The polymerization mechanism of the bacterial cell division protein FtsZ

Scheffers, Dirk-Jan; Driessen, A.J.M.

Published in:
FEBS Letters

DOI:
10.1016/S0014-5793(01)02855-1

Publication date:
2001

FEBS Letters 506 (2001) 6–10 FEBS 25254

Minireview

The polymerization mechanism of the bacterial cell division protein FtsZ

Dirk-Jan Scheffers[1], Arnold J.M. Driessen*

Department of Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Received 20 July 2001; revised 23 August 2001; accepted 23 August 2001

First published online 5 September 2001

Edited by Julio Celis

Abstract Bacteria and archaea usually divide symmetrically by formation of a septum in the middle of the cell. A key event in cell division is the assembly of the FtsZ ring. FtsZ is the prokaryotic homolog of tubulin and forms polymers in the presence of guanine nucleotides. Here, we specifically address the polymerization of FtsZ and the role of nucleotide hydrolysis in polymer formation and stabilization. Recent structural and biochemical results are discussed and a model for FtsZ polymerization, similar to that for tubulin, is presented.

Key words: FtsZ; Polymerization; Cell division; Tubulin

1. Introduction

Cell division is a crucial event in the life of every organism. Most bacteria and archaea divide symmetrically in a process that is subject to extensive regulation to ensure that both newly formed daughter cells contain a copy of the chromosome (for a recent review, see [1]). By far the best-characterized cell division protein is FtsZ. *FtsZ* is the first cell division gene to act during the division cycle in *Escherichia coli* and has been identified in most prokaryotic species, with the exception of *Chlamydia*, a *Ureaplasma* species, and *Crenarchaea* [1,2]. FtsZ is conserved across the evolutionary divide, being essential for division of chloroplasts and mitochondria in some eukaryotes [3,4]. Pioneering work in the early 1990s provided important clues for FtsZ function. Immunolocalization demonstrated that in *E. coli*, FtsZ is present in a ring structure at the division site [5], which was later confirmed in other bacteria and archaea (see [1]). The appearance of the FtsZ ring coincides with the termination of DNA replication, but a direct signal for ring assembly has not yet been identified [6]. The FtsZ ring behaves dynamically during division and remains attached to the leading edge of the constricting septum [7]. Formation of the FtsZ ring is essential for correct localization of the division plane. A striking example is the *ftsZ26* mutant, which forms a FtsZ spiral rather than a ring, giving rise to cells with spiral constrictions [8]. After formation of the FtsZ ring, all other cell division proteins localize to the division site in an ordered manner. The ring structure prompted the suggestion that FtsZ is a cytoskeletal protein [5], which was augmented by the discovery that FtsZ is the homolog of the eukaryotic cytoskeletal protein tubulin. In this minireview, recent insights in the mechanism of FtsZ polymerization are discussed.

2. Structural similarities between FtsZ and tubulin

The homology between FtsZ and tubulin was suggested on the basis of functional and structural data. First, FtsZ is a GTPase that contains the tubulin-signature nucleotide-binding motif GGGTGS/TG [9–11]. Secondly, FtsZ is capable of nucleotide-dependent assembly into filaments [12,13]. The elucidation of the structures of FtsZ1 from the thermophilic archaeon *Methanococcus jannaschii* [14] and the α,β-tubulin heterodimer from bovine brain [15] provided conclusive evidence for their homology (Fig. 1). FtsZ and tubulin form a distinct class of GTPases and a common nomenclature for their secondary structure elements has been developed [16]. Recent work has provided evidence for similar roles of two loop regions in tubulin and FtsZ. First, loop T3 contacts the γ-phosphate in α-tubulin [15]. The loss of the γ-phosphate is thought to result in a conformational change caused by the displacement of loop T3 [17]. A molecular dynamics simulation of FtsZ has identified a conformational change of loop T3 induced by the nucleotide γ-phosphate, which was confirmed by fluorescence spectroscopy [18]. Secondly, in the α,β-tubulin dimer [15] as well as in the microtubule (MT) [17], loop T7 and the start of helix 8 (T7-loop region) form contacts with the nucleotide bound to another monomer. For FtsZ, a similar interaction was postulated based on a molecular modeling of its crystal structure in reconstructed electron micrographs of FtsZ protofilaments [19]. The interaction of the T7-loop region of FtsZ with the guanine nucleotide on the adjacent subunit is supported by mutagenesis and biochemical data. Various mutations that inhibit *E. coli* FtsZ GTPase activity, map in the T7-loop region, which is on the opposite side of the nucleotide-binding site in the monomer (Table 1). T7-loop region mutants with inhibited GTP hydrolysis activity also suppress the activity of wild-type FtsZ when mixed (D.-J. Scheffers et al., submitted). A conserved Asp residue in the T7-loop region was found to be involved in the coordina-

*Corresponding author. Fax: (31)-50-3632154.
E-mail address: a.j.m.driessen@biol.rug.nl (A.J.M. Driessen).

[1] Present address: Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK.

Abbreviations: GTP-γ-S, guanosine-5′-*O*-(3-thiotriphosphate)

PII: S0014-5793(01)02855-1

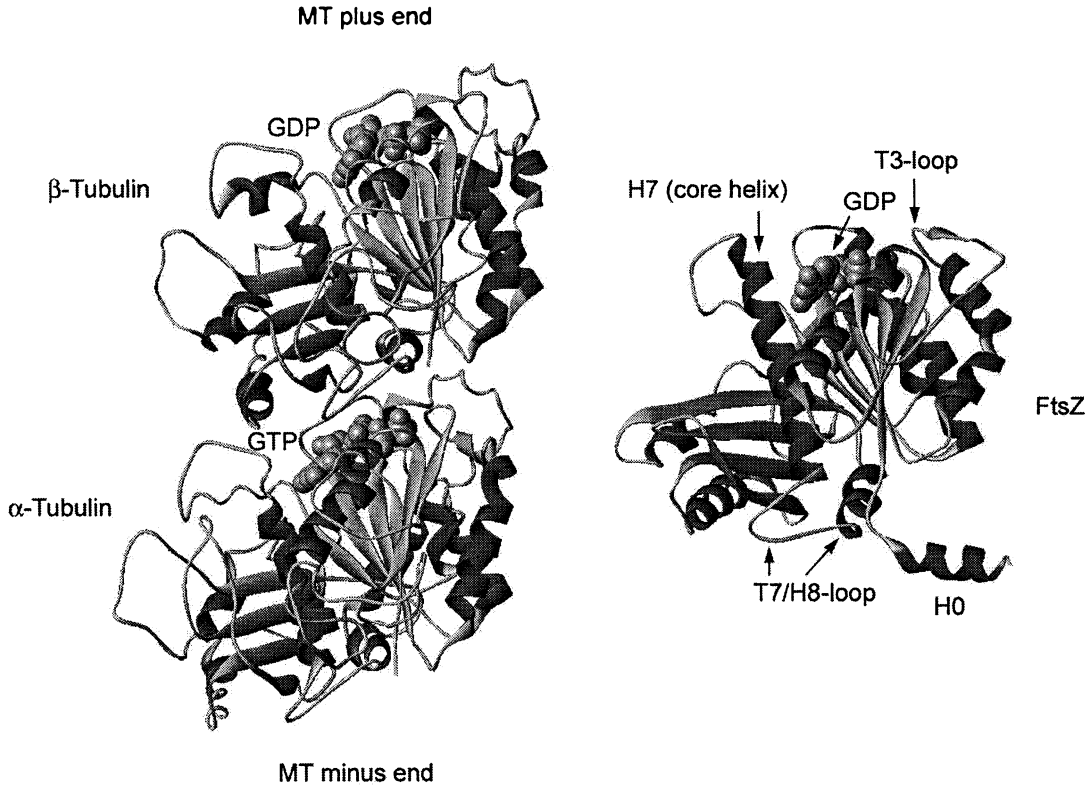

Fig. 1. Structures of the α,β-tubulin heterodimer (PDB entry 1TUB, [15]) and FtsZ (PDB entry 1FSZ, [14]). Some of the secondary structure elements discussed in the text are assigned for FtsZ according to the unifying secondary structure assignment of Nogales et al. [16]. The orientation of the α,β-tubulin heterodimer in the MT is indicated.

tion of the cation involved in nucleotide hydrolysis [20]. Therefore, it appears that the active site for GTP hydrolysis is formed through interaction of two FtsZ monomers.

3. Multimeric state of FtsZ in vivo and in vitro

In the cell, about 75% of FtsZ is present as multimers with more than 25 subunits. This was shown by gel filtration of *E. coli* cytosolic extracts, which also revealed that the self-association is concentration-dependent and cold-sensitive [21]. In vitro, FtsZ self-association is concentration-dependent, and shifted to the monomeric state by the addition of GDP [22,23]. The multimers are likely to contain GDP at the interface between the subunits, since purified FtsZ retains 0.5–0.7 mol GDP per mol FtsZ [9,20,22]. The presence of Mg^{2+} induces non-cooperative FtsZ self-association with one Mg^{2+} bound per FtsZ monomer, similar to tubulin [23]. The assembly of FtsZ into polymers is promoted by the pres-

Table 1
Single amino acid replacements that inhibit *E. coli* FtsZ GTPase activity

Replacement	Location in the *FtsZ* structure
N43D [51]	loop T2 (NBS)[a]
D45A [36], D45N [51]	loop T2 (NBS)
A70T (*ftsZ1*)[b] [36,52]	loop T3 (contacts GTP γ-phosphate)
D86K [36]	helix H3
G105S (*ftsZ84*) [9,44]v	loop T4 (NBS)
T108A (ftsZ3) [52]	loop T4 (NBS)
N165D, N165Y [51]	loop T6 (NBS)
M206C[c]	loop T7 (AS)[d]
N207C[c], N207D [51]	loop T7 (AS)
D209A [36], D209C[c], D209E[c], D209N[c]	loop T7 (AS)
D212A[36], D212C [20], D212E [20], D212G (*ftsZ2*) [34,53], D212N [20]	loop T7 (AS)
D269A [36]	loop between S8 and H10[e]

[a]NBS: Nucleotide-binding site: in direct contact with the nucleotide in the monomeric crystal structure [14].
[b]The original names of mutants, identified in screens for *ftsZ* mutants that were temperature-sensitive (*ftsZ84*) or resistant to cell division inhibitors (*ftsZ1*, *ftsZ2* and *ftsZ3*), are given in brackets.
[c]D.-J. Scheffers et al., submitted.
[d]AS: Active site for GTP hydrolysis, formed through interaction with the nucleotide-binding site on another FtsZ monomer at the interface in the protofilament (see text).
[e]This loop is present at the interface of two FtsZ monomers in the protofilament.

ence of crowding proteins that are not related to cell division [24]. Analysis of the analytical ultracentrifugation data suggests the presence of long straight FtsZ filaments [23,24]. However, microscopical studies of polymers formed with GDP show predominantly curved polymers (see below).

4. FtsZ polymer morphology

In vitro, FtsZ polymers can form a variety of structures, depending on the polymerization conditions applied. With GTP, FtsZ assembles into thin protofilaments [12,13,25–27]. Recently, similar polymers were found using guanylyl-(α,β)-methylene-diphosphonate, a GTP analogue that is hydrolyzed slowly by FtsZ [28]. The dynamic assembly of polymers is coupled to GTP consumption: when GTP runs out, polymers disassemble [26,29]. The protofilaments can further assemble into polymer sheets and bundles when stimulated by millimolar amounts of Mg^{2+} [29] and, more so, Ca^{2+} [19,29,30], or the polycation DEAE-dextran [25]. This probably relates to an effect of cations on the negatively charged polymers [31]. The Ca^{2+}-induced sheets are composed of an antiparallel arrangement of double protofilaments [19]. Similar bundles of FtsZ filaments were reported when FtsZ was polymerized in the presence of the cell division protein ZipA, which interacts directly with FtsZ [32,33]. At conditions that induce bundling, either high cation concentrations or the presence of ZipA, FtsZ GTPase activity is reduced, resulting in prolonged polymer presence ([29]; Piet de Boer, personal communication).

With GDP, FtsZ assembles as curved filaments and minirings [25,27,28]. Helical tubules of FtsZ polymers were reported after polymerization with GDP or GTP in the presence of DEAE-dextran [27,34]. Similar tubes were found with FtsZ1 from *M. jannaschii*. Polymerization with GTP and Ca^{2+} induces the formation of 'closed' tubes consisting of six helically twisted, antiparallel-arranged pairs of protofilaments. With Mg^{2+} GDPPCP, 'open' tubes were identified that are made up of three groups of four protofilament pairs, which are helically twisted around each other, leaving space that appeared to be filled up by additional filaments [35]. These 'open' tubes consist of curved protofilaments, while MTs consist of 13 to 14 straight protofilaments [35]. Long-spirals and closed-circular hoops were observed with FtsZ mutant D209A at 10 mM EDTA or with GTP [36]. Finally, cation-induced polymerization without added nucleotide has been observed for Asp212 mutants that retain the bound GTP during purification [20].

5. FtsZ dynamics: GTP hydrolysis

The relation between FtsZ polymerization and GTP hydrolysis has been the subject of numerous studies. FtsZ GTPase activity is Mg^{2+}-dependent and is stimulated by KCl [11]. Studies on FtsZ from five bacterial species and one archaeon showed that all FtsZ species display a protein concentration-dependent GTPase activity [22,37–40]. The GTPase activity was found to be stimulated at concentrations of FtsZ that allowed polymer assembly, similar to tubulin [41]. This implies that FtsZ GTP hydrolysis is self-activated, with an active site that is formed by interaction of two monomers. Assembly of the active site is likely to be accompanied by immediate GTP hydrolysis, since the nucleotide extracted from FtsZ in polymers is mainly GDP, even at conditions that do not allow for a high rate of GTP turnover [42]. The self-activation phenomenon prompted the suggestion that the well-conserved R214 at the T7-loop region acts as an 'arginine-finger' that enhances GTPase activity, but this could not be confirmed [22]. An R214C mutant suggests a role for the positive charge at this position for the coordination of the release of γ-phosphate after GTP hydrolysis (D.-J. Scheffers et al., submitted). Recently, it has been stated that FtsZ polymers in fact contain GTP [43]. However, this study did not rule out the possibility that the polymer contains bound GDP and P_i.

The G105S mutation of the temperature-sensitive *ftsZ84* mutant alters the FtsZ nucleotide specificity from a GTPase to an ATPase [44]. Mutation of the corresponding residue in *Caulobacter crescentus* FtsZ conveyed a dominant-lethal phenotype [45]. A similar phenotype was obtained with a double-alanine mutant of the D212/R214 corresponding residues [45], again stressing the role of the loop-T7/H8 region. Various mutations that severely affect GTP hydrolysis activity do not inhibit the formation of a functional FtsZ ring [36,46,47], suggesting that the high GTPase capacity is not essential for FtsZ function.

It is still unclear why KCl, and not NaCl, has such an influence on GTP hydrolysis, and hence, polymer stability [11,26,40]. Other factors known to reduce the GTPase activity of FtsZ, such as high concentrations (>2.5 mM) of Mg^{2+} and Ca^{2+}, induce bundling of the FtsZ polymers [29,40]. Although it is still unresolved whether polymer bundling has a role in vivo, it is interesting to note that ZipA, which interacts directly with the extreme C-terminus of FtsZ, induces FtsZ polymer bundling in vitro [32,33] and reduces the FtsZ GTPase activity (Piet de Boer, personal communication).

6. FtsZ dynamics: polymerization

Given the structural homology between FtsZ and tubulin, data on FtsZ dynamics are usually interpreted according to existing models for MT polymerization. The dynamic instability model for MT dynamics, coined by Mitchison and Kirschner [48], postulates that single MTs never reach a steady-state length, but undergo phases of polymerization and depolymerization, which interconvert infrequently (for review, see [49,50]). MTs are polar polymers that contain a fast-growing plus end and a slow-growing minus end, with the β-subunit of the tubulin dimer oriented towards the plus end and the α-subunit towards the minus end. During polymerization, GTP-containing tubulin subunits add predominantly to the MT plus end with concomitant hydrolysis of the GTP that was exposed at the plus end. The position of the hydrolyzed GTP at the monomer interface in the MT prevents its exchange [16]. The stability of the MT relies on the presence of GTP-containing tubulin subunits at the MT plus end, the so-called GTP-cap [48]. MT depolymerization would be the result of the loss of GTP from one of the MT subunits at the minimal cap.

Since single FtsZ protofilaments are too small to be visualized by light microscopy, it has not yet been possible to determine whether FtsZ polymers contain similar plus and minus ends, or how polymers grow and disassemble. Modeling of the structure of FtsZ in low-resolution images of FtsZ polymer sheets [19] suggests that FtsZ polymers are ordered, possibly containing both a plus and minus end. The plus end would contain an exposed nucleotide-binding site, while the

minus end may contain the catalytic site formed by the loop-T7 region (Fig. 1).

The clear link between GTP hydrolysis and polymerization was first shown when the presence of FtsZ polymers in solution was found to be coupled to GTP hydrolysis, with polymer loss coinciding with GTP depletion [26]. FtsZ polymerizes above a critical protein concentration [26] that corresponds to the concentration required for GTP hydrolysis [37]. Mg^{2+} is not required for polymerization, but is needed for the dynamic behavior of FtsZ polymers as GTP hydrolysis is Mg^{2+}-dependent [29]. Factors that influence the GTP hydrolysis rate directly affect the polymer stability [26,29,30].

Recently, isodesmic assembly of single FtsZ protofilaments of 10–100 subunits with GTP and GMP-CPP was described [28] without self-activation or critical concentration[2]. The difference between this finding and previous reports may be the size of the polymers studied, with the concentration-dependent polymerization in fact reflecting the assembly of laterally associated FtsZ protofilaments.

The question whether the FtsZ polymer contains GTP or GDP is still a matter of debate in the field. One hypothesis is that GTP hydrolysis occurs after polymer formation, driving a conformational change that transforms the polymers from a straight to curved form [27]. This event may drive constriction of the FtsZ-ring. Both for FtsZ and tubulin, straight and curved polymer conformations are associated with the presence of either GTP or GDP. However, curved filaments at MT ends appear only during rapid MT depolymerization (see [49]), and the curvature of GDP-bound FtsZ filaments is incompatible with that of the cell surface [35].

The isodesmic polymers are thought to consist of GTP-bound FtsZ, with slow, lagging GTP hydrolysis occuring gradually throughout the polymer [28]. GTP hydrolysis is generally determined as the amount of phosphate released after hydrolysis, providing a measure of GTP turnover. This method, however, does not identify whether a lag is caused by hydrolysis or slow release of P_i. Recently, FtsZ polymerized with [γ-^{32}P]GTP was shown to retain the label, providing evidence that the polymers contain either GTP or GDP plus P_i [43]. We favor the latter explanation for various reasons. First, FtsZ incubated with [α-^{32}P]GTP rapidly hydrolyzes GTP even at conditions of low GTP turnover [42]. The bound nucleotide in the FtsZ polymers is predominantly GDP. Secondly, FtsZ polymers formed with Ca^{2+} can be stabilized with guanosine-5′-*O*-(3-thiotriphosphate) (GTP-γ-S). Such polymers do not exchange the bound GDP, which resembles the inability of nucleotide exchange in the MT [42]. Thirdly, although FtsZ rapidly hydrolyzes the GTP, the release of the formed P_i is slow (D.-J.S. and A.J.M.D., unpublished). These observations make it unlikely that the FtsZ polymer contains GTP, but are consistent with a model in which the active site for GTP hydrolysis is assembled during polymerization (discussed above). GTP hydrolysis is then coupled to the addition of subunits to the polymer, similar to tubulin.

[2] The absence of a critical concentration for polymerization was concluded from a comparison of polymer sedimentation data with theoretical curves for cooperative assembly of polymers from an all-monomer starting solution. However, it is clearly established that FtsZ in solution is never all-monomeric, but exists in various multimeric states [22–24].

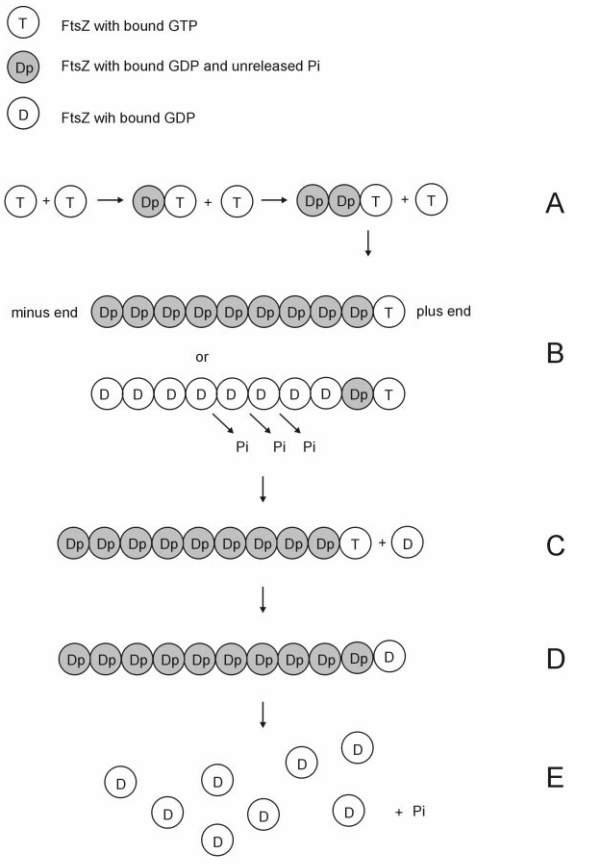

Fig. 2. A model for FtsZ polymerization. GTP-containing subunits associate, and upon association, the GTP in the newly formed active site is hydrolyzed (A). This process continues and leads to the formation of vectorial polymers (B), which either contain GDP or GDP and P_i bound to most subunits and an exposed GTP at the polymer plus end. Since GTP-binding strongly increases the affinity of the FtsZ–FtsZ interaction [28], the addition of GTP-bound FtsZ to the polymer is highly favored. This changes when GTP runs out. Addition of GDP-containing FtsZ results in hydrolysis of the exposed GTP at the plus end (C) and destabilization of the polymer (D), which results in depolymerization (E). Lateral interactions of the polymers are not incorporated in this model.

7. Conclusion: a model for FtsZ polymerization

On the basis of the current evidence, we would like to propose a model for FtsZ polymerization which closely resembles tubulin polymerization (Fig. 2). GTP-bound FtsZ is incorporated into polymers that contain a plus end with the nucleotide-binding site and a minus end with the T7-loop region. Addition of FtsZ subunits leads to rapid hydrolysis of the GTP that is present in the newly formed active site. The FtsZ polymer is stable as long as a GTP-containing monomer is present at the plus end. This model fits the observation that the addition of GDP to polymers formed with limiting amounts of GTP leads to immediate polymer disassembly, whereas GTP-γ-S stabilizes the polymers [42]. Addition of GDP-bound FtsZ to the polymer leads to hydrolysis of the exposed GTP and blocks addition of further subunits, as does the addition of GTP-γ-S-bound FtsZ. However, the exposed GDP renders the polymer unstable, whereas exposed GTP-γ-S mimics GTP. One of the most interesting questions remaining

involves the fate of formed P_i. It may very well be that not GTP hydrolysis, but the release of P_i, constitutes the trigger for a conformational change [18] that destabilizes the polymer or leads to the straight/curved transition. It will be important to define the rate-limiting step in polymerization, and to understand the link between the formation of protofilaments and lateral polymer interactions.

Since most of the available data on FtsZ polymerization were obtained in vitro, it is very well possible that factors that govern FtsZ-ring formation in vivo are being overlooked. Studies in the presence of other cell division proteins, known to modulate FtsZ [1], will shed further light on the mechanism of FtsZ polymerization in the bacterial cell.

Acknowledgements: We would like to thank Dr. Piet de Boer (Case Western Reserve University, Cleveland, OH, USA) for discussion and Dr. Tanneke den Blaauwen (Swammerdam Institute for Life Sciences, University of Amsterdam, NL) for critically reading the manuscript. This work was supported by Grant 805-33-222 from the Division of Earth and Life Sciences (ALW) of the Dutch Organization for the Advancement of Scientific Research (NWO).

References

[1] Margolin, W. (2000) FEMS Microbiol. Rev. 24, 531–548.
[2] Bernander, R. (2000) Trends Microbiol. 8, 278–283.
[3] Osteryoung, K.W., Stokes, K.D., Rutherford, S.M., Percival, A.L. and Lee, W.Y. (1998) Plant Cell 10, 1991–2004.
[4] Beech, P.L., Nheu, T., Schultz, T., Herbert, S., Lithgow, T., Gilson, P.R. and McFadden, G.I. (2000) Science 287, 1276–1279.
[5] Bi, E. and Lutkenhaus, J. (1991) Nature 354, 161–164.
[6] den Blaauwen, T., Buddelmeijer, N., Aarsman, M.E.G., Hameete, C.M. and Nanninga, N. (1999) J. Bacteriol. 181, 5167–5175.
[7] Sun, Q. and Margolin, W. (1998) J. Bacteriol. 180, 2050–2056.
[8] Addinall, S.G. and Lutkenhaus, J. (1996) Mol. Microbiol. 22, 231–237.
[9] RayChaudhuri, D. and Park, J.T. (1992) Nature 359, 251–254.
[10] de Boer, P.A.J., Crossley, R. and Rothfield, L.I. (1992) Nature 359, 254–256.
[11] Mukherjee, A., Dai, K. and Lutkenhaus, J. (1993) Proc. Natl. Acad. Sci. USA 90, 1053–1057.
[12] Mukherjee, A. and Lutkenhaus, J. (1994) J. Bacteriol. 176, 2754–2758.
[13] Bramhill, D. and Thompson, C.M. (1994) Proc. Natl. Acad. Sci. USA 91, 5813–5817.
[14] Löwe, J. and Amos, L.A. (1998) Nature 391, 203–206.
[15] Nogales, E., Wolf, S.G. and Downing, K.H. (1998) Nature 391, 199–203.
[16] Nogales, E., Downing, K.H., Amos, L.A. and Lowe, J. (1998) Nat. Struct. Biol. 5, 451–458.
[17] Nogales, E., Whittaker, M., Milligan, R.A. and Downing, K.H. (1999) Cell 96, 79–88.
[18] Diaz, J.F., Kralicek, A., Mingorance, J., Palacios, J.M., Vicente, M. and Andreu, J.M. (2001) J. Biol. Chem. 276, 17307–17315.
[19] Löwe, J. and Amos, L.A. (1999) EMBO J. 18, 2364–2371.
[20] Scheffers, D.J., de Wit, J.G., den Blaauwen, T. and Driessen, A.J.M. (2001) FEBS Lett. 494, 34–37.
[21] Justice, S.S., Garcia-Lara, J. and Rothfield, L.I. (2000) Mol. Microbiol. 37, 410–423.
[22] Sossong Jr., T.M., Brigham-Burke, M.R., Hensley, P. and Pearce Jr., K.H. (1999) Biochemistry 38, 14843–14850.
[23] 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.
[24] Rivas, G., Fernandez, J.A. and Minton, A.P. (2001) Proc. Natl. Acad. Sci. USA 98, 3150–3155.
[25] Erickson, H.P., Taylor, D.W., Taylor, K.A. and Bramhill, D. (1996) Proc. Natl. Acad. Sci. USA 93, 519–523.
[26] Mukherjee, A. and Lutkenhaus, J. (1998) EMBO J. 17, 462–469.
[27] Lu, C., Reedy, M. and Erickson, H.P. (2000) J. Bacteriol. 182, 164–170.
[28] Romberg, L., Simon, M. and Erickson, H.P. (2001) J. Biol. Chem. 276, 11743–11753.
[29] Mukherjee, A. and Lutkenhaus, J. (1999) J. Bacteriol. 181, 823–832.
[30] Yu, X.-C. and Margolin, W. (1997) EMBO J. 16, 5455–5463.
[31] Tang, J.X. and Janmey, P.A. (1996) J. Biol. Chem. 271, 8556–8563.
[32] RayChaudhuri, D. (1999) EMBO J. 18, 2372–2383.
[33] Hale, C.A., Rhee, A.C. and de Boer, P.A.J. (2000) J. Bacteriol. 182, 5153–5166.
[34] Trusca, D., Scott, S., Thompson, C. and Bramhill, D. (1998) J. Bacteriol. 180, 3946–3953.
[35] Löwe, J. and Amos, L.A. (2000) Biol. Chem. 381, 993–999.
[36] Lu, C., Stricker, J. and Erickson, H.P. (2001) BMC Microbiol. 1, 7.
[37] Lu, C., Stricker, J. and Erickson, H.P. (1998) Cell Motil. Cytoskeleton 40, 71–86.
[38] Wang, X. and Lutkenhaus, J. (1993) Mol. Microbiol. 9, 435–442.
[39] Wang, X. and Lutkenhaus, J. (1996) Mol. Microbiol. 21, 313–319.
[40] White, E.L., Ross, L.J., Reynolds, R.C., Seitz, L.E., Moore, G.D. and Borhani, D.W. (2000) J. Bacteriol. 182, 4028–4034.
[41] David-Pfeuty, T., Erickson, H.P. and Pantaloni, D. (1977) Proc. Natl. Acad. Sci. USA 74, 5372–5376.
[42] Scheffers, D.J., den Blaauwen, T. and Driessen, A.J. (2000) Mol. Microbiol. 35, 1211–1219.
[43] Mingorance, J., Rueda, S., Gómez-Puertas, P., Valencia, A. and Vicente, M. (2001) Mol. Microbiol. 41, 83–91.
[44] RayChaudhuri, D. and Park, J.T. (1994) J. Biol. Chem. 269, 22941–22944.
[45] Wang, Y., Jones, B.D. and Brun, Y.V. (2001) Mol. Microbiol. 40, 347–360.
[46] Addinall, S.G., Cao, C. and Lutkenhaus, J. (1997) J. Bacteriol. 179, 4277–4284.
[47] Bi, E. and Lutkenhaus, J. (1992) J. Bacteriol. 174, 5414–5423.
[48] Mitchison, T. and Kirschner, M. (1984) Nature 312, 237–242.
[49] Desai, A. and Mitchison, T.J. (1997) Annu. Rev. Cell Dev. Biol. 13, 83–117.
[50] Nogales, E. (2000) Annu. Rev. Biochem. 69, 277–302.
[51] Wang, X., Huang, J., Mukherjee, A., Cao, C. and Lutkenhaus, J. (1997) J. Bacteriol. 179, 5551–5559.
[52] Dai, K., Mukherjee, A., Xu, Y. and Lutkenhaus, J. (1994) J. Bacteriol. 175, 130–136.
[53] Bi, E. and Lutkenhaus, J. (1990) J. Bacteriol. 172, 5602–5609.