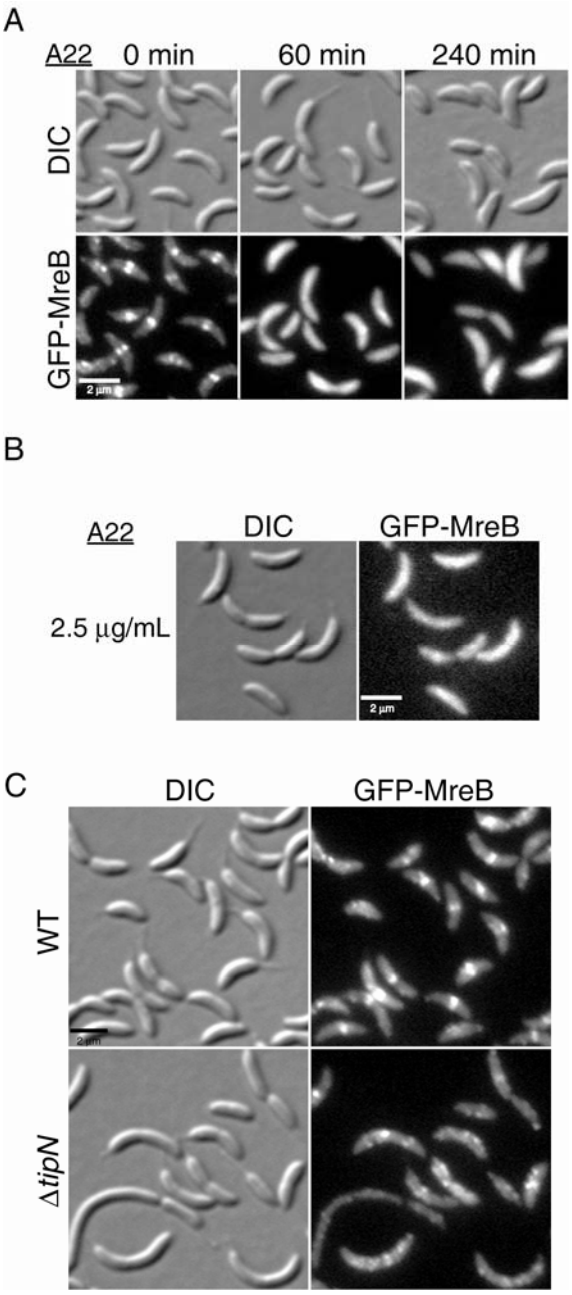

Figure S4. TipN Is Required for the Proper Localization of MreB

(A) Two hours after the addition of 0.3% xylose to induce *gfp-mreB* expression, PYE cultures of LS3814 cells were treated with 10 µg/mL of A22 for up to 240 min. As previously described (Gitai et al., 2005), A22 disrupted GFP-MreB localization.

(B) Two hours after the addition of 0.3% xylose, LS3814 cells grown in liquid $M2G^{+}$ were spotted on an $M2G^{+}$ agarose-padded slide containing 2.5 μg/mL of A22. Within 5 min, exposure to 2.5 μg/mL of A22 caused a disruption in GFP-MreB localization similar to the one caused by 10 μg/mL of A22 under otherwise similar conditions.

(C) The localization pattern of GFP-MreB is abnormal in a Δ*tipN* cell population. GFP-MreB localization was examined in asynchronous populations of LS3814 cells (wild-type) and of CJW1422 cells (Δ*tipN*). In the wild-type background, GFP-MreB forms a band-like structure at the division plane of dividing cells whereas, in the Δ*tipN* background, the GFP-MreB band-like structure was largely disrupted.