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A flexible C-terminal linker is required for proper FtsZ assembly *in vitro* and cytokinetic ring formation *in vivo*

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SUMMARY

Assembly of the cytoskeletal protein FtsZ into a ring-like structure is required for bacterial cell division. Structurally, FtsZ consists of four domains: the globular N-terminal core, a flexible linker, 8–9 conserved residues implicated in interactions with modulatory proteins, and a highly variable set of 4–10 residues at its very C terminus. Largely ignored and distinguished by lack of primary sequence conservation, the linker is presumed to be intrinsically disordered. Here we employ genetics, biochemistry and cytology to dissect the role of the linker in FtsZ function. Data from chimeric FtsZs substituting the native linker with sequences from unrelated FtsZs as well as a helical sequence from human beta-catenin indicate that while variations in the primary sequence are well tolerated, an intrinsically disordered linker is essential for *B. subtilis* FtsZ assembly. Linker lengths ranging from 25–100 residues supported FtsZ assembly, but replacing the *B. subtilis* FtsZ linker with a 249-residue linker from *A. tumefaciens* FtsZ interfered with cell division. Overall, our results support a model in which the linker acts as a flexible tether allowing FtsZ to associate with the membrane through a conserved C-terminal domain while simultaneously interacting with itself and modulatory proteins in the cytoplasm.

INTRODUCTION

The prokaryotic cytoskeletal protein FtsZ plays a central role in bacterial cell division. In rod-shaped bacteria such as *Bacillus subtilis* and *Escherichia coli*, FtsZ assembles into a ring shaped structure (Z ring) at midcell that establishes the location of the future division site and serves as a platform for assembly of the division machinery. As cell division progresses, the Z ring constricts to begin separation of the newly forming daughter cells. The Z ring is required for recruitment of downstream cell division proteins, including those required for cross wall synthesis, to the nascent division site (for reviews see: Adams and Errington, 2009; Kirkpatrick and Viollier, 2011; Lutkenhaus et al., 2012).

High-resolution microscopy suggests that the Z ring consists primarily of heterogeneously distributed short filaments (~100 nm in length) held together via lateral interactions (Fu et al., 2010; Strauss et al., 2012). FtsZ protofilaments are believed to associate with the membrane through interactions with FtsA and other, less widely conserved, cell division proteins. In addition, several studies have shown that FtsZ filaments targeted to the membrane in *in vitro* liposomes are able to generate visible distortions where FtsZ is bound and suggest the force being applied is solely through FtsZ (Osawa et al., 2008; Osawa et al., 2009). Computational modeling suggests that GTP-hydrolysis-dependent bending of FtsZ filaments generates sufficient force to drive inward cell-wall growth during division (Hsin et al., 2012).

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In vitro, FtsZ assembly into single stranded “protofilaments” is stimulated by GTP (Romberg and Levin, 2003; Erickson et al., 2010). FtsZ, a tubulin homolog, binds to GTP as a monomer. Polymerization leads to the formation of active sites for GTP hydrolysis at the interface between FtsZ subunits. FtsZ protofilaments are able to associate laterally to form filament bundles, spirals, tubes, and sheets depending on the buffer conditions. Lateral interaction potential varies between FtsZs from different species and is dependent in part on the charge of the variable residues at the very C-terminus of the protein (Mukherjee and Lutkenhaus, 1999; Gueiros-Filho and Losick, 2002; Popp et al., 2009; Gündoğdu et al., 2011; Buske and Levin, 2012).

The FtsZ monomer is divided into 5 domains: an unstructured N-terminal peptide, a highly conserved globular core, an unstructured C-terminal linker (CTL), a conserved set of ~11 residues referred to here as the C-terminal constant region (CTC), and a small, highly variable group of residues at the extreme C-terminus of FtsZ termed the C-terminal variable region (CTV) (Vaughan *et al.*, 2004; Erickson et al., 2010; Buske and Levin, 2012). For simplicity, we treat the N-terminal peptide and core as a single unit. The core contains residues required for GTP binding and hydrolysis as well as residues involved in longitudinal interactions between subunits (Lu et al., 2001; Redick et al., 2005). *In vitro*, the core has also been shown to be sufficient for filament formation and has also been implicated in protofilament bundling (Wang *et al.*, 1997). The very C-terminus of FtsZ, a 15 residue region in *E. coli* FtsZ and a 17 residue region in *B. subtilis* FtsZ that includes both the CTC and the CTV, has been implicated in interactions between FtsZ and a host of modulatory proteins. Such proteins include FtsA, ZipA and MinC in *E. coli* and EzrA and SepF in *B. subtilis* (Ma and Margolin, 1999; Mosyak et al., 2000; Yan et al., 2000; Haney et al., 2001; Singh et al., 2007; Shen and Lutkenhaus, 2009; Król et al., 2012; Szwedziak et al., 2012). The CTV also appears to be a key determinant of lateral interaction potential. We have designated the region containing the CTC and CTV (residues 366–382 of *B. subtilis* FtsZ) the “grappling hook peptide” (GHP) to reflect its role in mediating interactions between modulatory proteins in both the cytoplasm and the plasma membrane.

While recent work has begun to clarify the role of the core, the CTC and the CTV in FtsZ assembly and Z-ring integrity, the function of the unstructured C-terminal linker has remained largely mysterious. The CTL spans the gap between the core and the CTC, suggesting it may be required to maintain a flexible connection between FtsZ protofilaments and modulatory proteins bound to the GHP of FtsZ subunits. Phylogenetic analysis of FtsZs across domains of life showed that there is little sequence conservation of the CTL even amongst bacteria from the same taxonomic phylum.

Intriguingly, while the length of the CTL can range 2 to 330 residues (Vaughan et al., 2004), it is ~50–100 residues long in the majority of FtsZs sequenced that contain all structural domains. An exception to this rule is FtsZs from the Alpha-Proteobacteria, in which linkers are significantly longer, between 119 amino acids in *Rickettsia prowazekii* and 251 amino acids in *Agrobacterium tumefaciens*. Amongst the model organisms for bacterial cell division, the CTL of *B. subtilis* and *E. coli* is ~50 residues, while that of *Caulobacter crescentus*, a member of the Alpha-Proteobacteria, is 176. Notably, the CTL is irresolvable on crystal structures of bacterial FtsZ (Leung et al., 2004; Oliva et al., 2007; Haydon et al., 2008; Läppchen et al., 2008; Raymond et al., 2009; Matsui et al., 2012), and therefore has been presumed to be an intrinsically disordered peptide (IDP) (Erickson et al., 2010). Previous work suggests the CTL is flexible with a contour length of 17 nm, and an average end-to-end distance of 5.2 nm for the relaxed peptide (Ohashi et al., 2007).

Here we employ genetics, cell biology, and biochemistry to clarify the role of the FtsZ CTL. Our data indicate the CTL is required for protofilament assembly *in vitro* and formation of a

curved ring *in vivo*, suggesting a role for the CTL in the geometry of longitudinal interactions between individual subunits. While changes in linker length were generally tolerated *in vitro*, increases in length greater than 100 residues led to disruptions in the frequency and position of FtsZ assembly *in vivo*. Changes in the linker's primary sequence had little impact on assembly *in vivo* or *in vitro* when retained as an IDP, whereas replacing the linker with an inflexible domain from human beta-catenin was not tolerated. Notably, our findings in *B. subtilis* closely mirror those from the Erickson laboratory working in *E. coli* (Gardner et al., in submission), who similarly determined CTL sequence to be unimportant while length being crucial. Taken together our findings have significant implications for our understanding of FtsZ assembly dynamics at the molecular level, as well as the role of various regions of FtsZ in assembly of a productive cytokinetic ring.

RESULTS

The FtsZ linker is required for normal function in *B. subtilis*

Deletion analysis indicated that the linker region of FtsZ was essential for efficient assembly *in vitro* and ring formation *in vivo*. As a first step towards assessing the role of the linker in FtsZ activity, we generated an FtsZ mutant in which the linker was deleted, leaving only the core followed immediately by the CTC and CTV domains (FtsZ Δ CTL50) (Fig. 1). Transmission electron microscopy (TEM) of wild-type FtsZ assembled in our standard FtsZ polymerization buffer (50 mM MES, pH 6.5, 50 mM KCl, 2.5mM MgCl₂, 1mM EGTA, 1mM GTP) indicated the present of filament bundles and sheets (Fig. 2A and B), consistent with our previous findings (Buske and Levin, 2012). In contrast, FtsZ Δ CTL50 appeared impaired for filament formation and filament bundling. By TEM we observed primarily short oligomers (<25 nm) interspersed amongst single- or double-stranded filaments of FtsZ Δ CTL50 5 minutes after initiation of polymerization by GTP (Fig. 2C). 15 minutes after addition of GTP, FtsZ Δ CTL50 filaments became more pronounced and longer, suggesting the rate of filament formation was delayed compared to wild type, though many small oligomers could still be observed also suggesting gross filament formation was diminished (Fig. 2D).

FtsZ Δ CTL50 assembly was GTP-dependent, as we observed a modest increase in 90° light scattering signal following the addition of 1 mM GTP (Fig. 2E). Here, as previously, we use light scattering to measure increase in polymer mass and FtsZ bundling (Mukherjee and Lutkenhaus, 1999; Buske and Levin, 2012), and define assembly as a global term to include head-to-tail polymerization into protofilaments and lateral interactions. Consistent with its limited assembly in TEM micrographs, the FtsZ Δ CTL50 light scattering signal was 37-fold lower than that of wild-type FtsZ in the presence of GTP. Similarly, the critical concentration (C_c) of FtsZ Δ CTL50 was 1.08 μ M compared to 0.68 μ M for wild type, suggesting a reduction in cooperative assembly. We also observed that time needed to reach a peak light scattering value was on the order of minutes whereas wild-type FtsZ was on seconds. The delay in filament formation suggested that head-to-tail assembly of filaments was impaired without the FtsZ linker. The GTPase activity of FtsZ Δ CTL50 was 1.48 GTP/FtsZ/min, significantly lower than the 3.82 GTP/FtsZ/min measured for wild type (Table 1). Notably, CD spectra indicated that FtsZ Δ CTL50 folded normally despite the loss of the linker, eliminating a trivial explanation for its defect in polymerization (Fig. S1).

In vivo, FtsZ Δ CTL50 was unable to support either FtsZ assembly or division. Taking advantage of a strain that allowed for depletion of wild type FtsZ, we expressed FtsZ Δ CTL50 as the sole copy of FtsZ in *B. subtilis* cells under the control of an isopropyl 1-thio- β -D-galactopyranoside (IPTG) - inducible promoter at the *amyE* locus (see "Experimental Procedures"). Expression FtsZ Δ CTL50 in the absence of wild-type FtsZ led to the rapid demise of cells: cell density as measured by absorbance at 600 nm (A_{600})

declined ~2-fold four hours after reaching a peak density of 0.4 following induction of FtsZ Δ CTL50 and depletion of wild-type FtsZ (Fig. 3A). It should be noted we were only able to observe growth when cells were grown in inducer at a starting A_{600} twice that of all other constructs used in the study, suggesting that expression of FtsZ Δ CTL50 had an immediate and deleterious impact on cell viability.

Immunofluorescence microscopy of FtsZ Δ CTL50 cells 2.5 hours post induction in the absence of wild-type FtsZ supported the idea that the linker was required for proper FtsZ assembly and the formation of an intact cytokinetic ring. Cells were filamentous ($> 80 \mu\text{m}$, the length of our microscopy field) and FtsZ was mostly in punctate dots (88% of cells) throughout the cytoplasm in contrast to the Z rings of cells expressing wild-type FtsZ (Fig. 2F, arrow). Strikingly, in 30% of cells FtsZ Δ CTL50 formed long and relatively straight filaments that extended longitudinally through the cell similar to what we observed *in vitro* and suggested defects in the geometry of FtsZ Δ CTL50 assembly. Co-expression of FtsZ Δ CTL50 with wild-type FtsZ resulted in a dominant lethal phenotype and loss of cell viability when assessed by dilution plating assay (Fig. S2).

Together, these findings suggested the linker played a critical role in FtsZ filament assembly counter to previous reports indicating that *E. coli* FtsZ mutants lacking the entire CTR readily assemble *in vitro* (Wang et al., 1997; Yu and Margolin, 1997). In light of this discrepancy, we examined the ability of *B. subtilis* and *E. coli* FtsZ core constructs lacking the entire C-terminal region (CTR; *B. subtilis* residues 316–382) to assemble *in vitro* under our standard conditions (Fig. S4). Somewhat surprisingly, both the *B. subtilis* and *E. coli* core domains were defective in both protofilament formation and lateral interactions, counter to the earlier studies (Wang et al., 1997; Yu and Margolin, 1997; Singh et al., 2007). A potential explanation for this discrepancy may be buffer conditions: while earlier studies employed 10 mM MgCl_2 or CaCl_2 , MgCl_2 was at 2.5 mM in our standard conditions. Divalent cations have been shown to promote the formation of stabilizing lateral interactions between protofilaments most likely through charge shielding of the core (Mukherjee and Lutkenhaus, 1999).

FtsZ function is independent of its linker sequence

We next determined if the linear sequence of the FtsZ linker was critical for function. The linker exhibited little or no conservation between even closely related bacterial species, suggesting that its presence and potentially its flexible nature, but not its precise sequence, were critical for function. To explore this possibility we generated an FtsZ mutant in which the sequence of linker was randomly scrambled (FtsZ CTLScr), keeping the same amino acids as wild-type *B. subtilis* FtsZ but changing their order. In addition we swapped the 50-residue *B. subtilis* linker with the equivalent 50 residues from the *E. coli* linker to yield a linker of 54 residues (CTLE) and with the first 46 amino acids of the 251 residue long linker from *Agrobacterium tumefaciens* FtsZ to yield a linker of 50 residues (CTLA50) (Fig. 1).

Our data suggested that linker sequence had a small impact on FtsZ assembly *in vitro*. FtsZ CTLScr showed no evidence of compromised protofilament assembly and readily formed single stranded protofilaments and small bundles 3–5 filaments thick as seen by TEM (Fig. 4B). In agreement with TEM data, the 90° light scattering assays of FtsZ CTLScr indicated robust, GTP-dependent assembly to ~60% that of FtsZ WT (Fig. 4E). This level of assembly was consistent with the smaller-than-wild-type bundles we observed by EM but significantly (95–98%) higher than that of FtsZ mutants that were incapable of forming lateral interactions (Buske and Levin, 2012). Evidence of bundling was also seen by a lowered GTPase rate of 2.68 GTP/FtsZ/min. The C_c of CTLScr was $0.88 \mu\text{M}$, similar to the wild-type value of $0.68 \mu\text{M}$ (Table 1).

When FtsZ CTLScr was expressed as the sole copy in *B. subtilis*, cells were able to divide normally and Z rings localized to midcell as wild type (Fig. 4F). Here, and in all cells types that displayed the ability to form Z rings, we employed measurement of the length to ring (L/R) ratio as proxy for cell size. As some cell types became filamentous, using interseptal distance underestimated the average length of cells in a given population due to an inability to distinguish between partial and completed septa in these longer cells. FtsZ CTLScr-expressing cells had an average 5.48 $\mu\text{m}/\text{Z ring}$ compared to 5.82 $\mu\text{m}/\text{Z ring}$ for wild type (Fig. 3B).

Replacing the native linker with heterologous sequences had modest impacts on FtsZ assembly, particularly lateral interactions between single stranded protofilaments. Both FtsZ CTLE and FtsZ CTLA50 readily assembled in the presence of GTP (Fig. 4C and D); however, the structures appeared to be primarily single stranded by TEM. Similarly, the light scattering signals for CTLE and CTLA50 were ~ 62 fold and 37-fold lower than FtsZ WT, respectively, consistent with a reduction in lateral interactions and the formation of higher order structures. Finally, the GTPase activity of CTLE and CTLA50 was also elevated relative to wild-type FtsZ, 5.04 GTP/FtsZ/min and 5.51 GTP/FtsZ/min for CTLE and CTLA50 (Table 1), a phenotype that is also consistent with a reduction in lateral interaction potential (Buske and Levin, 2012). The apparent C_c was near wild type for both chimeras.

In vivo, FtsZ CTLE and FtsZ CTLA50 all appeared to support normal division, despite their reduction in lateral interaction potential *in vitro*. Expression of FtsZ CTLE and FtsZ CTLA50 in the absence of wild-type FtsZ fully supported normal division and FtsZ localization (Fig. 4F) with near wild-type L/R ratios (Fig. 3B). Cells expressing these FtsZ chimeras displayed growth rates near that of wild type (Fig. 3A). Some irregular cell wall staining seen in these mutants we attributed to lysozyme treatment for IFM, as we observed similar staining in wild-type cells (Fig. S3A). The ability of these mutants to support normal division despite reduced lateral interaction potential was counter to our previous findings that ability to form lateral FtsZ interactions *in vitro* affected cell division *in vivo* with a CTV mutant (Buske and Levin, 2012). This finding will be addressed in the discussion.

Linker length is an important determinant for normal FtsZ function

To determine the role of linker length in FtsZ function we generated FtsZ chimeras of different length and examined the impact of these changes on FtsZ assembly *in vitro* and *in vivo*. Reducing the length of the native FtsZ linker by deleting the last 25 residues (FtsZ ΔCTL25), significantly altered FtsZ assembly *in vitro*. Purified FtsZ ΔCTL25 formed short, irregular protofilaments with little evidence of lateral interactions (Fig. 5B). Consistent with reduced assembly, FtsZ ΔCTL25 assembly peaked at a level ~ 79 fold lower than wild-type FtsZ in 90° light scattering assays (Fig. 5E and F). FtsZ ΔCTL25 hydrolyzed GTP at a rate of 4.66 GTP/FtsZ/min, higher than wild-type FtsZ and FtsZ CTLScr, presumably due to a reduction in stabilizing lateral interactions. Interestingly, the C_c for FtsZ ΔCTL25 was 0.30 μM , about half that for wild type (Table 1).

Surprisingly, despite significantly altering FtsZ assembly dynamics *in vitro*, reducing the length of the linker by 50% had little effect on FtsZ ring formation or division *in vivo*. Expression of FtsZ ΔCTL25 in *B. subtilis* fully supported Z-ring assembly and cell division. Cells were able to grow at a rate near that of wild type (Fig. 3A), and FtsZ ΔCTL25 assembled into apparently normal FtsZ rings that were appropriately localized to midcell (Fig. 5G). The L/R ratio of FtsZ ΔCTL25 -expressing cells was also near wild type at 5.62 $\mu\text{m}/\text{Z ring}$ (Fig. 3B).

In a complementary set of experiments we generated FtsZ chimeras with longer linkers by swapping the native linker sequence with either the entire linker (FtsZ CTLA249) from *A. tumefaciens* (FtsZ CTLA249) or the first 96 residues (FtsZ CTLA100) to make a total linker length of 100 residues (FtsZ CTLA100) (Fig. 1). Both FtsZ CTLA249 and FtsZ CTLA100 assemble into single-stranded protofilaments in the presence of GTP as observed by TEM (Fig. 5C and D). However, there were few, if any, bundles apparent in any field of view, suggesting a reduction in lateral interactions. Consistent with this view FtsZ CTLA249 and FtsZ CTLA100 exhibited ~43 and ~33 fold decreases in signal relative to wild-type FtsZ in 90° light scattering assays (Fig. 5E and F). The apparent critical concentrations for both chimeras were near wild type; FtsZ CTLA249 had a C_c of 0.80 μM and FtsZ CTLA100 a C_c of 0.78 μM . GTPase activities were 4.40 and 5.09 GTP/FtsZ/min for FtsZ CTLA249 and FtsZ CTLA100, respectively (Table 1).

Despite the near wild-type *in vitro* assembly dynamics of the two chimeras, only FtsZ CTLA100 was able to support division *in vivo*. Cells expressing FtsZ CTLA100 were essentially wild type with regard to growth rate, FtsZ ring assembly, and localization with a wild-type L/R ratio of 5.44 $\mu\text{m}/\text{Z}$ ring (Fig. 3). In contrast, cells expressing FtsZ CTLA249 as their only copy of FtsZ were unable to divide and formed extremely long filaments with what appeared to be partial invaginations of the cell wall. Cell growth was significantly reduced as A_{600} reached a peak value of ~0.5 four hours post-induction (Fig. 3A). Cells expressing FtsZ CTLA249 were also not viable in a plating assay, though co-expression with wild-type FtsZ did restore some growth (Fig. S2B). Although FtsZ rings were present in FtsZ CTLA249 cells, their localization appeared somewhat irregular, with closely spaced rings frequently followed by longer lengths of cells with no rings at all (Fig. 5G). The L/R of FtsZ CTLA249 cells was 4.66 $\mu\text{m}/\text{Z}$ ring, which was not statistically significantly different from cells expressing wild-type FtsZ (Fig. 3B).

The FtsZ linker must be flexible and unstructured

To determine if linker flexibility and disorder was essential for FtsZ function, we replaced the linker from *B. subtilis* FtsZ with residues 398–455 from the human beta-catenin protein (FtsZ CTLH) (Fig. 1). This region of beta-catenin was selected both for its rigid nature — it encompasses ~ 4 of the 12 alpha-helical Armadillo repeats from the protein — and for its neutral charge, to eliminate potentially interfering effects from electrostatic interactions (Xing et al., 2008).

Our data suggested that linker flexibility was critical for proper FtsZ function both *in vitro* and *in vivo*. We observed only small oligomers of FtsZ CTLH by TEM and there was only a negligible increase in signal following the addition of GTP to a 90° light scattering assay (~136 times lower than FtsZ WT) (Fig. 6B and C). Although it was unable to assemble to a significant degree, FtsZ CTLH hydrolyzed GTP at a rate of 0.71 GTP/FtsZ/min. The apparent C_c for FtsZ CTLH was 0.70 μM (Table 1). Together, these findings suggested that FtsZ CTLH was still able to bind GTP but was significantly impaired for hydrolysis. We could not attribute assembly defects toward any large changes in FtsZ subunit secondary structure as CD spectra of FtsZ CTLH was nearly identical to that of wild type (Fig. S1).

Consistent with its impaired assembly *in vitro*, FtsZ CTLH was unable to support division in the absence of wild-type FtsZ (Fig. 6D, bottom panel). Cells expressing FtsZ CTLH in the absence of wild-type FtsZ were extremely filamentous and unable to divide. Immunofluorescence microscopy indicated that instead of forming a coherent ring, FtsZ CTLH localized in small punctae that were distributed randomly throughout the filaments (Fig. 6D, bottom panel). This punctate localization pattern was consistent with the inability of FtsZ CTLH to assemble *in vitro* (Fig. 6B). We also observed some crosswalls in cells that we attributed to remnants of previous cell divisions before wild-type FtsZ was depleted, as

we saw similar crosswalls in cells in which no FtsZ was expressed (Fig. S3B). Expression of FtsZ CTLH also had a deleterious effect on growth as the growth curve reveals cells only reached a peak A_{600} of ~0.2 3 hours after induction followed by a slow 1.7-fold decrease in cell density over 4 hours (Fig. 3A). Co-expression with wild-type FtsZ was also dominant lethal as indicated by dilution plating (Fig. S2B).

DISCUSSION

Previous studies of FtsZ have largely ignored its C-terminal linker, in large part because this region is irresolvable in crystal structures. Our data suggest the presence of a flexible and disordered C-terminal linker is critical for protofilament assembly and the architecture of the cytokinetic ring. At the same time, the specific sequence composition and/or length of the CTL appear to be less critical, with FtsZ assembly and Z-ring formation being largely wild type in *B. subtilis* expressing FtsZ variants with heterologous CTLs up to 100 residues in lengths. Below we discuss the implications of these data in the context of our current understanding of FtsZ assembly and the ultrastructure of the FtsZ ring.

An intrinsically disordered linker is essential for FtsZ assembly and Z-ring formation

Somewhat unexpectedly, our data support a role for the flexible linker not only *in vivo*, but also in longitudinal interactions between FtsZ subunits *in vitro*. FtsZ Δ CTL50 exhibits a long delay in protofilament assembly (Fig. 2D and E) and an elevated critical concentration (Table 1), suggesting the linker is needed for efficient incorporation of subunits into a filament. Its delayed assembly dynamics provide a potential explanation for the long straight filaments formed by FtsZ Δ CTL50 *in vivo* (Fig. 2F), as do aberrant interactions with modulatory proteins via its C-terminal grappling hook peptide (GHP). At the same time, we cannot discount possibility that in the absence of the linker, the GHP itself sterically interferes with subunit interactions. A role for the unstructured linker in coordinating longitudinal interactions between FtsZ subunits assembly is supported by data indicating that a chimeric FtsZ in which the linker has been replaced with a helical repeat (FtsZ CTLH) is unable to assemble *in vitro* or *in vivo* (Fig. 6).

Conservation of an intrinsically disordered peptide

The inability of the helical repeat to functionally replace the FtsZ linker suggests that the ability of the linker to behave as an intrinsically disordered peptide (IDP) is a key determinant of linker functionality. Regardless of primary sequence, our FtsZ CTLScr, FtsZ CTLE, and FtsZ CTLA50 chimeras support cell division (Fig. 4), and all maintain disorder when run through multiple secondary structure predictors (Rost et al., 2004; Ishida and Kinoshita, 2008). Importantly, the lack of sequence conservation and the ability of highly divergent linkers to substitute for one another *in vivo* strongly argue against the linker as a site for interaction with modulatory proteins.

Although disorder is a key feature of the functional FtsZ linker, there appear to be some constraints on its size in the cell as a large increase in linker length has a negative impact on *B. subtilis* FtsZ assembly *in vivo*. While FtsZ CTLA249 assembles into ring-like structures *in vivo* (Fig. 5G), Z-ring localization was abnormal (4.66 μ m length-to-ring ratio) and cells were unable to constrict. Specifically, why FtsZ CTLA249 fails to support division in *B. subtilis*, despite its relatively normal *in vitro* assembly dynamics, is not clear. One possibility is the large linker interferes with interactions between FtsZ and modulatory proteins. Alternatively, the longer linker may provide too much freedom to FtsZ subunits, disrupting the ultrastructure of the FtsZ ring. It remains to be seen why such long linkers are present in FtsZs from the Alpha-Proteobacteria, where exceptionally long (119–330 amino acids) are the norm and where different sets of FtsZ-interacting proteins exists.

FtsZ linker charge impacts lateral filament interactions

Our results reinforce previous work implicating C-terminal charge as an important determinant of lateral interactions between FtsZ protofilaments. While CTLE and CTLA50 have little impact on protofilament assembly and Z-ring formation, both carry net positive charges well below wild type (wild-type FtsZ linker net charge +4; CTLE and CTLA50 net charge of +1.5 and -0.3, respectively) and display reduced lateral interaction potential (Fig. 4 C–E). The FtsZ CTLScr chimera, which retains the same charge composition as wild-type FtsZ, readily forms bundled structures *in vitro*, although its light scattering signal is only half that of wild type (Fig. 4E), suggesting that the position of charge within the linker may also contribute to lateral interactions. These findings are consistent with data indicating that CTV charge, as well as buffer pH and salt concentration, have a strong impact on FtsZ's lateral interaction potential, most likely by shielding negatively charged core domains on different protofilaments from one another (Pacheco-Gómez et al., 2011; Buske and Levin, 2012).

At the same time, the finding that FtsZ CTLE and CTLA50 are essentially wild type for localization and division, despite the absence of lateral interactions *in vitro*, is somewhat surprising based on our previous work suggesting that loss of lateral interaction potential, via changes in CTV charge, significantly undermines the integrity of the FtsZ ring (Buske and Levin, 2012). One explanation for the more severe phenotype of the CTV mutant (Bs FtsZ CTVE) is that the CTV, like the CTC, is required for interaction between FtsZ and a subset of modulatory proteins *in vivo*, and that loss of these interactions negatively impacts the stability of the FtsZ ring.

Alternatively, *in vivo*, CTV charge may be a more important determinant of lateral interaction potential than linker charge, either through a better ability to access adjacent protofilaments or its proximity to the CTC and associated modulatory proteins. In support of the former, a recent crystal structure showed that the CTV was bound to FtsA from *Thermotoga maritima* (Szwedziak et al., 2012). Similarly, it was also recently reported that mutations in CTV residues 377, 378, 380 and 381 significantly reduce interactions between FtsZ and the bundling protein SepF in *B. subtilis* (Król et al., 2012). Monitoring cell division in cells expressing the different FtsZ CTL and CTV mutants in the absence of these modulatory proteins will help clarify such questions.

The FtsZ linker is a flexible tether

We propose that the primary role of the linker is to allow FtsZ to be anchored in the membrane via interactions between its GHP and modulatory proteins. At the same time, the linker permits FtsZ filaments to maintain a sufficient distance from the membrane for protofilaments to form the curved structure necessary for assembly of the Z ring and division machinery needed for cytokinesis to proceed along the short axis of the cell, similar to what has been proposed by Erickson et al., (2010) (Fig. 7A). Based on the near-linear localization pattern of the FtsZ Δ CTL50 construct, linkerless FtsZ filaments instead remain in close proximity to the membrane unable to respond to its curvature in a manner that allows Z ring formation (Fig. 7B). A structural role for the linker as a flexible tether is consistent with our finding that relatively large changes in linker length are tolerated both for FtsZ assembly *in vitro* and Z-ring formation *in vivo*. FtsZ with linkers ranging in lengths from 25 residues up to 100 residues appear to be wild type for both assembly and cell division. The Erickson lab has reported a similar finding for *E. coli* FtsZ (Gardner et al., in submission).

A flexible linker may also be essential to allow FtsZ to adopt a conformation that allows it to position the CTV in a way that promotes lateral filament interactions, likely with the core of

adjacent filaments and similar to what we previously proposed (Buske and Levin, 2012) (Fig. 7A). In the case of native *B. subtilis* FtsZ, this can be accomplished in the absence of modulatory proteins due to its proclivity toward forming FtsZ bundles. In FtsZs where filaments do not bundle, the interaction between the GHP and modulatory proteins that promote bundling can be positioned near neighboring filaments through the linker.

While the precise nature of the mechanisms responsible for initiating FtsZ assembly at the nascent division site, modulating the architecture of the FtsZ ring, and driving constriction of the ring at the beginning of cytokinesis remain to be determined, our findings together with those of the Erickson lab (Gardner et al., in submission), reinforce the need to consider the role of the linker in all aspects of FtsZ dynamics.

EXPERIMENTAL PROCEDURES

General Methods

All *B. subtilis* strains are derivatives of the strain JH642 (Perego et al., 1988). Cloning and genetic manipulation were performed using standard techniques (Harwood et al., 1990; Sambrook and Russell, 2001). All cloning was done using the *E. coli* strain AG1111 derivative PL930 (Wang and Lutkenhaus, 1993). PL930 contains the low copy plasmid pBS58 expressing *E. coli* ftsQAZ, which facilitates cloning of *B. subtilis* FtsZ. Vent DNA polymerase was used for PCRs (New England Biolabs). Cells were grown in Luria-Bertani (LB) medium at 37 °C unless otherwise noted. Antibiotics were used at the following concentrations: ampicillin = 100 µg ml⁻¹, spectinomycin = 100 µg ml⁻¹, chloramphenicol = 100 µg ml⁻¹. Strains and plasmids used in this study are listed in Table 2. Primers used are listed in Table S1.

Cloning

The pPJ15 plasmid was made by Quick Change II site-directed mutagenesis (Agilent Technologies) of pPJ1 plasmid to delete *B. subtilis* FtsZ aa 316–365. All other FtsZ chimeras were constructed as follows. Novel restriction sites were introduced into the *B. subtilis* FtsZ sequence by site-directed mutagenesis (BamHI after aa 315 and XmaI before aa 366) to yield the pPJ19 plasmid. This resulted in the amino acid pairs GS and PG at the beginning and end of the FtsZ linker. Gene sequences were PCR amplified, restriction digested, and ligated into the pPJ19 plasmid between the BamHI and XmaI sites. FtsZ ΔCTL25 was made by PCR amplification of *B. subtilis* FtsZ amino acids 1–338 and ligating into pPJ19. Including the XmaI site, this resulted in a linker length of 25 amino acids. FtsZ CTLScr was created by assigning each linker codon a number and running it through a random number generator (www.random.org) to yield the resulting sequence: KQSTNTPQEKQPSIKVRRQQEVKPSLQKQENTVHIRRHSNAPTS DPPEEQ. A synthetic oligonucleotide encoding this sequence was made (Integrated DNA Technologies) and PCR amplified to insert into pPJ19. Including the restriction sites, the total linker length was 54 amino acids. The *E. coli* linker sequence (amino acids 318–366) was amplified from genomic DNA from strain MG1655 DNA and ligated into pPJ19. Including the restriction sites, the total linker length was 52 amino acids. FtsZ CTLH was made through the creation of a gBlocks™ Gene Fragments synthetic double-stranded oligonucleotide encoding human beta-catenin residues 398–455 (Integrated DNA Technologies). The oligonucleotide was then restriction digested and inserted into pPJ19. *A. tumefaciens* FtsZ1 linker sequences were amplified from genomic DNA from strain A136 (gift of W. Margolin). FtsZ CTLA249 contained amino acids 322–566 from *A. tumefaciens* FtsZ1 in addition to extra residues from the novel restriction sites resulting in a total linker of 249 residues. FtsZ CTLA100 contained amino acids 322–417 from *A. tumefaciens* FtsZ1 plus restriction sites yielding a

linker total length of 100 amino acids. FtsZ CTLA50 contained amino acids 322–367 from *A. tumefaciens* FtsZ1 along with restriction sites producing a linker of 50 residues.

pPJ27–35 were constructed by ligating restriction-digested full-length FtsZ chimera insert into the digested pDR67 plasmid with forward primers containing the *B. subtilis* ribosome binding site. All DNA sequences were confirmed by Sanger sequencing.

Strain Construction

B. subtilis strains PL3400-07 were constructed as previously described (Buske and Levin, 2012). Briefly, these strains allow depletion of wild-type FtsZ and expression of *B. subtilis* FtsZ variants. They were constructed by first transforming pPJ27–35 plasmids into wild-type JH642 cells. This allowed the insertion of a copy of FtsZ chimeras with the *B. subtilis* ribosome-binding site upstream of the start codon at the *amyE* locus under control of the isopropyl 1-thio- β -D-galactopyranoside-inducible P_{spac} promoter. Chromosomal DNA from the resulting strains was then transformed into competent PL2084 cells (JH642 *thrC::P_{xyI}-ftsZ_{BS} ftsZ_{BS}::spc, xylA::tet*), which have their only copy of native FtsZ expressed from a xylose-inducible promoter at the *thrC* locus. This strain permits depletion of wild-type FtsZ and is xylose-dependent for normal growth (Weart and Levin, 2003).

Growth Conditions

Growth conditions were as follows. *B. subtilis* strains PL3171 (FtsZ WT) and PL3400–3407 (FtsZ variants) were first grown from single colony overnight in LB containing spectinomycin and xylose to a final concentration of 0.5%. The next day, cells were diluted 1:100 in fresh LB medium supplemented with 0.5% xylose and grown to an A₆₀₀ of 0.4 – 0.5, at which point 1 ml of cell culture was harvested by centrifugation. Cells were then washed twice with a fresh 1 ml of LB medium and then diluted 1:100 into a fresh 20 ml of LB medium plus isopropyl 1-thio- β -D-galactopyranoside to a final concentration of 0.1 mM to induce expression of FtsZs. IPTG concentration was determined by titration in PL3171 to match FtsZ expression to that of wild-type JH642 cells. A₆₀₀ measures were taken every 30 min to monitor cell growth, and after 2.5 hours post-induction with IPTG cells were harvested and prepared for immunofluorescence.

Immunofluorescence Microscopy

Immunofluorescence was performed as described previously (Buske and Levin, 2012). An Olympus BX51 microscope with Chroma filters and a Hamamatsu OrcaERG camera were used for image capture. Images were processed using Openlab version 5.2.2 (Improvision) and Adobe Photoshop CS version 8.0 (Adobe Systems). All cell or ring measurements for collected images were obtained with a minimum population of 200 cells/strain. *B. subtilis* FtsZ was detected using affinity-purified polyclonal rabbit anti-FtsZ serum (Levin and Losick, 1996) in combination with goat anti-rabbit serum conjugated to Alexa488 (Life Technologies). Cell walls were visualized with wheatgerm agglutinin conjugated to tetramethylrhodamine (Invitrogen). Nucleoids were stained with DAPI.

Determination of Cell Length/FtsZ Ring Ratio

The cell length/FtsZ ring (L/R) ratio was calculated as the sum total length of a population of cells divided by the number of FtsZ rings in that population as described previously (Weart et al., 2007).

Dilution Plating

Strains 3171, 3400, 3404, and 3405 were used for dilution plating. Cells were grown overnight in LB medium containing ampicillin at 30 °C, and the following morning, cultures

were first grown from single colony overnight in LB containing spectinomycin and xylose to a final concentration of 0.5%. The next day, cells were diluted 1:100 in fresh LB medium supplemented with 0.5% xylose and grown to an A₆₀₀ of 0.4 – 0.5, at which point 1 ml of cell culture was harvested by centrifugation. Cells were then washed twice with a fresh 1 ml of LB medium upon which serial dilutions from 10⁻¹ to 10⁻⁸ were made in LB medium. 10 µl of each dilution was then plated in series using a multichannel pipette onto pre-warmed LB agar containing spectinomycin and 0.1 mM isopropyl 1-thio-β-D-galactopyranoside with or without 0.5% xylose. Liquid cultures were allowed to dry on the plates at room temperature for upwards of 6 h, and then plates were incubated at 37 °C overnight. The plates were then imaged, and relative growth was qualitatively assessed.

Protein Purification

FtsZ variants were cloned into the pET-21b(+) expression vector through *E. coli* strain AG1111. The resulting plasmids were minipreped and freshly transformed into C41(DE3) cells (Miroux and Walker, 1996) and consequently used for expression of protein. No frozen stocks were used. Briefly, 1 liter of LB medium was inoculated 1:100 with overnight culture from a single colony. Cells were grown at 37 °C until A₆₀₀ ~0.6, and then cells were induced with isopropyl 1-thio-β-D-galactopyranoside to a final concentration of 1 mM. Cells were grown for an additional 4 h at 37 °C, and then cells were harvested by centrifugation, and cell pellets were stored at –80 °C. Purification of FtsZs were performed as described previously (Buske and Levin, 2012) with the following modifications. FtsZ CTLE, CTLH CTLA249, CTLA100, and CTLA50 were all precipitated with one ammonium sulfate cut at 30%. The FtsZ was further purified on a MT-20 column manually packed with UNOsphere™ Q beads (Bio-Rad) with a linear gradient of 50–500 mM KCl in 50 mM Tris, pH 8.5, 1 mM EDTA, 10% sucrose. Peak fractions were analyzed by SDS-PAGE, pooled together, and dialyzed overnight twice, first in 1 liter of FtsZ dialysis buffer, pH 6.5 (50 mM MES, pH 6.5, 50 mM KCl, 2.5 mM MgCl₂, 1mM EGTA, 10% sucrose), then in 1 liter of FtsZ dialysis buffer, pH 7.5 (50 mM HEPES, pH 7.5, 50 mM KCl, 2.5 mM MgCl₂, 1mM EGTA, 10% sucrose). Protein preparations were concentrated in dialysis tubing using PEG 12,000, aliquoted, flash frozen on liquid N₂, and stored at –80 °C. Prior to use in an assay to remove any insoluble polymers, FtsZ aliquots were thawed on ice, then spun at 80,000 rpm in a TLA-100 rotor (Beckman, Inc.) for 10 minutes at 4 °C and the supernatant used. FtsZ was then quantified using Coomassie Plus™ Protein Assay Reagent (Fisher) with porcine tubulin (Cytoskeleton, Inc.) as a standard.

90° Angle Light Scattering Assay

Light scattering experiments were performed essentially as described earlier (Buske and Levin, 2012) using a DM-45 spectrofluorimeter (Olis). Assembly reactions contained 5 µM FtsZ in assembly buffer (50 mM MES, pH6.5, 50 mM KCl, 2.5mM MgCl₂, 1 mM EGTA, 1 mM GTP).

Electron Microscopy

Electron microscopy was performed essentially as described (Buske and Levin, 2012). 3 µM FtsZ was used because it was found to be the best concentration in which to visualize single FtsZ filaments. FtsZ was assembled with GTP as for light scattering, and samples were visualized using a JEOL 1200EX transmission electron microscope. FtsZ filament lengths were measured using ImageJ, and data were compiled in Microsoft Excel and Kaleidagraph.

GTPase Assay and Critical Concentration

GTPase activity was measured using the continuous, regenerative coupled GTPase assay of Ingberman and Nunnari (2005). Assays were conducted in buffer conditions identical to those

used for light scattering. Each reaction included 1–8 μM FtsZ, 1 mM GTP, 1 mM phosphoenolpyruvate, 250 μM NADH, 80 units/ml lactose dehydrogenase, and 80 units/ml pyruvate kinase. A linear decline of absorbance at 340 nm for NADH was observed at 30 °C for 5 min in a 96 well plate (path length calculated as 0.425 cm) using a SPECTRAmax Plus spectrophotometer (Molecular Devices). The raw data of absorbance per minute were then converted to activity using the extinction coefficient for NADH at 340 nm of $6220 \text{ M}^{-1} \text{ cm}^{-1}$ and the path length. The raw data were then exported to Microsoft Excel for analysis. The critical concentration was determined as the X-intercept of a plot of the GTP consumed per minute versus FtsZ concentration as has been done previously (Chen et al., 2012).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LIST OF ABBREVIATIONS

aa	amino acid
CTC	C-terminal tail
CTL	C-terminal linker
CTR	C-terminal region
CTV	C-terminal variable region
EDTA	ethylenediaminetetraacetic acid
GHP	grappling hook peptide
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IDP	intrinsically disordered peptide
IFM	immunofluorescence microscopy
IPTG	isopropyl 1-thio- β -D-galactopyranoside
MES	2-(N-morpholino)ethanesulfonic acid
SDS-PAGE	sodium dodecyl sulfate – polyacrylamide gel electrophoresis
TEM	transmission electron microscopy
WT	wild type

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FIGURE 1. FtsZ Constructs

Schematic of the different *B. subtilis* FtsZ constructs used in this study, drawn to scale. FtsZ ΔCTL50 is a complete deletion of the linker, leaving only the core and conserved CTC and CTV. FtsZ ΔCTL25 is a deletion of the last 25 linker residues. FtsZ CTLScr is a randomly generated scramble of the native linker sequence. FtsZ CTLH has the linker replaced with helical repeats (residues 398–455) from human beta-catenin. FtsZ CTLE has the native *B. subtilis* linker replaced with residues 318–366 from *E. coli* FtsZ. FtsZ CTLA249 is a replacement of the native linker with residues 322–566 from *A. tumefaciens* FtsZ, while FtsZ CTLA100 and CTLA50 are replacements with residues 322–417 and 322–367, respectively.

FIGURE 2. The FtsZ linker is required for cell division in *B. subtilis*

Note: Buffer conditions for all *in vitro* work are: 50 mM MES pH 6.5, 50 mM KCl, 2.5 mM MgCl_2 , 1 mM EGTA, 1 mM GTP. FtsZ is used at a concentration of 3 μ M for all electron microscopy to facilitate visualization of single stranded polymers. For 90° angle light scattering and GTPase assays FtsZ concentration is always 5 μ M. A–C. Electron micrographs of 3 μ M FtsZ assembled in the presence of GTP. Bar = 100 nm. A. Bundled rings of FtsZ. B. Ordered sheets of FtsZ filaments on the same grid as A. C. FtsZ Δ CTL50 assembles mostly into small oligomers with occasional short, single or double-stranded filaments positioned randomly 5 minutes after addition of GTP. D. FtsZ Δ CTL50 filaments 15 minutes after addition of GTP. Note increase in filament length compared to 5 minutes. E. Representative traces of 5 μ M FtsZ assembled in presence of GTP and measured by 90° angle light scattering. Time course is over 60 minutes to denote delay in peak light scattering signal displayed by FtsZ Δ CTL50 compared to FtsZ WT. FtsZ Δ CTL50 shows a ~37 fold decreases in light scattering signal relative to FtsZ. F. Immunofluorescence microscopy of *B. subtilis* cells expressing *ftsZ*, or *ftsZ* Δ CTL50 as the sole copy of FtsZ. FtsZ Δ CTL50 fails to form Z rings and support cell division instead showing punctate localization or forming strands throughout the cytoplasm. Bar = 5 μ m.

FIGURE 3. FtsZ chimeras and their effect on cell growth

A. Growth curves monitoring optical density of *B. subtilis* cells post-induction of chimeric FtsZs with IPTG at 37°C. B. Chimeric FtsZs that form Z rings exhibit regular Z-ring localization. Length-to-ring ratios were measured for all *B. subtilis* cell types that showed the formation of Z rings. There is no significant difference between groups as determined by one way ANOVA analysis with $\alpha = 0.001$.

FIGURE 4. The sequence of the FtsZ linker does not affect protofilament assembly or cell division *B. subtilis*

A-D. Electron micrographs of 3 μ M FtsZ assembled in the presence of GTP. Arrows indicate individual filaments. Bar = 100 nm. A. Bundled rings of FtsZ. Same as Fig. 2A to allow direct comparison of wild type with FtsZ chimeras. B. FtsZ CTLScr assembles into filaments able to associate laterally and form ordered sheets and bundles 3–5 protofilaments thick. C. FtsZ CTLE forms predominantly single stranded filaments with little evidence of lateral interactions. D. FtsZ CTLA50 also forms predominantly single- or double-stranded filaments. E. Representative traces of 5 μ M FtsZ assembled in presence of GTP and measured by 90° angle light scattering. FtsZ CTLScr displays a high signal, 60% that of wild-type FtsZ, indicating it is able to form lateral interactions. FtsZ CTLE and CTLA50

show ~62 and ~37 fold decreases in light scattering signal relative to FtsZ, respectively. The low light scattering signals, coupled with the electron micrographs, indicate FtsZ CTLE and CTLA50 primarily form single-stranded protofilaments. F. Immunofluorescence microscopy of *B. subtilis* cells expressing *ftsZ*, *ftsZ CTLSer*, *ftsZ CTLE*, or *ftsZ CTLA50* as the sole copy of FtsZ. All FtsZ chimeras are able to support cell division normally and display properly positioned Z rings. Bar = 5 μ m.

FIGURE 5. FtsZ linker length does not affect protofilament formation but is important for cell division

A–D. Electron micrographs of 3 μ M FtsZ assembled in the presence of GTP. Bar = 100 nm. A. Bundled rings of FtsZ. Same as Fig. 2A to allow direct comparison of wild type with FtsZ chimeras. B. FtsZ Δ CTL25 assembles into short single-stranded filaments unable to associate laterally. C. FtsZ CTL249 forms predominantly single stranded filaments with little evidence of lateral interactions. D. FtsZ CTLA100 also forms predominantly single-stranded filaments with little evidence of lateral interactions. E. Representative traces of 5 μ M FtsZ assembled in presence of GTP and measured by 90° angle light scattering. FtsZ Δ CTL25 displays a signal ~79 fold lower than that of wild-type FtsZ. FtsZ CTLA249 and CTLA100 show ~43 and ~33 fold decreases in light scattering signal relative to FtsZ,

respectively, indicative of single-stranded filaments. F. Same plot as in E but scaled to highlight GTP-dependent increase in light scattering of FtsZ Δ CTL25, FtsZ CTLA249, and FtsZ CTLA100. G. Immunofluorescence microscopy of *B. subtilis* cells expressing *ftsZ ftsZ* Δ CTL25, *ftsZ CTLA249*, or *ftsZ CTLA100* as the sole copy of FtsZ. The short linker in FtsZ Δ CTL25 supports normal cell division and Z-ring localization. Cells expressing *ftsZ CTLA249* are able to form Z rings, but cells cannot divide and instead are filamentous. A linker of intermediate length does not affect cell division, as cells expressing *ftsZ CTLA100* are able to divide normally. Bar = 5 μ m.

FIGURE 6. A flexible and disordered linker is essential for FtsZ assembly and cell division
 A-B. Electron micrographs of 3 μ M FtsZ assembled in the presence of GTP. Bar = 100 nm.
 A. Bundled rings of FtsZ. Same as Fig. 2A to allow direct comparison of wild type with FtsZ chimeras. B. FtsZ CTLH is unable to assemble into protofilaments, instead seemingly forming small oligomers. C. Representative traces of 5 μ M FtsZ assembled in presence of GTP and measured by 90° angle light scattering. FtsZ CTLH displays a signal ~136 fold lower than wild-type FtsZ, indicating it is very poor at assembling into protofilaments. D. Immunofluorescence microscopy of *B. subtilis* cells expressing *ftsZ* or *ftsZ_{CTLH}* as the sole copy of FtsZ show the cells are unable to divide and FtsZ is punctate throughout the cell.

FIGURE 7. The FtsZ linker is a flexible tether

A. FtsZ filaments (tan) are anchored to the membrane through the interaction between its grappling hook peptide (GHP; green) and FtsA (light blue). As the filament ends move further away from the membrane due to the intrinsic bend of the filament, the FtsZ linker (dark blue) extends and takes on a conformation which allows the GHP to remain tethered to FtsA. Lateral FtsZ filament interactions are also capable of forming through the positioning of the GHP near the N-terminal core of adjacent subunits by the linker. B. A FtsZ Δ CTL50 filament without the linker is able to anchor to the membrane through its GHP. However, in the absence of the linker, the filament remains near the membrane in a straighter positioning

that cannot respond to the membrane curvature and form a proper Z ring. The FtsZ Δ CTL50 filament is also unable to form bundles.

Table 1

FtsZ GTPase Turnover Rates and Critical Concentrations

FtsZ species	C _c (μM)	GTP/FtsZ/min	FtsZ Polymers at pH 6.5, 50 mM KCl
WT	0.68	3.82	Filament bundles and rings
ΔCTL50	1.08	1.48	Single filaments
ΔCTL25	0.30	4.66	Single filaments
CTLScr	0.88	2.68	Filament bundles
CTLH	0.70	0.71	Small oligomers
CTLE	0.64	5.04	Single filaments
CTLA249	0.80	4.40	Single filaments
CTLA100	0.78	5.09	Single filaments
CTLA50	0.64	5.51	Single filaments

Table 2

Bacterial strains and plasmids used in this work

Strain or plasmid	Genotype and/or features	Source
<i>B. subtilis</i>		
JH642	<i>B. subtilis trpC2 pheA1</i>	(Perego et al., 1988)
PL 2084	<i>JH642 thrC::P_{xyI}-ftsZ, ftsZ::spc, xylA::tet</i>	(Weart and Levin, 2003)
PL 3171	<i>PL 2084 amyE::P_{spac}-ftsZ</i>	(Buske and Levin, 2012)
PL 3400	<i>PL 2084 amyE::P_{spac}-ftsZΔCTL50</i>	This study
PL 3401	<i>PL 2084 amyE::P_{spac}-ftsZ ΔCTL25</i>	This study
PL 3402	<i>PL 2084 amyE::P_{spac}-ftsZCTLScr</i>	This study
PL 3403	<i>PL 2084 amyE::P_{spac}-ftsZCTLE</i>	This study
PL 3404	<i>PL 2084 amyE::P_{spac}-ftsZCTLH</i>	This study
PL 3405	<i>PL 2084 amyE::P_{spac}-ftsZCTLA249</i>	This study
PL 3406	<i>PL 2084 amyE::P_{spac}-ftsZCTLA100</i>	This study
PL 3407	<i>PL 2084 amyE::P_{spac}-ftsZCTLA50</i>	This study
<i>E. coli</i>		
MG1655	<i>F⁻ λ⁻ilvG⁻ rfb-50 rph-1</i>	
AG1111	<i>DZR200- MC1061 F⁺lacIQ lacZM15 Tn10 (tet)</i>	(Ireton et al., 1993)
C41 (DE3)	<i>F⁻ ompT hsdSB (rB⁻ mB⁻) gal dcm (DE3)</i>	(Miroux and Walker, 1996)
PL930	<i>AG111 + pB5S8</i>	(Weart and Levin, 2003)
Plasmids		
pPJ1	pET21b(+)-ftsZ stop	(Buske and Levin, 2012)
pPJ15	pET21b(+)-ftsZΔCTL50 stop	This study
pPJ19	pET21b(+)-ftsZ946-51 BamHI 1090-95 XmaI stop	This study
pPJ21	pET21b(+)-ftsZΔCTL25 stop	This study
pPJ22	pET21b(+)-ftsZCTLScr stop	This study
pPJ23	pET21b(+)-ftsZCTLE stop	This study
pPJ24	pET21b(+)-ftsZCTLH stop	This study
pPJ25	pET21b(+)-ftsZCTLA249 stop	This study
pPJ26	pET21b(+)-ftsZCTLA100 stop	This study
pPJ27	pET21b(+)-ftsZCTLA50 stop	This study
pPJ28	pDR67-ftsZΔCTL50 stop	This study
pPJ29	pDR67-ftsZ ΔCTL25 stop	This study
pPJ30	pDR67-ftsZCTLScr stop	This study
pPJ31	pDR67-ftsZCTLE stop	This study
pPJ32	pDR67-ftsZCTLH stop	This study
pPJ33	pDR67-ftsZCTLA249 stop	This study
pPJ34	pDR67-ftsZCTLA100 stop	This study
pPJ35	pDR67-ftsZCTLA50 stop	This study