Structural changes in FtsZ induced by intermolecular interactions between bound GTP and the T7 loop

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ABSTRACT

FtsZ is a prokaryotic homolog of tubulin, and is a key molecule in bacterial cell division. FtsZ with bound GTP polymerizes into tubulin-like protofilaments. Upon polymerization, the T7 loop of one subunit is inserted into the nucleotide-binding pocket of the second subunit, which results in GTP hydrolysis. Thus, the T7 loop is important for both polymerization and hydrolysis in the tubulin/FtsZ family. Although X-ray crystallography revealed both straight and curved conformations of tubulin, only a curved structure was known for FtsZ. Recently, however, FtsZ from Staphylococcus aureus (SaFtsZ) has been shown to have a very different conformation from the canonical FtsZ structure. The present study was performed to investigate the structure of SaFtsZ by mutagenesis experiments; the effects of amino acid changes in the T7 loop on the structure as well as on GTPase activity were studied. These analyses indicated that FtsZ changes its conformation suitable for polymerization and GTP hydrolysis by movement between N- and C-subdomains via intermolecular interactions between bound nucleotide and residues in the T7 loop.

FtsZ is an essential bacterial cell division protein and is a prokaryotic homolog of the tubulin family (1,2). FtsZ assembles into FtsZ-like protofilaments by head-to-tail association (3), and forms during cell division a contractile ring-like structure known as the Z-ring at the midpoint of the cell anchored to the cytoplasmic membrane by several accessory proteins (2).

Tubulin is known to alternate between a curved conformation and a straight microtubule conformation depending on the bound nucleotide in the assembly/disassembly cycle. The structures of tubulin have been studied extensively (4-10). In particular, the structures of the straight and curved protofilaments have both been determined by electron microscopy (7) and X-ray crystallography (8). In addition to bends at the subunit interface, the two subdomains within each tubulin subunit show marked rotations when changing from the straight to curved protofilament conformation (9). A structure of capped tubulin dimers is now available (10) and this has a subdomain conformation identical to that in curved protofilaments, suggesting that the curved subdomain conformation is the relaxed state.

Protofilaments of FtsZ were also found to form straight and curved structures in relation to the bound nucleotide, as determined by electron microscopy (11,12). A molecular dynamics simulation suggested that bending the H7 helix of FtsZ acts as a nucleotide-regulated molecular switch.
switch in the assembly/disassembly cycle of cell division (13). *Escherichia coli* FtsZ (*EcFtsZ*) with GTP may switch the conformation *in vitro*, and flexibility between subdomains is important for GTPase activity in *EcFtsZ* (14). The dynamics simulation also suggested that GTP-bound FtsZ dimer has a more rigid molecular formation than GDP-bound dimer, and the simulation predicted that the GDP-FtsZ filament is much more curved than the relatively straight GTP-FtsZ filament (15).

The enzymatic domain of FtsZ is known to act as a self-activating GTPase (16,17). It is composed of two globular subdomains (N- and C-terminal subdomains) separated by a central core helix (H7 helix) and a synergy loop (T7 loop). There is a nucleotide-binding pocket in the N-terminal subdomain (residues 13 – 173). The C-terminal subdomain is likely a GTPase-activating subdomain (residues 223 – 310). For polymerization, the T7 loop in one subunit is inserted into the nucleotide-binding pocket of the next subunit (18). Mutations of Asn207, Asp209, and Asp212 in the T7 loop of *EcFtsZ* severely affected GTP hydrolysis (19), suggesting that these catalytic residues in the T7 loop are poised to attack the γ-phosphate of GTP for GTP hydrolysis in the FtsZ polymer. In this way, GTPase activity is highly dependent on the polymerization of FtsZ (3,19).

A number of structures of bacterial and archaeal FtsZs have been determined by X-ray crystallography (3,20-22). All of these structures were very similar irrespective of the bound nucleotide and the slight differences observed seemed to be related to interspecies differences (22). Recently, however, *Staphylococcus aureus* FtsZ (*SaFtsZ*) has been shown to have a markedly different structure by sliding/rotation between two subdomains with concomitant conformational change of the T7 loop (23). This structure seemed to be related to the polymerization state; the T7 loop of the “upper” subunit in *SaFtsZ* was inserted more deeply into the nucleotide-binding pocket of the “lower” subunit than any other structure determined to date. Therefore, the *SaFtsZ* structure has been interpreted as representing a straight protofilament conformation, while other structures are curved (and relaxed) conformations (23). This view is reinforced by the observation that *SaFtsZ* bound with PC190723, which inhibits GTPase activity by stabilizing the polymer formation (24), has the same conformation (23,25,26). Very recently Ying Li *et al.* showed that our FtsZ structure is consistent with their mutagenesis work (27). The rotation of the subdomains from curved FtsZ to straight FtsZ is in approximately the same direction as that of tubulin, but larger in magnitude. A structural change induced by intermolecular interaction had been proposed to explain FtsZ assembly (28,29).

In the present study, we analyzed the molecular characteristics of *SaFtsZ* by investigating the effects of the amino acid change in the T7 loop on the structure as well as on the GTPase activity. For this purpose, three mutants were prepared: a chimera mutant (T7Bs), the T7 loop of which has been replaced by that of *Bacillus subtilis* FtsZ (BsFtsZ), and two truncated mutants (ΔT7GAN and ΔT7GAG) in which the T7 loop was shortened. In addition, we also determined the co-crystallized structure of *SaFtsZ*-guanosine 5'-O-(3-thiotriphosphate) (GTPγS). Here, we discuss these structural features in relation to the polymerization and GTPase activities of FtsZ.

**EXPERIMENTAL PROCEDURES**

*FtsZ mutagenesis – Full-length* (*SaFtsZ*) and enzymatic domain (residues 12 – 316, *SaFtsZt*) of FtsZ from *S. aureus* Mu50 were amplified and expressed previously (23). Mutant *SaFtsZs* were prepared using a Stratagene Quick-Change mutagenesis kit (Agilent Technologies) with *SaFtsZ* expression vectors as a template. The T7 loop (VSGEV) of *SaFtsZ* was replaced with the T7 loop (TPGLI) of *B. subtilis* FtsZ (designated as T7Bs). Residues 204 – 208 (SGEVN) in the T7 loop of *SaFtsZ* were substituted for GAG and GAN (designated as ΔT7GAG and ΔT7GAN, respectively). The DNA sequences were confirmed using an ABI 310 Genetic Analyzer (Applied Biosystems).

*Protein expression and purification of SaFtsZ mutants –* Protein expression and purification of *SaFtsZ* mutants were performed as described previously (23). Purified proteins were concentrated to 5.0 mg/mL using Vivaspin 20-10K ultrafiltration devices (GE Healthcare) in
storage buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl).

Refolding of T7Bs mutant and SaFtsZ – Cell lysate of T7Bs in lysis buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10% (v/v) glycerol, 0.5 mg/mL lysozyme, and 0.1 mg/mL DNase I) was sonicated and then centrifuged at 40000 × g for 30 min at 10°C. The precipitate was resuspended in denaturing buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 6 M guanidine hydrochloride) and dialyzed against 3 L of dialysis buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10% (v/v) glycerol) three times to yield the refolded T7Bs. The dialyzed resuspension was centrifuged at 40000 × g for 30 min at 10°C. The purification procedures using chromatography were the same as described above (23). The refolded SaFtsZ was obtained according to the previously described procedure (23).

Crystallization and structure determination – All crystals were obtained under the conditions shown in Table 1 and appeared after a few weeks at 20°C. X-ray diffraction data of ΔT7GAG-GDP, ΔT7GAN-GTP, and refolded T7Bs were collected on beamline BL41XU of SPring-8 (Hyogo, Japan) under cryogenic conditions at –173°C. These diffraction data were processed and scaled with either the HKL-2000 program package (30) (ΔT7GAG-GDP and T7Bs) or XDS (31) (ΔT7GAN-GTP). X-ray diffraction data of ΔT7GAN-GDP and refolded SaFtsZ-GTPγS were collected on beamline BL1A and BL17A of Photon Factory (Tsukuba, Japan), respectively, under cryogenic conditions at –173°C. Diffraction data of ΔT7GAN-GDP and SaFtsZ-GTPγS were processed and scaled with XDS (31). Molecular replacements of T7Bs, ΔT7GAN-GTP, and SaFtsZ-GTPγS were performed with 3VOA (SaFtsZt) (23) as the search model using Molrep in the CCP4 suite (32,33). Molecular replacements of ΔT7GAG-GDP and ΔT7GAN-GDP were performed with 2VXY (B. subtilis FtsZ; BsFtsZ) (34) and ΔT7GAG-GDP as the search model using Molrep in the CCP4 suite, respectively. The structures were modified manually with Coot (35), and refined with Phenix (36).

The data collection and refinement statistics are summarized in Table 1. Structures were displayed using PyMOL (37). Coordinates and structure factors of T7Bs, ΔT7GAG-GDP, ΔT7GAN-GTP, ΔT7GAN-GTP, and SaFtsZ-GTPγS have been deposited in the RCSB Protein Data Bank under the accession numbers 3WGJ, 3WGG, 3WGL, 3WGM, and 3WGN, respectively.

GTPase assay – SaFtsZ GTPase assay was performed as described previously (23). GTP was added to 5 µM proteins in reaction buffer (50 mM MOPS, pH 7.2, 5 mM MgCl2, 200 mM KCl) at a final concentration of 100 µM to start the reaction at 25°C. Then, 65 µM EDTA was added to the sample at various time points to stop the reaction. The samples were mixed with BIOMOL GREEN (BIOMOL Research Laboratories) and incubated at 25°C for 30 min before measurement.

RESULTS

Crystals of FtsZ mutants – Although ΔT7GAG was crystallized in only one form, ΔT7GAN had several different crystal forms with different unit cell parameters (Table 2). Two of them were studied and their structures were determined at resolutions of 3.07Å and 2.18Å, respectively (Table 1). ΔT7GAN and T7Bs were refined at resolutions of 2.80Å and 2.18Å, respectively (Table 1). The space groups of ΔT7GAN and two ΔT7GAN crystals were P21, while T7Bs crystal was P1. The crystals of all mutants contained two molecules in the asymmetric unit and there were no significant differences in these two molecules in each crystal. All of these mutants crystallized as straight protofilaments forming longitudinal interactions (described in detail in a later section). The electron density maps of T7Bs indicated the absence of bound nucleotide in the nucleotide-binding pocket of both independent molecules in the crystal, whereas a calcium ion was coordinated with the conserved residues in the T7 loop. While the electron density maps of ΔT7GAN (ΔT7GAG-GDP) and one of ΔT7GAN (ΔT7GAN-GDP) crystals indicated the presence of GDP in the nucleotide-binding pocket, GTP and a magnesium ion were observed in the nucleotide-binding pocket of the other ΔT7GAN crystal (ΔT7GAN-GTP). All mutagenized residues were identified in the electron density maps.

Structural comparison of FtsZ mutants with BsFtsZ and SaFtsZ – Figure 1 shows the crystal structures of all mutants superposed on both BsFtsZ (left) and SaFtsZ (right). All pictures in
Figure 1 were drawn after superposition of the N-terminal subdomains (residues 13 – 173). Although the N-terminal subdomains were superposed well in all mutants, the overall structures were considerably different (Figure 1 and Table 3) due to the differences in relative orientation of the subdomains. The C-terminal subdomains of four mutants were moved downward (i.e., from the BsFtsZ-type to SaFtsZ-type) in the order of ΔT7GAN-GDP < ΔT7GAG-GDP < T7Bs < ΔT7GAN-GTP, as judged by the overlap with BsFtsZ or SaFtsZ (Figure 1 and Table 3).

The conformation of T7Bs is shown in Figure 1C. Although the amino acid sequence of the T7 loop in T7Bs was replaced by that of BsFtsZ from SaFtsZ, the overall conformation of T7Bs showed the same structural features as SaFtsZ (r.m.s.d. of 0.39 Å for backbone Cα atoms of C-terminal subdomains as in Table 3) and was different from that of BsFtsZ (r.m.s.d. of 7.54 Å). The main chain of the T7 loop in T7Bs also converged well with that in SaFtsZ (r.m.s.d. of 0.50 Å).

Figure 1B shows structural comparisons of ΔT7GAN-GDP with BsFtsZ and SaFtsZ. Although the conformation of the N-terminal subdomain in ΔT7GAN-GDP was almost the same as those in the two FtsZs, the overall structure was not the same as either structure (Figure 1B, and Table 3) due to the differences in relative orientation of the subdomains. This is also the case for ΔT7GAN-GDP (Figure 1A). There were almost no structural differences between ΔT7GAN-GDP and ΔT7GAG-GDP (r.m.s.d. of 0.74 Å, Table 3). The H6-H7 loop and T7 loop in both ΔT7GAN-GDP and ΔT7GAN-GDP were located in different positions from those in SaFtsZ but at similar positions to those in BsFtsZ (Figures 1A and 1B). The H7 helix in ΔT7GAN-GDP and ΔT7GAN-GDP was shifted by about one helical pitch in comparison with that in SaFtsZ (Figures 1A and 1B). To evaluate these changes in orientation, we used the DynDom program, which determines hinge axes by comparing two conformations (38). This program indicated that the subdomain orientation of ΔT7GAN-GDP and ΔT7GAN-GDP was also more similar to BsFtsZ than SaFtsZ (Table 3).

The structure of ΔT7GAN-GTP was different from any of these ΔT7 mutants bound with GDP.
Although crystal packing of \( \Delta T_{7GAG} \)-GTP was more similar to that of \( \Delta T_{7GAN} \)-GTP was less than 1 Å². These results suggest that flipping the T7 loop out of the GTP-binding pocket also reduces the molecular interaction. Thus, the interactions between adjacent molecules in \( \Delta T_{7GAN} \) mutant crystals were strongly correlated with the subdomain orientation in the monomer.

**GTPase activities of \( \Delta T_{7GAN} \) mutants** – To clarify the characteristics of T7 loop mutants, we measured their GTPase activities (Figure 3). The GTPase activity of \( \Delta T_{7Bs} \) was similar to that of \( \Delta T_{7GAG} \). In contrast, GTPase activities of \( \Delta T_{7GAG} \) and \( \Delta T_{7GAN} \) were markedly reduced. In particular, it is noteworthy that \( \Delta T_{7GAN} \), which retains all known catalytic residues, showed almost complete loss of GTPase activity. GTPase activity is highly dependent on the polymerization of FtsZ (3,19). Thus, lost of GTPase activities in two \( \Delta T_{7} \) mutants is likely to be due to defects in their polymerization activities.

**Structure of \( \Delta T_{7GAN} \)–Refolded \( \Delta T_{7GAN} \)** – Refolded \( \Delta T_{7GAN} \) was co-crystallized with GTPγS and its structure was determined at 2.6 Å (Figure 4C and Table 1). The space group of GTPγS complex was P1. The crystal contained two molecules in the asymmetric unit. The electron density map of the crystal clearly showed the presence of GTPγS in the nucleotide-binding pocket of all independent molecules. However, the presence of a calcium ion could not be identified. The complex had a head-to-tail association with a molecular interval of 44.3 Å. There was no major conformational difference between the GTPγS complex and GDP complex determined previously (23) (r.m.s.d. of 0.59 Å, Figure 4C). The packing interaction was also the same as seen in \( \Delta T_{7GAG} \)-GDP (Figures 4A and 4B). The catalytic residues in the nucleotide-binding pocket, Asn208, Asp210, and Asp213, and Asp46, were also at the same locations as \( \Delta T_{7GAG} \)-GDP (Figures 4A and 4B). However, the electron densities of side chains of Asn44 and Gln48 in the pocket were too poor to determine their positions.

**DISCUSSION**

Although both straight and curved conformations of tubulin have been observed by X-ray crystallography, only a curved structure was known for FtsZ, possibly because it is more stable than any other states. However, FtsZ from *S. aureus* (\( \Delta T_{7GAN} \)) has recently been shown to have a very different conformation from the canonical FtsZ structure determined previously (23). Briefly, the structure of \( \Delta T_{7GAN} \) was different from the canonical FtsZ structure (e.g. \( B_{s}FtsZ \), PDB ID: 2RHL) in that the C-terminal subdomain slid down by about one helical pitch along the central H7 helix. This conformational change seemed to allow more intimate molecular association in the polymer and to enable the T7 loop of one molecule to intrude deeply into the GTPase pocket of the second molecule. The structure of FtsZ from *Staphylococcus epidermidis* deposited very recently (PDB ID: 4M8I), showed that its structure and longitudinal interactions were very similar to those of \( \Delta T_{7GAN} \). Possibly, some molecular interactions in these FtsZ crystals make the straight-polymer type crystal more stable.

It has been suggested that the GTPase activity of FtsZ depends on the polymerization and several residues in the T7 loop were proposed to be responsible not only for the GTPase activity but also for polymerization (19,40). Therefore, in the present study, we investigated the effects of mutation of the T7 loop in \( \Delta T_{7GAN} \) on the FtsZ structure as well as on the GTPase activity. The chimeric mutant \( \Delta T_{7Bs} \) had the sequence of \( \Delta T_{7GAG} \) in which the T7 loop sequence (VSGEV) was replaced by that of \( B_{s}FtsZ \) (TPGLI). As expected, \( \Delta T_{7Bs} \) retained the wild-type level of GTPase activity (Figure 3) and the structure was very similar to \( \Delta T_{7GAG} \) rather than \( B_{s}FtsZ \) (Figure 1C), i.e., \( \Delta T_{7Bs} \) crystallized in the form of assembled straight protofilaments, with a subdomain conformation very similar to that of polymerized \( \Delta T_{7GAN} \). These observations indicated that the species-specific difference in amino acid residues in T7 loop is not directly related to the change in FtsZ conformation and clearly excluded the possibility that the difference in amino sequence in the T7 loop was responsible for the different structure of \( \Delta T_{7GAN} \).

In contrast, the structure of \( \Delta T_{7GAG} \)-GDP was markedly altered to a structure similar to that of \( B_{s}FtsZ \) (Figure 1B and Table 3). The tip of the T7 loop in this mutant was shortened from the sequence SGEVN of \( \Delta T_{7GAN} \) to GAG.
Furthermore, ΔT7\textsubscript{GAN}-GDP in which the important residue for GTPase activity (Asn208) is retained also showed the same change (Figure 1A and Table 3). Thus, the length of the T7 loop seems to be very important for the SaFtsZ-type structure. Although ΔT7\textsubscript{GAN}-GDP and ΔT7\textsubscript{GAN}-GDP exhibited crystal packing with polymer-like molecular associations, their interactions were rather loose, consistent with the observation that they showed no GTPase activity. The T7 loop in the “upper” molecule was not deeply inserted into the nucleotide-binding pocket of the “lower” molecule (Figure 2A). Moreover, the S9 strand and H10 helix in the “upper” molecule were distant from the C-terminal subdomain in the “lower” molecule due to the overall conformational change (Figure 2A). Thus, the interaction surfaces between adjacent molecules in the crystals of these ΔT7 mutants were markedly reduced.

ΔT7\textsubscript{GAN} was also crystallized in GTP-bound form (ΔT7\textsubscript{GAN}-GTP). Interestingly, while the structure of ΔT7\textsubscript{GAN}-GDP was changed to the BsFtsZ-type, ΔT7\textsubscript{GAN}-GTP showed features closer to SaFtsZ (Table 3). The H7 helix in ΔT7\textsubscript{GAN}-GTP had slid down by about one helical pitch with regard to the BsFtsZ-type structure. Two residues in the H7 helix (Phe183 and Asp187) interacted with the guanine ring in GDP form, but only one residue (Phe183) retained stacking interactions with the guanine ring in ΔT7\textsubscript{GAN}-GTP. To our knowledge, this is the first observation of a nucleotide-dependent large conformational change of FtsZ; previous crystallographic studies did not detect any significant structural differences between FtsZ-GDP and FtsZ-GTP in the monomeric form (22). In the present study, we did not observe a major conformational difference between the GTP\textsubscript{S} complex and GDP complex determined previously (23) (r.m.s.d. of 0.59 Å, Figure 4). The crystal structures of tubulin bound with GDP and GTP also showed no conformational change due to nucleotide exchange (41).

Previous molecular dynamics simulation analysis in the monomeric form suggested that GDP to GTP exchange invokes a subtle conformational change in the nucleotide-binding pocket that tends to align the top portion of the H7 helix along the longitudinal axis of the protein (42). The conformational changes observed in ΔT7\textsubscript{GAN}-GDP and ΔT7\textsubscript{GAN}-GTP were, although more significant, somewhat in accordance with this prediction. The conformational change induced by GDP to GTP exchange should be attributed primarily to the γ-phosphate and Mg\textsuperscript{2+} ion in the nucleotide-binding pocket. However, the present crystal structure suggests that addition of γ-phosphate does not seem to have such a large effect directly on the FtsZ structure within one molecule. This large conformational change may be induced via interactions with the T7 loop of the second molecule.

The γ-phosphate interacted with the catalytic residues (Asp210 and Asp213) in the T7 loop and H8 helix of the “upper” molecule via water molecules in our GTP-bound model of SaFtsZ (23). This model was confirmed by the structure of SaFtsZ bound with GTP\textsubscript{S} (Figure 4A). The crystal packing of ΔT7\textsubscript{GAN}-GTP showed a similar head-to-tail association as seen in SaFtsZ. The crystal structure of ΔT7\textsubscript{GAN}-GTP also had interactions between γ-phosphate and the side chains of Asp210 and Asp213 via water molecules (Figure 5B). These interactions between the T7 loop and H8 helix in the “upper” molecule and the residues around the nucleotide-binding pocket in the “lower” molecule were not detected in the crystal of ΔT7\textsubscript{GAN}-GDP (Figures 2 and 5). Thus, it is likely that nucleotide exchange (addition of γ-phosphate) exerts an influence on the “upper” molecule via interaction with the T7 loop and induces the change in the subdomain orientation of the “upper” molecule, which leads to polymerization and GTP hydrolysis.

The structure of ΔT7\textsubscript{GAN}-GTP shows that the start and end residues of the T7 loop in ΔT7\textsubscript{GAN}-GTP were located at similar positions to those of the wild-type. Despite the similar molecular associations in the polymer, the interactions at the tip of the T7 loop were very different between ΔT7\textsubscript{GAN}-GTP and the wild-type (Figures 4B and 5B). Due to the replacement of residues (204)SGEVN(208) with GAN at the tip of the T7 loop, the chain trace of the T7 loop was significantly changed and Asn208 occupied a very different position in ΔT7\textsubscript{GAN}-GTP from that in the wild-type (Figures 4B and 5). It is likely that conserved residues, such as Asn208, Asp210,
and Asp213, help the “upper” molecule interact with the bound γ-phosphate via the T7 loop and induce the conformational change, but because of truncation of the tip of the T7 loop, ΔT7GAN·GTP does not leave Asn208 at the proper position for GTP hydrolysis.

It may be worth mentioning that the downshift of C-terminal subdomain in ΔT7GAN·GTP was even larger than that in SaFtsZ (Figure 1D and Table 3). The larger shift of the C-terminal subdomain of ΔT7GAN·GTP may be attributed to truncation of the tip of the T7 loop, which leaves more empty space at the region surrounded by the T7 loop and S9 strand. To maximize van der Waals interactions, the C-terminal subdomain may slide down further. This may reflect the intrinsic nature of the FtsZ molecule to adjust itself by changing the subdomain movement.

In this study, we observed several distinct conformations by changing the amino residues in T7 loop of SaFtsZ. In the case of ΔT7GAN, we obtained two different conformations depending on the bound nucleotides. These results strongly suggested that the T7 loop triggers the whole structural change by rearranging the relative orientation between subdomains. It has been suggested that the T7 loop is a synergy loop with important roles not only for polymerization (19,40) but also for the molecular switch between straight and curved conformations induced by binding of GTP (11,12).

Recently, it was proposed that a hydrolysis-dependent conformational switch at the T3 loop of FtsZ leads to longitudinal bending between subunits (27). However, based on the results of the present study as well as on the structures deposited previously by other groups (PDB ID: 2RHL, 2RHO, 2Q1X, and 2Q1Y) (43,44), nucleotide exchange appears not to directly induce a structural change in the monomer, including the T3 loop. Rather, the present results suggest that the intermolecular interactions between bound GTP and the T7 loop of the second molecule induce the structural change in the molecule from the R (Relaxed) state to the T (Tense) state. This structural change allows more intimate interactions of the bound nucleotide with the catalytic residues of the second molecule in the straight polymer, resulting in GTP hydrolysis. After hydrolysis, the molecule returns to the R state and to the curved conformation (Figure 6). The reorganization of the FtsZ subdomains is transmitted from one monomer to the other, and thus the intramolecular change may affect the intermolecular conformation as seen in tubulin polymers (9,29).
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**FOOTNOTES**

†T.M. and X. H. contributed equally to this work.

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3 The abbreviations used are: BsFtsZ, FtsZ from *Bacillus subtilis*; EcFtsZ, FtsZ from *Escherichia coli*; GTPγS, guanosine 5′-O-(3-thiotriphosphate); SaFtsZ, FtsZ from *Staphylococcus aureus*.

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**FIGURE LEGENDS**

**FIGURE 1.** Structures of T7 mutants superposed on *BsFtsZ* (PDB ID: 2RHL mol A) (left) or *SaFtsZ* (PDB ID: 3VOA) (right). All pictures were drawn after superposing the N-terminal domain (residues 13 – 173). Note that *BsFtsZ* and *SaFtsZ* (shown in gray) have very different subdomain orientations; the C-terminal subdomain of *SaFtsZ* is downshifted by about one helical pitch along the central H7 helix. GDP and GTP bound to T7 mutants are represented as sky blue and pink sticks, respectively. GDPs bound to background molecules are represented as yellow sticks. Calcium and magnesium ions bound to T7 mutants are represented as magenta and deep blue spheres, respectively. (A) Δ*T7GAN*-GDP is shown in orange. (B) Δ*T7GAG*-GDP is shown in blue. (C) T7*Bs* is shown in green. (D) Δ*T7GAN*-GTP is shown in wheat.

**FIGURE 2.** Longitudinal interactions of Δ*T7GAN* in crystals. (A) Two molecules of Δ*T7GAN*-GDP are shown in orange and yellow with GDP in sky blue sticks. (B) Two molecules of Δ*T7GAN*-GTP are shown in wheat and gray. GTP and magnesium ion are represented as pink sticks and a deep blue sphere, respectively. (C) For comparison, two molecules of *SaFtsZ* bound with GDP (PDB ID: 3VOA) are also shown in red and salmon. GDP and calcium ion are represented as sky blue sticks and green sphere. Side chains of Asn25, Asp46, Gln48, Phe183, Asp187, Asn208, Asp210, and Asp213 are also shown as stick representations.

**FIGURE 3.** GTPase activities of FtsZ mutants given in the relative Pi values released. GTPase activities of *SaFtsZt* are in filled triangles as a control. T7*Bs*, Δ*T7GAG* and Δ*T7GAN* are represented as filled rectangles, open circles, and crosses, respectively. T7*Bs* was obtained in the soluble fraction only in the truncated form (Materials and methods). Therefore, the relative activity of T7*Bs* was calculated using *SaFtsZt* as a control, while Δ*T7GAG* and Δ*T7GAN* were calculated against *SaFtsZ*. The error bars represent
the relevant standard deviations from three independent experiments.

**FIGURE 4.** Close-up view of the GTPase site. (A) The “upper” and “lower” molecules of *Sa*FtsZ-GTP\_γS are shown in teal and cyan, respectively. (B) The “upper” and “lower” molecules of *Sa*FtsZ-GDP are shown in salmon and red, respectively. (C) Superposition of *Sa*FtsZ-GDP molecule (red) and *Sa*FtsZ-GTP\_γS molecule (cyan) showing that they have similar conformations. The side chains of Asn44, Asp46, Gln48, Phe183, Asn208, Asp210, and Asp213 are shown in stick representations. GTP\_γS, GDP, and calcium ion are represented as purple stick, blue stick, and green sphere, respectively.

**FIGURE 5.** Close-up views of longitudinal interactions in the crystal. Intermolecular interfaces of (A) ΔT7\_GAN-GDP and (B) ΔT7\_GAN-GTP are shown. (A) Two molecules of ΔT7\_GAN-GDP are shown in orange and yellow. (B) Two molecules of ΔT7\_GAN-GTP are shown in wheat and gray. GDP bound to ΔT7\_GAN-GDP, and GTP and a magnesium ion bound to ΔT7\_GAN-GTP are represented as sky blue stick, pink stick, and deep blue sphere, respectively. Side chains of Asn25, Asp46, Gln48, Phe183, Asp187, Asn208, Asp210, and Asp213 are shown as sticks.

**FIGURE 6.** Proposed mechanism of straight-to-curved conformational switch. (A) FtsZ bound with GDP is a soluble monomer in the R (Relaxed) state. (B) When FtsZ is bound to GTP, FtsZ is assembled. (C) The intermolecular interactions between bound GTP and the T7 loop of the second molecule induce a structural change of the molecule from the R to the T (Tense) state to form the straight protofilament. (D) In the straight protofilament, the catalytic residues in the upper subunit hydrolyze GTP to GDP and an intermediate state of FtsZ with GDP in the straight protofilament is formed. (E) After releasing γ-phosphate, the FtsZ molecule returns to the R state, and the straight protofilament changes to a curved protofilament. Finally, the curved protofilament is disassembled to monomeric FtsZ.
Table 1. Summary of data collection and refinement statistics

| PDB code | ΔT7\textsubscript{GAG-GDP} | ΔT7\textsubscript{GAN-GDP} | ΔT7\textsubscript{GAN-GTP} | T7\textsubscript{Bs} | SaFtsZ-GTP\textsubscript{γS} |
|----------|---------------------------|---------------------------|---------------------------|---------------------|---------------------------|
| 3WGK     | 3WGL                      | 3WGM                      | 3WGJ                      | 3WGN                |                           |

**Sample conditions**

| Crystallization conditions | ΔT7\textsubscript{GAG-GDP} | ΔT7\textsubscript{GAN-GDP} | ΔT7\textsubscript{GAN-GTP} | T7\textsubscript{Bs} | SaFtsZ-GTP\textsubscript{γS} |
|---------------------------|---------------------------|---------------------------|---------------------------|---------------------|---------------------------|
| 0.2 M Sodium Acetate      | 0.1 M Sodium HEPES pH 7.2 | 0.1 M tri-Sodium citrate  | 0.1 M Lithium chloride   | 0.1 M Sodium Isopropanol acetate |
| 0.1 M Tris pH 8.5          | 7.2                       | 7.5                       | 20%(w/v)                 | 30%(w/v)            |
| 30%(w/v) PEG4000          | 27.5%(w/v) PEG2000        | 20%(w/v)                  | 10%(v/v)                 |
| 15% (v/v) ethylene glycerol| 15% (v/v) ethylene glycerol| 15% (v/v) ethylene glycerol| 10% (v/v) ethylene glycerol|

**Data Collection**

| Beamline | Wavelength (Å) | Space group | Unit cell parameters |
|----------|----------------|-------------|---------------------|
| SPring-8 BL41XU | 1.0000       | P2\textsubscript{1} | a, b, c (Å) |
| PF BL1A     | 1.1000       | P2\textsubscript{1} | 44.3, 95.9, 72.1 |
| SPring-8 BL41XU | 1.0000       | P2\textsubscript{1} | 44.9, 97.2, 72.6 |
| SPring-8 BL41XU | 1.0000       | P1          | 44.5, 89.6, 66.6 |
| PF BL17A   | 0.9800       | P1          | 44.0, 43.9, 86.5 |
|           |               |             | 44.3, 44.2, 73.3 |
|           |               |             | α, β, γ (°)       |
|           | β = 100.9     | β = 102.4    | 96.9, 102.3, 109.4 |
|           |               | β = 96.1     | 82.8, 82.8, 82.3 |

10% (v/v) glycerol

5 mM GTP\textsubscript{γS}
**T7 loop controls FtsZ structure and functions**

| Resolution range (Å) | 50.0 – 2.80 | 50.0 – 3.07 | 50.0 – 2.10 | 50.0 – 2.18 | 50.0 – 2.61 |
|----------------------|--------------|--------------|--------------|--------------|--------------|
| (Å)                  | (2.90 – 2.80) | (3.25 – 3.07) | (2.22 – 2.10) | (2.22 – 2.18) | (2.76 – 2.61) |

| Completeness (%) | 99.7 (99.7) | 99.0 (97.9) | 98.4 (95.1) | 98.1 (96.2) | 98.4 (96.7) |
|------------------|--------------|--------------|--------------|--------------|--------------|

| Completeness (%) | 99.7 (99.7) | 99.0 (97.9) | 98.4 (95.1) | 98.1 (96.2) | 98.4 (96.7) |
|------------------|--------------|--------------|--------------|--------------|--------------|

| Completeness (%) | 99.7 (99.7) | 99.0 (97.9) | 98.4 (95.1) | 98.1 (96.2) | 98.4 (96.7) |
|------------------|--------------|--------------|--------------|--------------|--------------|

| Completeness (%) | 99.7 (99.7) | 99.0 (97.9) | 98.4 (95.1) | 98.1 (96.2) | 98.4 (96.7) |
|------------------|--------------|--------------|--------------|--------------|--------------|

| Completeness (%) | 99.7 (99.7) | 99.0 (97.9) | 98.4 (95.1) | 98.1 (96.2) | 98.4 (96.7) |
|------------------|--------------|--------------|--------------|--------------|--------------|

| <I/σ(I)> (%) | 36.3(3.6)* | 10.22 (1.90) | 8.13 (1.98) | 19.9 (5.0)* | 11.8 (2.4) |
|---------------|-------------|--------------|-------------|-------------|-------------|

| R<sub>merge</sub> (%) | 9.3(43.0) | 15.1 (75.5) | 11.8 (77.8) | 14.1 (38.9) | 9.1 (61.0) |
|------------------------|------------|--------------|-------------|-------------|-------------|

| Multiplicity | 3.8 (3.7) | 3.4 (3.5) | 3.8 (3.8) | 2.8 (2.2) | 3.8 (3.8) |
|--------------|-----------|----------|---------|--------|--------|

| No. of observed reflections | 55,808 | 38,611 | 116,012 | 81,417 | 62,356 |
|-----------------------------|-------|--------|--------|-------|-------|

| No. of unique reflections | 14,645 | 11,397 | 30,298 | 29,546 | 16,267 |
|---------------------------|--------|--------|--------|--------|--------|

**Refinement**

| Model for MR | 2VXY | 3WGK | 3VOA | 3VOA | 3VOA |
|--------------|------|------|------|------|------|

| Resolution (Å) | 37.45 – 2.80 | 48.58 – 3.07 | 44.82 – 2.09 | 40.49 – 2.18 | 43.5 – 2.61 |
|----------------|--------------|--------------|--------------|-------------|-------------|

| Overall R<sub>work</sub> (%) | 20.0 | 20.9 | 19.3 | 18.5 | 22.8 |
|-------------------------------|------|------|------|------|------|

| Overall R<sub>free</sub> (%) | 24.3 | 26.5 | 22.7 | 23.5 | 28.0 |
|-------------------------------|------|------|------|------|------|

| Total atoms | 4434 | 4433 | 4437 | 4589 | 4450 |
|-------------|------|------|------|------|------|

| No. of protein atoms | 4378 | 4377 | 4330 | 4388 | 4386 |
|----------------------|------|------|------|------|------|

| No. of ligand atoms | 56 | 56 | 64 | - | 64 |
|---------------------|---|---|---|---|---|

| No. of metal atoms | - | - | 2 | 2 | - |
|--------------------|---|---|---|---|---|

| No. of solvent atoms | - | - | 41 | 199 | - |
|----------------------|---|---|----|----|---|

| Average B-factors (Å<sup>2</sup>) | | | | | |
### Protein Structure Analysis

| Component          | Protein Atoms | Ligand Atoms | Metal Atoms | Solvent Atoms | RMSD from Ideal Bond Lengths (Å) | RMSD from Ideal Bond Angles (°) |
|--------------------|---------------|--------------|-------------|---------------|---------------------------------|---------------------------------|
|                    | 76.1          | 66.1         | -           | -             | 0.005                           | 0.956                            |
|                    | 42.2          | 34.9         | -           | -             | 0.004                           | 0.911                            |
|                    | 22.9          | 17.2         | -           | -             | 0.005                           | 1.023                            |
|                    | 29.8          | -            | 21.5        | -             | 0.011                           | 1.189                            |
|                    | 38.2          | -            | -           | -             | 0.012                           | 1.591                            |

### Ramachandran Plot

| Region              | Favored Region | Allowed Region | Outlier Region |
|---------------------|----------------|----------------|----------------|
|                     | 582 (96.4%)    | 22 (3.6%)      | 0 (0%)         |
|                     | 596 (98.8%)    | 7 (1.2%)       | 0 (0%)         |
|                     | 594 (99.3%)    | 4 (0.7%)       | 0 (0%)         |
|                     | 592 (98.0%)    | 12 (2.0%)      | 0 (0%)         |
|                     | 583 (96.5%)    | 21 (3.5%)      | 0 (0%)         |

*Values in parentheses are for the highest resolution shells.

$^1 R_{merge} = \sum_h \sum_j |<I>-I_{h,j}|/\sum_h \sum_j I_{h,j}$, where $<I>$ is the mean intensity of symmetry-equivalent reflections.

$^2 R_{work} = \sum |F_{obs} - F_{cal}| / \sum F_{obs}$, where $F_{obs}$ and $F_{cal}$ are observed and calculated structure factor amplitudes, respectively.

$^3 R_{free}$ value was calculated for R factor, using only an unrefined subset of reflection data.

$^4$ Diffraction data were processed and scaled with the HKL-2000 program package (24), others were processed and scaled with XDS (31).
Table 2. Crystals of $\Delta T7_{GAN}$

| Sample conditions | $\Delta T7_{GAN}$ | $\Delta T7_{GAN}$-GDP | $\Delta T7_{GAN}$-GTP |
|-------------------|-------------------|-----------------------|-----------------------|
| **Crystallization conditions** | 0.1 M Sodium HEPES pH 6.8 | 0.1 M Sodium HEPES pH 7.2 | 0.1 M Sodium HEPES pH 7.5 |
| PEG2000MME | 27.5% (w/v) | 30% (w/v) | 27.5% (w/v) |
| **Cryoprotectant** | 15% (v/v) ethylene glycerol | 15% (v/v) ethylene glycerol | 15% (v/v) ethylene glycerol |
| **Data Collection** | PF BL17A | PF BL1A | SPring-8 BL41XU |
| **Wavelength (Å)** | 1.0000 | 1.1000 | 1.0000 |
| **Space group** | $P2_1$ | $P2_1$ | $P2_1$ |
| **Unit cell parameters** | a, b, c (Å) | 43.8, 88.0, 68.6 | 44.9, 97.2, 72.6 | 44.5, 89.6, 66.6 |
| $\alpha, \beta, \gamma$ (°) | $\beta = 98.5$ | $\beta = 102.4$ | $\beta = 96.1$ |
Table 3. RMS conformational (left side) and rotational (right side) differences between each pair of structures. \(^{a,b,c}\)

|                 | BsFtsZ | \(\Delta T7_{GAN}^{\text{GDP}}\) | \(\Delta T7_{GAG}^{\text{GDP}}\) | SaFtsZ | T7\(_{Bs}\) | \(\Delta T7_{GAN}^{\text{GTP}}\) |
|-----------------|--------|-------------------------------|-------------------------------|--------|-----------|-------------------------------|
| \(\Delta T7_{GAN}^{\text{GDP}}\) | 3.10 Å  | -                             | N/D \(^d\)                   | 25.1°  | 25.1°     | 25.1°                         |
| \(\Delta T7_{GAG}^{\text{GDP}}\) | 3.21 Å  | 0.74 Å                        | -                             | 26.5°  | 25.7°     | 21.7°                         |
| SaFtsZ          | 7.21 Å  | 4.77 Å                        | 4.96 Å                       | -      | N/D \(^d\) | N/D \(^d\)                   |
| T7\(_{Bs}\)     | 7.54 Å  | 4.90 Å                        | 5.18 Å                       | 0.39 Å | -         | N/D \(^d\)                   |
| \(\Delta T7_{GAN}^{\text{GTP}}\) | 8.47 Å  | 5.69 Å                        | 5.95 Å                       | 1.88 Å | 1.80 Å    | -                             |

\(^a\) The conformational differences were calculated by the backbone \(C\alpha\) coordinates of C-terminal subdomain (residues 223 – 310) after superposition of the N-terminal subdomain (residues 13 – 173).

\(^b\) The conformational and rotational differences were determined using PyMOL (37) and DynDom (38), respectively.

\(^c\) SaFtsZ is the GDP-bound form of FtsZ from *Staphylococcus aureus* (PDB ID: 3VOA) and BsFtsZ is the GDP-bound form of FtsZ from *Bacillus subtilis* (PDB ID: 2RHL).

\(^d\) N/D means no dynamic domains were found by DynDom (38).

\(\Delta T7_{GAN}^{\text{GDP}}\) and \(\Delta T7_{GAG}^{\text{GDP}}\) pair and SaFtsZ and T7\(_{Bs}\) pair are practically identical as shown by the values in rectangles.
FIGURE 1

A $\Delta T7_{GAN}$ GDP

B $\Delta T7_{GAC}$ GDP

C $T7_8$s

D $\Delta T7_{GAN}$ GTP

Superposed on B$sFtsZ$

Superposed on S$aFtsZ$
FIGURE 3
FIGURE 4

A SaFtsZ-GTP$_\gamma$S

B SaFtsZ

C GTP$_\gamma$S, GDP

Ca T7 loop
FIGURE 5

A \( \Delta T7_{GAN} \)-GDP

B \( \Delta T7_{GAN} \)-GTP
**FIGURE 6**

A. monomer

- H6-H7 loop
- C-terminal subdomain
- N-terminal subdomain
- GTP
- GDP

B. assembled

C. straight protofilament

D. intermediate state

E. curved protofilament

free phosphate

hydrolysis
Structural change in FtsZ induced by intermolecular interactions between bound GTP and the T7 loop.
Takashi Matsui, Xuerong Han, Jian Yu, Min Yao and Isao Tanaka

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