Dynamic FtsZ polymerization is sensitive to the GTP to GDP ratio and can be maintained at steady state using a GTP-regeneration system

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In vitro polymerization of the essential bacterial cell division protein FtsZ, in the presence of GTP, is rapid and transient due to its efficient binding and hydrolysis of GTP. In contrast, the in vivo polymeric FtsZ structure which drives cell division – the Z-ring – is present in cells for extended periods of time whilst undergoing constant turnover of FtsZ. It is demonstrated that dynamic polymerization of Escherichia coli FtsZ in vitro is sensitive to the ratio of GTP to GDP concentration. Increase of GDP concentration in the presence of a constant GTP concentration reduces both the duration of FtsZ polymerization and the initial light-scattering maximum which occurs upon addition of GTP. It is also demonstrated that by use of a GTP-regeneration system, polymers of FtsZ can be maintained in a steady state for up to 85 min, while preserving their dynamic properties. The authors therefore present the use of a GTP-regeneration system for FtsZ polymerization as an assay more representative of the in vivo situation, where FtsZ polymers are subject to a constant, relatively high GTP to GDP ratio.

There have been multiple studies of the dynamics of FtsZ polymerization in vitro. In the presence of GDP (Rivas et al., 2000, 2001; Sossong et al., 1999) or in the presence of GTP under particular conditions where only single protofilaments are formed (Romberg et al., 2001), polymerization is proposed to be isodesmic. Under conditions where FtsZ forms more complex polymers, polymerization and the GTPase activity are co-operative (Caplan & Erickson, 2003; Mukherjee & Lutkenhaus, 1999; White et al., 2000).

The technique of right-angled light-scattering has proved useful for real-time monitoring of FtsZ polymerization (Mingorance et al., 2001; Mukherjee & Lutkenhaus, 1999; Romberg et al., 2001; Scheffers et al., 2000, 2002; White et al., 2000). In a typical assay using E. coli FtsZ, GTP provided at 40-fold molar excess is completely hydrolysed within 15 min, and depolymerization starts to occur within 8 min of addition of GTP (Mukherjee & Lutkenhaus, 1999). Since GTP hydrolysis has been shown to occur immediately upon FtsZ polymerization (Scheffers & Driessen, 2002), GDP levels will increase throughout the reaction, as GTP levels decrease. The effects of these changes in nucleotide concentration as the assay progresses are likely to be complex since GDP-bound and GTP-bound FtsZ molecules have different polymerization characteristics (Mukherjee & Lutkenhaus, 1999; Rivas et al., 2000, 2001; Romberg et al., 2001; Sossong et al., 1999). Indeed, previous work has demonstrated that addition of GDP in at least 10 times excess (compared to GTP concentration) causes rapid depolymerization of FtsZ/FtsZ2 co-polymers which are

INTRODUCTION

The FtsZ protein has a fundamental role in bacterial cell division (Lutkenhaus & Addinall, 1997), is highly conserved throughout the eu-bacteria and appears to be required for division of chloroplasts, some archaea and some mitochondria (Beech et al., 2000; Osteryoung & McAndrew, 2001; Vitha et al., 2001; Wang & Lutkenhaus, 1996). FtsZ is a cytoplasmic protein which, at a particular stage in the Escherichia coli cell cycle, locates to the cell centre, forming a polymeric ring (the Z-ring) around the inner circumference of the cytoplasmic membrane (Addinall et al., 1996; Bi & Lutkenhaus, 1991; Dai & Lutkenhaus, 1991; Den Blaauwen et al., 1999; Pla et al., 1991). Invagination of the division septum then follows the shape of the Z-ring as it reduces in diameter until septation is complete (Addinall & Lutkenhaus, 1996; Bi & Lutkenhaus, 1991, 1992). FtsZ is strongly related to α/β-tubulin in terms of three-dimensional structure (Lowe & Amos, 1998), the possession of GTPase activity (de Boer et al., 1992; Mukherjee et al., 1993; RayChaudhuri & Park, 1992) and the ability to polymerize in a nucleotide-dependent manner in vitro (Erickson et al., 1996; Mukherjee & Lutkenhaus, 1994). Indeed, FtsZ forms a variety of polymeric structures in vitro depending on experimental conditions (Erickson et al., 1996; Lowe & Amos, 1999, 2000; Mukherjee & Lutkenhaus, 1994; Yu & Margolin, 1997); however, all of these represent different arrangements of linear protofilaments.

Abbreviation: Z-ring, FtsZ ring.
otherwise relatively stable (Mukherjee et al., 2001). It has also been demonstrated that FtsZ polymers formed in the presence of 10 mM calcium (which have reduced dynamic properties and reduced GTPase activity) are destabilized by addition of GDP (Scheffers et al., 2000).

Observation of the dynamics of Z-rings suggests that the in vivo polymerization of FtsZ has different properties to that so far observed in vitro. Assembly of Z-rings can occur very quickly [e.g. less than 1 min (Addinall et al., 1997); 1–3 min (Sun & Margolin, 1998)], indicating rapid initial polymerization. Subunits within the ring turn over with an approximate half-life of 30 s (Stricker et al., 2002), thus the intact Z-ring is a very dynamic structure. However, Z-rings can be present for up to 50 min in actively dividing cells of E. coli K-12 (Den Blaauwen et al., 1999) and Z-rings in ftsI temperature-sensitive filamentous cells (which do not constrict and can persist for multiple generations; Addinall et al., 1996; Pogliano et al., 1997) were shown to be equally as dynamic as those in dividing cells (Stricker et al., 2002). Polymers in the Z-ring therefore undergo prolonged continuous, rapid turnover (Stricker et al., 2002) in the presence of a constant, relatively high ratio of cellular GTP concentration ([GTP]) to GDP concentration ([GDP]) (Neuhard & Nygaard, 1987).

In order to better understand the mechanism of in vivo polymerization of FtsZ, we have quantified the effect of alterations in the [GTP]/[GDP] ratio on dynamic FtsZ polymerization in vitro. We show that decreasing the [GTP]/[GDP] ratio reduces both the length of the FtsZ polymerization reaction (which we designate \(t_0\)) and the initial light-scattering maximum (delta). Our approach to quantification of FtsZ polymerization monitored using light-scattering is further validated by our ability to detect lengthening of \(t_0\) in the presence of GTP\(\cdot\)S. We have used a GTP-regeneration system to prolong the polymerization steady-state period, allowing maximal GTPase activity and polymerization at constant GTP concentration for periods of up to 85 min, whilst maintaining the dynamic nature of the polymers. Our assay therefore more closely mimics the apparent in vivo situation.

**METHODS**

**Strains and plasmids.** Strains of E. coli and plasmids used in this study are described in Table 1. Standard phage P1 transduction was used for strain construction as indicated.

**Protein purification and manipulation.** We have used three methods for purification of FtsZ from W3110 pKD126. The method of Mukherjee & Lutkenhaus (1998a) produced FtsZ with GTPase activity of 3–7 moles GTP hydrolysed per mole FtsZ per minute (mol mol\(^{-1}\) min\(^{-1}\)). However, we found this preparation to have co-purifying acetyl kinase activity (Fig. 2a). An alternative method (Lu & Erickson, 1998) yielded protein with a GTPase activity of 2–3 mol mol\(^{-1}\) min\(^{-1}\) which lacked co-purifying acetyl kinase activity (data not shown). We have combined these methods to purify FtsZ, which routinely has a GTPase activity of approximately 3 mol mol\(^{-1}\) min\(^{-1}\) and lacks a co-purifying acetyl kinase activity, as follows. The method of Mukherjee & Lutkenhaus (1998a) was used except (1) the sonicated lysate was incubated with DNase I at 10 \(\mu\)g ml\(^{-1}\) for 60 min at 4 °C, (2) the gel filtration step was omitted (and 3) pooled, dialysed DEAE column fractions were subjected to a 20 % ammonium sulphate cut which was discarded) before a final 30 % ammonium sulphate pellet was solubilised and dialysed into storage buffer. The 20 % ammonium sulphate cut removes the acetyl kinase activity from the FtsZ preparation and can be performed either before or after the DEAE chromatography (data not shown) with similar results. FtsZ concentration was measured with the Bio-Rad protein assay using BSA as a standard and a conversion factor of 0.82 as described by Lu & Erickson (1998).

**Light-scattering assay.** Our standard FtsZ polymerization assay is based on that of Mukherjee & Lutkenhaus (1999). Such polymerization has been described as ‘dynamic’ due to the rapid initial polymer formation, high GTPase activity and depolymerization in response to the reduction in [GTP] ([Mukherjee & Lutkenhaus, 1998b, 1999]). We also use the term dynamic to distinguish this assay from others where FtsZ is polymerized in the presence of high calcium ion concentration (e.g. Scheffers et al., 2000). This reduces the GTPase activity and increases the extent of polymer bundling (Mukherjee & Lutkenhaus, 1999). Right-angled light-scattering was measured at a constant 30°C, using a Jasco FP-750 Spectrofluorimeter (Fig. 2) or a Cary Eclipse Fluorescence Spectrophotometer (Figs 1 and 3; Table 2), with both excitation and emission wavelengths set at 350 nm. In both cases, a thermostattable cuvette holder was used. Unless stated otherwise, a 125 \(\mu\)l reaction consisting of 50 mM MES-NaOH (pH 6.5), 10 mM MgCl\(_2\), 50 mM KCl, 0.5 mM GTP and 8.3 \(\mu\)M FtsZ was initially made up omitting the GTP. This mixture was transferred to a Starna submicro fluorimeter cuvette.

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Genotype/derivation</th>
<th>Source</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Strains</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>W3110</td>
<td>(\lambda^-) IN(rrnD-rrnE1) rph-1</td>
<td>M. M. Khattar, The American University of Beirut, Lebanon</td>
<td>Bachmann (1972)</td>
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<td>RBB1095</td>
<td>(\mathrm{thi}^-\ \mathrm{thr}^-\ \mathrm{leuB6\ hisF159\ rpsL136\ lacY(G-155)}) DE3([lac]74 (\Delta(cheA\ cheW\ tar\ cheR\ cheB\ cheY\ cheZ):ZeoR\ ackA::Tn10\ phoA^-2)</td>
<td>M. Eisenbach, The Weizmann Institute of Science, Rehovot, Israel</td>
<td>Barak et al. (1998)</td>
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<tr>
<td>SGA79</td>
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<td>W3110 × P1 [RBB1095]</td>
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<td>Plasmid</td>
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<tr>
<td>pKD126</td>
<td>(\mathrm{ftsZ}) gene from E. coli cloned downstream of (\mathrm{tac}) promoter</td>
<td>A. Mukherjee, The University of Kansas Medical Center, USA</td>
<td>Dai et al. (1994)</td>
</tr>
</tbody>
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Table 1. Strains and plasmids used in this study
Table 2. Quantification of the effects of varying the ratio of GTP to GDP, ATP, ADP and GTP:S on FtsZ polymerization

Concentration of competitor nucleotide phosphate added along with 0.5 mM GTP is indicated in column 2. The \( t_0 \), delta and baseline parameters of light-scattering (Fig. 1b) are expressed as percentages of the GTP-only experiment (set to 100% in each experiment). Each value represents the mean of three independent experiments plus and minus standard error.

<table>
<thead>
<tr>
<th>Competitor</th>
<th>Conc (mM)</th>
<th>( t_0 ) (%)</th>
<th>Delta (%)</th>
<th>Baseline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDP</td>
<td>0.5</td>
<td>67.1 ± 4.4</td>
<td>74.5 ± 6.0</td>
<td>103.5 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>46.6 ± 1.1</td>
<td>56.3 ± 5.1</td>
<td>104.3 ± 1.1</td>
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<tr>
<td>ADP</td>
<td>0.5</td>
<td>97.1 ± 3.2</td>
<td>78.9 ± 8.1</td>
<td>99.9 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>96.8 ± 2.9</td>
<td>68.7 ± 8.1</td>
<td>96.4 ± 2.9</td>
</tr>
<tr>
<td>ATP</td>
<td>0.5</td>
<td>93.4 ± 4.7</td>
<td>79.6 ± 5.4</td>
<td>97.9 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>74.0 ± 7.6</td>
<td>60.8 ± 10.6</td>
<td>99.3 ± 4.1</td>
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<tr>
<td>GTP:S</td>
<td>0.5</td>
<td>113.4 ± 4.3</td>
<td>79.7 ± 4.2</td>
<td>98.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>101.3 ± 4.6</td>
<td>51.6 ± 0.7</td>
<td>103.4 ± 1.2</td>
</tr>
</tbody>
</table>

(100 μl ‘geometric volume’; Optiglass) pre-warmed at 30°C. After 5 min (or once a consistent baseline level of light-scattering was obtained), 10 μl GTP (6-25 mM) was added, the sample was mixed thoroughly and light-scattering was monitored thereafter. Similarly, when GTP was tested in combination with ATP, ADP, GDP or GTP:S, a stock mixture of GTP plus the other nucleotide phosphate was prepared such that addition of 10 μl resulted in final concentrations presented in Table 2. Components of the GTP-regenerating system (acetate kinase at 0-013 units ml\(^{-1}\) and acetyl phosphate at concentrations indicated in the text) were included in the polymerization reaction before the baseline was established. For Fig. 3(c), CaCl\(_2\) was included in the reaction mixture at 10 mM, FtsZ was present at 5 μM and the voltage setting on the Cary Eclipse fluorimeter was reduced from 500 to 450 to ensure that the light-scattering signal remained below threshold levels.

**Data analysis.** Light-scattering data points were taken every 5 or 10 s but are plotted here every 30 or 60 s for clarity. Only experiments performed on the same day are plotted together, although we generally found increases in light-scattering values to be consistent from day to day.

For each experiment described in Table 2, reaction buffer and FtsZ were made up in a pool and then split into three. After a baseline had been obtained, either GTP or a mixture of GTP with another nucleotide phosphate was added, and the light-scattering was recorded as above. Thus each experiment had its own GTP-only (0.5 mM final concentration) control reaction. The ‘baseline’ is the level of light-scattering before GTP is added and after all GTP has been hydrolysed (Mukherjee & Luktenhaus, 1999) and represents a further internal control. The ‘delta’ is the maximal increase in light-scattering in response to addition of GTP (Mukherjee & Luktenhaus, 1999). The ‘\( t_0 \)’ is the time from addition of GTP to the point at which half-maximal light-scattering value is reached as the trace returns to baseline levels, and is a measure of the longevity of the polymerization reaction. Values for baseline, delta and \( t_0 \) were obtained by plotting individual traces on identical axes and measuring these parameters by hand as illustrated in Fig. 1(b). This allowed any slight underlying slopes in the traces to be accurately accounted for. Baseline and delta values (measured in arbitrary light-scattering units) and \( t_0 \) values (in seconds) represent the mean of three separate experiments (± standard error) and are expressed as a percentage of the control GTP-only reaction.

**GTPase assay.** We routinely measured GTPase activity by TLC using \( [\gamma-\text{32P}]\text{GTP} \) (Amersham) essentially as described by RayChaudhuri & Park (1992) and Yu & Margolin (1997). In the presence of acetyl phosphate, we used the malachite green assay for phosphate production which has been used previously to assay FtsZ GTPase (Rivas et al., 2001; Romberg et al., 2001). This was preferable to using radiolabelled GTP since, in the presence of the GTP-regeneration system, GDP molecules are recycled.

**Negative stain electron microscopy.** FtsZ polymers were visualized by negative stain electron microscopy essentially as described previously (Lu & Erickson, 1998; Mukherjee & Luktenhaus, 1998a). Briefly, 10 μl polymerization reactions were applied to carbon-coated copper grids, left for 1 min, then blotted off. Negative staining was performed immediately by applying and blotting off 10 μl 2% uranyl acetate, repeating this once then allowing the grids to dry. Grids were examined using a Philips 400 transmission electron microscope.

**RESULTS**

**In vitro FtsZ polymerization is sensitive to the [GTP] to [GDP] ratio**

We tested the assumption that, as the [GTP]/[GDP] ratio falls during a dynamic *in vitro* FtsZ polymerization reaction, this would alter the behaviour of FtsZ. We performed preliminary experiments where the [GTP]/[GDP] ratio was varied from 1/1 to 1/7 (while keeping the GTP concentration constant at 0.5 mM – see Methods), and found that both the light-scattering peak level and the duration of the polymerization reaction were reduced by increasing the GDP concentration (Fig. 1a). Increase in light-scattering upon addition of 0.5 mM GTP was completely abolished when a ratio of 1/7 [GTP]/[GDP] was used (Fig. 1a). We wished to quantify the effects of changing the [GTP]/[GDP] ratio more accurately and so decided to measure three parameters of the polymerization reaction (Fig. 1b) in experiments repeated in triplicate, with internal controls (see Methods). We chose to study the 1/1 and 1/2 [GTP]/[GDP] ratios since these still resulted in FtsZ polymerization, giving a substantial light-scattering signal above background levels, while showing the effects of increasing GDP concentration (Fig. 1a). As further controls, we performed identical experiments with ADP or ATP in place of GDP and we tested the effect of the non-hydrolysable GTP analogue GTP:S in the same assay.

The duration of FtsZ polymerization (\( t_0 \)) is highly sensitive to the [GTP]/[GDP] ratio. At a 1/1 [GTP]/[GDP] ratio, \( t_0 \) was reduced to 67% of the control (GTP only) level and at a 1/2 [GTP]/[GDP] ratio \( t_0 \) fell to 47% (Table 2). In contrast, ADP had no effect on \( t_0 \) and ATP had minimal effect (74% at 1/2 ratio; Table 2). The value of the maximal increase in light-scattering (delta) was sensitive to addition of all nucleotide phosphates tested. Delta dropped to 74–80% of control levels when the ratio was 1/1 and 52–69% at a 1/2 ratio (Table 2). Baseline light-scattering levels were constant throughout each experiment. Therefore, a decrease in the
[GTP]/[GDP] ratio significantly decreases the total amount and the persistence of FtsZ polymers.

A slowing of FtsZ depolymerization due to GTP$_\gamma$S has been demonstrated in the presence of 10 mM calcium (Scheffers et al., 2000). Under these conditions, FtsZ polymers have significantly reduced GTPase activity and polymers are much more stable. Our approach to quantification of dynamic FtsZ polymerization is validated by our ability to detect a significant increase (13%) in the $t_{0.5}$ in the presence of GTP$_\gamma$S at a 1:1 ratio with GTP (Table 2). Previous attempts to detect the effects of GTP$_\gamma$S under similar conditions were unsuccessful (Scheffers et al., 2000).

A GTP-regeneration system can extend the steady-state period of FtsZ polymerization, without altering its dynamic properties

Having established that the [GTP]/[GDP] ratio has significant influence on dynamic FtsZ polymerization, we tested whether FtsZ polymerization could be extended by use of a GTP-regeneration system consisting of acetyl phosphate and acetate kinase (MacNeal et al., 1977; Purich et al., 1982). In such a way, the [GTP]/[GDP] ratio could be maintained constant for long periods of time. Using FtsZ purified by the method of Mukherjee & Lutkenhaus (1998a), we found that addition of 0.013 U acetate kinase ml$^{-1}$ and 0.5 mM acetyl phosphate increased the $t_{0.5}$ of FtsZ polymerization approximately twofold (Fig. 2a, open circles) compared to the control reaction (Fig. 2a, closed circles).

We were able to extend $t_{0.5}$ up to approximately ninefold by adding increased amounts of acetyl phosphate, with the steady-state period of polymerization in the longest experiments lasting 45 and 85 min (2 and 4 mM acetyl phosphate, respectively; data not shown). Importantly, reactions extended by addition of the GTP-regeneration system returned to baseline levels in the same manner as the control (e.g. Fig. 2a, b), indicating that the dynamic nature of the assay (see Methods) had not been compromised. We also found that the GTPase activity of FtsZ was not altered by the presence of the phosphate-donor compound (acetyl phosphate) of the GTP-regeneration system (data not shown; see Methods). Together these indicate that depolymerization occurs when GTP becomes limiting, exactly as in control reactions (Mukherjee & Lutkenhaus, 1998b, 1999), but that this occurs after exhaustion of acetyl phosphate.

Interestingly, we found that polymerization of this preparation of FtsZ was extended to a similar extent by addition of acetyl phosphate alone (Fig. 2a, filled triangles) and that the $t_{0.5}$ of the polymerization was dependent upon the concentration of added acetyl phosphate (Fig. 2b). This was evidently due to contamination of the FtsZ preparation with acetate kinase, since FtsZ purified from strain SGA79 [which has a Tn10 insertion in ackA (Barak et al., 1998), the gene encoding acetate kinase] required addition of both components of the GTP-regeneration system for an increase of $t_{0.5}$ (data not shown). As described in Methods, we have found that a combination of two previous FtsZ purification

![Fig. 1. Effects of changes in the [GTP]/[GDP] ratio on FtsZ polymerization. (a) FtsZ polymerization was initiated by the addition of 0.5 mM GTP only (○) or 0.5 mM GTP together with GDP at 0.5 mM (■), 1 mM (□), 2 mM (◆) and 3.5 mM (○). Initial baseline values were set to zero for this plot and the time axis starts when GTP or nucleotide phosphate mixtures were added. (b) The full GTP-only light-scattering trace from (a) (reduced in scale by 50%) is annotated with baseline, delta and $t_{0.5}$ – see Methods for parameter definitions.](image-url)
We have demonstrated that an increase of GDP activity and causes bundling of polymers, resulting in hugely increased light-scattering (Mukherjee & Lutkenhaus, 1999; Scheffers et al., 2000; Yu & Margolin, 1997). We therefore invoked a GTP-cap model for FtsZ polymerization, similar to that for tubulin. In this model, exchange of guanine nucleotide phosphates takes place at the ends of polymers rather than by diffusion from within polymers. An explanation for the observed effect of GDP in our experiments would be that both GDP and GTP bind to FtsZ monomers and the end subunits of polymers with similar affinities. [Previous data show that GDP binds to FtsZ approximately half as well as GTP, and that GDP can compete with GTP for FtsZ binding (de Boer et al., 1992; Mukherjee et al., 1993; Scheffers et al., 2000; RayChaudhuri & Park, 1992).] Addition of GTP or GTP-bound FtsZ to the end of a polymer would allow polymerization to continue, whereas addition of GDP or GDP-bound FtsZ would promote depolymerization. Indeed, since in vivo experiments demonstrate continuous, rapid turnover of subunits within the Z-ring (Stricker et al., 2002), addition of GDP or

**DISCUSSION**

We have demonstrated that an increase of [GDP] in the presence of constant [GTP] significantly reduces the delta and $t_{0.5}$ of FtsZ polymerization (Table 2). In doing so, we have also demonstrated that with appropriate controls and careful measurement, dynamic polymerization of FtsZ under various circumstances can be compared in a quantitative manner. Indeed, our measurements were able to detect a subtle effect on dynamic FtsZ polymerization caused by addition of GTP/S (Table 2) which previously went (Scheffers et al., 2000) undetected. Experiments where [GTP] was reduced while [GDP] was increased (with total guanine nucleotide phosphate concentration kept constant) gave more precipitous reductions in $t_{0.5}$ and delta (data not shown). However, since it has previously been demonstrated that reduction in [GTP] alone reduces the $t_{0.5}$ of FtsZ polymerization (Mukherjee & Lutkenhaus, 1999), we chose to present data where [GTP] is constant.

GTP is hydrolysed immediately upon FtsZ polymerization while release of phosphate from the polymers lags behind (Scheffers & Driessen, 2002). FtsZ subunits within polymers have GDP plus phosphate at their active site and therefore are effectively in their GTP-bound configuration (Scheffers & Driessen, 2002). Scheffers & Driessen (2001, 2002) have therefore invoked a GTP-cap model for FtsZ polymerization, similar to that for tubulin. In this model, exchange of guanine nucleotide phosphates takes place at the ends of polymers rather than by diffusion from within polymers. An explanation for the observed effect of GDP in our experiments would be that both GDP and GTP bind to FtsZ monomers and the end subunits of polymers with similar affinities. [Previous data show that GDP binds to FtsZ approximately half as well as GTP, and that GDP can compete with GTP for FtsZ binding (de Boer et al., 1992; Mukherjee et al., 1993; Scheffers et al., 2000; RayChaudhuri & Park, 1992).] Addition of GTP or GTP-bound FtsZ to the end of a polymer would allow polymerization to continue, whereas addition of GDP or GDP-bound FtsZ would promote depolymerization. Indeed, since in vivo experiments demonstrate continuous, rapid turnover of subunits within the Z-ring (Stricker et al., 2002), addition of GDP or

**FtsZ polymer morphology and the effects of calcium are not altered by the GTP-regeneration system**

We wanted to establish whether use of the GTP-regeneration system altered certain well-established properties of FtsZ polymers. Firstly, we examined the morphology of FtsZ polymers using negative stain electron microscopy as described previously (Lu & Erickson, 1998; Mukherjee & Lutkenhaus, 1998a). We found no significant difference in the types or apparent lengths of polymers formed in the presence or absence of the GTP-regeneration system (Scheffers & Driessen, 2000; Mukherjee & Lutkenhaus, 1999a; Yu & Margolin, 1997). We therefore invoked a GTP-cap model for FtsZ polymerization, similar to that for tubulin. In this model, exchange of guanine nucleotide phosphates takes place at the ends of polymers rather than by diffusion from within polymers. An explanation for the observed effect of GDP in our experiments would be that both GDP and GTP bind to FtsZ monomers and the end subunits of polymers with similar affinities. [Previous data show that GDP binds to FtsZ approximately half as well as GTP, and that GDP can compete with GTP for FtsZ binding (de Boer et al., 1992; Mukherjee et al., 1993; Scheffers et al., 2000; RayChaudhuri & Park, 1992).] Addition of GTP or GTP-bound FtsZ to the end of a polymer would allow polymerization to continue, whereas addition of GDP or GDP-bound FtsZ would promote depolymerization. Indeed, since in vivo experiments demonstrate continuous, rapid turnover of subunits within the Z-ring (Stricker et al., 2002), addition of GDP or

**Fig. 2.** Extension of FtsZ polymerization using a GTP-regeneration system. (a) Light-scattering was used to monitor polymerization of FtsZ only (●), FtsZ plus 0·013 U acetate kinase (AcK) ml$^{-1}$ and 0·5 mM acetyl phosphate (AcP) (○) or FtsZ plus 0·5 mM AcP (△). (b) Light-scattering was used to monitor polymerization of FtsZ only (●) and FtsZ plus the following concentrations of AcP in mM: 0·25 (○); 0·5 (△); 0·75 (△); 1·0 (●); 1·5 (□). GTP (0·5 mM) was added to all reactions at time = 0 s. Final baseline values were set to zero and the time axis starts when GTP is added for both (a) and (b).

 protocols (Lu & Erickson, 1998; Mukherjee & Lutkenhaus, 1998a) results in FtsZ with consistently high GTPase activity and no detectable acetate kinase contamination (data not shown).

![Fig. 2](image-url)
GDP-bound FtsZ to a growing polymer may cause events similar to microtubule catastrophe. Hence in our experiments, the dynamic polymerization reaction is effectively quenched by an increase in the concentration of GDP.

An alternative explanation for decrease in both $t_0.5$ and delta in the presence of increased GDP could be that mean polymer length is reduced. The concentration of polymer ends would be elevated and therefore GTP would be hydrolysed more quickly. As yet, there is no established method for measurement of FtsZ polymer lengths in such dynamic assays; however, we do not favour this explanation because GTPase activity is not increased in assays with added GDP (data not shown).

Control experiments were performed using ATP or ADP in place of GDP. It has previously been demonstrated that ATP does not bind to FtsZ and cannot compete out binding of GTP to FtsZ (de Boer et al., 1992; Mukherjee et al., 1993; RayChaudhuri & Park, 1992). Therefore, we were surprised to find that, although neither ATP or ADP affected the duration of FtsZ polymerization, both had negative effects on the delta value similar to that of GDP. It is possible that ADP and ATP can occupy the nucleotide-binding site on FtsZ transiently and that this is enough to interfere with the very fast initial polymerization (the kinetics of which are largely undescribed). Nevertheless, we note that GDP and GTP$_7$S, both of which are known to bind to FtsZ, had the most severe effects on delta.

Acetate kinase and acetyl phosphate represent a highly efficient system for GTP regeneration which maintains the GTP to GDP ratio at 200 to 1 or greater, even when 90% of acetyl phosphate has been converted to acetate (Purich et al.,...
In this study, we have shown that acetate kinase and acetyl phosphate do not significantly alter the dynamic properties of FtsZ characterized thus far in vitro (Mukherjee & Lutkenhaus, 1998b, 1999). In the light of our results which demonstrate that FtsZ polymerization is sensitive to the [GTP]/[GDP] ratio, and since binding of GDP by FtsZ favours bending and depolymerization of polymers (Lu et al., 2000), a regeneration system is preferable to adding high concentrations of GTP to a polymerization reaction. The latter prolongs the polymerization steady state to some extent (Mukherjee & Lutkenhaus, 1999) but GDP will still accumulate. Other methods which prolong the existence of FtsZ polymers in vitro, such as addition of 10 mM calcium or reduction of magnesium concentration (Mukherjee & Lutkenhaus, 1999; Scheffers et al., 2000), serve to reduce the FtsZ GTPase activity and thus the dynamic nature of the polymers.

The intracellular [GTP]/[GDP] ratio in Salmonella typhimurium is approximately 7/1 (Neuhard & Nygaard, 1987) and is likely to be similar in E. coli. Since levels of GTP are unlikely to be affected (even locally at the cell centre) by the strong GTPase of FtsZ (Stricker et al., 2002), the Z-ring therefore must assemble, constrict and disassemble in conditions of consistently high [GTP]/[GDP] ratio. Consequently, models for mechanisms of initiation of Z-ring assembly and initiation of Z-ring constriction must take this into account, together with the observed constant turnover within the Z-ring (Stricker et al., 2002). We assert that the gradual build-up of GDP in in vitro polymerization assays used so far is unlike the in vivo situation. We therefore present the FtsZ polymerization in the presence of a GTP-regeneration system as a more in vivo-like assay for FtsZ polymerization which will be useful to identify and characterize proteins which affect critical aspects of the control of cell division.

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REFERENCES


Addinall, S. G., Cao, C. & Lutkenhaus, J. (1997). Temperature shift experiments with an ftsZ84(Ts) strain reveal rapid dynamics of FtsZ localization and indicate that the Z ring is required throughout septation and cannot reoccupy division sites once constriction has initiated. J Bacteriol 179, 4277–4284.


