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Dynamic assembly of FtsZ regulated by GTP hydrolysis

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FtsZ forms a cytokinetic ring, designated the Z ring, that directs cytokinesis in prokaryotes. It has limited sequence similarity to eukaryotic tubulins and, like tubulin, it has GTPase activity and the ability to assemble into various structures including protofilaments, bundles and minirings. By using both electron microscopy and sedimentation, we demonstrate that FtsZ from *Escherichia coli* undergoes a strictly GTP-dependent polymerization and the polymers disappear as the GTP is consumed. Thus, FtsZ polymerization, like that of tubulin, is dynamic and regulated by GTP hydrolysis. These results provide the basis for the dynamics of the Z ring and favor a model in which the Z ring is formed by a nucleation event.

Keywords: cytokinesis/cytoskeleton/FtsZ/GTPase/tubulin

Introduction

FtsZ is an essential cell division protein that forms a cytokinetic ring that directs cell division in bacteria (Bi and Lutkenhaus, 1991). This ring, designated the FtsZ or Z ring, has been studied most extensively in *Escherichia coli* but appears to direct the formation of the septum in all prokaryotic organisms (reviewed in Lutkenhaus and Addinall, 1997). Evidence suggests that the Z ring is a cytoskeletal element that is functionally analogous to the contractile ring in eukaryotic cells. Interestingly, FtsZ has limited but significant homology to eukaryotic tubulins, suggesting that it is an ancestral homolog (Mukherjee and Lutkenhaus, 1994; Erickson, 1995). Like tubulin, FtsZ is a GTPase (de Boer *et al.*, 1992; RayChaudhuri and Park, 1992; Mukherjee *et al.*, 1993) and undergoes GTP/GDP-dependent polymerization (Mukherjee and Lutkenhaus, 1994; Erickson *et al.*, 1996).

In rapidly growing cells, the Z ring is formed shortly after the previous division by recruitment of FtsZ from the cytoplasm (Bi and Lutkenhaus, 1991; Addinall *et al.*, 1996; Pogliano *et al.*, 1997). About half way through the cell cycle, it contracts at the leading edge of the invaginating septum. It is clear that the Z ring is required throughout septation and directs the ingrowth of the septum (Addinall and Lutkenhaus, 1996a; Addinall *et al.*, 1997b). It does this in part by tethering other cell division proteins to restrict their enzymatic activity spatially to the septum (Addinall and Lutkenhaus, 1996b; Ma *et al.*, 1996; Addinall *et al.*, 1997a). Another function may be to provide energy for invagination of the septum (Bramhill, 1994; Erickson *et al.*, 1996).

Of interest is how the Z ring is formed. It has been postulated that the Z ring is a cytoskeletal element that is formed through self-assembly (Bi and Lutkenhaus, 1991; Lutkenhaus, 1993). Although the Z ring could represent the accumulation of FtsZ without it actually being in a structure (structures have not been detected in sections examined by electron microscopy), the examination of the ability of various derivatives of FtsZ to make additional patterns besides the ring is best explained by assembly into a structure. For example, FtsZ26 makes spiral structures that result in spiral septa (Addinall and Lutkenhaus, 1996a) and an FtsZ–green fluorescent protein (GFP) fusion makes spirals and long filaments when overproduced (Ma *et al.*, 1996).

If the Z ring is a cytoskeletal element, then a critical event in the assembly is the nucleation event. It has been proposed that the Z ring is positioned and assembled in response to the activation of a nucleation site at midcell (Lutkenhaus, 1993). Consistent with this proposal, Z rings and spirals appear to grow bidirectionally from a single point, suggesting that there is one nucleation site per Z ring (Addinall and Lutkenhaus, 1996a). Also, immunostaining for FtsZ reveals spots of fluorescence at early stages during the recovery of filamentous cells of an *ftsZ84* (Ts) mutant, indicating that Z rings are nucleated at single sites (Addinall *et al.*, 1997b). Likewise, spots of fluorescence have been observed with overproduction of an FtsZ–GFP fusion (Ma *et al.*, 1996).

The GTPase activity of FtsZ has a dramatic dependence on the FtsZ concentration, suggesting that self-association is involved (de Boer *et al.*, 1992; Mukherjee *et al.*, 1993; Wang and Lutkenhaus, 1993, 1996). However, the role of GTP hydrolysis in the assembly of FtsZ is unknown due to the various promoting agents added to the assembly reaction. These promoting agents, DEAE–dextran, which also promotes tubulin assembly (Erickson and Voter, 1975), and cationic phospholipids promote FtsZ assembly in the presence of either GDP or GTP (Mukherjee and Lutkenhaus, 1994; Erickson *et al.*, 1996). An additional report (Bramhill, 1994) describing GTP-specific assembly of FtsZ was later reported to be due to a large drop in the pH, indicating that it was due to acid precipitation (Erickson *et al.*, 1996). Thus, determination of the role of GTP hydrolysis in the dynamics of FtsZ assembly requires conditions that do not involve these promoting agents.

In this study, we have isolated FtsZ from *E.coli* and determined conditions for polymerization that allow us to examine the role of GTP hydrolysis. We have found conditions in which FtsZ readily polymerizes in a strictly GTP-dependent manner and, importantly, these polymers turn over upon GTP hydrolysis.

Results

FtsZ polymerization

The aim of this study was to examine the role of GTP hydrolysis in FtsZ polymerization. Since the GTPase activity of our previous preparation of FtsZ displayed a remarkable lag (Mukherjee *et al.*, 1993), we used an alternative purification buffer that included 10% glycerol and KCl instead of NaCl. The purified FtsZ had 0.7 mol of GDP (average of two experiments) bound per mol of FtsZ, and the GTPase activity did not display any discernible lag (Figure 1). Whereas our previous preparation of FtsZ readily polymerized at pH 7.2 with GTP or GDP in the presence of DEAE–dextran (Mukherjee and Lutkenhaus, 1994), we found that it did not polymerize readily without this promoting agent even though it displayed GTPase activity. We find that our present preparation of FtsZ readily polymerizes without DEAE–dextran.

Using negative staining with uranyl acetate and electron microscopy, we looked for conditions in which our new preparation of FtsZ would polymerize in the absence of DEAE–dextran and found that at slightly acidic pH, FtsZ readily forms polymers. Incubation of FtsZ at 5 μM (200 μg/ml) with 1 mM GTP and 10 mM $MgCl_2$ at pH 6.0 resulted in polymerization into ribbon-like structures (Figure 2). However, upon the inclusion of 200 mM KCl in the reaction buffer, the ribbon-like structures are no longer seen; instead protofilaments ~7 nm wide and bundles of protofilaments which range up to 20 nm in width are seen (Figure 2). Upon lowering the KCl concentration to 50 mM, the FtsZ polymers formed are similar to that seen with 200 mM KCl (data not shown). When polymerization was carried out at pH 6.5 with and without 200 mM KCl, a dense network of polymers was readily seen, which resembled that observed at pH 6.0 with 200 mM KCl (Figure 2). Thus, at pH 6.5, unlike at pH 6.0, the addition of KCl did not affect the structure of the polymers.

Significantly, the formation of FtsZ polymers at both pH 6.0 and 6.5, irrespective of the presence of KCl, is strictly GTP dependent and does not occur with GDP or ATP (see later and data not shown). The ribbon-like structures seen at pH 6.0 without KCl must result from a combination of low ionic strength and acidic pH and, therefore, are not likely to be physiologically relevant. To determine if the polymers formed at pH 6.5 are only formed at a slightly acidic pH, we also checked for GTP-dependent polymerization at pH 7.2. At pH 7.2, we observed polymers, although not as readily, similar to those seen at pH 6.5 (data not shown). Erickson *et al.* (1996) also reported assembly of FtsZ at pH 7.0. Since the only difference appeared to be abundance, we carried out subsequent experiments on FtsZ polymerization at pH 6.5.

GTP hydrolysis renders FtsZ polymers their dynamic nature

Owing to the many similarities between FtsZ and tubulin (Lutkenhaus, 1993; Mukherjee and Lutkenhaus, 1994; Erickson, 1995), it has been suggested that the GTPase activity of FtsZ plays a role in polymerization similar to that in tubulin. In previous reports with DEAE–dextran (Mukherjee and Lutkenhaus, 1994; Erickson *et al.*, 1996) and cationic lipids (Erickson *et al.*, 1996), it was not possible to assess this, since in both instances polymerization was also promoted by GDP. However, with the demonstration that FtsZ polymerization at pH 6.5 is strictly GTP dependent, study of the role of GTP hydrolysis in FtsZ polymerization now becomes possible. As a first step, we carried out experiments in which we followed the fate of the FtsZ polymers by electron microscopy under GTP hydrolyzing conditions, from early time points when GTP is present until later when GTP is exhausted.

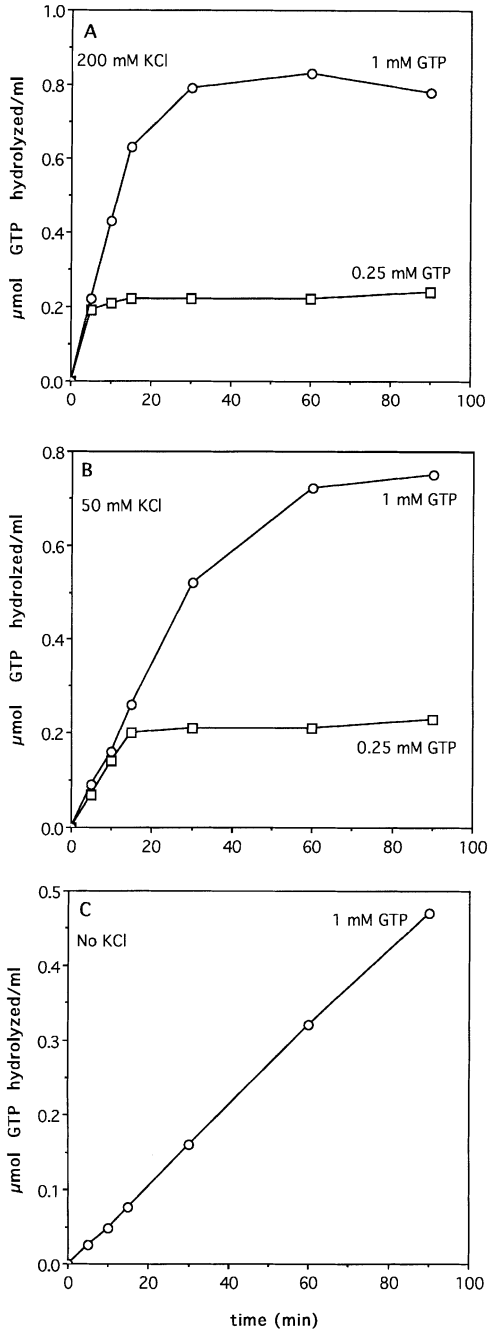

Fig. 1. FtsZ GTPase activity at different GTP and KCl concentrations. Reaction mixtures containing 5 μM (200 μg/ml) FtsZ in polymerizing buffer with different concentrations of [γ-^{32}P]GTP (as indicated) and KCl (200 mM, **A**; 50 mM, **B**; and none, **C**) were incubated at 30°C. GTPase activity was determined as described in Materials and methods.

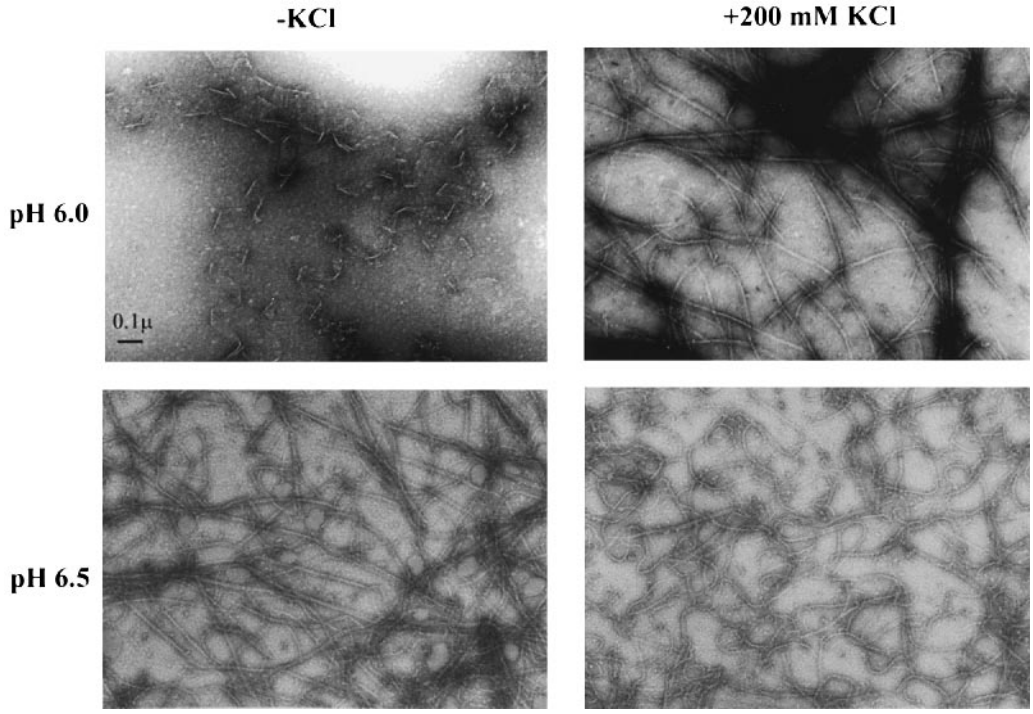

Fig. 2. Polymerization of FtsZ at different pHs with and without KCl. FtsZ was diluted to 200 μg/ml (5 μM) in polymerizing buffer as indicated. The reaction was started by shifting the samples to 30°C and adding 1 mM GTP. At 15 min, samples were taken and polymers visualized by electron microscopy.

FtsZ was incubated at 5 μM (200 μg/ml) in polymerization buffer with 200 mM KCl and 1 mM GTP at 30°C and aliquots of the reaction mixture were withdrawn at various times and applied to grids for electron microscopy. In a parallel experiment, the GTPase activity was measured. As shown in Figure 3, FtsZ polymers are abundant at 10 min, but by 20 min the polymers are few and short and by 60 min no polymers are seen. The GTPase activity under identical conditions (Figure 1A) is linear for 15 min, but by 30 min ~80% of the GTP is consumed and the hydrolysis rate plateaus. In another experiment, the concentration of GTP was reduced to 0.25 mM. The polymers were present at 5 min but no polymers were seen from 10 min onwards (Figure 3). The GTPase activity at this concentration of GTP is linear for 5 min and is then curtailed as the GTP is consumed (Figure 1A). Thus the polymers are observed until the GTP is exhausted.

The rate of GTP hydrolysis by FtsZ is affected by the concentration of KCl (Mukherjee *et al.*, 1993), and lowering it from 200 to 50 mM reduces the initial rate by ~2.5-fold (compare Figure 1A and B). Therefore, if similar experiments to those described above are carried out with 50 mM KCl instead of 200 mM, the GTP would last longer and consequently the FtsZ polymers should persist longer as well. Indeed, with 1 mM GTP and 50 mM KCl, abundant filaments were observed at 20 min and were still present at 40 min, although they were shorter and less frequent (Figure 3). None were seen at 90 min. The abundance of polymers at 40 min with 50 mM KCl resembles that at 20 min with 200 mM KCl. With 0.25 mM GTP and 50 mM KCl, the polymers, although not so abundant, are observed at 20 min (Figure 3), whereas with 200 mM KCl none are seen at 10 min. Therefore, with 50 mM KCl, the polymers persist about twice as long as with 200 mM KCl. This persistence correlates with the presence of GTP as estimated from the GTPase activity under these conditions (Figure 1). Furthermore, in the absence of any KCl, the initial rate of GTPase activity is 10-fold less than at 200 mM KCl and the GTP is not exhausted even at 60 min (Figure 1C). A dense network of FtsZ polymers is still observed at 60 min (data not shown). These experiments, therefore, clearly demonstrate that FtsZ polymers persist as long as GTP is present and disappear rapidly (depolymerize) as soon it is consumed. An intermediate stage when polymers are fewer and shorter can also be observed. These results demonstrate that the FtsZ polymers are dynamic in nature due to the hydrolysis of GTP.

Quantitative assay of FtsZ polymerization

Since electron microscopy revealed that at pH 6.5 FtsZ readily forms a dense network of polymers, we tested whether these could be sedimented by centrifugation. To a reaction mixture containing 200 μg/ml FtsZ at pH 6.5 and 50 mM KCl, 1 mM GTP was added and immediately spun at 25°C in a Beckman TL-100 for various times at 80 000 r.p.m. The amount of FtsZ recovered in the pellet was 51% with a 15 min spin (Table I). With a 10 min spin, this percentage was approximately the same but it decreased with a 5 min spin and it did not increase with longer times (data not shown). With GDP or ATP, 5% or less of the FtsZ was recovered in the pellet compared with 10% without any nucleotide (Table I). Omitting Mg^{2+}, which is required for GTP hydrolysis (de Boer *et al.*, 1992; RayChaudhuri and Park, 1992; Mukherjee

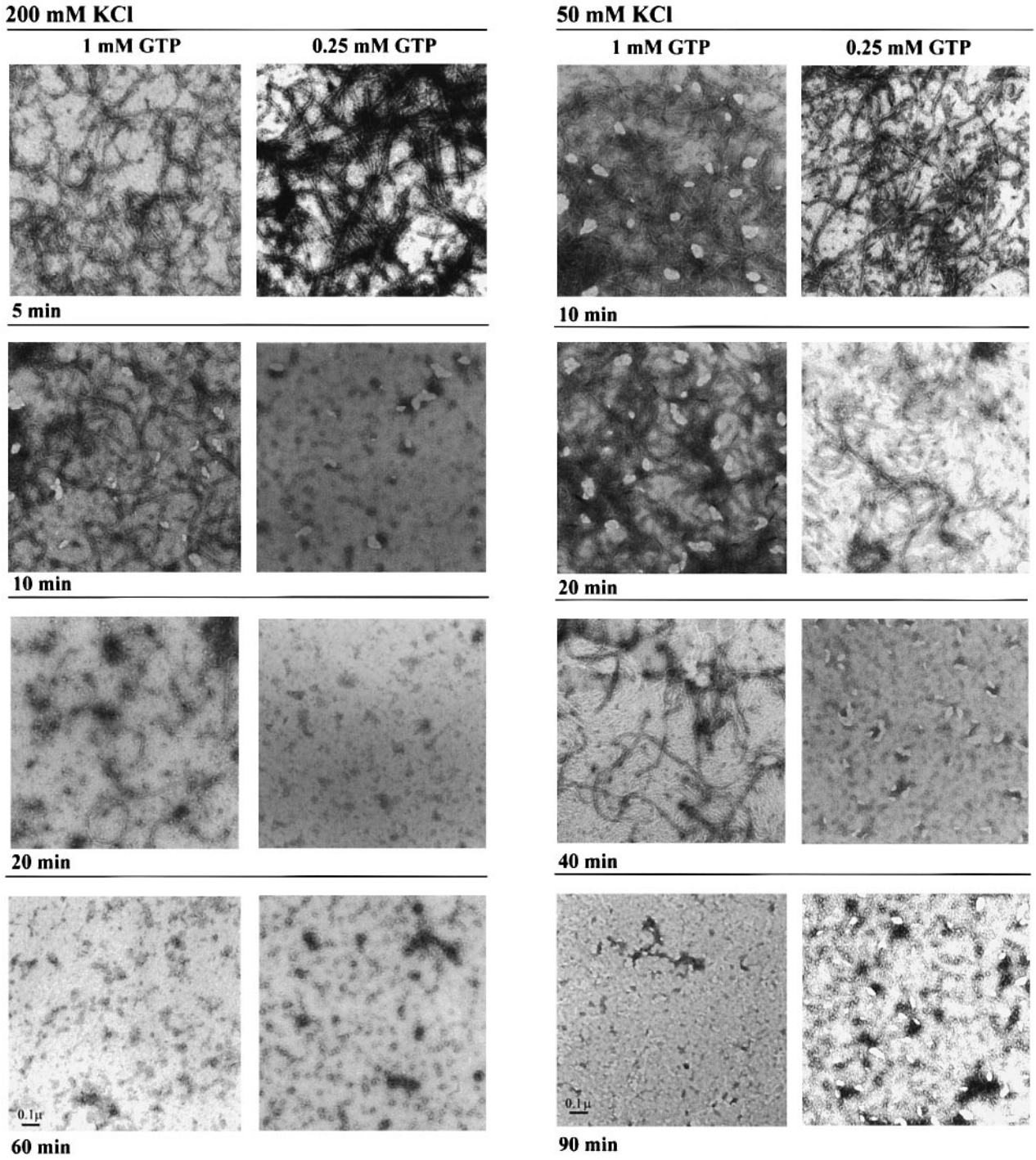

Fig. 3. Role of GTP hydrolysis in the stability of FtsZ polymers. FtsZ was diluted to 200 μg/ml (5 μM) in polymerizing buffer as indicated. Polymerization was initiated by shifting the reaction mixture to 30°C and adding GTP at the indicated concentrations. At the indicated times, samples were taken and the polymers visualized by electron microscopy.

et al., 1993), resulted in 24% of the FtsZ in the pellet. The amount recovered in the pellet without Mg^{2+} was constant for at least 60 min, indicating that it was quite stable (data not shown). Examination of such a reaction by electron microscopy revealed the presence of typical polymers (data not shown). Thus, polymerization of FtsZ as assayed by sedimentation and electron microscopy is strictly GTP dependent but does not require hydrolysis.

The dynamic nature of FtsZ polymers can be monitored by sedimentation

We tested if the dynamic nature of FtsZ polymers observed by electron microscopy can be assayed quantitatively by sedimentation. The experiment was performed with 50 mM KCl and 1 mM GTP so that the GTP would last throughout the centrifugation for 15 min at 25°C since GTP hydrolysis would be ongoing. In plotting the data for this experiment,

Table I. Amount of FtsZ recovered in the pellet with various nucleotides

Nucleotide	% FtsZ in pellet
None	10
ATP	4
GDP	5
GTP	51
GTP (–$MgCl_2$)	24

To a 100 µl reaction mixture containing 5 µM (200 µg/ml) FtsZ in polymerizing buffer with 50 mM KCl, 1 mM nucleotide was added and immediately centrifuged, and the protein was assayed in the pellet as described in Materials and methods.

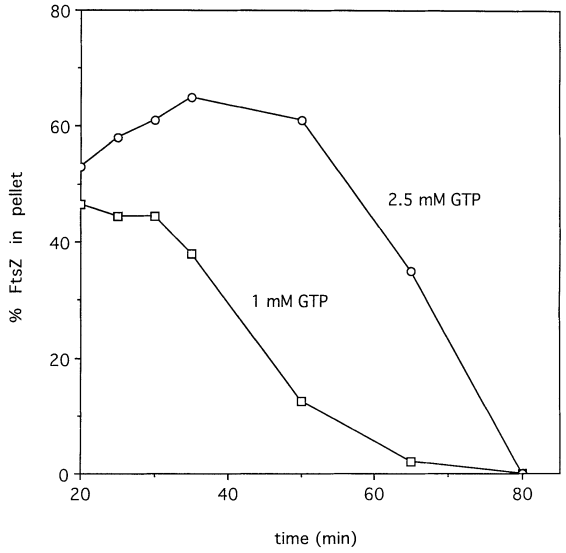

Fig. 4. Monitoring FtsZ polymers by centrifugation. FtsZ was diluted to 5 µM (200 µg/ml) into polymerizing buffer and polymerization initiated by adding 1 mM GTP and shifting to 30°C. At the indicated times, reaction mixtures were centrifuged and the amount of FtsZ in the pellet determined. The time in this plot reflects the 20 min added to each sample for the centrifugation (15 min) and sample preparation (5 min). The data were adjusted by subtracting the amount of FtsZ in the pellet (<5%) obtained with GDP.

20 min was added to each sample to reflect the 15 min for centrifugation and the 5 min that elapsed from starting the experiment to loading the rotor and starting the spin. As seen in Figure 4, ~45% of the FtsZ is recovered in the pellet for the first 30 min, after which time the amount recovered rapidly decreases. It should be noted that at 40 min polymers can still be seen by electron microscopy; however, they are fewer and shorter than at earlier times (Figure 3). Thus, the decline in the amount of FtsZ recovered in the pellet parallels the disappearance of FtsZ polymers observed by electron microscopy. If the starting GTP concentration was increased to 2.5 mM, then as much as 65% of the FtsZ was recovered in the pellet (Figure 4). In addition, it was possible to recover FtsZ in the pellet for a longer time.

Determination of the critical concentration for FtsZ assembly

The critical concentration for assembly of FtsZ was determined by measuring the mass of FtsZ polymer as a function of the initial FtsZ concentration using the sedimentation assay. FtsZ at different concentrations was

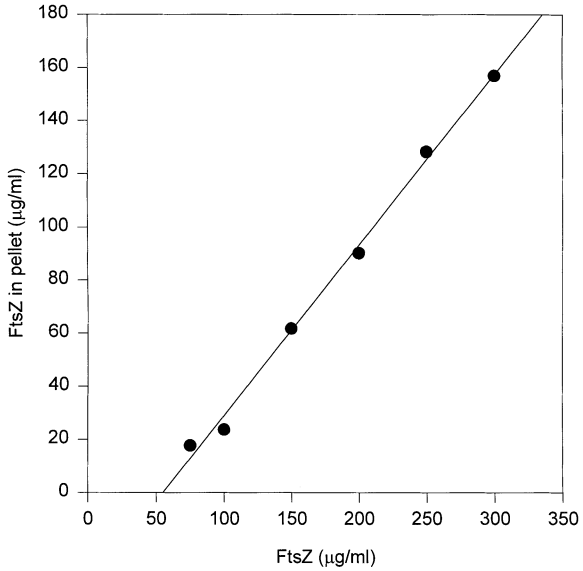

Fig. 5. Determination of the critical concentration for FtsZ polymerization. FtsZ was diluted to the indicated concentrations in polymerizing buffer at pH 6.5, 50 mM KCl at 25°C. The polymerization was initiated by the addition of 1 mM GTP and the samples were centrifuged immediately. The FtsZ in the pellet was then determined. The data were adjusted by subtracting the amount of FtsZ in the pellet (<5%) obtained with GDP.

incubated in polymerizing buffer (50 mM KCl) and the samples centrifuged. The FtsZ in the pellet was determined and plotted against the initial concentration (Figure 5). The value for the critical concentration obtained from this plot is 1.4 µM (55 µg/ml). This experiment was repeated by starting the reaction with FtsZ at 300 µg/ml and GTP at 2.5 mM, incubating for 5 min at 30°C and then diluting to various FtsZ concentrations before centrifugation. The critical concentration determined by this approach was very similar, 1.6 µM (65 µg/ml).

FtsZ polymers recycle

Since FtsZ polymers depolymerize upon the depletion of GTP, we wanted to test if repolymerization would occur if GTP is added back. FtsZ (5 µM) was incubated in polymerizing buffer with 200 mM KCl and 0.25 mM GTP for 20 min, at which time 1 mM GTP was added. Samples for electron microscopy were prepared at various time points. As observed earlier (Figure 2), a dense network of polymers was present at 5 min which disappeared by 10 min (Figure 6). When 1 mM GTP was added at 20 min, polymers readily reappeared within 5 min (see 25 min picture of Figure 6). By 40 min, the polymers were shorter and less abundant, and by 60 min the polymers were no longer seen. These observations demonstrate that FtsZ can cycle through rounds of polymerization and confirm that FtsZ polymers are dynamic and regulated by GTP hydrolysis.

Discussion

Our major finding is that FtsZ polymerization is dynamic and regulated by GTP hydrolysis. This result provides a mechanism for the dynamic properties of the Z ring *in vivo*; its ability to assemble at the division site, to contract during septation and to disassemble after division

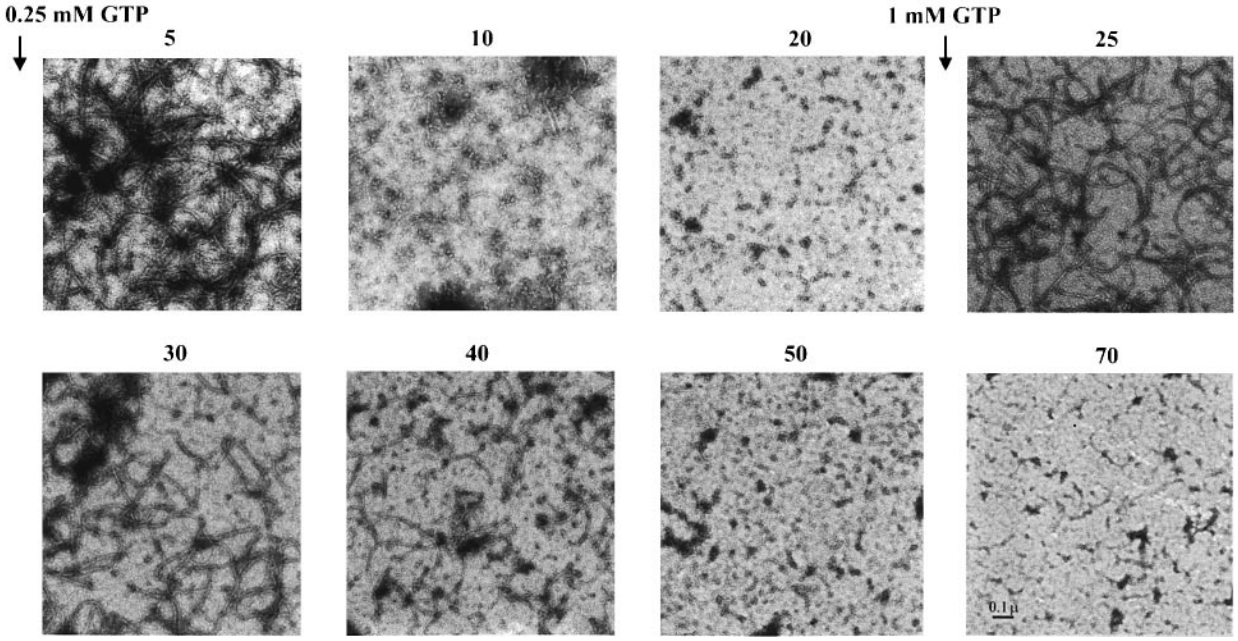

Fig. 6. FtsZ polymers can recycle. FtsZ was diluted to 5 μM (200 μg/ml) in polymerizing buffer (pH 6.5, 200 mM KCl). Polymerization was initiated by shifting to 30°C and adding 0.25 mM GTP. After 20 min, 1 mM GTP was added and the incubation continued. At the indicated times, samples were examined by electron microscopy.

is complete. Therefore, the results support the suggestion that the Z ring is a cytoskeletal element as first proposed (Bi and Lutkenhaus, 1991). In addition, the present results further extend the functional homolgy between FtsZ and eukaryotic tubulins, strengthening previous suggestions that they are homologs, a suggestion based largely upon limited sequence similarity (Mukherjee and Lutkenhaus, 1994; Erickson, 1995).

In the present study, we manipulated the GTP and KCl concentrations to alter the time of the reaction at which the GTP would be consumed. Under these various conditions, we always observed polymers as long as GTP was present that disappeared as the GTP was exhausted. Our results are similar to those obtained by Carlier and Pantaloni (1978) for tubulin polymerization in the presence of limiting GTP. Thus, the dynamic properties of FtsZ polymers are regulated by nucleotide hydrolysis as are tubulin and actin polymers.

Importantly, FtsZ polymerization can be quantitated by centrifugation, an independent method confirming the polymerization. Furthermore, we determined that the critical concentration for FtsZ assembly is 1.5 μM (60 μg/ml) at 25°C in 50 mM KCl. Also, we observe that 45–60% of the FtsZ is sedimented, as opposed to the nearly 100% observed by Bramhill and Thompson (1994). Their inability to determine a critical concentration is also consistent with their sedimentation results being due to acid precipitation rather that polymerization. Interestingly, the critical concentration we observed for FtsZ assembly is similar to that (100 μg/ml) reported for yeast tubulin (Davis *et al.*, 1993).

The rate of GTP hydrolysis by FtsZ in our study is ~9/min under optimal conditions (200 mM KCl, Figure 1A). This rate is ~100 times greater than the rate of GTP hydrolysis observed for tubulin during the steady-state phase of microtubule polymerization (0.06/min, Erickson and O'Brien, 1992). This comparison suggests that FtsZ polymers turn over much faster than microtubules or that the GTPase activity of FtsZ is not tightly coupled to polymerization under the present conditions.

The level of FtsZ in a fast growing cell is ~20 000 molecules, corresponding to a concentration of 0.5 mg/ml (Bi and Lutkenhaus, 1991). This concentration of FtsZ is significantly higher than that needed to obtain polymerization but similar to the concentration of tubulin in eukaryotic cells. Therefore, it is likely that other proteins are interacting with FtsZ to limit polymerization. It is known that overproduction of FtsA and ZipA, two proteins that interact directly with FtsZ, can block cell division and, for FtsA, block FtsZ localization (Hale and de Boer, 1997; Wang *et al.*, 1997). Thus these proteins may influence FtsZ polymerization under physiological conditions; however, it is likely that they function after FtsZ in cell division (Addinall and Lutkenhaus, 1996b; Addinall *et al.*, 1996). With the assembly conditions we report here, it should be possible to begin to identify additional components involved in regulating FtsZ assembly.

A model for formation of the Z ring was proposed based in part upon analogy with microtubule assembly (Lutkenhaus, 1993). In this model, FtsZ self-assembles in response to the activation of a nucleation site at midcell. Our observations that FtsZ assembles into polymers under physiological conditions and that the polymers turn over with GTP hydrolysis support the model as it provides a basis for the dynamic properties of the Z ring. With microtubules, dynamic instability is observed and best explained by the GTP cap model (Carlier *et al.*, 1984; reviewed in Erickson and O'Brien, 1992). In this model, GTP hydrolysis lags behind assembly, resulting in a cap of GTP-containing subunits. If hydrolysis catches up to the growing tip, catastrophe can occur and the microtubule undergoes disassembly. It is possible that FtsZ polymerization is regulated in a similar fashion, although we have no evidence for dynamic instability with FtsZ polymers

as we have only done bulk measurements and not observed the behavior of individual polymers.

It is clear that the Z ring directs cytokinesis (Addinall and Lutkenhaus, 1996a) although the mechanism by which the Z ring functions to bring about this event is not clear. It is possible that the Z ring provides cytomusculature for cytokinesis through depolymerization of FtsZ polymers or, with the aid of a motor protein, cause adjacent, membrane-anchored FtsZ polymers to slide past each other. In addition to this possible function though, it is clear that the Z ring functions as a cytoskeleton to organize spatially proteins that are responsible for synthesis of the septum (Addinall and Lutkenhaus, 1996b; Addinall *et al.*, 1997a).

Materials and methods

Overproduction and purification of FtsZ

FtsZ was purified as described (Mukherjee *et al.*, 1993), with the following modifications. *Escherichia coli* W3110 transformed with pKD 126 (Dai *et al.*, 1994) was grown overnight at 37°C in Luria broth with ampicillin (100 μg/ml). The overnight culture was diluted 100-fold into 3 l of fresh medium and grown with shaking at 37°C. At OD_{600} of 0.3, 0.5 mM isopropl-β-D-thiogalactopyranoside (IPTG) was added and the culture grown for another 3 h. The culture was then chilled, harvested at 4°C, washed once with 20 mM Tris–HCl (pH 7.9) and the pellet stored frozen at –70°C. From this step onwards, all procedures were carried out at 4°C. The frozen pellet was thawed in 30 ml of 50 mM Tris–HCl (pH 7.9), 50 mM KCl, 1 mM EDTA, 10% glycerol (buffer A) and lysed by passage through a French press. The lysate was spun at 10 000 r.p.m. for 10 min to remove debris and unbroken cells, and the supernatant fractionated with 30% ammonium sulfate (16.6 g/100ml of extract). The ammonium sulfate precipitate was recovered by centrifugation at 12 000 r.p.m. for 20 min, resuspended in buffer A and dialyzed extensively against the same buffer. The dialyzed sample was passed through a DEAE column (bed volume, 25 ml) equilibrated with buffer A, washed with 5 vols of buffer A and eluted with 200 ml of a 50 mM–1 M KCl gradient. The pooled peak fractions (eluting at 200–250 mM KCl) of the DEAE column were then loaded (a maximum of 50 mg of protein) onto a Sephacryl S-300HR (Pharmacia) column (1.6 cm× 70 cm) and eluted with buffer A. The pooled fractions were concentrated using Centriprep 10 to 8–10 mg/ml, dialyzed against 50 mM HEPES-NaOH (pH 7.2), 0.1 mM EDTA and 10% glycerol, aliquoted and stored frozen at –70°C. The total yield of FtsZ was between 80 and 100 mg per 3 l of culture.

GTPase assay

Reaction mixtures were incubated under different conditions as described in the text and GTPase assayed exactly as described before (Mukherjee *et al.*, 1993).

Assay of GDP bound to FtsZ

The amount of GDP bound to FtsZ was measured by the method of Field *et al.* (1996). FtsZ [200 μg (5 nmol)] in a 20 μl volume was denatured by adding 180 μl of 8 M urea in 50 mM HEPES-NaOH (pH 7.2). After leaving it at room temperature for 10 min, it was placed in a boiling water bath for 1 min. The denatured FtsZ was then placed on a Centricon filter (10 000 kDa cut off) and the tube in which FtsZ was denatured was washed once with 100 μl of 50 mM HEPES-NaOH (pH 7.2) and added to the filter. After filtration by centrifugation, the filter was washed twice with 50 μl of the above buffer. The filtrate was analyzed for the presence of GDP by FPLC using a 1 ml Mono-Q column (Pharmacia) equilibrated with 100 mM NH_4HCO_3. Elution was carried out with a gradient of 100–500 mM NH_4HCO_3 for 30 min at a flow rate of 1 ml/min. GTP and GDP standards (1–100 nmol) were run under similar conditions.

Polymerization of FtsZ

FtsZ at the concentrations indicated was incubated at 25 or 30°C in polymerizing buffer. This buffer consisted of 50 mM MES-NaOH (pH 6.5, or as indicated), 10 mM $MgCl_2$ and 0–200 mM KCl (as indicated). GTP was added at the concentration indicated. All additions were made at room temperature, with GTP added last. Polymerization was monitored by electron microscopy or sedimentation.

Visualization of FtsZ polymers by electron microscopy

A 10 μl aliquot was withdrawn from reaction mixtures incubated under different conditions as described in the text and placed on a carbon-coated copper grid (300 mesh size) for 2 min. It was then blotted dry, negative-stained with 1% uranyl acetate and immediately blotted dry again. The grids were then viewed in a JEOL transmission electron microscope (Model 100 CXII).

Centrifugation of FtsZ polymers

For centrifugation of FtsZ polymers, reaction mixtures were incubated in polycarbonate centrifuge tubes for a Beckman TLA 100.2 rotor under different conditions as described in the text. FtsZ polymers were pelleted by centrifugation at 80 000 r.p.m. in a Beckman TLA 100.2 rotor for 15 min. The supernatant was withdrawn carefully and the pellet, which cannot be seen, was suspended in the appropriate volume of 50 mM MES-NaOH (pH 6.5), 10 mM $MgCl_2$ and assayed for protein by the Bio-Rad protein assay kit using bovine serum albumin as a standard.

Acknowledgements

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