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FtsZ protofilaments use a hinge-opening mechanism for constrictive force generation

Ying Li¹, Jen Hsin^{2,†}, Lingyun Zhao^{3,†}, Yiwen Cheng^{1,†}, Weina Shang¹, Kerwyn Casey Huang², Hong-Wei Wang^{3,4}, and Sheng Ye^{1,5,*}

¹Life Sciences Institute, Zhejiang University, Hangzhou, 310058 Zhejiang, P.R. China

²Department of Bioengineering, Stanford University, Stanford, CA 94305, United States

³School of Life Sciences, Tsinghua University, Beijing, 100084 Beijing, P.R. China

⁴Tsinghua-Peking Center for Life Sciences, Center for Structural Biology, School of Life Sciences, Tsinghua University, Beijing 100084, P.R. China

⁵Institute of Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, 310058 Zhejiang, P.R. China

Abstract

The essential bacterial protein FtsZ is a GTPase that self-assembles into a structure at the division site termed the “Z-ring”. During cytokinesis, the Z-ring exerts a constrictive force on the membrane by utilizing the chemical energy of GTP hydrolysis. However, the structural basis of this constriction remains unresolved. Here, we present the crystal structure of a GDP-bound *Mycobacterium tuberculosis* FtsZ (MtbFtsZ) protofilament, which exhibits a curved conformational state. The structure reveals a longitudinal interface that is important for function. The protofilament curvature highlights a hydrolysis-dependent conformational switch at the T3 loop that leads to longitudinal bending between subunits, which could generate sufficient force to drive cytokinesis.

In most bacteria, cytokinesis is initiated by the localization of the essential protein FtsZ, a GTPase and tubulin homolog that can form ring-like structures and generate constrictive forces (1). Hydrolysis of GTP bound to FtsZ protofilaments is thought to drive a straight-to-curved conformational change, and generates the constrictive force required for cell division (2, 3). While multiple crystal structures of FtsZ in different nucleotide states and as monomers and protofilaments are available (4–9), little is known about the structural mechanism underlying a hydrolysis-mediated conformational change.

We determined the crystal structure of MtbFtsZ-GDP to an Rfree factor of 26.7% with data to 2.9 Å resolution (Table S1, Fig. S1, S2). The MtbFtsZ-GDP subunits were arranged longitudinally in a head-to-tail manner and assemble into two continuous polymers that intertwine to form a left-handed, anti-parallel, double-stranded structure in the crystal lattice (Fig. 1A, S3). The polymer curvature is qualitatively similar to previous electron

*Corresponding author. sye@zju.edu.cn.

[†]J.H., L.Z., and Y.C. contributed equally to this work

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microscopy images of *Escherichia coli* FtsZ (EcFtsZ) (2), *Methanococcus jannaschii* FtsZ (MjFtsZ) (10), and *Pseudomonas aeruginosa* FtsZ (PaFtsZ) (11). The structure reveals an inter-subunit interface formed by highly conserved residues (Fig. 1B, S2, S4A), and is different from the previously observed “longitudinal interface” in a MjFtsZ-GTP dimer structure (Fig. S4B) (7). One amino acid at the center of the hydrophobic inter-subunit interactions is Leu269 (Fig. 1B, S4A), which was previously identified as a key residue in *E. coli* (Leu272) (12). However, the longitudinal contacts of the MjFtsZ dimer structure do not involve this residue (Leu297, Fig. S4B) (7).

To confirm the importance of the observed inter-subunit contacts, we created 11 point mutants in EcFtsZ based on our crystallographic observations, and characterized their division phenotypes (Table S2) using a complementation system in *E. coli* (13). As expected, Mutant L272E failed to complement and was dominant negative (Fig. S5) (12). All other point mutants designed to disrupt the MtbFtsZ inter-subunit interface by changing hydrophobic residues to acidic residues failed to complement, and three of them were also dominant negative (Fig. S5). Further mutagenesis experiments revealed that Leu272, Ala181, and Phe137 are highly sensitive, with only L272I, L272M, and A181L able to complement (Fig. S6, S7). Double mutants combining two complementing mutations (L272I/A181L and L272M/A181L) did not complement (Fig. S8), while none of those combining two non-complementing mutations complemented (Fig. S9), indicating that the above point mutants affect FtsZ function by disrupting the inter-subunit interface. Thus, the observed inter-subunit interface is biologically relevant and the hydrophobic interactions play a pivotal role.

We observed that MtbFtsZ and EcFtsZ GTPase activities *in vitro* were dramatically affected by the interfacial mutations (Fig. 1C, 1D). As FtsZ GTPase activity depends on proper longitudinal assembly (14), we measured the GTP-dependent polymerizations of wild-type and mutated FtsZ. While wild-type MtbFtsZ and EcFtsZ both polymerized, mutants MtbFtsZ L269E in MtbFtsZ and L272E in EcFtsZ failed to polymerize (Fig. 1E), as did all of the point mutants modifying the interfacial hydrophobic residues (Fig. S10A, B). Assembly of FtsZ in the presence of GMPCPP showed similar results (Fig. S10C). Thus, the observed inter-subunit contacts belong to the longitudinal interface.

The MjFtsZ dimer structure (Fig. S4B) was initially proposed to reflect a longitudinal interface (7). However, a recently determined *Staphylococcus aureus* FtsZ (SaFtsZ) structure (4, 5) revealed a different inter-subunit interface from the MjFtsZ dimer (Fig. S4C, S4D). To distinguish which interface is biologically relevant, we designed two MjFtsZ point mutants, P313E and L297E (Fig. S2). P313 is involved in the inter-subunit contacts in the MjFtsZ dimer structure but L297 is not (7). Wild-type and P313E MjFtsZ were fully functional and polymerized with the addition of GTP, whereas L297E MjFtsZ had reduced GTPase activity and did not polymerize (Fig. S11A, S12A). A series of point mutants disrupting the SaFtsZ longitudinal interface were all inactive (Fig. S11B) and failed to polymerize (Fig. S12B). The SaFtsZ inter-subunit interface is similar to the tubulin longitudinal interface (15) and involves highly conserved residues shown to be functionally important in mutagenesis studies (12, 13), indicating that the SaFtsZ-GDP structure represents a genuine FtsZ protofilament.

A structural comparison of the MtbFtsZ-GDP dimer with the straight SaFtsZ-GDP dimer (4) revealed two noteworthy features (Fig. 2A). First, the MtbFtsZ-GDP longitudinal interface is similar to that in the SaFtsZ-GDP dimer, with only a slight degree of twist (Fig. S13). Second, the major difference stems from a remarkable straight-to-curved conformational change with 49.6° of bending between adjacent subunits. This conformational change suggests a “hinge-opening” motion pivoted around the inter-subunit interface of our

MtbFtsZ-GDP dimer. Such motion has been demonstrated to be feasible (16), and is in agreement with functional studies (17). MtbFtsZ formed shorter and more highly curved protofilaments in the presence of GDP compared with GTP (Fig. S14A). Disruptive mutants that did not polymerize with GDP also failed to polymerize with GTP (Fig. S14A, B). Thus, the observed inter-subunit interface is present in both straight and curved protofilaments and serves as a pivot point in the straight-to-curved conformational change. As a further test of the hinge-opening mechanism, disruptive mutants at the T7 loop only polymerized in the presence of GDP (Fig. S14A, B).

How does GTP hydrolysis trigger such inter-subunit bending? Despite the overall similarity between the GTP S-bound MtbFtsZ monomer (8) and our GDP-bound MtbFtsZ filament structures, each GDP-bound MtbFtsZ subunit displays a significant nucleotide-dependent structural difference at the T3 loop (Fig. 2B). The GTP γ -phosphate stabilizes the T3 loop conformation in a compact state (Tension or T state) (Fig. S15A) (8). The same T-state conformation is maintained in all GTP-bound and some GDP-bound FtsZ structures (7, 18, 19), and is necessary for the longitudinal assembly of a straight FtsZ protofilament (5), in which the T3 loop interacts extensively with the T7 loop of the top subunit. In contrast, in our GDP-bound MtbFtsZ structure, the T3 loop adopts a relaxed conformation (R state) in the absence of the γ -phosphate (Fig. S15B), indicating that it is flexible and can be in either state. This T3 loop conformational switch was previously predicted based on studies on MjFtsZ (20). The SaFtsZ-GDP structure (4, 5) also reveals that the GDP molecule bound between two SaFtsZ subunits is completely occluded. Since both α - and β -phosphates are negatively charged, the release of γ -phosphate by hydrolysis can transition the T3 loop from a T to an R state. This would weaken the FtsZ longitudinal interactions between the T3 and T7 loops, and further drive the hinge-opening event around the pivot point (Fig. 2C and Movie S1).

This hydrolysis-mediated structural transition has two functional consequences: generation of mechanical work and facilitation of turnover. FtsZ contains a flexible C-terminal that binds FtsA, which is attached to the membrane through its C-terminal amphipathic helix (21). The straight SaFtsZ (5) and our curved MtbFtsZ protofilament structures give rise to a model of FtsZ structural dynamics in which the bending between two longitudinal subunits causes a 17.5-Å displacement of the C-terminal Phe312 (Fig. S16). In the context of FtsZ division function, the straight-to-curved transition can exert constrictive forces on the envelope (Fig. 3A). To estimate the magnitude of force generated by GTP hydrolysis-induced bending of an FtsZ protofilament, we investigated MtbFtsZ-GDP dimer structures using all-atom molecular dynamics simulations. First, we conducted two repeat equilibrium simulations and demonstrated that the inter-subunit contacts in the MtbFtsZ-GDP dimer were well maintained (Fig. 3B). To probe the energetics and forces associated with the hinge-opening motion, we performed free-energy calculations of MtbFtsZ dimers in both GDP- and GTP-bound states (Fig. 3C). Both energy profiles along the hinge-opening pathway suggested the existence of intermediate conformations (Fig. 3C), but overall FtsZ-GDP preferred a more bent conformation than FtsZ-GTP (16), and this difference in conformational energy can generate constrictive force. For example, when a GDP-FtsZ dimer transitions from a straight ($\sim 170^\circ$) to a bent ($\sim 135^\circ$) dimer, 7 kcal/mol is released. Assuming this energy is fully utilized for constrictive force and using the estimations provided in (17), about 20 pN of force can be derived from such a hydrolysis-induced structural transition.

The straight-to-curved structural transition also greatly reduces the buried surface area from 2360 Å² for the SaFtsZ longitudinal interface (5) to 1040 Å² for the MtbFtsZ longitudinal interface, indicating weaker assembly for the curved protofilament than the straight state. These values are comparable with calculations of the transition between GTP-bound and

GDP-bound states in an MjFtsZ dimer (16), demonstrating that a hinge-opening motion and reduced buried surface area due to hydrolysis are likely common components of the FtsZ force generation mechanism regardless of differences in longitudinal interface. Therefore, a curved FtsZ protofilament is more prone to filament disassembly, in agreement with earlier studies showing that assembly of FtsZ-GDP is weaker than FtsZ-GTP, and that hydrolysis destabilizes FtsZ filaments (10, 22–24).

Taken together, our studies demonstrate a hinge-opening conformational change between a straight and curved FtsZ protofilament that supports the “hydrolyze and bend” model (3) and provides a structural basis for constrictive force generation in bacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Structure of a double-stranded MtbFtsZ-GDP polymer reveals key inter-subunit contacts

(A) Ribbon representation of a double-stranded MtbFtsZ-GDP polymer containing 24 subunits. The FtsZ subunits are colored in grey and cyan to distinguish the two protofilaments, and GDP molecules are colored in orange. (B) Detailed stereo-view of the inter-subunit contacts. The interactions, especially the hydrophobic contacts, are formed by highly conserved residues (Fig. S2). Among these residues, Leu269 of the top subunit is at the center of the hydrophobic interactions, forming van der Waals contacts with several hydrophobic residues of the bottom subunit. (C, D) GTPase activities of wild-type and mutant MtbFtsZ (C) or EcFtsZ (D). Means $\pm$ SD are shown ($n = 3$). (E) Electron microscopy analysis of GTP-dependent polymerization of MtbFtsZ (wild-type and L269E) and EcFtsZ (wild-type and L272E). Example protofilaments (PF) are highlighted with arrows.

Figure 2. GTP hydrolysis induces a "straight" to "curved" conformational switch at the longitudinal interface

(A) Superposition of the bottom subunit of a GDP-bound MtbFtsZ dimer (molecules A and B, this study) with that of a GDP-bound SaFtsZ dimer (PDB ID: 4DXD), showing a straight-to-curved bending at the longitudinal interface. Monomers in the MtbFtsZ dimer are shown in light red and dark grey for the top and bottom subunits, whereas those in the SaFtsZ dimer are shown in light green and light grey for the top and bottom subunits, respectively. (B) Superposition of MtbFtsZ monomers in the GDP-bound polymerized state (molecule A, this study; protein shown in light red with GDP in magenta) and the GTP S-bound monomeric state (PDB ID: 1RLU; protein shown in light green with GTP in blue), illustrating that the T3 loop of MtbFtsZ adopts two different conformations: GTP-dependent Tension (T) and Relaxed (R) states. (C) Proposed mechanism for hydrolysis-mediated conformational switch of the longitudinal interface.

Figure 3. Bending of FtsZ protofilaments produces an inward force on the membrane
(A) Schematic of membrane deformation due to the force produced by hydrolysis-induced FtsZ protofilament bending. **(B)** Inter-monomer contacts were maintained in two independent molecular dynamics simulations of the MtbFtsZ-GDP dimer, suggesting stability of the bent conformation. Each time course measures the closest distance between a pair of amino acids: L272(top)-F135(bottom) (blue), L272(top)-L167(bottom) (orange), and L272(top)-L176(bottom) (purple). **(C)** Potential of mean force measurements for MtbFtsZ-GDP (red) and MtbFtsZ-GTP (green) along the hinge-opening pathway demonstrate a preference of MtbFtsZ-GDP protofilaments for a bent conformation, and suggest several energetically feasible intermediate conformations.