Polymerization of nucleotide-free, GDP- and GTP-bound cell division protein FtsZ: GDP makes the difference

Sonia Huecas*, José Manuel Andreu*

Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain

Received 6 April 2004; revised 11 May 2004; accepted 18 May 2004

Available online 7 June 2004

Edited by Amy McGough

Abstract Stable, more than 98% nucleotide-free apo-FtsZ was prepared from purified *Methanococcus jannaschhi* FtsZ. This facilitates the study of the functional mechanisms of this FtsZ, an assembling GTPase, which shares a common fold with eukaryotic tubulin. Apo-FtsZ underwent cooperative magnesium-induced polymerization with a similar critical concentration and morphology related to that of reconstituted GTP-bound FtsZ, suggesting that the binding of GTP contributes insignificantly to the stability of the FtsZ polymers. On the other hand, reconstituted GDP-FtsZ polymerized with a larger critical concentration than GTP-FtsZ, indicating that GDP binding destabilizes FtsZ polymers. Upon GTP hydrolysis by FtsZ polymers, in the absence of a continued GTP supply and under macromolecular crowding conditions enhancing FtsZ polymerization, the straight GTP polymers disappeared and were replaced by characteristic helically curved GDP-bound polymers. These results suggest that the roles of GTP binding and hydrolysis by this archaeal FtsZ are simply to facilitate disassembly. In a physiological situation in GTP excess, GDP-bound FtsZ subunits could again bind GTP, or trigger disassembly, or be recognized by FtsZ filament depolymerizing proteins, allowing the Z-ring dynamics during prokaryotic cell division.

1. Introduction

FtsZ is an essential protein of the prokaryotic cell division machinery which assembles forming the cytokinetic Z-ring and recruits the other components of the septosome [1–4]. The bacterial cell division proteins are potential targets for new antibiotics [5,6]. FtsZ is a simpler structural homolog of eukaryotic tubulin, with which it forms a distinct family of assembling GTPases [7] proposed to share a similar protofilament structure [8]. Central to the functioning of nucleotide hydrolyzing protein assembly systems, including the actin and tubulin families, are the properties of their nucleotide triphosphate, nucleotide diphosphate and nucleotide-free states, which help to understand their dynamics and regulation. Possibly due to their homology, it has been frequently thought that the nucleotide interaction properties of tubulin and microtubules may also be found in FtsZ and its polymers; these properties include non-exchangeability of the nucleotide in polymers, treadmilling and dynamic instability [9]. However, this is not necessarily the case for the simpler FtsZ system. As for tubulin, the GTPase activity of FtsZ is induced by the contacts in the assembled polymer, however, in contrast to the lateral interactions of microtubule protofilaments, it has been proposed that the primary assembly product of FtsZ is a double-stranded filament, one or several of which might form the dynamic Z ring during prokaryotic cell division [10], and currently there is no proof that the FtsZ polymers have a microtubule-like dynamics.

In a study of the reversible unfolding of FtsZ by guanidinium chloride (GdmCl), we found that FtsZ from the thermophilic archaea *Methanococcus jannaschhi* releases its bound nucleotide (midpoint ~1.5 M GdmCl) without any detectable secondary structure change, whereas the unfolding of the protein takes place at higher denaturant concentrations (midpoint 3.1 M GdmCl); in contrast, FtsZ from *Escherichia coli* releases its nucleotide coinciding with an initial unfolding stage [11]. This suggested that nucleotide binding may not be essential for the structural stability of purified *M. jannaschii* FtsZ under our conditions. We have recently determined the energetics of polymerization of *M. jannaschii* FtsZ, and have shown that the hydrolysis switch from GTP-bound into GDP-liganded FtsZ involves the formation of curved polymers with an elongation affinity only 1–2 kcal mol^{-1} less favorable than the straight GTP-polymers [12]; we could also observe that purified FtsZ, which contained less than one equivalent of bound nucleotide, polymerized simply by magnesium addition without added nucleotide better than with GDP and magnesium (Huecas and Andreu, unpublished). Here, we report the novel finding of magnesium-induced polymerization of nucleotide-free FtsZ from *M. jannaschii*, in comparison with GTP- and GDP-reconstituted FtsZ. The results show that the main difference is found in the curved GDP-FtsZ polymers, and lead to the proposal that nucleotide triphosphate binding by this FtsZ is not essential for polymer stability, but that together with subsequent nucleotide hydrolysis it is a regulatory mechanism required for polymer disassembly.

2. Materials and methods

2.1. Preparation of FtsZ and nucleotide-free FtsZ from Methanococcus jannaschii

FtsZ (without a histidine tag) was overproduced in *E. coli* BL21(DE3) pLys and was purified as described [10,12]. The purified

* Corresponding authors. Fax: +34-91-5360432.
E-mail addresses: sonia@cib.csic.es (S. Huecas), j.m.andreu@cib.csic.es (J.M. Andreu).

0014-5793/$22.00 © 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.05.048

protein contained 0.45 ± 0.05 nucleotide bound (of which 80% is GTP and 20% is GDP), which increased to 0.92 ± 0.04 GDP bound upon equilibration with this nucleotide [12]. Nucleotide-free FtsZ (apo-FtsZ) was prepared by incubating FtsZ in 2.5 M GdmCl for 30 min at room temperature, followed by gel filtration in a 0.9×25 cm Sephadex G-25 column in 50 mM Mes, and 2.5 M GdmCl, pH 6.5, to separate the protein from the released nucleotide (monitored spectrophotometrically at 254 and 280 nm), and a second G-25 column in 50 mM Mes, 50 mM KCl, and 1 mM EDTA, pH 6.5 (Mes assembly buffer), in which GdmCl was eliminated from the protein, and subsequently concentrated with a Centricon YM10 (Millipore) filter in the cold. The concentration of apo-FtsZ was measured spectrophotometrically employing an extinction coefficient $\varepsilon_{280} = 6990\ M^{-1}\ cm^{-1}$, calculated for the protein (1 Trp, 1 Tyr) in buffer [13], which is practically coincident with a previous value calculated for this protein in 6 M GdmCl ($\varepsilon_{280} = 6970\ M^{-1}\ cm^{-1}$, $\varepsilon_{254} = 4275\ M^{-1}\ cm^{-1}$ [11]). Guanine nucleotides were measured spectrophotometrically with extinction coefficient $\varepsilon_{254} = 13620\ M^{-1}\ cm^{-1}$ ($\varepsilon_{280} = 8100\ M^{-1}\ cm^{-1}$) [11]. Apo-FtsZ was spectrophotometrically found devoid of nucleotide, but it was able to bind nucleotide when equilibrated in buffer with 10 μM GDP or GTP, reconstituting holo-FtsZ (note that even FtsZ fully unfolded by treatment with 6 M GdmCl is known to be able to bind again GDP or GTP upon removal of the denaturant [11]). Apo-FtsZ was immediately used or kept at 4 °C for less than 3 days. Apo-FtsZ polymers (Section 3) were analyzed by HPLC and no nucleotide was detected, which calibrating the sensitivity of the system and with the apo-FtsZ concentration employed, indicated less than 0.02 guanine nucleotide per FtsZ.

2.2. Miscellaneous procedures

Circular dichroism spectra were acquired at 25 °C as described [12]. Polymerization of FtsZ in Mes assembly buffer at 55 °C was initiated by addition of 10 mM $MgCl_2$, monitored by 90° light scattering, and the structure of the polymers formed was examined by electron microscopy. FtsZ polymers were quantified by isothermal pelleting [12] (6 min at $139\,000 \times g$ in a prewarmed TL100 Beckmann rotor with 7×20 mm tubes, except where otherwise indicated) and protein concentration measurement. Nucleotides were extracted from FtsZ with cold 0.5 M $HClO_4$ and the supernatant was analyzed by HPLC [12].

3. Results

3.1. Polymerization of nucleotide-free FtsZ. Comparison with FtsZ reconstituted with GTP and GDP

We questioned whether nucleotide binding is required for FtsZ polymerization. More than 98% nucleotide-free FtsZ from *M. jannaschii* (apo-FtsZ) was prepared by 2.5 M GdmCl treatment of FtsZ followed by removal of the released nucleotide and of the GdmCl (Section 2). The circular dichroism spectra of apo-FtsZ and FtsZ showed no significant difference (not shown; for the circular dichroism of FtsZ see [11]), indicating undetectable changes in the average secondary structure of nucleotide-free FtsZ.

Cooperative polymerization of apo-FtsZ was induced by 10 mM $MgCl_2$, under standard polymerization conditions for *M. jannaschii* FtsZ (Mes assembly buffer, pH 6.5, at 55 °C), as determined by quantitative sedimentation of the polymer (Fig. 1A). For a nucleated condensation polymerization system [14], such as FtsZ [12], essentially all the proteins above a given critical concentration assemble into large polymers which can be quantitatively sedimented; the critical concentration equals in good approximation the reciprocal of the apparent binding equilibrium constant of a new monomer to the polymer (polymer elongation affinity). Addition of 1 mM GTP to apo-FtsZ in order to reconstitute holo-FtsZ (Section 2) resulted in polymerization of FtsZ-GTP with a slightly lower critical concentration. In contrast, addition of 1 mM

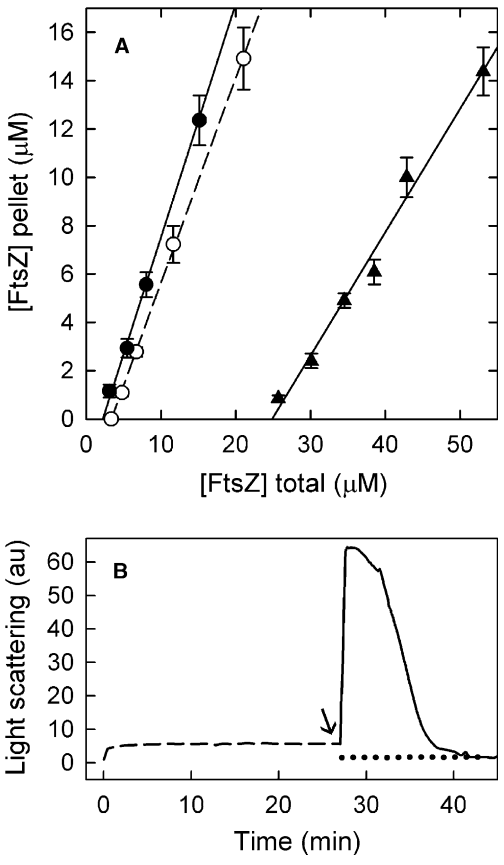

Fig. 1. (A) Polymerization of apo-FtsZ (○) and FtsZ reconstituted with 1 mM GTP (●) or with 1 mM GDP (▲) in Mes assembly buffer, pH 6.5, 10 mM $MgCl_2$ at 55 °C, measured by sedimentation. Increasing the centrifugation time of the GDP-FtsZ samples from 6 min (Section 2) to 15 min did not modify these results. (B) Polymerization of apo-FtsZ (12.8 μM) in Mes assembly buffer, pH 6.5, at 55 °C, monitored by light scattering. Polymerization was initiated by the addition of 10 mM MgCl2 (- - -) and where marked by the arrow, 1 mM GTP (—) or 1 mM GDP (· · ·) was added.

GDP resulted in polymerization of FtsZ with a sixfold higher critical concentration than with GTP (Fig. 1A and Table 1) (only half of the FtsZ-GDP protein in excess above the critical concentration apparently assembled under these conditions, but all of it assembled in Ficoll, Fig. 2A, or at pH 6.0 [12]). The critical concentration values of GTP- and GDP-reconstituted FtsZ were comparable to the corresponding values determined for the native protein (Table 1). Monitoring the time-course of polymerization by light scattering indicated the magnesium-induced formation of stable polymers from apo-FtsZ. Their light scattering was reproducibly reduced by GDP addition (Fig. 1B, dash and dotted lines). This effect is compatible with disassembly of GDP-FtsZ below its critical concentration (Fig. 1A) and it is qualitatively similar to previously observed rapid disassembly of GTP-FtsZ by GDP addition [10]. Addition of GTP to apo-FtsZ polymers resulted in a large increase in light scattering (about 30-fold larger than with apo-FtsZ) with a half-rise time <20 s (Fig. 1B, solid line). The relatively small light scattering of the apo-FtsZ polymers under these conditions can be related to their small width compared to GTP-FtsZ polymers (see below). Several minutes later the scattering decreased with

Table 1
Critical concentration for FtsZ polymerization in Mes assembly buffer, pH 6.5, in the presence and in the absence of 200 g/l Ficoll 70

	Cr (μM)	Cr (μM) + Ficoll
Apo-FtsZ	3.40 ± 0.25	0.17 ± 0.04
Apo-FtsZ + 1 mM GTP	2.20 ± 0.16 (1.30 ± 0.11)	0.16 ± 0.05 (0.14 ± 0.04)
Apo-FtsZ + 1 mM GDP	12.75 ± 0.88 (16.2 ± 1.0)	1.40 ± 0.08 (0.48 ± 0.06)

The values in brackets are from data with the native protein [12], shown here for comparison. The Cr values were determined from the intercepts on the *x*-axis, except in GDP without Ficoll (Fig. 1, triangles), where Cr was determined from the negative intercept on the *y*-axis, a procedure to correct for the partial assembly activity of the protein [28] under these conditions.

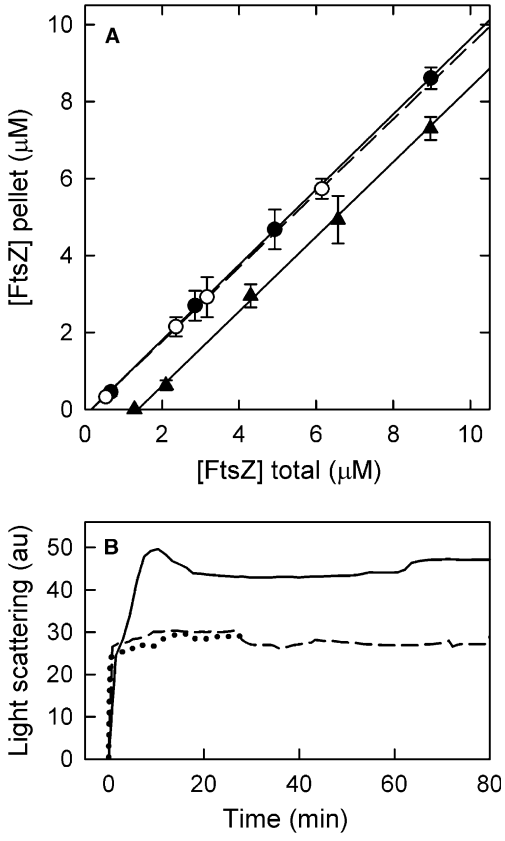

Fig. 2. (A) Polymerization of apo-FtsZ (○) and FtsZ reconstituted with 1 mM GTP (●) or with 1 mM GDP (▲) in Mes assembly buffer, pH 6.5, 200 g/l Ficoll 70, 10 mM $MgCl_2$ at 55 °C, measured by sedimentation. (B) Polymerization of apo-FtsZ (12.8 μM) in Mes assembly buffer, pH 6.5, 200 g/l Ficoll 70, at 55 °C, monitored by light scattering. Polymerization was initiated by the addition of 10 mM $MgCl_2$ (- - -), 10 mM $MgCl_2$ plus 1 mM GTP (—) or 10 mM $MgCl_2$ plus 1 mM GDP (· · ·).

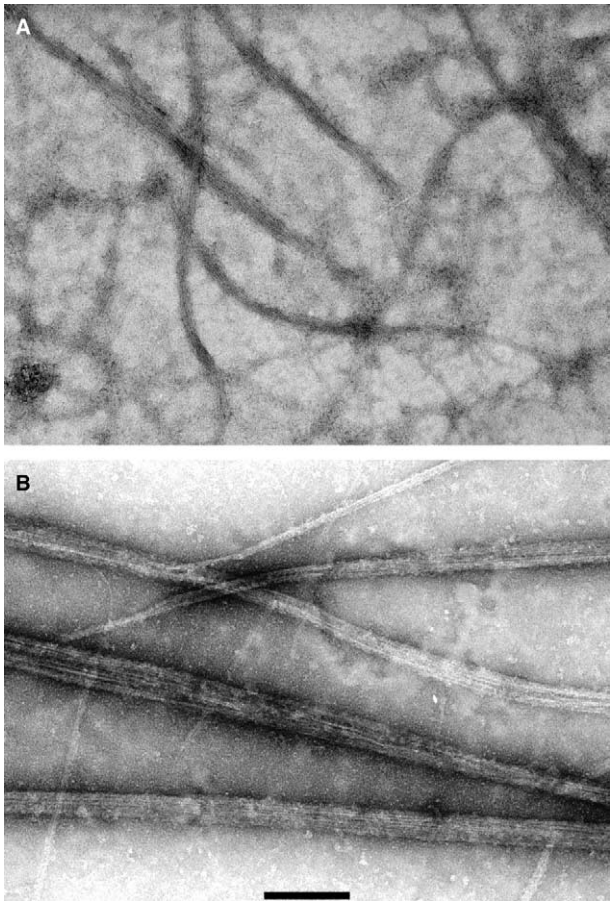

Fig. 3. Electron micrographs of polymers formed by apo-FtsZ (12.8 μM) (A) and by GTP-FtsZ (B) in Mes assembly buffer, pH 6.5, 10 mM $MgCl_2$ at 55 °C. The bar indicates 200 nm.

time to levels similar to GDP-FtsZ (Fig. 1B, solid line), following the characteristic pattern due to GTP hydrolysis by FtsZ under these conditions (see Fig. 1 in [10]). From these results, we concluded that apo-FtsZ cooperatively polymerizes with an apparent elongation affinity (2.9×10^5 M^{-1}) close to that of GTP-FtsZ (4.5×10^5 M^{-1}) and significantly higher than that of GDP-FtsZ (0.8×10^5 M^{-1}), and also that addition of nucleotide to apo-FtsZ semi-quantitatively reconstitutes the behavior of the native protein.

Similar experiments were made in buffer containing the macromolecular crowding agent Ficoll 70 (200 g/l), a procedure to enhance FtsZ assembly [12], which is considered to resemble the crowded intracellular environment [15]. The critical concentrations were shifted to lower values, and apo-FtsZ assembled with a critical concentration close to that of GTP-FtsZ and significantly lower than that of GDP-FtsZ polymers, which could be sedimented quantitatively under these conditions (Fig. 2A and Table 1). This confirmed the nucleotide effects, i.e., apo-FtsZ polymerizes with a similar elongation affinity than GTP-FtsZ, and GDP-FtsZ polymerizes with a lower apparent elongation affinity (but larger than without Ficoll). Monitoring polymerization with crowder at pH 6.5 by light scattering showed a large scattering increase induced by Mg^{2+}; the scattering was roughly stable during more than 1 h with GTP (Fig. 2B) instead of decreasing, which would be compatible with macromolecular crowding retarding the GTP hydrolysis, as for polymers of FtsZ from *E. coli* [15]. However, the large scattering intensity in Ficoll was found relatively insensitive to the structure of the different large polymers formed by apo-, GDP- or

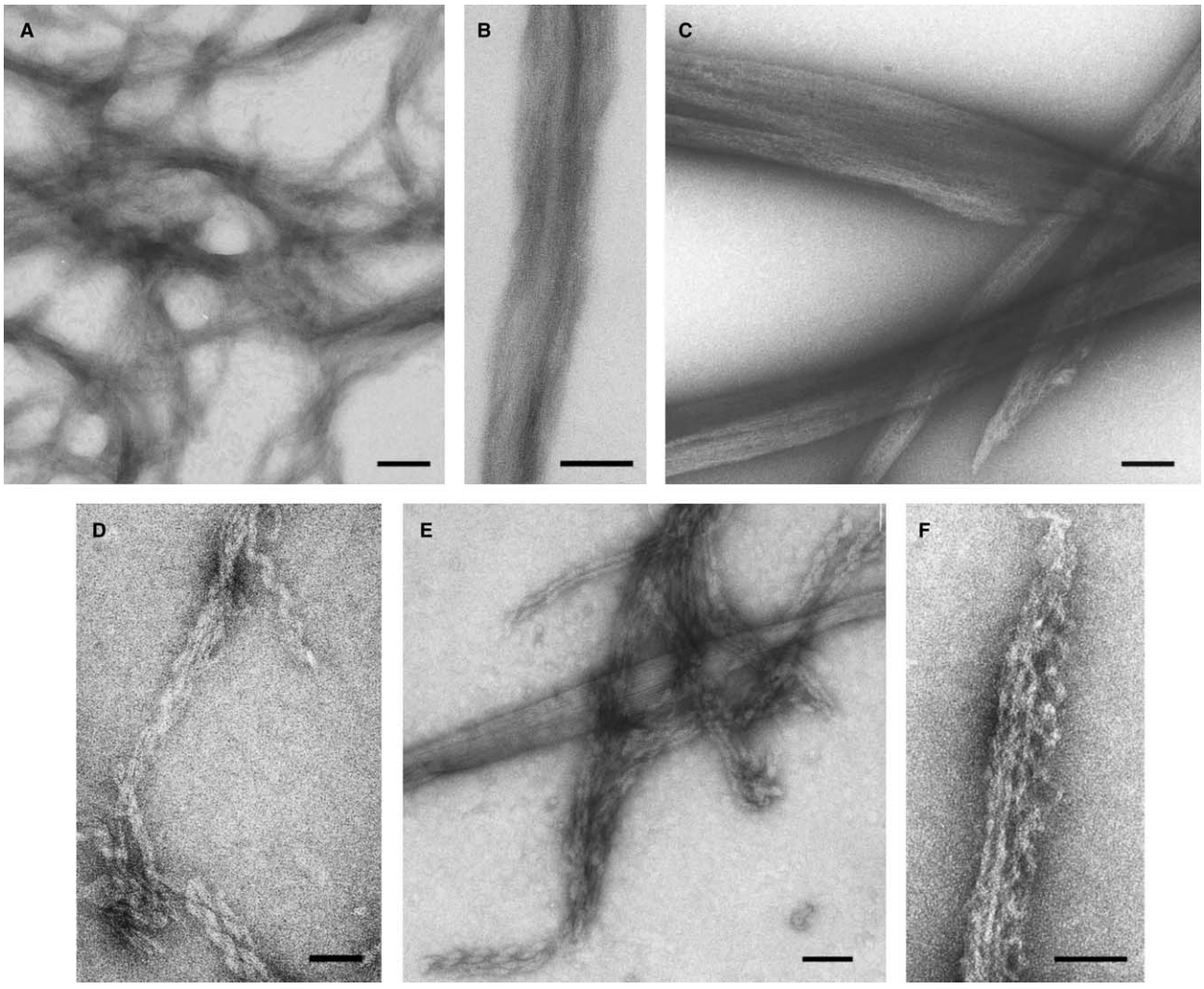

Fig. 4. Electron micrographs of polymers formed by apo-FtsZ (12.8 μM) in Mes assembly buffer, plus 200 g/l Ficoll 70, pH 6.5, 10 mM $MgCl_2$, at 55 °C, without nucleotide (A and enlarged image B), with 1 mM GTP (C) and with 1 mM GDP (D). In a different experiment, 1 mM GTP was added to preformed apo-FtsZ polymers under the same conditions, except pH 6.0, and the changes with time (GTP hydrolysis, Table 2) were followed starting from images of GTP-FtsZ polymers (such as C) and ending into GDP-FtsZ polymers (indistinguishable from D). At intermediate times of 30 and 40 min, images of mixed polymer populations were obtained (E and F, respectively). Bars indicate 100 nm.

GTP-FtsZ (see below), and was not useful for their comparison.

3.2. Morphology of FtsZ polymers

The polymers formed by apo-FtsZ in Mes assembly buffer with 10 mM $MgCl_2$ were examined by negative stain electron microscopy and found to consist of 15–40 nm wide filamentous polymers (Fig. 3A), most of which are thinner than the majority of the 25–80 nm wide polymers of reconstituted GTP-FtsZ observed under the same conditions (Fig. 3B); some isolated filaments could also be observed in both cases.

Table 2
Nucleotide hydrolysis by FtsZ polymers in Ficoll

	Time after adding 1 mM GTP		
	17 min	42 min	162 min
Polymer	46% GTP 54% GDP	39% GTP 60% GDP	7% GTP 93% GDP
Supernatant	63% GDP 34% GDP	36% GTP 64% GDP	0.5% GTP 99.5% GDP

The samples for HPLC were taken at different times after addition of 1 mM GTP to polymers of apo-FtsZ.

In the presence of Ficoll 70 (200 g/l), apo-FtsZ formed polymer bundles (Fig. 4A and B), which were similar, although somewhat less straight, than GTP-FtsZ bundles in Ficoll (Fig. 4C). In contrast, reconstituted GDP-FtsZ formed under same conditions, clearly different, helically curved ribbon-like polymers (Fig. 4D). In the absence of Ficoll, we could not reproducibly visualize GDP-FtsZ polymers by negative stain electron microscopy, as reported elsewhere [12]. These results showed that the morphology of apo-FtsZ polymers is related to GTP-FtsZ polymers, but markedly different from the curved GDP-FtsZ polymers.

3.3. Changes in FtsZ polymers induced by nucleotide binding and hydrolysis

Electron microscopy, after addition of nucleotides to preformed FtsZ polymers, permitted to observe the results of dynamic structural changes in FtsZ polymer solutions. Addi-

tion of GTP to apo-FtsZ polymers (Figs. 1B and 3A) resulted in the rapid acquisition of the characteristically wider morphology of GTP-FtsZ polymers (indistinguishable from Fig. 3B); subsequent GTP hydrolysis by these polymers (see Fig. 1B) resulted in lack of observation of any large polymers after 15 min.

Hydrolysis of GTP added to preformed apo-FtsZ polymers in Ficoll resulted, instead of depolymerization, in a slower change observed from the straight morphology of GTP polymers (Fig. 4C) into characteristically curved GDP-FtsZ polymers (indistinguishable from Fig. 4D). The observation of a population of all curved GDP-FtsZ polymers upon GTP exhaustion from the solution (Table 2) required more than 40 min, at pH 6.0 instead of pH 6.5 (probably due to a slower GTP hydrolysis at pH 6.5, see Fig. 2B). During the course of time, helically curved polymer sections gradually appeared among the straight polymers (Fig. 4E) and images suggestive of partially curved polymers could also be observed (Fig. 4F). These results confirmed the curved conformation of GDP-FtsZ polymers and showed that the curved polymers arise from the straight GTP-FtsZ polymer solutions upon GTP hydrolysis in the absence of a continued GTP supply. It should be cautioned that the available results do not prove whether these changes take place in individual polymers or require disassembly and assembly.

4. Discussion

The results of this work show that a bound nucleotide is not required for the practical structural stability of purified FtsZ from *M. jannaschii* at moderate temperatures (although nucleotide binding should thermodynamically stabilize the folded form of the protein). Furthermore, nucleotide binding is not essential for FtsZ polymerization, since nucleotide-free FtsZ undergoes cooperative magnesium-induced polymerization with an apparent elongation affinity similar to GTP-bound FtsZ. Therefore, GTP binding appears to contribute insignificantly to the stability of FtsZ polymers. This is a remarkable property, considering that the GTP binding site is at the axial contact interface between consecutive FtsZ monomers in the tubulin-like FtsZ model protofilament [8]. In addition, the rapid transformation of apo-FtsZ polymers into GTP-FtsZ polymers upon GTP addition (Fig. 1B) suggests accessibility of the nucleotide binding site in the apo-FtsZ polymers, rather than GTP-induced assembly of the bulk FtsZ in the solution via the small fraction of FtsZ monomers in equilibrium with polymers; this would support the proposal of a rapidly exchangeable nucleotide binding site in FtsZ polymers [16].

GDP-bound FtsZ has a smaller elongation affinity than apo-FtsZ and GTP-FtsZ, and it forms different, curved polymers. This is compatible with the effects predicted in a molecular dynamics study, which indicate movements of switch loop T3 and helix H3 potentially curving the protofilament in the GDP state with respect to the GTP state [17]. Curved filaments with GDP were previously observed in FtsZ from *E. coli*, which formed minirings onto cationic phospholipid monolayers and helical tubes with the polycation DEAE-dextran [18]. GDP-FtsZ from *E. coli* oligomerized in solution [19], and small arcs and rings were observed by electron microscopy [20], in contrast with the much longer polymers formed with GTP [19,20]. GDP binding apparently destabilizes large FtsZ polymers, making a functional polymerization difference with the GTP-bound and nucleotide-free FtsZ. Confirmation under the extreme environmental conditions of *M. jannaschii* [21] is not technically feasible. The availability of stable apo-FtsZ should facilitate nucleotide and Mg^{2+} binding studies. Polymerization without a bound nucleotide may be a property of this hyperthermophilic FtsZ, or also of FtsZs from mesophilic organisms; however, similar experiments with FtsZ from *E. coli* were hampered by the instability of this protein without bound nucleotide. The assembly of nucleotide-free actin has been studied with the aid of a stabilizing co-solvent [22]. Polymerization of nucleotide-free tubulin has not been documented to our knowledge; instead, the hydrolyzable GTP and coordinated magnesium ion of β-tubulin [23] control microtubule assembly, and the magnesium ion coordinated with the non-exchangeable GTP of α-tubulin is required for the stability of the αβ-tubulin dimer [24].

The use of nucleotide-free FtsZ is a procedure of studying the functional mechanisms of this protein. It seems that for this primitive archaeal FtsZ, in a physiological situation in excess GTP, the role of nucleotide binding followed by hydrolysis may be simply to facilitate disassembly from the GDP state. GTP hydrolysis by purified archaeal FtsZ polymers, in the absence of a continued GTP supply, leads to polymer curvature, and also to disassembly depending on the solution conditions. The bacterial Z-ring is very dynamic, with a turnover of FtsZ subunits in tens of seconds [25]. Hydrolysis of GTP may be an internal timer indicating the age of the FtsZ filaments and triggering disassembly, analogously to actin filaments in cells [26], rather than directly generating a constriction force at the septal site. Very recently, it has been shown that at steady state polymers of FtsZ from *E. coli* are predominantly GTP liganded, and that the intrinsic GTP hydrolysis reaction is rate-limiting for nucleotide turnover [27]. GDP-FtsZ polymers might form more or less extensively in vivo, depending on the rates of nucleotide hydrolysis, nucleotide and subunit exchange, and polymer disassembly. In a minimal case, a few GDP-bound FtsZ subunits would trigger disassembly by themselves or be recognized by FtsZ-filament depolymerizing proteins.

Acknowledgements: We thank Drs. J.F. Díaz and J. Mingorance for discussion, and Martín Alba for FtsZ purification. This work was supported by grants from MCyT BIO 2002-03665 and Red Temática de Investigación Cooperativa FIS C03/14.

References

[1] Lutkenhaus, J (2002) Curr. Opin. Microbiol. 5, 548–552.
[2] Buddelmeijer, N. and Beckwith, J. (2002) Curr. Opin. Microbiol. 5, 553–557.
[3] Errington, J., Daniel, R.A. and Scheffers, D.J. (2003) Microbiol. Mol. Biol. Rev. 67, 52–65.
[4] Romberg, L. and Levin, P.A. (2003) Annu. Rev. Microbiol. 57, 125–154.
[5] Wang, J., Galgoci, A., Kodali, S., Herath, K.B., Jayasuriya, H., Dorso, K., Vicente, F., Gonzalez, A., Cully, D., Bramhill, D. and Singh, S. (2003) J. Biol. Chem. 278, 44424–44428.
[6] Kenny, C.H., Ding, W., Kelleher, K., Benard, S., Dushin, E.G., Sutherland, A.G., Mosyak, L., Kriz, R. and Ellestad, G. (2003) Anal. Biochem. 323, 224–233.

[7] Nogales, E., Downing, K.H., Amos, L. and Löwe, J. (1998) Nature Struct. Biol. 5, 451–457.
[8] Lowe, J. and Amos, L.A. (1999) EMBO J. 18, 2364–2371.
[9] Howard, J. and Hyman, A.A. (2003) Nature 422, 753–758.
[10] Oliva, M.A., Huecas, S., Palacios, J.M., Martin-Benito, J., Valpuesta, J.M. and Andreu, J.M. (2003) J. Biol. Chem. 278, 33562–33570.
[11] Andreu, J.M., Oliva, M.A. and Monasterio, O. (2002) J. Biol. Chem. 277, 43262–43270.
[12] Huecas, S. and Andreu, J.M. (2003) J. Biol. Chem. 278, 46146–46154.
[13] Pace, N.C., Vajdos, F., Fee, L., Grimsley, G. and Gray, V. (1995) Protein Sci. 4, 2411–2423.
[14] Oosawa, F. and Asakura, S. (1975) Thermodynamics of the Polymerization of Protein. Academic Press, London.
[15] Gonzalez, J.M., Jiménez, M., Velez, M., Mingorance, J., Andreu, J.M., Vicente, M. and Rivas, G. (2003) J. Biol. Chem. 278, 37664–37671.
[16] Mingorance, J., Rueda, S., Gomez-Puertas, P., Valencia, A. and Vicente, M. (2001) Mol. Microbiol. 41, 83–91.
[17] Diaz, J.F., Kralicek, A., Mingorance, J., Palacios, J.M., Vicente, M. and Andreu, J.M. (2001) J. Biol. Chem. 276, 17307–17315.
[18] Lu, C., Reedy, M. and Erickson, H.P. (2000) J. Bacteriol. 182, 164–170.
[19] Rivas, G., Lopez, A., Mingorance, J., Ferrandiz, M.J., Zorrilla, S., Minton, A.P., Vicente, M. and Andreu, J.M. (2000) J. Biol. Chem. 275, 11740–11749.
[20] Romberg, L., Simon, M. and Erickson, H.P. (2001) J. Biol. Chem. 276, 11743–11753.
[21] Jones, W.J., Leigh, J.A., Mayer, F., Woese, C.R. and Wolfe, R.S. (1983) Arch. Microbiol. 136, 254–261.
[22] De la Cruz, E.M., Mandinova, A., Steinmetz, M.O., Stoffer, D., Aebi, U. and Pollard, T.D. (2000) J. Mol. Biol. 295, 517–526.
[23] Correia, J.J., Beth, A.H. and Williams, R.C. (1988) J. Biol. Chem. 263, 10681–10686.
[24] Menéndez, M., Rivas, G., Díaz, J.F. and Andreu, J.M. (1998) J. Biol. Chem. 273, 167–176.
[25] Stricker, J., Maddox, P., Salmon, E.D. and Erickson, H.P. (2002) Proc. Natl. Acad. Sci. USA 99, 3171–3175.
[26] Pollard, T.D. and Borisy, G.G. (2003) Cell 112, 453–465.
[27] Romberg, L. and Mitchison, T.J. (2004) Biochemistry 43, 282–288.
[28] Weisenberg, R.C. (1986) Ann. N.Y. Acad. Sci. 466, 543–551.