This is a repository copy of ZapE is a novel cell division protein interacting with FtsZ and modulating the Z-ring dynamics.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/161141/

Version: Published Version

Article:
Marteyn, B.S., Karimova, G., Fenton, A.K. orcid.org/0000-0003-4042-4580 et al. (10 more authors) (2014) ZapE is a novel cell division protein interacting with FtsZ and modulating the Z-ring dynamics. mBio, 5 (2). e00022-14. ISSN 2150-7511

https://doi.org/10.1128/mbio.00022-14

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike (CC BY-NC-SA) licence. This licence allows you to remix, tweak, and build upon this work non-commercially, as long as you credit the authors and license your new creations under the identical terms. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.
ZapE Is a Novel Cell Division Protein Interacting with FtsZ and Modulating the Z-Ring Dynamics

Benoit S. Marteyn, a,b Gouzel Karimova, c,d Andrew K. Fenton, a, b Anastasia D. Gazi, a, b Nicholas West, a Lhoussaine Touqui, i Marie-Christine Prevost, j Jean-Michel Betton, o Oemer Poyraz, d Daniel Ladant, k Kenn Gerdes, g Philippe J. Sansonetti, a, b, j Christoph M. Tang a

Institut Pasteur, Unité de Pathogénie microbienne Moléculaire, Paris, France; Institut National de la Sante et de la recherche Medicale Unité 786, Paris, France; Institut Pasteur, Unité de Biologie des Interactions Moléculaires, Paris, France; CNRS UMR3528, Unité de Biologie des Interactions Moléculaires, Paris, France; University of Newcastle, Centre for Bacterial Cell Biology, Newcastle-upon-Tyne, United Kingdom; Institut Pasteur, Unité de Défense Innée et Inflammation, Paris, France; Institut Pasteur, Imagoopole/FFMU, Paris, France; Institut Pasteur, Unité de Microbiologie Structurale, Paris, France; Karolinska Institutet, Department of Medical Biochemistry & Biophysics, Stockholm, Sweden; Collège de France, Paris, France; University of Oxford, Sir William Dunn School of Pathology, Oxford, United Kingdom

ABSTRACT Bacterial cell division requires the formation of a mature divisome complex positioned at the midcell. The localization of the divisome complex is determined by the correct positioning, assembly, and constriction of the FtsZ ring (Z-ring). Z-ring constriction control remains poorly understood and (to some extent) controversial, probably due to the fact that this phenomenon is transient and controlled by numerous factors. Here, we characterize ZapE, a novel ATPase found in Gram-negative bacteria, which is required for growth under conditions of low oxygen, while loss of zapE results in temperature-dependent elongation of cell shape. We found that ZapE is recruited to the Z-ring during late stages of the cell division process and correlates with constriction of the Z-ring. Overexpression or inactivation of zapE leads to elongation of Escherichia coli and affects the dynamics of the Z-ring during division. In vitro, ZapE destabilizes FtsZ polymers in an ATP-dependent manner.

IMPORTANCE Bacterial cell division has mainly been characterized in vitro. In this report, we could identify ZapE as a novel cell division protein which is not essential in vitro but is required during an infectious process. The bacterial cell division process relies on the assembly, positioning, and constriction of FtsZ ring (the so-called Z-ring). Among nonessential cell division proteins recently identified, ZapE is the first in which detection at the Z-ring correlates with its constriction. We demonstrate that ZapE abundance has to be tightly regulated to allow cell division to occur; absence or overexpression of ZapE leads to bacterial filamentation. As zapE is not essential, we speculate that additional Z-ring destabilizing proteins transiently recruited during late cell division process might be identified in the future.

RESULTS Identification of ZapE, required for growth in the absence of oxygen. While screening a library of Shigella flexneri mutants for their ability to colonize the gastrointestinal (GI) tract (9, 10), we identified a mutant that was defective for colonization with a transposon 12 bp upstream of a 1,128-bp open reading frame (ORF) (accession number b3232; Fig. 1A). The role of this ORF is known about cell division during growth under low-oxygen conditions, even though many bacteria thrive in anaerobic environments, particularly within mammalian hosts (7, 8).

Here we identify a further Z-ring-associated protein named ZapE which is required for cell division under low-oxygen conditions. ZapE is an ATPase present in Gram-negative bacteria that appears at the constraining Z-ring late in cell division. ZapE affects FtsZ multimerization and polymerization in vitro. In vivo ZapE recruitment correlates with Z-ring constriction.
ZapE is an ATPase that interacts with components of the divisome. ZapE is found in Gram-negative bacteria (see Table S1C in the supplemental material) and is predicted to contain putative Walker A (GGVGRKKwRwT) and Walker B nucleotide-binding sites (see Fig. S3B in the supplemental material). Therefore, we examined the capacity of ZapE to hydrolyze ATP and GTP. We found that purified recombinant ZapE hydrolyzed ATP but not GTP (Fig. 2C). Introduction of a single amino acid substitution in the ATP-binding site (in ZapE_{K84A}) abolished ATPase activity (Fig. 2C). In addition, we modeled the organization of the active site of ZapE by small-angle X-ray scattering (SAXS) analysis (Fig. 2D; see also Fig. S3C to E), using fold recognition methods based on the extended AAA+ ATPase domain of the ATP-dependent metalloprotease FtsH (PDB identification number [ID] 2CE7). As FtsZ is a known FtsH substrate (11, 12), the structural similarity between ZapE and FtsH suggests that ZapE might interact directly with FtsZ.

To further define the role of ZapE during cell division, we examined whether the protein interacts with components of the divisome, including FtsZ. Using a bacterial two-hybrid (BACTH) system (13), we identified potential interactions between ZapE and FtsQ, FtsL, FtsI, and FtsN (Fig. 3A) (P < 0.01 and P < 0.001, Student’s t test). In contrast, no interaction was observed with FtsA, which forms actin-like protofilaments (14), and FtsK, which coordinates DNA segregation with division (15). A similar result was obtained using ZapE from S. flexneri (M90T) as bait paired with these E. coli division proteins (Fig. 3A). Interactions of ZapE with FtsZ could not be examined by the BACTH system, as expression of both T18-zapE or T25-zapE and T18-ftsZ together was toxic (not shown and reference 16).

To confirm interactions identified by BACTH analysis, pull-down assays were performed with E. coli His-tagged ZapE bound to beads and green fluorescent protein-FtsQ (GFP-FtsQ), GFP-FtsL, GFP-FtsI, and GFP-FtsN expressed in E. coli (Fig. 3B); no interaction was observed when GFP was expressed on its own. In addition, pulldown assays with bound ZapE or ZapE_{K84A} isolated FtsZ-GFP from cell lysates (Fig. 3B), indicating that the ZapE-ATP binding site was not required for FtsZ interaction in vitro.

ZapE was found in the cytoplasm, and further localization by electron microscopy (EM) analysis revealed that most of the signal was detected in the vicinity of the bacterial inner membrane (see Fig. S4A in the supplemental material) (P < 0.001, Student’s t test); this observation is consistent with an interaction with FtsZ.

ZapE localizes to the constricting Z-ring toward the end of cell division. Next, we performed time-lapse microscopy to determine the temporal and spatial association of ZapE in relation to FtsZ at the single-cell level. To achieve this, ZapE-mCherry was expressed under the control of its native promoter (see Fig. S4B in the supplemental material) and visualized in the cell by electron microscopy (EM) analysis (Fig. 3C). We found that ZapE localized to the constriction zone of the midcell, indicating that ZapE is required for correct cell division.

ZapE was also dispersed during the early stages of cell division (Fig. 3C; see also Movie S1 in the supplemental material). Indeed, the appearance of ZapE at the midcell coincided with constriction of the Z-ring and with its subsequent disappearance (see Fig. S4C). In events leading up to the maximalization of Z-ring constriction (defined as t_{max}), FtsZ levels increased in a stepwise fashion as the diameter of the Z-ring diminished; the amount of ZapE present at the Z-ring increased progressively until maximal constriction of

| Strain | Description | C.I. |
|--------|-------------|-----|
| M90TmutW | Transposon mutant zapE | < 0.01 |
| M90T::ΔzapE | zapE mutant | 0.03 |
| M90T::ΔzapE + | complemented mutant | 0.31 |
| ΔzapE-GFP M90T | | |

(here designated ZapE) in colonization was confirmed by construction and analysis of a deletion mutant (M90T::ΔzapE) and by complementation (Fig. 1; see also Fig. S1A and Tables S1A and B in the supplemental material). Note, examination of tissues from infected animals demonstrated that S. flexneri M90T::ΔzapE displayed an elongated cellular phenotype in the GI tract (Fig. 1B).

To further characterize the contribution of ZapE to cell division, we examined the phenotype of an Escherichia coli MG1655 mutant (K12::ΔzapE) grown under a variety of conditions relevant to survival of bacteria in vivo. Strikingly, while E. coli lacking ZapE had no demonstrable growth defect in aerobic conditions, the mutant was defective for growth in anaerobiosis (Fig. 2A; see also Fig. S1B and C in the supplemental material); complementation analysis confirmed that loss of ZapE was responsible for the failure to grow in a low-oxygen environment. Additionally, growth at elevated temperatures (i.e., 42°C) led to an exaggerated elongation of E. coli lacking ZapE (Fig. 2B). The temperature-dependent elongated phenotype was also observed in Shigella— with or without a virulence plasmid (INV+), as well as in E. coli, comparing growth at 30°C and 42°C (Student’s t test; P < 0.001) (see Fig. S2A and B). The K12::ΔzapE phenotype was confirmed using a K12::ΔzapE::Km-ΔzapE strain in the presence of glucose but not of arabinose, allowing functional complementation of the phenotype (see Fig. S3A).

**FIG 1** ZapE is required during the *Shigella* infectious process *in vivo*. (A) Competitive index (C.I.) of a *Shigella flexneri* 5A zapE transposon mutant (M90TmutW), a zapE mutant (M90T::ΔzapE), and a complemented strain (M90T::ΔzapE+pazape::GFP M90T) *in vivo*. The C.I. assessed the ability of each mutant to colonize the rabbit ileal loop in comparison with the wild-type strain. A C.I. of 1 indicates no attenuation. Data represent averages of the results of three independent experiments. (B) Immunodetection of the M90T, M90T::ΔzapE, and M90T::ΔzapE+pazape::GFP strains in the rabbit ileal loop model. DNA was stained with DAPI (blue) and actin with RRX-phalloidin (red). Shigella strains were labeled using α-LPS polyclonal antibody (pAb) (green). Image acquisition was performed using a confocal microscope. Right-hand panels show enlarged areas. Bars are 5 μm.
the Z-ring occurred (Fig. 3D) (Student’s t test; \( P < 0.001 \)), demonstrating a clear correlation between the expression and localization of ZapE and Z-ring constriction in vivo.

**ZapE abundance modulates Z-ring stability.** We next examined whether modulating levels of ZapE within cells affected their shape and the appearance of Z-rings. Initially, the location of FtsZ-GFP during cell division was examined in either the presence or the absence of ZapE. FtsZ-GFP does not fully complement an ftsZ mutant, but localizes to the midcell, and does not impair normal cell division when expressed at basal levels in wild-type bacteria (18). Loss of ZapE in a strain expressing FtsZ-GFP led to marked filamentation, with the loss of ordered Z-rings in cells (Fig. 4A [rich media] and B [minimum media]; see also Fig. S5A and Movie S2 in the supplemental material [live microscopy]). This filamentation was more pronounced during growth at higher temperatures (Fig. 4A) and during the stationary phase (Fig. 4C). We confirmed that filamentation in cells lacking ZapE was more marked following overexpression of native FtsZ in a dose-dependent manner (Fig. 4D).

Loss of ZapE in a strain expressing FtsZ-mCherry led to the formation of multiple disorganized FtsZ rings found sporadically along the length of the extended bacteria compared to the wild-type strain (Fig. 5A). Furthermore, we examined the effect of overexpressing ZapE under the control of an IPTG (isopropyl-\( \beta \)-D-thiogalactopyranoside)-inducible promoter. In wild-type cells, ZapE overexpression also resulted in dose-dependent filamentation (Fig. 5B and C; see also Fig. S5B in the supplemental material). However, no Z-rings (detected with FtsZ-mCherry) were detected along the length of filaments when ZapE overexpression was induced with high levels of IPTG (Fig. 5A; see also Fig. S5C [as a control, without IPTG]), although additional adverse effects of ZapE overexpression could not be ruled out. This was not observed in cells overexpressing ZapE\(_{K84A}\), which exhibited normal bacterial shape and single ordered Z-rings (Fig. 5A). Taken together, these data are consistent with ZapE and FtsZ having antagonizing roles at the Z-ring inside cells.

**ZapE impairs the stability of polymerized FtsZ in vitro.** Next we examined the effect of ZapE on polymerized FtsZ in vitro using
purified recombinant proteins. As described previously (19), in the presence of GTP and Ca\(^{2+}\) purified FtsZ and FtsZ-GFP form small polymers \textit{in vitro} (Fig. 5D; see also Fig. S5D in the supplemental material). The addition of ZapE promoted the polymerization of FtsZ/FtsZ-GFP through large helical structures (Fig. 5D; see also Movie S3). These structures were no longer stable when ATP was added with ZapE after a 3-min incubation (Fig. 5D), while ZapE\(_{K84A}\) had no effect (Fig. 5D), indicating that active ZapE reduces the stability of FtsZ polymers in the presence of ATP through a molecular mechanism which remains to be defined.

**DISCUSSION**

Here, we identified and characterized a novel Z-ring-associated protein named ZapE, which is an ATPase found among Gram-negative bacteria (see Table S1C in the supplemental material). ZapE is not essential in \textit{E. coli} or in \textit{Shigella} during growth under standard laboratory conditions. However, in the absence of oxygen or at temperatures over 37°C, the contribution of ZapE to cell division becomes evident, with cells lacking this protein displaying a growth defect and elongated phenotype (Fig. 2A and B). The latter was detected in tissue sections of the GI tract infected with a \textit{Shigella} \textit{zapE}\(_{-}\)mutant; as a likely consequence of the cell division defect, \textit{zapE} is required for efficient colonization of GI track (Fig. 1A and B). Therefore, the identification of ZapE from studies of bacteria \textit{in vivo} indicates that examining bacteria in the environments that they encounter outside the laboratory could uncover the function of other accessory cell division proteins. In the future, studies of bacterial cell division under other growth conditions such as low pH, oxidative stress, or amino acid starvation will reveal novel aspects of cell division regulation allowing bacterial survival during host invasion.

Several ATPases targeting FtsZ during cell division such as...
FtsH, MinD, and KaiC are involved in cell division functions. FtsH is an ATP-dependent zinc metalloprotease targeting FtsZ in vitro (11), although this activity could not be confirmed in vivo (12). MinD belongs to the Min system involved in Z-ring positioning (20). KaiC inhibits Z-ring formation controlling the circadian clock in *Synechococcus elongatus* (21). None of these ATPases have proven functions in the dynamics of Z-ring constriction in vivo. Indeed, if starting events of cell division (Z-ring assembly and

---

**FIG 4** Effect of ZapE inactivation on K-12 shape upon FtsZ-GFP and FtsZ expression modulation. (A) Localization of FtsZ-GFP (pDSW230) in K-12 and K12::ΔzapE strains grown in rich media (LB) at 37°C or 42°C in the absence of IPTG until an OD$_{600}$ = 0.5 was reached. Bars are 5 μm. (B) Localization of FtsZ-GFP (pDSW230) in K-12 and K12::ΔzapE strains grown in minimum media (M9) at 37°C in the absence of IPTG until an OD$_{600}$ = 0.5 was reached. Bars are 2 μm. (C) FtsZ-GFP (pDSW230) localization in K-12 and K12::ΔzapE strains during the stationary phase performed in LB rich media at 37°C or 42°C. These observations are representative of the results of at least three independent experiments. Bars are 1 μm. (D) Effect of FtsZ-H$_6$ (WM971) overexpression on K-12 and K12::ΔzapE shape. Bacteria were grown in LB at 37°C in the presence of the indicated concentrations of IPTG until an OD$_{600}$ = 0.5 was reached. These observations are representative of the results of three independent experiments. Bars are 10 μm.
have been well characterized (22), events leading to the disappearance of the ring and completion of division have not been defined in as much detail and remain to be discussed (23). This might be explained by the transiency of the Z-ring constriction phenomenon. Consistently, ZapE fusion (mCherry) recruitment at the Z-ring could be seen in this study only by live microscopy (Fig. 3C; see also Movie S1 in the supplemental material).

We observed that in the presence of ATP and Ca\(^{2+}\), ZapE promoted bundling of FtsZ/FtsZ-GFP polymers into unstable three-dimensional structures (Fig. 5D; see also Movie S3 in the supplemental material). A structural homology between ZapE and FtsH was found by SAXS analysis (Fig. 2D). This observation should support further investigations on how ZapE directly or indirectly affects the effect of activity on Z-ring constriction, accounting for the destabilization observed \textit{in vitro} (Fig. 5D) and \textit{in vivo} upon ZapE overexpression (Fig. 4A).

The precise control of the ZapE level appears to be crucial for Z-ring stability and dynamics as we showed that either ZapE loss or overexpression (Fig. 4A) altered Z-ring stability and led to bacterial filamentation. Further studies should aim at defining ZapE
abundance regulation (expression and degradation) during the cell division process and its consequences for Z-ring stability to better characterize the role of ZapE in the Z-ring dynamic in vivo.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are described in Table S1A in the supplemental material. Shigella strains were grown in Trypticase soy (TCS) broth or on TCS agar plates supplemented with 0.01% Congo red (Sigma). E. coli strains were grown in LB media. Anaerobic growth experiments were performed in a Mini Macs MG-250 chamber (Don Withley). d-Glucose and 1-arabinose were used at a concentration of 0.2% to modulate the expression of genes cloned under the control of the pBAD33 promoter.

Expression plasmid construction. The pSUC vector construction was achieved by amplifying the mCherry fusion from the pmCherry-N1 vector using the SG150 and SG151 primer pair (see Table S1B in the supplemental material), introducing the BamHI and EcoRI restriction sites. The pSU19 vector was digested with BamHI and EcoRI restriction enzymes prior ligation of the digested mCherry amplified fragment, leading to the generation of the pSUC vector (see Fig. S4B). This expression vector allows the expression of mCherry protein fusions in the C terminus under the control of the promoter of the gene of interest in E. coli and in Shigella.

Expression of the FtsZ-mCherry fusion under the control of a lac promoter was performed using either the pDSW230 and pDSW231 construct (kindly provided by David Weiss) (described in Table S1A in the supplemental material). FtsZ-mCherry fusion expression under the control of an arabinose-inducible promoter was performed with pARK133 (described in Table S1A).

DNA manipulations. The initial transposon insertion in S. flexneri M90T in zapE was performed as described previously (9). The construction of inactivated zapE mutants was then performed in E. coli and S. flexneri.

ΔzapE mutant construction. In MG1655, E. coli K-12 P1vir page lysate was prepared on the JW3201 donor strain from the Keio collection (1, 24). In the JW3201 strain, the zapE ORF is substituted by the kanamycin (Km) resistance marker (ΔzapE::Km) (2, 24). The ΔzapE::Km cassette was introduced into MG1655 by P1 transduction (3, 25), and selection for kanamycin-resistant (Km+) colonies was made on LB plates containing kanamycin (50 μg/ml). After reisolation, several clones were verified by PCR to confirm the right chromosomal structure of the ΔzapE::Km deletion. One clone was chosen and named K12ΔzapE::Km.

K12ΔzapE was then obtained from K12ΔzapE::Km by removing the kanamycin resistance marker from the ΔzapE::Km cassette. In this ΔzapE::Km cassette, the antibiotic resistance marker is flanked by two direct frt repeats, which are the recognition targets for the site-specific recombinase FLP (4, 24). Therefore, to get rid of the resistance marker from the K12ΔzapE::Km chromosome, pCP20, a temperature-sensitive plasmid that encodes the FLP recombinase, was used (5, 26). Briefly, K12Δopi::Km cells were transformed with pCP20, and chloramphenicol-resistant (Cm+) colonies were selected at 30°C on LB plates containing the corresponding antibiotic (30 μg/ml). Several of these clones were grown overnight on antibiotic-free LB plates at 42°C. Ten independent colonies were selected, and after single-colony passage at 30°C, all 10 colonies were no longer Cm+ and Km+, indicating simultaneous loss of pCP20 and the kanamycin resistance marker from the bacterial chromosome. This FLP-catalyzed excision created an in-frame deletion of the zapE ORF, leaving behind a 102-bp scar sequence (ΔzapE::frt) (6, 24). To confirm the correct chromosomal structure of the ΔzapE::frt deletion, several Cm+ and Km+ clones were tested by PCR using the NWpr40 and NWpr41 primer pair (see Table S1B in the supplemental material). After confirmation, one clone was chosen and named K12ΔzapE.

Alternatively, the ΔzapE::Km cassette was introduced into MG1655 expressing pBAD33 (p3zapE) by P1 transduction (7, 25), and selection for kanamycin-resistant (Km+) colonies was performed on LB plates containing kanamycin (50 μg/ml). The K12ΔzapE::Km p3zapE strain was obtained.

In order to inactivate zapE in Shigella flexneri (M90T), a one-step chromosomal inactivation method was used to target homologous region for integration. Therefore, we generated PCR products with much longer flanking sequence using the K12ΔzapE null mutant as the template. The M90T was transformed with PCR products amplified from K12ΔzapE::Km mutant genomic DNA using primers NWpr23 and NWpr24 (see Table S1B in the supplemental material). The NWpr23 and NWpr24 primers were designed to include 50 bp of upstream and downstream sequence flanking zapE. This product was transformed into M90T::pKD46, which resulted in all kanamycin-resistant colonies containing the 1.50-kb kanamycin resistance gene as analyzed by PCR. Thus, an S. flexneri null mutant (M90TΔzapE) was successfully generated.

Expression, respectively, a zapE-GFP and a zapE-mCherry fusion under the control of the zapE promoter resulted in the complementation of the M90TΔzapE and K12ΔzapE mutants. In order to express a ZapE-GFP fusion, the zapE genes of Shigella and E. coli and their promoters (~500 bp) were amplified with the SG127 and SG128 primer pair (see Table S1B in the supplemental material) and cloned into pPFPV25 vector digested with the BamHI and Ndel restriction enzymes. The pzapE-GFP M90T and pzapE-GFP K-12 constructs were obtained and sequenced (Table S1A).

In order to express zapE-mCherry, the zapE gene and its promoter (~500 bp) were amplified with the SG219 and SG355 primer pair (see Table S1B in the supplemental material) and cloned in pSUC vector digested with the HindIII and XbaI restriction enzymes (see Fig. S4B). The K84A point mutation of zapE was performed using the SG114 and SG115 primer pair (see Table S1B). pzapE-mCherry and pzapE-K84A-mCherry constructs were obtained and sequenced (see Table S1A).

In order to overproduce the ZapE-His and ZapEK84A-His protein fusions in an IPTG-dependent manner, the corresponding ZapE DNA fragments were amplified by PCR prior cloning in the pKJ1 plasmid digested using the Ncol and BamHI restriction enzymes (see Table S1A in the supplemental material). zapE was amplified using the SG90 and SG91 primer pair (see Table S1B), and ZapEK84A was obtained using the SG114 and 1G115 primer pair to introduce a K84A single point mutation (see Table S1B). The resulting pzapE-His and pzapEK84A-His constructs were analyzed by PCR and sequenced.

Rabbit ligated ileal loop model. New Zealand White rabbits weighing 2.5 to 3 kg (Charles River Breeding Laboratories, Wilmington, MA) were used for experimental infections. For each animal, up to 12 intestinal ligated loops, each 5 cm in length, were prepared as described previously (8, 9, 27). For the evaluation of the competitive index (C.I.), equal quantities of the wild-type strain and of the mutant were injected in each loop (corresponding to a total dose of 10^6 CFU per loop). After 16 h, animals were sacrificed and the luminal fluid was aspirated and S. flexneri recovered. The C.I. was calculated as the proportion of mutant to wild-type bacteria recovered from animals divided by the proportion of mutant to wild-type bacteria in the inoculums, and results are expressed as the means of the results determined with at least 4 loops from two independent animals. The experimental protocol was approved by the French Ethic Committee Paris 1 (number 20070004, 9 December 2007).

For immunohistochemical staining, infected rabbit ileum samples were washed in phosphate-buffered saline (PBS), incubated at 4°C in PBS containing 12% sucrose for 90 min and then in PBS–18% sucrose overnight, and frozen in optimum cutting temperature (O.C.T.) compound (Sakura) on dry ice. Sections (7 μm in thickness) were obtained using a CM-3050 cryostat (Leica). Fluorescent staining was performed using a rabbit anti-Shigella lipopolysaccharide (LPS) primary antibody (P. Sonnetoi, Institut Pasteur) (1:200 dilution) and an anti-rabbit fluorescent isothiocyanate (FITC)-conjugated secondary antibody (1:1,000). Epithelium cell nuclei were stained with DAPI (4′,6-diamidino-2-
phenylindolone) (1:1,000) and actin stained with Rhodamine Red-X (RRX)–phalloidin (1:1,000). Image acquisition was performed using laser scanning confocal microscopy. Image analysis was performed using ImageJ software.

**Two-hybrid screen.** We used the BACTH system that is based on the interaction-mediated reconstitution of an adenylate cyclase (AC) enzyme in the otherwise defective DHM1 *E. coli* strain (9, 13, 28). This system is composed of two replication-compatible plasmids, pKT25 and pUT18, respectively encoding the intrinsically inactive N-terminal T25 domain and C-terminal T18 domain of the AC enzyme. *E. coli* and *Shigella zapE* was amplified using the NG1281 and NG1282 primer pair and cloned in pKT25 vector.

pKT25 and pUT18 plasmids were subsequently doubly transformed to DHM1 to search for the AC reconstitution that turns on pKT25 vector.

**TLC analysis.** ATPase and GTPase assays were performed in the presence of bovine serum albumin (BSA) (Sigma) (1.25 mg/ml), ATPγS or GTPγS (PerkinElmer) (30 μCi), ATP or GTP (Sigma) (50 μM), and 0.1 to 10 μg of purified ZapE-H6 and ZapF-β-galactosidase. The final reaction mixture volume was 20 μl in TMD buffer (25 mM Tris [pH 7.4], 10 mM MgCl2, 1 mM dithiothreitol [DTT]). The reaction was run during 10 min at 37°C and stopped by the addition of 20 μl methanol. When indicated, chromatography was performed on thin-layer chromatography (TLC) plates (Thomas Scientific), with a mobile phase containing a mixture of lithium chloride (LIC) and formic acid. After chromatography, a film was exposed on the plate and further developed. Radiolabeled protein fusions were analyzed on a GE Healthcare Bio-Rad Compute system (R2012 version with Image Processing Toolbox and Statistics Toolbox). Statistical analyses were performed using GraphPad Prism 5 software.

**Fluorescent protein fusion imaging.** In order to localize FtsZ-GFP and ZapE-mCherry and mutated versions of protein fusions in bacteria, the corresponding expression plasmids were transformed in *E. coli* K-12 MG1655 wild-type or ΔZapE strains, as indicated. The localization was performed on either fixed or living bacteria. The fixation of bacteria was performed by adding 4% paraformaldehyde (PFA) followed by a washing in PBS. The observation was performed using a Nikon Eclipse 80i or an

Marteyn et al.
epifluorescence microscope. The live observation of FtsZ-GFP and ZapE-mCherry during the cell division process was performed on an LB agarose (1%) pad using a 200 M Axioskop epifluorescence microscope (Zeiss) equipped with a Lambda LS 300W Xenon lamp and a CoolSnapHQ charge-coupled-device (CCD) camera.

In order to localize FtsZ-mCherry (pAKF133) in K-12 and K12::ΔzapE strains, bacteria were grown in an M9 minimum media at 37°C until an OD_{600} of 0.5 was reached and were further incubated on a minimal medium agar pad with 0.01% arabinose. The cell division process was observed using an inverted microscope (Nikon Ti) equipped with a 100X 1.4 numerical aperture (NA) PL-APo objective lens. Image stacks were acquired using Metamorph software (MDS) and a CCD camera (Photometrics CoolSnap HQ). A similar procedure was applied to observe the effect of ZapE-H₄ overexpression on FtsZ-mCherry localization, through the addition of IPTG (1 mM) within the minimal medium agar pad with 0.01% arabinose (no IPTG was added during the liquid culture growth).

Image reconstruction (see Movie S3 in the supplemental material) was performed using Imaris software (Bitplane).

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00022-14/-/DCSupplemental.

Movie S1, MOV file, 0.4 MB.
Movie S2, MOV file, 1.9 MB.
Movie S3, MOV file, 5.7 MB.
Figure S1, JPG file, 0.1 MB.
Figure S2, JPG file, 0.1 MB.
Figure S3, JPG file, 0.1 MB.
Figure S4, JPG file, 0.2 MB.
Figure S5, JPG file, 0.1 MB.
Table S1, DOCX file, 0.1 MB.
Text S1, DOCX file, 0.1 MB.

ACKNOWLEDGMENTS
We acknowledge access to the Protein Science Facility, Karolinska Institutet. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank David Weiss for supplying the various Fts protein fusions. We thank Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat for supplying the various Fts protein fusions. We thank Arne Lång, David Weiss for supplying the various Fts protein fusions. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat for supplying the various Fts protein fusions. We thank Arne Lång, David Weiss for supplying the various Fts protein fusions. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice. We thank Arndt Naujokat, Aurélie Naud, Émilie Echard for scientific discussions and advice. We gratefully thank Jeff Errington for his initial interest in this project. We thank William Margolin for biochemistry advice.
the antibiotic-resistance determinant. Gene 158:9–14. http://dx.doi.org/10.1016/0378-1119(95)00193-A.
27. Martínez E, Bartolomé B, de la Cruz F. 1988. pACYC184-derived cloning vectors containing the multiple cloning site and lacZ alpha reporter gene of pUC8/9 and pUC18/19 plasmids. Gene 68:159–162.
28. Valdivia RH, Falkow S. 1996. Bacterial genetics by flow cytometry: rapid isolation of Salmonella typhimurium acid-inducible promoters by differential fluorescence induction. Mol. Microbiol. 22:367–378. http://dx.doi.org/10.1046/j.1365-2958.1996.00120.x.
29. Sansonetti PJ, d’Hauteville H, Formal SB, Toucas M. 1982. Plasmid-mediated invasiveness of “Shigella-like” Escherichia coli. Ann. Microbiol. (Paris) 133:351–355.
30. Yu XC, Margolin W. 1997. Ca2+-mediated GTP-dependent dynamic assembly of bacterial cell division protein FtsZ into asters and polymer networks in vitro. EMBO J. 16:5455–5463. http://dx.doi.org/10.1093/emboj/16.17.5455.
31. Sliusarenko O, Heinritz J, Emonet T, Jacobs-Wagner C. 2011. High-throughput, subpixel precision analysis of bacterial morphogenesis and intracellular spatio-temporal dynamics. Mol. Microbiol. 80:612–627. http://dx.doi.org/10.1111/j.1365-2958.2011.07579.x.