ZipA is a MAP–Tau homolog and is essential for structural integrity of the cytokinetic FtsZ ring during bacterial cell division

Debabrata RayChaudhuri

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA
E-mail: draycha@opal.tufts.edu

This paper is dedicated to the memory of Dr Henry C.Wu who passed away prematurely on February 12, 1996

The first visible event in prokaryotic cell division is the assembly of the soluble, tubulin-like FtsZ GTPase into a membrane-associated cytokinetic ring that defines the division plane in bacterial and archaeal cells. In the temperature-sensitive ftsZ84 mutant of Escherichia coli, this ring assembly is impaired at the restrictive temperature causing lethal cell filamentation. Here I present genetic and morphological evidence that a 2-fold higher dosage of the division gene zipA suppresses thermosensitivity of the ftsZ84 mutant by stabilizing the labile FtsZ84 ring structure in vivo. I demonstrate that purified ZipA promotes and stabilizes protofilament assembly of both FtsZ and FtsZ84 in vitro and cosediments with the protofilaments. Furthermore, ZipA organizes FtsZ protofilaments into arrays of long bundles or sheets that probably represent the physiological organization of the FtsZ ring in bacterial cells. The N-terminal cytoplasmic domain of membrane-anchored ZipA contains sequence elements that resemble the microtubule-binding signature motifs in eukaryotic Tau, MAP2 and MAP4 proteins. It is postulated that the MAP–Tau-homologous motifs in ZipA mediate its binding to FtsZ, and that FtsZ–ZipA interaction represents an ancient prototype of the protein–protein interaction that enables MAPs to suppress microtubule catastrophe and/or to promote rescue.

Keywords: cytokinesis/FtsZ protofilaments/FtsZ ring/MAPs/ZipA

Introduction

Most bacteria divide by forming a septum across the middle of the cell. In Escherichia coli, the process of cell division or cytokinesis involves the participation of at least nine essential gene products, FtsA, I, K, L, N, Q, W, Z and ZipA (reviewed by Bramhill, 1997; Erickson, 1997; Lutkenhaus and Addinall, 1997; Rothfield and Justice, 1997). Among the dedicated bacterial division proteins, FtsZ plays a pivotal role at the earliest known stage of cytokinesis. It assembles into a ring structure at the division site on the inner face of the cytoplasmic membrane and provides a cytoskeletal scaffold that recruits other division proteins to form the contractile septal ring essential for cell constriction (Bi and Lutkenhaus, 1991; Lutkenhaus and Addinall, 1997; Rothfield and Justice, 1997). FtsZ is universally present in eubacteria and archaea (Bramhill, 1997; Erickson, 1997; Lutkenhaus and Addinall, 1997), with the surprising exception of Chlamydia trachomatis (Stephens et al., 1998). It has also been identified as a nuclear-encoded chloroplast protein in Arabidopsis thaliana (Ostergaard and Vierling, 1995) and in the moss Physcomitrella patens (Strepp et al., 1998).

FtsZ is present at ~15 000 molecules per E. coli cell (Bi and Lutkenhaus, 1991; Lu et al., 1998). Cytoplasmic FtsZ becomes localized to the medial division site as a circumferential ring at an early stage in the division cycle and remains associated with the leading edge of the invaginating septum during cytokinesis (Bi and Lutkenhaus, 1991; Levin and Losick, 1996; Lutkenhaus and Addinall, 1997; Pogliano et al., 1997). In concert with septal progression, the FtsZ ring constricts, and after division, FtsZ is again cytoplasmic in the newborn cells (Bi and Lutkenhaus, 1991). The cell cycle signal that initiates membrane targeting and subsequent ring assembly of FtsZ remains unknown. The failure of the thermosensitive ftsZ84 mutant to divide correlates with the rapid disappearance of FtsZ rings in the mutant E. coli cells at 42°C (Addinall et al., 1997a), underscoring the central role of FtsZ assembly in cell division.

FtsZ is a tubulin-like GTPase and forms polymers that are structural homologs of microtubular protofilaments, protofilament sheets and rings (de Boer et al., 1992; RayChaudhuri and Park, 1992, 1994; Mukherjee et al., 1993; Mukherjee and Lutkenhaus, 1994; Erickson et al., 1996). Recent crystallographic studies have validated this nexus between FtsZ and tubulin, and have shown that the three-dimensional structure of FtsZ is virtually identical to the structures of α- and β-tubulin (Lowe and Amos, 1998; Nogales et al., 1998a,b). Several recent studies have documented the localization of other Fts proteins (Addinall et al., 1997b; Boyle et al., 1997; Khattar et al., 1997; Lutkenhaus and Addinall, 1997, and references therein; Weiss et al., 1997; Buddelmeijer et al., 1998; Wang and Lutkenhaus, 1998; Wang et al., 1998; Yu et al., 1998) and ZipA (Hale and de Boer, 1997) to the FtsZ ring and this assemblage constitutes the septal ring organelle (septalsome) (Holland, 1987) that catalyzes cell division. Despite these advances, some of the outstanding questions in cell division concern the mechanisms of FtsZ ring nucleation and growth at the division site, and of temporal recruitment of other division proteins to the FtsZ ring. A full understanding of bacterial cytokinesis would require a detailed knowledge of the molecular interplay between FtsZ and other division proteins.

To delineate protein–protein interactions between FtsZ and other components participating in septal ring assembly, I undertook an unbiased genome-wide search for dominant extragenic suppressors of the thermosensitive ftsZ84 muta-
tion. I found that one of the most frequently arising suppressor isolates is the essential division gene zipA (Hale and de Boer, 1997). I present evidence that suppression does not involve any mutational alteration in zipA, but that a 2-fold higher zipA gene dosage suppresses thermosensitivity of the ftsZ84 mutant by stabilizing the labile FtsZ84 ring structure at high temperature. I show that purified ZipA promotes both FtsZ and FtsZ84 polymerization in vitro, and organizes FtsZ protofilaments into arrays of long bundles or sheets. ZipA thus plays an essential role in imparting structural organization and integrity to the FtsZ ring during cell division.

I have found that the membrane-proximal sequence in the N-terminal cytoplasmic domain of ZipA contains motifs that resemble the microtubule (MT)-binding signature motifs in classical MT-associated proteins (MAPs) such as Tau, MAP2 and MAP4 (Lee et al., 1988; Lewis et al., 1988; Gustke et al., 1994). MAPs are a family of structural proteins that stabilize MT assembly (Desai and Mitchison, 1997; Drewes et al., 1998). The unexpected functional and sequence similarities between ZipA and MAPs reinforce our notion that the regulation of MT assembly in eukaryotes is an appropriate paradigm to understand FtsZ ring dynamics, and as a corollary, ongoing molecular analysis of bacterial cell division should enhance our understanding of the evolution of the eukaryotic cytoskeleton.

Results

The cell division gene zipA is the most frequently isolated ftsZ84 suppressor locus

My strategy for a genome-wide suppressor search against the ftsZ84-ts allele was based on the red plaque complementation assay of Maurer et al. (1984). This method involves formation of partial diploids by lysogenization of mutant bacteria with lambda clones that either carry a complementing gene or a dominant suppressor locus. A mutagenized λEMBL3 genomic library constructed using JFL100(ftsZ84-ts) chromosomal DNA yielded a total of 13 suppressor phage clones that allowed the JFL100(ftsZ84-ts)[λ+] tester strain to grow at 42°C. Analyses of the suppressor clones by restriction mapping and by Southern hybridization (see Materials and methods) revealed that 11 phage clones were related in having various lengths of overlapping chromosomal DNA in their inserts (not shown). Of the two remaining clones, one is a unique extragenic suppressor unrelated to all other isolates, while the other clone is a wild-type revertant of the ftsZ84 locus.

The insert in the suppressor clone λIB-9-2 (one of 11 related isolates) hybridized to the Kohara clone 417 (Noda et al., 1991), that carries DNA from ~54.3–54.5 min of the E.coli genome (not shown). I cloned the suppressor locus from λIB-9-2 and from a related clone λIB-17-1 into the single copy mini-F plasmid pFA-1 (Koop et al., 1987), and identified DRC13(ftsZ84-ts)-complementing open-reading frames in both constructs as the essential division gene zipA, residing at 54.5 min on the E.coli chromosome (Berlyn, 1998). The temperature-resistant DRC13 colonies showed a mixed morphology when examined by phase-contrast microscopy: normal rods, short rounded and small spherical cells, as well as filaments (~10%) were present (not shown).

The zipA sequence derived from both plasmids revealed one mismatch with the published sequence (DDBJ/EMBL/GenBank accession No. U74650) (Hale and de Boer, 1997); the 211th residue in our zipA sequence was Leu instead of the reported Ala. Sequencing the zipA locus, amplified from MC1000 chromosomal DNA by polymerase chain reaction (PCR), showed Leu as the 211th residue, similar to the zipA sequence (DDBJ/EMBL/GenBank accession No. AE000329) derived from the K12 strain MG1655 used in the E.coli genome sequencing project (Blattner et al., 1997). Therefore, I conclude that the zipA structural gene in the suppressor clones does not carry any mutation and that two copies of the wild-type zipA gene (one at the normal chromosomal locus and one carried by the integrated suppressor phage or by a single-copy plasmid) allow growth of the ftsZ84 mutant at the non-permissive temperature. Besides λIB-9-2 and λIB-17-1, full-length zipA fragments could be amplified from another six related phage clones by PCR (not shown). It seems reasonable to assume that zipA is the suppressor locus in these clones as well, making it the most frequently arising suppressor in the red plaque screen.

Ectopic expression of an additional copy of zipA from Plac suppresses ftsZ84 thermosensitivity without raising the FtsZ84 level

To demonstrate unequivocally whether an extra copy of the wild-type zipA gene is sufficient to suppress the thermosensitivity of DRC13(ftsZ84-ts) mutant cells, the lysogenic lambda derivative λDB322(lacIqPlac::zipA), which carries zipA under the control of lac promoter (Hale and de Boer, 1997), was integrated at the lambda att site of DRC13. As shown in Figure 1, DRC13 failed to grow at 42°C (Figure 1A), whereas the DRC13(λDB322) lysogen could form small colonies at 42°C even in the absence of isopropyl-β-D-galactopyranoside (IPTG) induction, due to leaky expression of zipA from Plac (Figure 1B). When zipA expression was induced with IPTG, the mutant colonies growing at 42°C were distinctly larger, with optimum suppression occurring at 25 μM IPTG (Figure 1B). At 50 μM IPTG, the colony size of the DRC13 lysogen at 42°C was diminished, indicating that higher zipA expression leads to a reduction in the efficiency of suppression (Figure 1B). This is consistent with the previous finding that zipA expression from λDB322 at 100 μM IPTG causes a division block in the strain PB103 (Hale and de Boer, 1997). Growth of both MC1000 and congenic DRC13 with or without chromosomally integrated Plac::zipA was similar at 30°C in the absence or presence of 5–50 μM IPTG (Figure 1A and B). These results firmly establish that increased zipA expression, albeit within a narrow range, can allow growth of the thermosensitive ftsZ84 cells at the restrictive temperature.

To date, most known suppressors of ftsZ84 cause upregulation of FtsZ84 expression to compensate for the reduced activity of the mutant protein (Powell and Court, 1998; also see Discussion). To assess whether an additional copy of zipA may engender elevated FtsZ84 expression, the levels of FtsZ84 protein in DRC13 as well as in DRC13(λDB322) cultures, grown with or without IPTG at 30°C, were analyzed by immunoblotting with anti-FtsZ antibody. The same blot was simultaneously probed with
Fig. 1. Genetic suppression of DRC13(ftsZ84-ts) by the lysogenic phage DB322 [lac P Plac::zipA bla] and the level of FtsZ84 expression after IPTG induction of chromosomal Plac::zipA. (A) Growth of the wild-type strain MC1000 and the congenic ftsZ84-ts derivative DRC13 on LB agar (supplemented with 0.3% NaCl) at 30 and 42°C after 22 h incubation. (B) Growth of MC1000(λDB322) and congenic DRC13(λDB322) at 30 and 42°C on LB agar (0.3% NaCl) containing 50 µg/ml Amp and different concentrations of IPTG. The plates were incubated for 22 h. (C) A Western blot of total cell extracts of DRC13 (lane 1) and of DRC13(λDB322) (lanes 2–6) immunostained with anti-FtsZ and anti-GroEL polyclonal antibodies. Each of lanes 1–6 received 40 µg total cell protein. DRC13(λDB322) cultures were induced with different concentrations of IPTG for 3 h at 30°C. The concentrations of IPTG relevant for different lanes are: lanes 1 and 2, no IPTG; 3, 5 µM; 4, 25 µM; 5, 50 µM; 6, 100 µM. Lane 7 contained purified FtsZ84 (65 ng) and GroEL (100 ng) as standards. GroEL immunostaining served as gel loading controls for different lanes.

anti-GroEL antibody as an internal control for sample loading. As shown in Figure 1C, the levels of FtsZ84 were essentially similar in DRC13, and in DRC13(λDB322) cultures grown with or without 5–100 µM IPTG, indicating that increased levels of ZipA do not lead to increased levels of FtsZ84.

ZipA stabilizes thermolabile FtsZ84 ring assembly in vivo

To ascertain if zipA-mediated ftsZ84 suppression involves stabilization of FtsZ84 ring assembly at 42°C, FtsZ localization in E. coli cells was examined by indirect immunofluorescence microscopy using anti-FtsZ antibody. As shown in Figure 2, DRC13(ftsZ84-ts) cells showed a dramatic loss in localization of FtsZ at 42°C. Within 5 min after shift-up, even small cells exhibited a punctate pattern of fluorescence distributed along the cell length (Figure 2B and B'), instead of the sharply defined equatorial FtsZ rings seen at 30°C (Figure 2A and A'). This indicates that FtsZ84 rings undergo rapid thermal destabilization into oligomers that generate diffuse fluorescence. After 120 min at 42°C, DRC13 filaments still displayed punctate fluorescent dots but of diminished brightness (Figure 2C and C'), perhaps suggesting progressive dissolution of the FtsZ84 oligomers. These results are consistent with the previous finding of the disappearance of FtsZ84 rings within a minute after shift-up of the mutant cells to 42°C (Addinall et al., 1997a).

To examine the ability of the FtsZ84 protein to assemble into cytokinetic rings at an elevated ZipA level,
DRC13(λDB322)[ftsZ84-ts(Plac::zipA)] cells were grown in the presence of 20 µM IPTG at both 30 and 42°C. After 180 min of induction at 30°C, the culture showed a mixed population of elongated cells and short rounded (ovoid shape) cells with clearly visible FtsZ84 rings (Figure 2D and D’). When DRC13(λDB322) was shifted to 42°C and the culture induced with IPTG for 90 min, there was a striking difference in FtsZ84 localization in comparison with DRC13, as well as a dramatic alteration in cell shape, with a significant number of short ovoid and small spherical cells in the culture (Figure 2E and E’, F and F’). DAPI staining showed that the major fraction of spherical cells contained nucleoids, while a smaller number of spherical cells were anucleate minicells arising from polar septation (Figure 2E’ and F’). Extra copies of ZipA induced in DRC13(λDB322) led to a variety of FtsZ84 localization patterns at 42°C: a rod-shaped cell harboring three FtsZ84 rings, one at midcell and two at the cell quarters (Figure 2E; the cell identified by double arrows); bipolar or unipolar FtsZ84 rings and fluorescent dots in ovoid cells (Figure 2E and F; two cells undergoing shape transition indicated by single arrows in 2E); medial FtsZ84 rings and discernible polar fluorescence in two cylindrical cells (Figure 2F; the cells marked by single arrows). Cells with both medial and distinct bipolar rings, as well as a low percentage (~5%) of filamentous cells, were also present in the culture at 42°C (not shown).

When MC1000(λDB322)[wt(Plac::zipA)] was induced with 20 µM IPTG at 30°C for 90 min, medial FtsZ84 rings formed in normal size cells (Figure 2G and G’). In contrast, when a similar culture was induced with IPTG for 180 min, the cells became ovoid and smaller, and displayed either medial FtsZ84 rings or unipolar/bipolar rings and fluorescent dots (Figure 2H and H’). This closely resembles the phenotype of DRC13(λDB322) cells induced for 90 min at 42°C (Figure 2E and F). Cultures of both MC1000 and congeneric DRC13 lysogens, grown with or without 20 µM IPTG for 6–20 h at 30°C, contained predominantly small spherical cells with FtsZ localized as unipolar or bipolar fluorescent spots (not shown). This rod-to-sphere shape transition is in marked contrast to the extensive filamentation reported for the λDB322 lysogens of PB103 or its derivatives induced with 100 µM IPTG (Hale and de Boer, 1997). The contrasting phenotypes of λDB322 lysogens observed in the two studies upon inducing Plac::zipA expression to different extents are likely the result of different stoichiometric ratios between ZipA and FtsZ, rather than a consequence of dissimilar strain backgrounds.

An increase in the frequency of FtsZ rings in cells (Figure 2) without an increase in the FtsZ level (Figure 1C) has not been previously observed (Ward and Lutkenhaus, 1985; Lutkenhaus and Addinall, 1997). The occurrence of complete FtsZ rings at the cell quarters of an apparently predivisional cell (Figure 2E) is novel and suggests that the future division sites are nucleation-competent in the mother cell and can support stable FtsZ assembly at an increased ZipA level. Alternatively, the quarter site rings may represent assemblies that occurred subsequent to the failure of the medial ring to constrict in the mother cell cycle, and hence constitute rings that formed in the daughter cell cycle.

**ZipA promotes FtsZ and FtsZ84 polymerization and cosediments with the polymers**

To corroborate the genetic and immunofluorescent localization data that ZipA stabilizes FtsZ84 ring assembly, the effect of purified His<sub>S</sub>-ZipA on both FtsZ and FtsZ84 polymerization was examined in vitro. Since sedimentation provides a facile assay to follow dynamic polymerization of FtsZ in the presence of GTP (Yu and Margolin, 1997; Mukherjee and Lutkenhaus, 1998), the effect of ZipA on such an assembly was studied using this assay. Figure 3A shows a profile of His<sub>S</sub>-ZipA purification from over-expressing cells to >95% purity. The amounts of FtsZ sediments after 12 min of assembly from reactions supplemented with or without purified ZipA (FtsZ:ZipA ratio ~1:1) (Figure 3B, upper panel, lanes 1 and 2) were essentially similar to that obtained from a reaction that had ZipA but no GTP (Figure 3B, upper panel, lane 3). A small amount of FtsZ sedimented in the absence of both GTP and ZipA (Figure 3B, upper panel, lane 9), similar to the GTP-independent FtsZ sedimentation observed previously (Mukherjee and Lutkenhaus, 1998). FtsZ sedimentation promoted by ZipA in the absence of GTP is consistent with the fact that FtsZ binds ZipA on Western blots in the absence of any exogenously added GTP (Hale and de Boer, 1997; Figure 3C).

In contrast to FtsZ, the effect of ZipA on FtsZ84 polymerization was dramatic. As shown in Figure 3B (upper panel), FtsZ84 self-assembly in the presence of GTP (lane 4) or ATP (lane 6) was negligible in comparison with FtsZ assembly with GTP (lane 1). However, when ZipA was present (FtsZ84:ZipA ratio ~1:1), the amounts of FtsZ84 recovered in the pellets from both GTP- and ATP-containing reactions after 12 min assembly were ~4- to 5-fold higher compared with FtsZ (Figure 3B, upper panel, lanes 5 and 7). Unlike FtsZ, FtsZ84 mutant protein does not copurify with any bound nucleotide (RayChaudhuri and Park, 1992); nevertheless, ZipA promoted significant polymerization of nucleotide-free FtsZ84 without GTP or ATP in the assembly reaction (Figure 3B, upper panel, lane 8). No detectable FtsZ84 sedimented in the absence of both nucleotides and ZipA (Figure 3B, upper panel, lane 10). FtsZ84 assembly in the presence of ATP was examined because, unlike wild-type FtsZ, the mutant protein binds ATP and displays a significant ATPase activity in vitro (RayChaudhuri and Park, 1994).

To determine whether ZipA co-sedimented with FtsZ or FtsZ84 protofilaments, the Western blot depicted in Figure 3B upper panel was probed with anti-His<sub>S</sub> monoclonal antibody to detect the presence of His<sub>S</sub>-ZipA in the pellet fractions. The lower panel (Figure 3B) shows that ZipA cosedimented with both FtsZ and FtsZ84 polymers but to markedly different extents. The amounts of ZipA that cosedimented with FtsZ from reactions supplemented with or without GTP were within a 2-fold range of each other (Figure 3B, lower panel, lanes 2 and 3). In contrast, ~8- to 10-fold higher ZipA cosedimented with FtsZ84 from both GTP- and ATP-containing reactions (compare lanes 5 and 7 with lanes 2 and 3, Figure 3B, lower panel). Interestingly, the amount of ZipA that pelleted together with FtsZ84 in the absence of any nucleotide (lane 8) was ~1.5- to 2-fold higher than the amounts of ZipA that cosedimented with FtsZ in the presence or absence of GTP (lanes 2 and 3). His<sub>S</sub>-ZipA did not sediment by itself to
any significant extent in the absence of FtsZ or FtsZ84, indicating that ZipA does not self-assemble into higher order structures (not shown). These results indicate that ZipA has a high propensity to associate with the FtsZ84 polymer lattice, presumably owing to an altered conformation of FtsZ84 relative to FtsZ (RayChaudhuri and Park, 1994). The robust association of ZipA molecules (at ~2-fold higher expression level) with the FtsZ84 protofilaments would stabilize the weakened intersubunit bonds in the FtsZ84 ring at the restrictive temperature. The polymerization results thus provide a biochemical explanation for ftsZ84 suppression by zipA.

**ZipA stabilizes FtsZ protofilament assembly in vitro**

FtsZ assembly in the presence of GTP is dynamic in nature because of the attendant GTP hydrolysis during assembly (Yu and Margolin, 1997; Lu et al., 1998; Mukherjee and Lutkenhaus, 1998). To determine whether ZipA can stabilize FtsZ polymers that form in the presence of GTP, a set of FtsZ assembly reactions with or without ZipA were allowed to proceed for 25 min to allow extensive polymer turnover and the pelleted fractions were analyzed by Western blotting. In the absence of ZipA, no sedimentable FtsZ could be detected (Figure 3C, right panel, lane 2), whereas in the presence of ZipA, detectable levels of FtsZ were recovered in the pellets (Figure 3C, right panel, lanes 3 and 4). In the absence of both GTP and ZipA, there was no FtsZ in the pellet fraction after 25 min incubation in the assembly buffer (Figure 3C, right panel, lane 1). Prior to anti-FtsZ immunostaining of the Western blot, [35S]FtsZ affinity overlay revealed that ZipA was present in the pellets from reactions containing both FtsZ and ZipA (Figure 3C, left panel, lanes 3 and 4). These results demonstrate that ZipA binds to and stabilizes FtsZ polymers.

**ZipA promotes FtsZ assembly into arrays of long narrow protofilament bundles or sheets**

To visualize the effect of ZipA on FtsZ assembly, the protein polymers were negatively stained with uranyl acetate and examined by transmission electron microscopy. Incubation of 5 µM FtsZ in the assembly buffer with 1 mM GTP for 10 min resulted in its polymerization into straight or slightly curved protofilaments or protofilament pairs of variable lengths (one paired filament marked by a double arrowhead) (Figure 4A). Such single and paired protofilaments have been recently designated as the active FtsZ polymer with GTPase activity (Lu et al., 1998). The average diameter of a single protofilament in Figure 4A is ~5–6 nm, similar to that reported previously (Erickson et al., 1996; Yu and Margolin, 1997). The negatively stained image also reveals a number of minirings with a distinct lumen (shown by arrows, Figure 4A), having an average diameter of ~14–15 nm. This contrasts with the markedly larger FtsZ minirings (average diameter ~24 nm) observed previously at the curved ends of protofilament sheets (Erickson et al., 1996). The difference in the

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**Fig. 3.** Purification of His6-ZipA and its effect on in vitro polymerization of FtsZ and FtsZ84. (A) Purification profile of overexpressed His6-ZipA after detergent extraction from membranes with 1% OG and chromatography on a Ni2+-chelate column. Lane 1, OG extract; lane 2, 50 mM imidazole wash; lane 3, protein molecular mass standards (New England Biolabs); lane 4, 150 mM imidazole wash; lane 5, 500 mM imidazole eluate containing His6-ZipA indicated by an arrow. Lanes 1 and 5 contained ~25 and ~2.5 µg protein, respectively. Proteins were visualized on the 12% SDS-polyacrylamide gel by staining with Coomassie Brilliant Blue R-250. (B) A Western blot analysis of co-sedimentation of FtsZ or FtsZ84 (upper panel) and His6-ZipA (lower panel) in the presence or absence of NTPs. FtsZ or FtsZ84 was incubated with or without His6-ZipA in the presence or absence of NTPs in the assembly buffer and the polymers were sedimented by ultracentrifugation. The pelletted protein samples were Western blotted and immunostained sequentially with polyclonal anti-FtsZ antibody (upper panel); colorimetric detection with AP and NBT/BCIP and with monoclonal anti-His6 antibody (lower panel); chemiluminescent detection with HRP and luminol). Lane 1, FtsZ+GTP; lane 2, FtsZ+ZipA+GTP; lane 3, FtsZ+ZipA; lane 4, FtsZ84+GTP; lane 5, FtsZ84+ZipA+GTP; lane 6, FtsZ84+ATP; lane 7, FtsZ84+ZipA+ATP; lane 8, FtsZ84+ZipA; lane 9, FtsZ alone; lane 10, FtsZ84 alone. The assembly reactions (25°C, 10 min) contained FtsZ, FtsZ84 or ZipA, each at 200 µg/ml, and GTP/ATP at 1 mM final concentration. (C) Stabilization of GTP-dependent FtsZ polymerization by His6-ZipA. FtsZ was polymerized without (lane 1) or with 1 mM GTP (lanes 2–4). The reactions analyzed in lanes 1 and 2 did not receive ZipA, whereas His6-ZipA was added to the other two reactions (lanes 3 and 4). The assembly reactions (25°C) were allowed to proceed for 25 min before analysis. The Western blot of pelleted protein samples was affinity-probed with [35S]FtsZ to detect ZipA (left panel). The blot, without stripping off the bound [35S]FtsZ probe, was next immunostained with anti-FtsZ antibody to assess FtsZ in the pellet fractions (right panel). The [35S]FtsZ-bound ZipA bands as well as the lower FtsZ bands in lanes 3 and 4 reacted with the antibody, whereas no FtsZ could be detected in lanes 1 and 2 (right panel). FtsZ was used at 200 µg/ml and His6-ZipA at 200 (lane 3) or 400 µg/ml (lane 4) in the assembly reactions.
ZipA stabilizes FtsZ ring structure

**Fig. 4.** Electron microscopy analysis of the effect of His<sub>6</sub>-ZipA on FtsZ polymerization in the presence of GTP. (A) Micrograph of FtsZ polymers assembled at 200 µg/ml (5 µM) protein in the presence of 1 mM GTP at 25°C. Straight or curved single protofilaments (5–6 nm diameter) and paired protofilaments (one pair shown by double arrowheads) of various lengths are visible. The field also reveals a number of minirings with distinct lumens (shown by four separate arrows). The average diameter of minirings in the micrograph is ~14–15 nm. Scale bar, 125 nm. (B) An identical FtsZ polymerization reaction as in (A) but supplemented with His<sub>6</sub>-ZipA at 200 µg/ml. ZipA dramatically promotes the lateral association of FtsZ protofilaments into a network of long bundles or sheets. The width of the protofilament arrays ranges between 65 and 100 nm. Minirings appear to be absent. Scale bar, 200 nm.

The diameter of the bundles/sheets ranged between 65 and 100 nm, indicating a lateral alignment of ~10–20 protofilaments per polymer array (Figure 4B). This finding is significant because it has been argued that the phylogenetically relevant polymer of FtsZ may be narrow protofilament sheets or bundles (Erickson et al., 1996). The micrograph in Figure 4B indicates that ZipA-promoted polymerization, as analyzed by sedimentation (Figure 3), is not due to artifactual aggregation or precipitation of FtsZ, but is the result of a specific structural effect of ZipA on protofilament assembly.

Based on the calculations of Erickson et al. (1996), I estimate that a total of ~15 000 FtsZ molecules per *E. coli* cell could form two to three ZipA-stabilized bundles/sheets, each an average of 10–15 protofilaments-thick and encircling the 0.6-µm diameter cell once. This estimate agrees with the two or three FtsZ84 rings seen in a subpopulation of DRC13(λDB322) cells that contain normal FtsZ84 level but extra copies of ZipA (Figure 2E and F). Therefore, although *E. coli* cells have the capacity to form multiple FtsZ rings, the chromosomal level of a stability factor like ZipA normally restricts assembly to a single ring, which may be in dynamic equilibrium with unassembled FtsZ in the cytoplasm.

**ZipA contains sequence elements that resemble the MT-binding motifs in Tau, MAP2 and MAP4**

The finding that ZipA is a crucial stability determinant of the FtsZ ring structure led me to examine its sequence for motifs that could provide a biochemical underpinning for its cellular function. *Escherichia coli* ZipA contains a highly basic region (aa 26–48), with a net charge of +8, immediately adjacent to the N-terminal transmembrane domain. FtsZ, like tubulin, is an acidic protein with a pI of 4.5. Thus, it seems an attractive possibility that the membrane-proximal basic region in ZipA may electrostatically interact with FtsZ during the ring assembly on the inner membrane. A close inspection revealed an adjacent sequence motif in ZipA (aa 45–70) that bears a resemblance to the C-terminal, MT-binding, 31-residue pseudorepeats in the non-motor structural MAPs such as Tau, MAP2 and MAP4 (Lee et al., 1988; Lewis et al., 1988; Gustke et al., 1994; Drewes et al., 1998). Figure 5A shows an alignment of *E. coli* and *Yersinia pestis* ZipA residues (*Y. pestis* contig 519; Sanger Centre sequencing project) with the first MT-binding repeat in mammalian MAPs and Tau, as well as the Tau homolog PTL-1a from...
the nematode Caenorhabditis elegans. Interestingly, PTL-1 does not show any significant homology to the mammalian Tau proteins outside the repeat motifs (McDermott et al., 1996). Unlike multiple (three to four) C-terminal repeats in the MAP–Tau proteins from higher eukaryotes, the MAP homolog MHP1 in yeast Saccharomyces cerevisiae contains a single repeat motif (Irminger-Finger et al., 1996), that has also been aligned with that in ZipA (Figure 5A).

Unlike the basic repeats in MAP, Tau and PTL-1 proteins, the MAP–Tau repeat (MTR) homology domain (residues 1211–1266) in yeast MTP1 is overall acidic but is preceded by a basic region (Irminger-Finger et al., 1996). Similarly, although the MTR motif in E.coli and Y.pestis ZipA is acidic (Figure 5A), it is juxtaposed to a highly basic region (aa 26–48) in both orthologs.

ZipA contains a 103-residue long central region (aa 26–128) and the PRR is predicted to be separated by a short stretch of β-strands and α-helices (aa 65–82), following which the PRR (aa 86–188), as expected, is devoid of any secondary structure. In contrast, the C-terminus of ZipA, beginning at residue 192, shows a high propensity to form α-helices and β-strands. Therefore, the domain organization and the extended structure of the N-terminal half of ZipA appear very similar to that of the assembly domain comprising the central PRR and the C-terminal repeats in MAPs and Tau (Figure 5B).

Genomics of zipA

Database searches revealed one full-length ZipA ortholog, the open reading frame (ORF) HI1101 (DDBJ/EMBL/GenBank accession No. L42023) from Haemophilus influenzae, that shows 28% identity and 47% similarity with the E.coli ZipA sequence. Though a comparison of ZipA amino acid sequences from E.coli and H.influenzae reveals that the identity in the N-terminal MAP–Tau-homologous regions (aa 45–70) is low between the two orthologs, the charge and the predicted secondary structure in the N-terminal halves of the two proteins are similar (not shown). In addition, partially sequenced ORFs (varying from 33 to 255 residues in length) from Gram-negative bacterial genomes such as Salmonella typhimurium, Actinobacillus actinomycetemcomitans, Pseudomonas aeruginosa, Y.pestis and the hyperthermophilic Thermotoga maritima show between 25 and 100% identity to either the N- or the C-terminal regions of E.coli ZipA (Microbial Genomes database at NCBI; http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html). In contrast, convincing homology is not seen with contigs or full-length ORFs from the completely or partially sequenced Gram-positive and archaeal genomes. Gene products with significant homology to ZipA are also absent in the Gram-negative bacteria Helicobacter pylori, Borrelia burgdorferi and Treponema pallidum, indicating that ZipA is conserved in a subset of Gram-negative genomes. It is conceivable that bacteria and archaea that lack ZipA orthologs may nevertheless contain proteins that stabilize the FtsZ ring in an analogous manner as ZipA, but their interactions with the ring could be mediated by different structural motifs.
Discussion

A model for combinatorial regulation of cytokinetic FtsZ ring assembly

The similarity between the atomic structures of FtsZ and tubulin, the dynamic nature of FtsZ polymerization, and the present finding that ZipA, an essential stability determinant of the FtsZ ring structure, is a MAP homolog, encourage a model for FtsZ assembly based on the paradigm of MT dynamics (Kirschner and Mitchison, 1986; Desai and Mitchison, 1997). One important property of MT dynamic instability is growth of MTs from nucleating centers at concentrations of tubulin below the critical concentration (Cc) for polymerization. Below Cc, any non-nucleated polymer that forms is unstable and will disappear, whereas if a nucleated polymer shrinks back to the nucleating site, there is a finite probability of regrowth. This property of polymer growth below Cc may also apply to FtsZ behavior in the cell. If the FtsZ ring assemblies by nucleation at the division site, the amount of FtsZ that is recruited to the membrane at the start of assembly would be at or below the Cc, and the growing polymers would be in dynamic equilibrium with soluble FtsZ. The maturation of the FtsZ ring structure will depend on the transition probabilities between polymer growth and shrinkage. Such probabilities would be affected by the concentration of FtsZ, by mutations such as ftsZ84-ts, and by interacting proteins such as ZipA. A high concentration of FtsZ or ZipA would increase the probability of nucleated FtsZ foci to mature into rings by either pushing the equilibrium toward assembly at high monomer concentration, or by stabilizing the growth of nucleated FtsZ oligomers via protein–protein interactions, respectively.

As with MTs, GTP hydrolysis during FtsZ assembly governs the dynamic nature of the polymers (Yu and Margolin, 1997; Lu et al., 1998; Mukherjee and Lutkenhaus, 1998; Figures 3 and 4). Besides GTP, the polymerization dynamics of MTs are influenced by MAPs that stabilize the polymers, whereas stathmin, the kinesins and MT-severing katanin destabilize the MT lattice (Desai and Mitchison, 1997). It is tempting to propose that the FtsZ ring dynamics in bacterial cells is likewise regulated by the opposing actions of interacting proteins that either impart stability to the FtsZ ring, such as ZipA and FtsW (Boyle et al., 1997; Khattar et al., 1997), or destabilize it, such as FtsA (Wang and Gayda, 1990; Dai and Lutkenhaus, 1992; Dewar et al., 1992) and FtsK (Draper et al., 1998). The mutual antagonism of proteins recruited to the FtsZ ring would form the basis for combinatorial regulation of division ring constriction in concert with ingrowth of the nascent septum (Figure 6).

Direct protein–protein interaction between ZipA and FtsZ84 stabilizes thermolabile FtsZ84 ring assembly

Attempts to identify genes that interact with ftsZ have led to a variety of genetic configurations that suppress heat-sensitivity of the ftsZ84 mutation, but suppression in each case appears to be the result of increased expression of the FtsZ84 protein and is not due to any specific physical interaction of the suppressor with the mutant target (Powell and Court, 1998 and references therein). In fact, conditional lethality of ftsZ84 can be suppressed by its own overexpression, indicating that lethality arises from reduced activity of FtsZ84 (Phoenix and Drapeau, 1988; D.RayChaudhuri, unpublished results).

In contrast to these genetic situations, 2-fold higher zipA gene dosage restores high temperature growth of the ftsZ84 mutant cells by physically stabilizing the thermolabile FtsZ84 ring assembly, without raising the steady-state cellular level of FtsZ84. This is the first example that the heat-sensitive lesion in FtsZ84 can be ameliorated by direct physical interaction with another essential division protein, such as ZipA. It is significant that the suppressor screen did not yield any clone carrying...
other known fts genes, even though the majority of the division proteins have been shown to localize to the FtsZ ring (Lutkenhaus and Addinall, 1997; also see Introduction).

**ZipA is the earliest recruit to the FtsZ ring in the septalsome assembly pathway**

ZipA was previously shown to be localized at midcell as a ring in both young *E.coli* cells as well as in deep cytokinesis, suggesting that it remains associated with the leading edge of the invaginating septum throughout cytokinesis (Hale and de Boer, 1997). The interaction between ZipA and FtsZ and the pattern of ZipA localization suggested that ZipA colocalizes with FtsZ to the septum. Using depletion strains to study the interdependence of FtsZ and ZipA localization, Hale and de Boer (1999) have recently shown that ZipA localization depends on FtsZ and have concluded that ZipA probably joins the FtsZ ring structure after its assembly. The presence of zipA, to the exclusion of all other known division genes, in the majority of the suppressor clones isolated in this study strongly indicates that ZipA forms the earliest association with FtsZ during ring assembly and is not recruited subsequent to the maturation of the FtsZ ring. Analogous to the dynamic behavior of MTs (Kirschner and Mitchison, 1986; Desai and Mitchison, 1997). I suggest that the nascent FtsZ oligomers nucleated at the division sites are unstable because the subunit interactions would potentiate FtsZ GTPase activity causing depolymerization, but ZipA binding imparts stability to the oligomers and allows their growth into stable rings (Figure 6). Because most other Fts proteins presumably associate with preassembled FtsZ rings, as inferred from the formation and the stability of the rings under conditions of thermal inactivation or depletion of these proteins, they are not expected to rectify the FtsZ defect which manifests in the extreme lability of the cytokinetic rings at the restrictive temperature (Addinall et al., 1997a; Figure 2).

**Is ZipA an ancient homolog of the Tau family of eukaryotic MAPs?**

ZipA shows structural and functional similarities to structural MAPs such as Tau, MAP2 and MAP4. It promotes and stabilizes FtsZ and FtsZ84 assembly in *vivo* and *in vitro*, organizes FtsZ protofilaments into long bundles or sheets and cosediments with the protofilaments, and displays an ability both to augment polymerization and to bind to the FtsZ/FtsZ84 polymers in a nucleotide-independent manner (Figures 2–4). Similarly, MAPs and Tau promote and stabilize MT assembly in the form of bundles and can bind to the MT lattice in a nucleotide-insensitive manner (Drechsel et al., 1992; Gustke et al., 1994; Trinczek et al., 1995; Desai and Mitchison, 1997; Drewes et al., 1998). These structural and functional similarities between ZipA and the MAP–Tau proteins, coupled with the striking structural resemblance between their respective polymeric targets, FtsZ protofilaments and MTs (Erickson et al., 1996; Lowe and Amos, 1998; Nogales et al., 1998a,b), make a compelling case for ZipA being a primitive MAP–Tau-like protein playing an essential role in the dynamics of the bacterial division ring.

MAPs stabilize MTs in a complex manner by either suppressing the frequency of growing-to-shrinking transitions (catastrophe) or by promoting the transition from depolymerization to polymerization (rescues) (Desai and Mitchison, 1997). To address the molecular mechanism of ZipA function, a future challenge would be to understand whether the GTP-dependent dynamic nature of FtsZ polymers derives from the MT-like dynamic instability behavior, or from subunit treadmilling, or from a combination of both these processes.

**Future directions**

A model for regulation of septal ring assembly (Figure 6) based on the stability of the primary scaffold—the FtsZ polymer—offers a simple framework to categorize the functions of various division proteins whose biochemical activities remain unknown. The finding that ZipA is essential to ensure structural integrity of the FtsZ ring strongly encourages a stability-based model. The stage is now set to approach biochemical reconstitution of septalsome assembly by analyzing FtsZ binding by various division proteins, and their effects, alone or in combination, on the dynamics of FtsZ assembly. This could help unveil the biochemical activities of these proteins as well as reveal the network of protein–protein interactions that underlies the assembly of the division machine.

**Materials and methods**

**Media and strains**

Cells were grown in Luria–Bertani (LB) medium which, where relevant, was supplemented with 50 or 100 µg/ml ampicillin (Amp). LB medium contained 0.5% NaCl unless indicated otherwise. *Escherichia coli* K12 strains used were: NM539 (supF hsdR [r− m−] lacY [A1306]), DB5564 (F′ thr leu lac Y117 Ts808 supE) (Maurer et al., 1984), JFL100 [F′ ily his thyA deo araAm (lac-125)(Am) galU (Am) tyrT (supF32-tv) ftsZ84-ts] (Lutkenhaus et al., 1980), MC1000 [F′ araD139 Δ(arAB-BC-leu)7679 galU galK Δ(lacX74 rpsL) thi] (Silhavy et al., 1984), DRC13 (ftsZ84-ts derivative of MC1000), and KD1067 (arg his mutD5 Su-) (Degnen and Cox, 1974). ADB322 is lacF Phlac::zipA blu imm21 (Hale and de Boer, 1997).

**Genetic and recombinant DNA methods**

P1 transduction for strain construction and lambda techniques were as described by Silhavy et al. (1984). Recombinant DNA techniques were based on those of Sambrook et al. (1989). Radioactive probes for Southern hybridization were prepared by nick-translation using [γ-32P]dithiothreitol (TTP (New England Nuclear). A 3.8 kb BamH1–Sal1 fragment and a 4.4 kb Sau3A fragment from the suppressor clone JIB-9-2, cloned into pBR322, were used as probes to determine the relatedness of the inserts in the suppressor isolates.

**Construction and in vivo mutagenesis of λ genomic library**

A genomic library was constructed by cloning a partial Sau3A1 digest of the JFL100(ftsZ84-ts) chromosomal DNA (~15–18 kb average fragment size) into the BamH1 sites of λEMBL3 (Promega). The recombinant λEMBL3 DNA pool was packaged *in vitro* and amplified on NM539 to a titer of ~3–5×1012 P.f.u./ml. The amplified library was mutagenized in three separate batches by growing the phage in the *mutD5* mutant strain KDI1067. Briefly, fresh 108 cells of KDI1067 (grown overnight in M9 minimal medium) was mixed with 5×108 recombinant phage and allowed to grow in LB medium for 2.5 h with aeration. The extent of mutagenesis was scored by the number of clear plaques formed by λ phage after an identical passage through the *mutD5* strain. The clear plaques arose at a frequency of ~4–5%.

**Red plaque method for isolation of suppressor λ clones**

This method is based on the red plaque complementation assay described previously (Maurer et al., 1984). Suppression was tested in partial diploids of JFL100(ftsZ84-ts)λ*+* tester strain that was lysogenized with mutagenized λEMBL3 library. The mutagenized phage library was first plated on DB5564 at 37°C (~1.5×1012 phage per plate) to allow plaque
formation. The plates were then sterilized by inversion over CHCl₃ for 15–20 min, exposed to a UV dose of 340 ergs/mm², and overlaid with a mixture of 3 ml soft LB agar, 0.3 ml 20% (w/v) maltose, 0.1 ml of 0.4% (w/v) 2,3,5-trinitro tetrazolium–HCl and 0.3 ml freshly grown JFL100(λ) tester cells (Klett ~50) to allow lysisogenization in situ. The plagues were UV-irradiated to stimulate homologous recombination with the λ prophage. Suppression was observed after 48–72 h as the growth of tester bacteria in red clusters at 42°C in the vicinity of a plaque. The visual detection of suppression is enhanced in this assay by the inclusion of maltose and the tetrazolium dye which is reduced to an insoluble red formazan by growing cells. The ‘red plaques’ were purified on DB5654 and restested for suppression over two to three cycles.

**Overexpression and purification of His₆-ZipA**

The zipA structural gene was amplified by PCR from MC1000 chromosome and cloned between the NdeI and Xhol sites of pET-15b (Novagen) to generate pET-15ZIP. The insert was sequenced to ensure that no mutational error occurred during PCR. To overproduce His₆-ZipA, BL21DE3(pLysS) cells (Studier et al., 1990), transformed with pET-15ZIP, were grown at 37°C in LB supplemented with 100 µg/ml ampicillin and 25 µg/ml chloramphenicol to an optical density of 0.1 at 650 nm. Cells were harvested, resuspended in 0.02 culture volume of buffer A (25 mM Tris–HCl pH 7.5, 100 mM NaCl, 2 mM EDTA) containing a protease inhibitor cocktail (Boehringer Mannheim), and subjected to three cycles of freeze-thawing followed by brief sonication. The cell extract was centrifuged at 20000 g (4°C, 30 min) to pellet membranes which were resuspended in buffer A containing 1% glycerol and protease inhibitors. The overproduced His₆-ZipA was incubated at 25°C with the membrane fractions and was extracted with 1.2% (w/v) octyl-β-D-glucopyranoside (OG) for 2 h at 4°C. The OG extract was clarified by ultracentrifugation and the supernatant containing His₆-ZipA was dialyzed against buffer B (25 mM MOPS–KO₃ pH 6.5, 500 mM NaCl, 5% glycerol, 0.36% OG). The dialyzed fractions (~3–5 mg protein) were loaded on a 1 ml nickel-chelate affinity column (ProBond resin, Invitrogen), equilibrated with buffer B and the column was washed sequentially with 5 ml volumes of buffer B containing imidazole at final concentrations of 1, 50 and 150 mM. The protein was eluted with 5 ml of 500 mM imidazole. The His₆-ZipA fractions were pooled, dialyzed against buffer C (25 mM MOPS–KO₃ pH 6.5, 50 mM KCl, 5% glycerol, 0.36% OG), and stored at ~70°C.

**Western and affinity blotting with [35S]FtsZ**

Protein minigels were Western blotted onto PVDF or nitrocellulose membranes (Millipore) at 100 V for 1 h using the Bio-Rad minigel transfer module. For chemiluminescent detection with anti-His₆ monoclonal antibody (Qiagen), the manufacturer’s protocol was followed and the blot developed with goat anti-mouse secondary antibody-herosardish peroxidase (HRP) conjugate (Promega) and enhanced ECL substrates (Amer sham). Anti-FtsZ polyclonal antibody (RayChaudhuri and Park, 1992) was used at a dilution of 1:15 000, anti-GroEL antibody (Epicentre Technologies) was used at a dilution of 1:2500, and the immunoreactive bands were detected with goat anti-rabbit secondary antibody-alkaline phosphatase (AP) conjugate (Sigma) and NBT/BCIP substrates. For affinity detection of ZipA on nitrocellulose (0.2 µ) blots (Hale and de Vruijl, 1990), [35S]FtsZ was used as a probe. FtsZ was induced from the T7 promoter on pET-32a′ plasmid (RayChaudhuri and Park, 1992), in BL21DE3(pLysS) cells growing in methionine- and cysteine-free medium, and labeled for 15 min with 10 μCi/ml 35S-Translabel (ICN) in the presence of 200 µg/ml rifampicin (Studier et al., 1990). Under these conditions, FtsZ was the predominant labeled band in the soluble fraction of induced cells and was used as a probe without further purification. To detect ZipA, the blot was incubated with 1000 c.p.m./ml [35FtSZ (250 000 c.p.m/ml) for 16 h at 4°C. The radioactivity associated with the ZipA bands was detected using the Storm PhosphorImager (Molecular Dynamics).

**Polymerization of FtsZ and FtsZ2 in the presence of His₆-ZipA**

FtsZ and FtsZ2 proteins were purified essentially as described previously (RayChaudhuri and Park, 1992), except that the ion-exchange step involved fast pressure liquid chromatography on a 1 ml Mono-Q HR 5/5 column (Pharmacia) and the proteins were eluted with a 0–500 mM KCl gradient. FtsZ or FtsZ2 (each at 200 µg/ml = 5 µM protein) was incubated at 25°C in 50 µl assay reactions consisting of 50 nM MOPS–KO₃ pH 6.5, 50 mM KCl, 5 mM MgCl₂, 0.04% OG and 1 mM GTP (pH pre-adjusted to 7.0). The presence of the detergent OG did not affect FtsZ polymerization. To study the effect of ZipA on polymerization, purified His₆-ZipA was added to a final concentration of 200 or 400 µg/ml. All additions were at 25°C and GTP/ATP was added as the final reactant to appropriate reactions. Reactions were centrifuged (25°C) immediately or after 13 min in a TLA 100.2 rotor (Beckman) at 80 000 r.p.m. for 10 min to sediment the polymers (Mukherjee and Lutkenhaus, 1998). The supernatant was aspirated and the pellet was resuspended in 50 mM MOPS–KO₃ pH 6.5, 5 mM MgCl₂, and 0.05% Triton X-100. Proteins in the supernatant and the pellet fractions were analyzed by immunostaining the Western blots. The total polymerization period included the 10 min centrifugation time plus 2 min needed to load the reaction tubes in the rotor and starting centrifugation.

**Indirect immunofluorescence microscopy**

Immunofluorescence microscopy of E.coli cells was carried out essentially as described by Hiraga et al. (1998). Cells growing at 30 or 42°C in LB were fixed with 80% methanol and immunostained with anti-FtsZ antibody (1:10 000 dilution), followed by goat anti-rabbit secondary antibody-FITC conjugate (Zymed Laboratories; 1:1000 dilution). Cells were then stained with 1 µg/ml 4′, 6-diamidino-2-phenyl-indole (DAPI) to visualize nucleoids. Microscopy was performed using an Olympus BX60 microscope with an Olympus 100× 1.25 Ph3 oil-immersion objective. An Olympus MWIB photobeuthe transmitting a wavelength of 360–370 nm or a wavelength of 460–490 nm was used to stimulate DAPI or FITC fluorescence, respectively, and images were captured with C-4742-95 digital CCD camera (Hamamatsu). The Image-Pro Plus v3.0 software (Media Cybernetics) was used to acquire images and to control an automatic light shutter (UniBlitz Model D122 35 mm shutter; Universal Imaging Corporation), and images were imported into Adobe Photoshop v3.0.5 (Adobe Systems) for the composition of Figure 2.

**Electron microscopy**

Aliquots (5 µl) of FtsZ assembly reactions supplemented with or without His₆-ZipA (FtsZ and ZipA, each present at 200 µg/ml) were applied to carbon-coated copper grids (300 mesh size) that had been subjected to glow discharge to render the surface hydrophilic. After 2 min, the drops were blotted dry, the grids were washed with 2 drops of 0.1 M ammonium formate, and negatively stained with 1% aqueous uranyl acetate (pH unadjusted). The grids were examined under a Philips CM-10 electron microscope and photographed at 39 000× or at 52 000×. Electron micrographs were converted to digital images using a UM3scanned (Supervista) and Adobe Photoshop v3.0.5.

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ZipA stabilizes FtsZ ring structure


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