Chapter 3

Non-hydrolysable GTP-γ-S stabilizes the FtsZ polymer in a GDP-bound state

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Summary

FtsZ, a tubulin homologue, forms a cytokinetic ring at the site of cell division in prokaryotes. The ring is thought to consist of polymers that assemble in a strictly GTP-dependent way. GTP, but not guanosine-5’-O-(3-thiotriphosphate) (GTP-γ-S), has been shown to induce polymerization of FtsZ, whereas in vitro Ca²⁺ is known to inhibit the GTP hydrolysis activity of FtsZ. We have studied FtsZ dynamics at limiting GTP concentrations in the presence of 10 mM Ca²⁺. GTP and its non-hydrolysable analogue GTP-γ-S bind FtsZ with similar affinity, whereas the non-hydrolysable analogue guanylyl-imidodiphosphate (GMP-PNP) is a poor substrate. Preformed FtsZ polymers can be stabilized by GTP-γ-S and are destabilized by GDP. As more than 95% of the nucleotide associated to the FtsZ polymer is in the GDP form, it is concluded that GTP hydrolysis by itself does not trigger FtsZ polymer disassembly. Strikingly, GTP-γ-S exchanges only a small portion of the FtsZ polymer-bound GDP. These data suggest that FtsZ polymers are stabilized by a small fraction of GTP-containing FtsZ subunits. These subunits may be located either throughout the polymer or at the polymer ends, forming a GTP-cap similar to tubulin.
Introduction

FtsZ is a key protein in the cell division process of *Escherichia coli* and other prokaryotes, forming a structural element known as the Z-ring at the site of cell division (for reviews see (18, 84, 85, 123). The Z-ring assembles more or less simultaneously with the termination of DNA replication, well before constriction of the cell wall is visible by microscopy (36), constricts during invagination of the cell envelope and disappears when cell division is completed. The Z-ring is critical for the localization of other protein components of the cell division machinery to the division site. These include FtsA (5, 89), FtsI and FtsW (143, 154), FtsN (3), FtsK (163), FtsQ (20, 27) and FtsL (56). The Z-ring is likely to be tethered to the cytoplasmic membrane by ZipA (58).

Recently, it was suggested that the Z-ring serves as a track for the motor protein MukB involved in chromosome partitioning (76).

FtsZ is a GTPase (34) (117) (100) with limited but significant sequence homology to tubulins (47). The structural similarities between *α* and *β*-tubulins and FtsZ (106, 108) (77, 78) suggest that FtsZ is a prokaryotic homologue of tubulin. In *vitro*, FtsZ is able to form filaments in a GTP-dependent manner which are thought to resemble the structure of the Z-ring in *vivo* (19) (101) (51). In the absence of polymerization promoting agents, such as DEAE-dextran, polymerization of FtsZ is strictly dependent on GTP and turnover is regulated by GTP hydrolysis (102, 104). The formation of FtsZ polymers is influenced by the presence of millimolar amounts of Ca$^{2+}$ (160).

Binding of GTP-analogues to FtsZ

FtsZ polymerization is strictly GTP dependent (102, 104), and cannot be induced by non-hydrolysable GTP analogue guanosine-5′-O-(3-thiotriphosphate) (GTP-γ-S) (19, 160). Only in the presence of DEAE-dextran is GDP capable of inducing polymerization (51, 101). GDP binds to FtsZ with a twofold lower affinity than GTP (100). The polymeric form of FtsZ predominantly contains bound GDP. Strikingly, most of the FtsZ remains in the GDP bound state when the polymers are stabilized by GTP-γ-S. The results are discussed in terms of a model in which FtsZ polymers are stabilized by a GTP cap.

Results
and 1D), whereas GTP-γ-S (Fig. 1B and 1D) was as effective as a competitor as GTP (Fig. 1A and 1D). These results suggest that FtsZ binds GTP-γ-S and GTP with almost similar affinities, while GMP-PNP is not recognized as a substrate.

To ensure that GTP-γ-S is not hydrolysed by FtsZ, the protein was incubated at 30 °C with GTP-γ-[35S] or [α-32P]-GTP in the presence and absence of 10 mM Ca2+. Hydrolysis was assessed by means of thin layer chromatography. Although substantial hydrolysis of [α-32P]-GTP could be detected, no hydrolysis of GTP-γ-[35S] occurred for the duration of the experiments described in the following sections (not shown). GTP-γ-S is therefore a true non-hydrolysable GTP analogue for FtsZ.

**FtsZ polymers are stabilized by GTP-γ-S and destabilized by GDP**

Polymerization of the FtsZ homologue tubulin can be driven by non-hydrolysable GTP analogues such as guanylyl-(α,β)-methylene-diphosphonate (GMPCPP) and GMP-PNP (ref. 37, and references therein), and tubulin polymers are stabilized by GDP in the absence of GTP (24). We used light scattering at a 90° angle (104) to study the effect of GDP and GTP-γ-S on the stability of the GTP-induced FtsZ polymers. First, reaction conditions were determined that allow monitoring of FtsZ polymer formation at limiting amounts of GTP. In the presence of 10 mM Ca2+, FtsZ polymers are stabilized because of the inhibition of GTP hydrolysis (104). Therefore, FtsZ dynamics were followed at GTP concentrations of 10-200 µM in the presence of 10 mM Ca2+ (Fig. 2). Polymers assembled rapidly at all GTP concentrations tested. In the absence of Ca2+, the light scattering was markedly reduced (not shown), confirming the observation of Mukherjee and Lutkenhaus (104). At GTP concentrations above 100 µM, FtsZ polymers persisted for at least 10 min (Fig. 2, traces 1 and 2). At lower GTP concentrations, a transient pattern was observed (Fig. 2, traces 3-5), indicative for a rapid polymerization reaction followed by slow depolymerization. To follow the depolymerization dynamics in further experiments, a concentration of GTP (20 µM) was used that was in slight excess to FtsZ (12.5 µM). Next, GTP, GDP or GTP-γ-S were added at various concentrations after 70 sec of polymerization, i.e. at the maximal scattering signal and just before the onset of substantial polymer disassembly. Addition of buffer alone
GTP-γ-S stabilizes FtsZ polymers

Addition of GTP to preformed polymers led to an increase in light scattering and a prolonged polymer persistence (not shown). Addition of GTP-γ-S also led to prolonged polymer persistence (Fig. 3A), and this phenomenon was maximal at 50 µM GTP-γ-S. At 200 µM, GTP-γ-S even caused an increase in the light scattering, indicative of either slow polymer growth or enhanced polymer bundling. GTP-γ-S-stabilized polymers remained stable for at least 3 h (not shown), whereas addition of GTP-γ-S to FtsZ in the absence of GTP did not induce polymerization (compare Fig. 5). In contrast to GTP-γ-S, GDP caused a rapid loss of the light scattering signal, indicating that GDP promotes polymer disassembly (Fig. 3B). These data suggest that, unlike microtubules (Carlier and Pantaloni, 1978), FtsZ polymers cannot be stabilized with GDP.

To confirm our observation that FtsZ polymers can be stabilized by GTP-γ-S, polymer presence was verified by electron microscopy and sedimentation analysis. FtsZ was polymerized with 20 µM GTP for 2 min. Subsequently GTP, GDP or GTP-γ-S were added at 200 µM. For electron microscopy, the samples were processed after an additional 15 min of incubation at 30 °C. In the presence of 200 µM GTP (Fig. 4A) or GTP-γ-S (Fig. 4B), bundles of FtsZ polymers were clearly visible by negative stain electron microscopy. Such structures were not observed when 200 µM GDP was added (Fig. 4C). The dots with a diameter of about 150 nm, visible after the addition of GDP, resemble the structures observed with FtsZ expressed in Chinese Hamster Ovary cells, which have a diameter of 200-500 nm as visualized by labelling with fluorescent secondary antibodies (162). These dots may represent FtsZ aggregates that cannot be recovered by sedimentation. Similar dots were also observed when FtsZ depolymerization in the presence of Ca2+ was monitored with differential interference contrast microscopy (M. Dogterom and D.J. Scheffers, unpublished). For sedimentation analysis, the samples were transferred to centrifuge tubes and the FtsZ polymers were sedimented and analyzed by SDS-PAGE, Coomassie brilliant blue staining and densitometric quantification. Although FtsZ polymers could be pelleted after the addition of 200 µM GTP or GTP-γ-S, hardly any polymers could be detected in the samples that had received 200 µM GDP (Table 1). Both methods independently confirm that the addition of GTP-γ-S causes the stabilization of preformed FtsZ polymers.

GTP-γ-S acts as trap for GTP-induced FtsZ polymers

Although GTP-γ-S and GDP are not capable of inducing FtsZ polymer formation (19) (160) (102, 104), our results indicate that the presence of these nucleotides affects the polymerization induced by GTP. This was tested by the addition of 20 µM GTP to FtsZ (12.5 µM) incubated with GTP-γ-S or GDP at various concentrations. Pre-incubation with GTP-γ-S or GDP for 70 s (Fig. 5) or longer (not shown), did
not result in any significant level of light scattering. Polymerization was induced by the subsequent addition of 20 µM GTP. The rate of polymerization decreased whereas the persistence of the FtsZ polymers increased with the concentration of GTP-γ-S present (Fig. 5). GDP, on the contrary, blocked the induction of polymerization by GTP in a concentration-dependent manner (Fig. 5). These data indicate that GTP-γ-S: (i) competes with GTP for FtsZ binding and subsequent polymerization and (ii) stabilizes the GTP-induced FtsZ polymers by trapping the protein in the polymerized state.

To assess the effect of GDP and GTP-γ-S on the nucleotide-bound state of the FtsZ polymers, [α-32P]-GTP was used to polymerize FtsZ, and the fate of the radioactive label was studied. FtsZ-bound nucleotide was separated from the free nucleotide by a rapid ammonium sulphate precipitation step, followed by nucleotide extraction using perchloric acid. Bound nucleotides were analyzed by thin layer chromatography (TLC). A control experiment with BSA showed low-level background nucleotide precipitation compared to FtsZ (Fig. 6B, protein fraction, P), independent of the

**FtsZ polymers contain bound GDP that is retained in the presence of GTP-γ-S**

Figure 3. Stabilization of FtsZ polymers by GTP-γ-S. Light scattering was performed as described in the text. FtsZ (12.5 µM) was incubated in polymerization buffer at 30 °C and the baseline was recorded for 180 sec. GTP (20 µM) was added to induce polymer formation as indicated by an asterisk. After 70 s of polymerization, GTP-γ-S (A) or GDP (B) was added to the solution (at point #) at the concentration indicated (µM).

Figure 4. Electron microscopy of stabilized FtsZ polymers. FtsZ (12.5 µM) was incubated in polymerization buffer at 30 °C with 20 µM GTP. After 2 min, GTP (A), GTP-γ-S (B), or GDP (C) was added at a concentration of 200 µM. After 15 min of incubation at 30 °C, the material was further processed for the electron microscopy. Bar, 100 nm.
GTP-$\gamma$-S stabilizes FtsZ polymers

Table 1. The effect of the addition of various nucleotides on preformed FtsZ polymers.

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>FtsZ pelleted (pmol)</th>
<th>$[\alpha^{32}\text{P}]-\text{GDP}$ bound (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>36 ± 11</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>GTP</td>
<td>279 ± 65</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>GTP-$\gamma$-S</td>
<td>290 ± 34</td>
<td>77 ± 14</td>
</tr>
<tr>
<td>GDP</td>
<td>38 ± 58</td>
<td>14 ± 3</td>
</tr>
</tbody>
</table>

FtsZ (12.5 µM, 625 pmol total) was polymerized with 20 µM $[\alpha^{32}\text{P}]-\text{GTP}$ in polymerization buffer at 30 °C. After 2 min, FtsZ polymers were chased with 200 µM cold nucleotide. After a 5 min incubation, polymers were sedimented in an air-driven ultracentrifuge for 10 min. The amount of protein and radioactivity in the pellet was determined as described in Experimental procedures. Values are the results of three independent experiments with indicated standard errors of the means. The non-specific nucleotide sedimentation amounted to 1.6 pmol $[\alpha^{32}\text{P}]-\text{GTP}$ and was subtracted from the values.

presence of Ca$^{2+}$ (not shown). GTP hydrolysis was observed only in the presence of FtsZ (Fig. 6B, solution fraction, S). After incubation with 20 µM $[\alpha^{32}\text{P}]-\text{GTP}$ for 15 s or 2 min, a large fraction of the nucleotide could be co-precipitated with FtsZ (Fig. 6A), which was predominantly (more than 95%) $[\alpha^{32}\text{P}]-\text{GDP}$. As under these conditions a large fraction of the FtsZ present is polymerized, we conclude that most of the polymerized FtsZ is in the GDP-bound state. Recovery of FtsZ-bound $[\alpha^{32}\text{P}]-\text{GTP}$ was possible under conditions at which the hydrolysis of GTP was prevented by omission of Ca$^{2+}$ and Mg$^{2+}$ from the reaction mixture and the presence of EDTA and EGTA (not shown).

Next, the FtsZ polymers formed with 20 µM radiolabelled GTP were chased after 2 min with 200 µM cold nucleotide. In a control using buffer, the bound $[\alpha^{32}\text{P}]-\text{GDP}$ was retained by FtsZ for at least 10 min (Fig. 6A). Addition of GDP or GTP (Fig. 6A) resulted in the release of 65 % and 60 %, respectively, of the bound $[\alpha^{32}\text{P}]-\text{GDP}$ after 10 min. Surprisingly, in the presence of excess GTP-$\gamma$-S, only a small fraction (15 %) of the FtsZ-bound $[\alpha^{32}\text{P}]-\text{GDP}$ was released after 10 min (Fig. 6A). As this assay does not discern between the polymerized and depolymerized form of FtsZ, the nucleotide bound state of FtsZ was also determined after sedimentation of the FtsZ polymers. Polymerization was induced with 20 µM $[\alpha^{32}\text{P}]-\text{GTP}$, followed by a chase with 200 µM cold nucleotide after 2 min. After 3 min, the FtsZ polymers were sedimented and analyzed for the bound radiolabelled nucleotide. With a GDP chase, hardly any FtsZ was found to sediment, but with GTP or GTP-$\gamma$-S, more than 40 % of the FtsZ could be recovered in the pellet fraction (Table 1). The GTP-$\gamma$-S chased sample contained significantly more bound $[\alpha^{32}\text{P}]-\text{GTP}$ than the GTP chased samples (Table 1). These experiments demonstrate that GTP-$\gamma$-S stabilizes the FtsZ polymer in the GDP-bound
state while GDP destabilizes the FtsZ polymer with free exchange of bound nucleotide.

Discussion

In this report, we show that preformed FtsZ polymers can be stabilized by the non-hydrolysable GTP analogue GTP-γ-S, and that polymerized FtsZ predominantly contains GDP as the associated nucleotide. Polymer stabilization by GTP-γ-S does not result in a major loss of the GDP associated with FtsZ polymers. Excess GDP promotes polymer disassembly, accompanied by a major exchange between the FtsZ-bound GDP and the free nucleotide pool. A similar exchange of FtsZ-bound GDP is found when polymers persist as a result of the addition of GTP, indicating polymer recycling. These findings have important implications for the mechanism of FtsZ polymerization and depolymerization. We hypothesize that FtsZ polymers consist of FtsZ subunits that contain GDP, and that these polymers are stabilized at their ends by a capping structure that contains GTP-bound FtsZ.

Various in vitro methods have provided evidence that FtsZ polymers are formed in a GTP-dependent manner and persist as long as GTP is present (102, 104, 160). Modulation of the GTP hydrolysis activity of FtsZ by changes in the salt composition, or the Mg²⁺ or Ca²⁺ concentration, is immediately reflected in the
time that polymers are stable (104). Apart from their effect on polymer persistence, Mg$^{2+}$ and Ca$^{2+}$ influence the appearance of FtsZ polymers. At 10 mM Mg$^{2+}$, the GTPase activity of FtsZ is reduced compared with 2.5 mM Mg$^{2+}$, and FtsZ filaments show slight bundling. With 10 mM Ca$^{2+}$, pronounced bundling of the FtsZ filaments occurs (104, 160). FtsZ filaments seem to have a tendency to form lateral associations in the presence of polyvalent cations, similar to other negatively charged polymers (133). At conditions of high GTP hydrolysis activity, polymer turnover may be too fast to permit filament bundling. The effect of Ca$^{2+}$ is probably two-fold, i.e. Ca$^{2+}$ reduces the GTPase activity of FtsZ and promotes lateral filament association into sheets and bundles (78).

Both structural data on the Methanococcus jannaschii FtsZ (77) and functional studies on E. coli FtsZ (161) have led to the suggestion that FtsZ is a Ca$^{2+}$-binding protein. Interestingly, FtsZ polymers can also be stabilized by the essential cell division protein ZipA, which shows structural and functional similarity to microtubule-associated proteins (MAPs) such as Tau, MAP2 and MAP4 (116). ZipA-stabilized polymers exhibit the bundling that is typically observed in the presence of 10 mM Ca$^{2+}$ (104, 116, 160). Although the in vivo relevance of the effect of Ca$^{2+}$ on FtsZ polymerization remains unresolved, Ca$^{2+}$ provides an important tool to modulate the FtsZ polymerization in vitro possibly by mimicking the role of ZipA. The presence of 10 mM Ca$^{2+}$ allows the detection of FtsZ polymers at limiting amounts of GTP. This tempoziation of FtsZ polymer dynamics enables the dissection of the various steps in the polymerization and depolymerization processes.

Our analysis shows that the subunits of the FtsZ polymer predominantly contain GDP. This suggests that GTP hydrolysis itself is not affected by Ca$^{2+}$. It has been proposed that GTP hydrolysis is not required for FtsZ polymerization, but is needed for depolymerization (102). As GTP hydrolysis occurs very rapidly under the conditions used to polymerize FtsZ, independent of the presence of Ca$^{2+}$, our data favour a model in which FtsZ polymerization triggers GTP hydrolysis similar to tubulin (81). The effect of Ca$^{2+}$ on the turnover of GTP may be the result of a reduced nucleotide exchange rate caused by filament bundling.

Our data show that GTP hydrolysis does not initiate FtsZ depolymerization. Because the polymerized FtsZ contains GDP, although the polymers do not persist in the absence of GTP and cannot be formed with GDP alone, it appears that polymers are stabilized by some structure that contains GTP. The presence of such a stabilising structure is also suggested by the observation that FtsZ polymers become stabilized by GTP-$\gamma$-S. GTP-$\gamma$-S is not capable of inducing polymerization and cannot be hydrolysed. It binds FtsZ with an affinity similar to that of GTP, and when it interacts with a preformed FtsZ polymer, it may lock the subunits in a conformation that resembles the GTP-bound state. As GTP hydrolysis is required for depolymerization (102), these locked polymers will be unable to disassemble. Complete trapping of polymers in a sample occurs only at concentrations at which GTP-$\gamma$-S effectively competes with GTP. For instance, preincubation of FtsZ with GTP-$\gamma$-S results in slow polymerization after GTP addition (Fig. 5). The slowly growing polymers are eventually trapped by GTP-$\gamma$-S when the GTP runs out. The destabilising effect of GDP on preformed FtsZ polymers further suggests the presence of a GTP-containing stabilising structure. A high concentration of GDP immediately blocks the addition of GTP-bound FtsZ to the growing FtsZ polymers. As a consequence, the entire polymer will disassemble.

What would a GTP-containing stabilising structure look like? GTP-$\gamma$-S binding throughout the entire polymer may stabilize the FtsZ polymer. This possibility seems unlikely as polymers, stabilized with GTP-$\gamma$-S, do not readily exchange bound GDP for GTP-$\gamma$-S, as evidenced by the retention of bound GDP. With tubulin, the exchangeable GTP site on the
tubulin dimer (E site) is only accessible in the dimeric state, but not in the tubulin polymer (152) (93). Based on the structural similarities between microtubules and FtsZ filaments (106) (78), it may be expected that at least part of the nucleotide-binding sites in the FtsZ polymer would be inaccessible. Another possibility is that FtsZ polymers are stabilized at their ends by a GTP containing “cap”, similar to the capping model described for microtubules (96) for a review, see (37). This model seems more likely because the retention of most of the bound GDP by the FtsZ filaments demonstrates that FtsZ is trapped as a polymer that does not bind large amounts of GTP-\(\gamma\)-S. Either the polymer ends that still contain non-hydrolysed GTP are capable of exchanging the GTP for GTP-\(\gamma\)-S or FtsZ subunits containing GTP-\(\gamma\)-S are slowly added onto existing FtsZ polymers. FtsZ subunits with GTP-\(\gamma\)-S bound at the polymer ends would resemble FtsZ in the GTP-bound conformation and stabilize the entire polymer if FtsZ polymers disassemble from the ends like tubulin (37, and references therein). In this respect, the wealth of structural data showing the great similarity between FtsZ and tubulins (77, 78, 106-108) strongly suggests that both proteins have similar mechanisms of polymer formation and stabilization.

It has not been possible to stabilize the FtsZ polymers with GTP-\(\gamma\)-S in the absence of Ca\(^{2+}\). This is possibly caused by an altered (enhanced) rate of polymer turnover without Ca\(^{2+}\). Although the amounts of Ca\(^{2+}\) used in our experiments are above the concentration of free Ca\(^{2+}\) in vivo (0.1 to 1.0 \(\mu\)M, see ref 54), Ca\(^{2+}\) probably mimics an in vivo modulator of FtsZ polymerization and depolymerization. ZipA is a likely candidate for such a modulator, although it is not essential for formation of the Z-ring (57). ZipA localizes to the Z-ring immediately after its formation, but before the localization of FtsA (57). ZipA is present at the division site throughout the entire division process (58). In the cell, there are about 10 FtsZ molecules per ZipA molecule (58). ZipA binds to FtsZ (58) and stabilizes the FtsZ polymer (116). A model can be envisioned in which ZipA binds to the ends of small FtsZ polymers, reducing the rate of GTP hydrolysis and thus allowing the formation of a GTP cap. With small polymers, interfacial sliding of polymer ends can be envisioned, providing an active mode of ring constriction as proposed by the sliding protofilament model (18). Constant polymer turnover during cell division instead of polymer stabilization seems unlikely as it would require a substantial amount of GTP.

In conclusion, this work demonstrates that FtsZ polymers contain GDP and that a GTP-containing structure, which we propose to be a cap, stabilizes these polymers. The further analysis of the formation of this cap, its position within the FtsZ polymers and the modulation by ZipA, and the question how Z-ring constriction is mediated pose future experimental challenges.

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Experimental procedures

Protein purification

FtsZ was overproduced in *E. coli* BL21(DE3) (130) from plasmid pRRE6 (a generous gift from N. Nanninga, University of Amsterdam, the Netherlands), a derivative of pET11B (Novagen) with the *ftsZ* gene under control of the lac promoter. FtsZ was purified as described (102) with the following modification: after ammonium sulphate precipitation, the protein material was resuspended in buffer A (50 mM Tris/HCl, pH 7.9, 50 mM KCl, 1 mM EDTA, 0.1 mM PMSF, and 10 % glycerol) and loaded on a Source 30Q column (Amersham Pharmacia Biotech, bed volume 10 ml) equilibrated with buffer A. The column was washed with
Buffer A containing 100 mM KCl, and FtsZ was eluted with 100 ml of a linear gradient of 100 to 500 mM KCl. Fractions containing pure FtsZ (> 95%, eluting between 200-250 mM KCl) were pooled and dialysed against 50 mM HEPES/KOH, pH 7.2, 0.1 mM EDTA, 0.1 mM PMSF, and 10 % glycerol. Samples were aliquoted, frozen in liquid nitrogen and stored at –80 °C.

**GTP binding**

GTP binding was monitored by the covalent cross-linking of [α-32P]GTP (3000 Ci/mmol) to FtsZ as has been described previously (34). Purified FtsZ (3 µg) was incubated in 20 µl of 50 mM MES/NaOH, pH 6.5, 100 mM KCl, 4 mM MgCl2, 1 mM EDTA, 0.1 mM EGTA, and 0.5 mM DTT. To the suspension, 0.1 µM [α-32P]GTP and non-labelled nucleotides were added as indicated in the text. Samples were incubated 15 min at 0 °C, then subjected to photoaffinity cross-linking for 5 min at 0 °C using a 254-nm lamp at a distance of 2 cm. Samples were analyzed by SDS-PAGE on 12 % gels. Gels were dried using a 254-nm lamp at a distance of 2 cm. Samples were then incubated in 200 µM GTP, GDP or GTP-[γ-S], further incubated for 5 min and followed by sedimentation using an A-100 18° rotor in a Beckman airfuge at 28 psi for 10 min at room temperature. The amount of FtsZ present in the pellets was determined by SDS-PAGE, Coomassie Brilliant Blue staining and densitometric scanning. The amount of [α-32P]-GDP bound to sedimented FtsZ was determined by liquid scintillation counting.

**Sedimentation of FtsZ polymers**

The sedimentation of FtsZ polymers was carried out as described previously (102) with minor modifications. FtsZ (12.5 µM) was polymerized with 20 µM GTP (or [α-32P]-GTP, 33 Ci mol⁻¹) at 30 °C in 100 µl of polymerization buffer (50 mM MES/NaOH, pH 6.5, 50 mM KCl, 5 mM MgCl2, and 10 mM CaCl2). After 2 min, the polymers were chased with 200 µM GTP, GDP or GTP-[γ-S], further incubated for 5 min and followed by sedimentation using an A-100 18° rotor in a Beckman airfuge at 28 psi for 10 min at room temperature. The amount of FtsZ present in the pellets was determined by SDS-PAGE, Coomassie Brilliant Blue staining and densitometric scanning. The amount of [α-32P]-GDP bound to sedimented FtsZ was determined by liquid scintillation counting.

**Light Scattering**

Light scattering at 90° was measured using an Aminco Bowman Series 2 spectrometer (SLM Instruments). Both the excitation and emission wavelengths were set at 350 nm, with slit widths of 2 nm. FtsZ (12.5 µM) was incubated in 200 µl of polymerization buffer in a fluorescence cuvette with a 1 cm path length. The sample was maintained at 30°C. After 180 s of data collection, polymerization was induced with GTP at concentrations indicated. Subsequent additions of nucleotides were performed as described in the text, with sample volume increase below 2 %.

**Thin Layer Chromatography**

FtsZ (12.5 µM) in polymerization buffer was equilibrated at 30°C, and polymerization was induced with 20 µM of [α-32P]-GTP (33 Ci mol⁻¹). After 2 min, competing nucleotides were added to 200 µM. At various time intervals, samples of 200 µl were taken and were transferred to 200 µl ice-cold 75 % ammonium sulphate in 50 mM MES/NaOH, pH 6.5, 50 mM KCl. Samples were incubated on ice for at least 5 min to precipitate the FtsZ, which was recovered by centrifugation for 10 min at 20 000 g at room temperature. Pelleted FtsZ was resuspended in 30 µl of 50 mM MES/NaOH, pH 6.5, and 50 mM KCl. Bound nucleotides were extracted by the addition of 15 µl of 14 % (w/w) perchloric acid and 9 mM EDTA and incubation for 20 min on ice. The precipitated protein was removed by centrifugation for 5 min at 20 000 g at room temperature, and 45 µl of the supernatant was added to 22.5 µl of 1 M KHCO3 and 1 M KOH to adjust the pH of the samples. Samples were clarified by 3 min centrifugation at 20 000 g. Subsequently, 5 µl of the supernatant was spotted on CEL 300 PEI/UV254 plates (Macherey Nagel) and analyzed by TLC in 0.65 M KH2PO4/H3PO4, pH 3.5. Plates were dried and exposed to Kodak Biomax MR film. Autoradiographs were densitometrically scanned and analyzed using SigmaScan software (Jandel Corp).

**Electron Microscopy**

FtsZ (12.5 µM) was polymerized with 20 µM GTP at 30 °C in 100 µl of polymerization buffer. After 2 min the polymers were chased with 200 µM GTP, GDP, or GTP-[γ-S]. After 15 min of polymerization, a 3 µl aliquot of the polymerization reaction was applied on a 400 mesh carbon-coated glow-discharged grid for 2 min and blotted dry by placement of a filter paper to the side of the grid. The grid was subsequently negatively stained for 1 min by 3 µl of 1 % aqueous solution of uranyl acetate and blotted dry. The grids were viewed in a Philips 400T transmission electron microscope (a generous gift of A. Knoester, Shell Research and Technology Center Amsterdam).