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The FtsH Protein Accumulates at the Septum of *Bacillus subtilis* during Cell Division and Sporulation

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The *ftsH* gene encodes an ATP- and Zn^{2+}-dependent metalloprotease which is anchored to the cytoplasmic membrane via two transmembrane segments in such a way that the very short amino- and the long carboxy termini are exposed to the cytoplasm. Deletion of the *ftsH* gene in *Bacillus subtilis* results in a pleiotropic phenotype such as filamentous growth. This observation prompted us to ask whether *ftsH* is involved in cell division. A translational fusion was constructed between the complete coding region of *ftsH* and *gfp*$^+$ the latter carrying five point mutations to obtain enhanced fluorescence. We detected that the FtsH protein accumulates in the midcell septum of dividing cells, and during sporulation first in the asymmetrically located septa of sporulating cells and later in the membrane which engulfs the forespore. These observations revealed a new function of FtsH.

Early in the *Escherichia coli* and *Bacillus subtilis* cell cycles, a complex of several cell division proteins assembles into a ring-like structure at the future site of septation (reviewed in references 7 and 17). This septation complex is formed at a medial position and includes the conserved proteins FtsZ and FtsA (2, 6, 15, 16) as well as enzymes required to synthesize septal peptidoglycan (17, 29, 30). These proteins remain at the cell midpoint until late in the cell cycle, when a so far unknown signal triggers the onset of septal biogenesis, and a ring composed of FtsZ molecules constructs as the septum grows inward (1, 6, 19).

In *B. subtilis*, the onset of sporulation is marked by the relocalization of the division site from the midcell position to sites near both cell poles, producing two daughter cells differing in size, the smaller forespore and the larger mother cell (recently reviewed in reference 25). This alteration in the site of division is mediated by the assembly of two apparently identical complexes of cell division proteins, one near each pole of the early sporangium (15). After the bipolar assembly of cell division complexes, one is activated, synthesizing the asymmetrically positioned sporulation septum which separates the mother cell from the forespore. The second potential division complex remains inactive and is later disassembled. Then, the mother cell membrane migrates around the forespore starting near the edge of the septum, and the membranes fuse when they meet on the distal side of the forespore. This process has been designated engulfment.

The *ftsH* gene was detected in *E. coli* as a temperature-sensitive mutant forming filaments during growth at the restrictive temperature (21). This gene encodes an ATP- and Zn^{2+}-dependent metalloprotease with a molecular mass of about 70 kDa (reviewed in reference 23). The FtsH protein is anchored in the cytoplasmic membrane via two transmembrane segments where both the short N and the long C terminus with the ATP- and Zn^{2+}-binding sites are exposed to the cytoplasm. Several substrate proteins have been identified in *E. coli*, among them the regulator proteins σ^{32} and λ CII and the uncomplexed integral membrane proteins SecY and subunit α of the F_1F_0 ATPase (4, 12, 13, 26). In *B. subtilis*, the *ftsH* gene has been identified as a salt-sensitive insertion mutant (11). In contrast to *E. coli*, the *ftsH* gene of *B. subtilis* is not essential but the mutant cells exhibit a pleiotropic phenotype including filamentous growth (10). This observation prompted us to ask whether *ftsH* might be involved in cell division. To approach this question, a translational *ftsH-gfp* fusion was constructed and microscopically analyzed.

Isolation of a modified version of GFP. To improve the sensitivity of green fluorescent protein (GFP) detection, the folding mutations of GFPuv (F99S, M153T, and V163A) (9) were combined with the chromophore mutations of GFPmut1 (F64L and S65T) (8) by site-directed mutagenesis of the *gfpuv* gene of pMS5, resulting in the *gfp*$^+$ gene of pMN402 (details will be published elsewhere). The absorption and emission maxima of GFP$^+$ were determined as 491 and 512 nm, respectively, and are very similar to those of all other class 2 GFPs (28). Using wild-type GFP as a reference, the fluorescence of *E. coli* expressing GFPuv, GFPmut1, and GFP$^+$ was increased

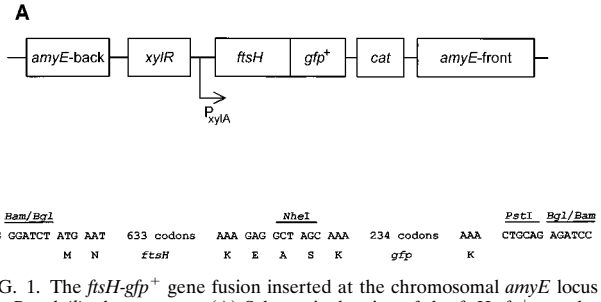

FIG. 1. The *ftsH-gfp*$^+$ gene fusion inserted at the chromosomal *amyE* locus of the *B. subtilis* chromosome. (A) Schematic drawing of the *ftsH-gfp*$^+$ translational fusion fused to the xylose-regulatable promoter P_{xylA} and sandwiched between *amyE*-front and *amyE*-back (not drawn to scale). (B) Partial DNA sequence of the *ftsH-gfp*$^+$ fusion. Indicated is the DNA sequence at the immediate beginning of *ftsH* with the first two codons, the hybrid *Bam*HI-*Bgl*II site, the last two codons of *ftsH* (K and E), two foreign codons introduced by the *Nhe*I restriction site (A and S), the first and the last codon of *gfp*, and the downstream *Pst*I and the hybrid *Bgl*II-*Bam*HI sites.

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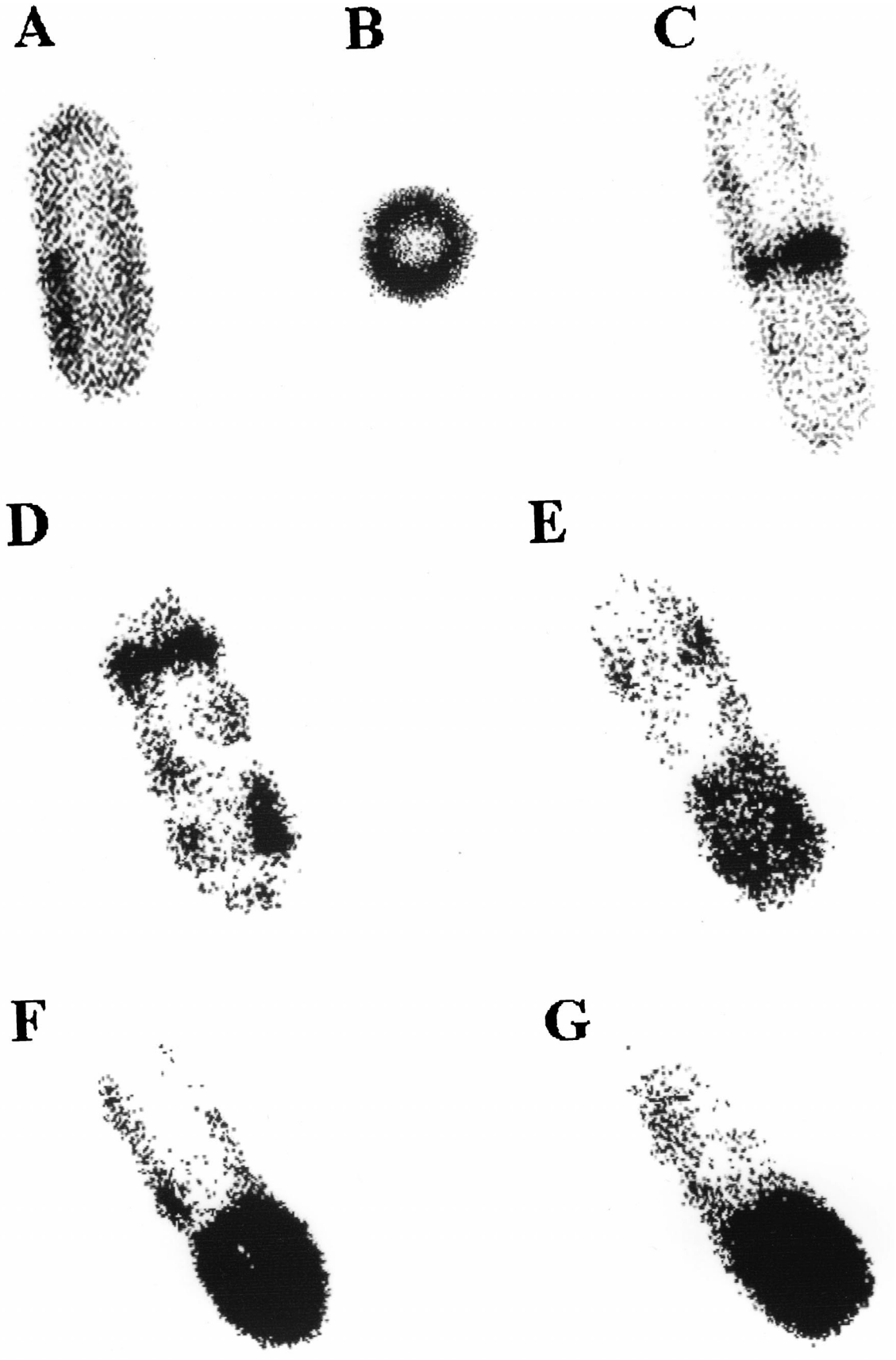

FIG. 2. FtsH-GFP$^+$ localization in live cells. Cells were grown either in Luria broth (A to C) or in DS medium (22) (D to G) in the presence of 0.1% xylose to induce *ftsH-gfp*$^+$. FtsH-GFP was visualized in live cells as follows. Cells from 1.5 ml of growth medium were sedimented, resuspended in 200 μl of Tris-HCl buffer (pH 7.4), and mixed with 500 μl of 4% agarose; 10 to 20 μl was applied to a glass slide, covered with a coverglass, and allowed to cool for ~1 min. A Leica (Heidelberg, Germany) TCS/SP confocal scanning laser microscope equipped with a argon-ion laser for excitation at 488 nm was used. Detection occurred at 510 nm, and the data from the channel were collected with fourfold averaging at a resolution of 512 by 512 pixels and processed using Corel Photo Point 7. Original magnification, ×20,000.

about 16-, 35-, and 130-fold, respectively (data not shown). This demonstrates that *gfp*$^+$ is superior to both parent genes for in vivo detection.

Construction of an *ftsH-gfp*$^+$ translational fusion. To obtain a gene fusion between *ftsH* and *gfp*, the coding region of *gfp* was fused in frame to the penultimate amino acid residue of FtsH. This hybrid gene was constructed as follows. First, the polylinker of pUC18 was replaced by a 28-bp linker carrying the three restriction sites *Bam*HI-*Eco*RV-*Bgl*II (pEH01). Then, *ftsH* was amplified using chromosomal DNA as template, flanked by *Bam*HI and *Bgl*II sites, and inserted into pEH01, resulting in pEH02. Third, the 3′-terminal 378-bp *Eco*RI-*Bgl*II fragment of *ftsH* was generated by PCR, and its stop codon was replaced by recognition sequences for *Nhe*I and *Pst*I (pWW01). Fourth, the coding region of *gfp* was PCR amplified using pMN402 as the template, flanked by *Nhe*I and *Pst*I sites, and fused in-frame to *ftsH* (pFtsH-GFP). In a last step, the hybrid gene was recovered as a 2.7-kb *Bgl*II fragment and ligated into *Bam*HI-linearized expression vector pX (14) and subsequently integrated at the *amyE* locus of strain 1012 (14). Expression of the chimeric protein is controlled by P_{xylA} and *xylR* as described previously (14); a schematic representation of the hybrid gene integrated at the *amyE* locus is presented in Fig. 1. The resulting *B. subtilis* strain WW02 carries both wild-type and *ftsH-gfp*$^+$ alleles. When the *ftsH-gfp*$^+$ gene was expressed in an *ftsH* knockout, cells did not exhibit fluorescence. This finding indicates that the fusion protein might be unstable by itself or, more likely, cannot form the oligomeric structure described (24). In all subsequent experiments, the *B. subtilis* strain WW02 was grown in the presence of 0.1% xylose to induce expression of the *ftsH-gfp*$^+$ gene.

The FtsH-GFP protein accumulates in the midcell septum of dividing *B. subtilis* cells. So far, there are no data concerning the localization of FtsH within the bacterial cell. Therefore, we asked whether FtsH is equally distributed within the *B. subtilis* cell or whether it might accumulate at specific sites, e.g., during cell division or during sporulation. To determine the location within the cell, we first analyzed bacterial cells carrying the *ftsH-gfp*$^+$ fusion growing in the exponential phase in Luria broth at 37°C. In nondividing cells, FtsH-GFP is more or less equally distributed around the cell, though there seems to be some concentration of fluorescence in one region (Fig. 2A). Microscopic examination of cells in cross section revealed a strong fluorescence in the cell envelope (Fig. 2B). Both pictures are fully in agreement with published data that FtsH is anchored within the cytoplasmic membrane (27).

In dividing cells, we detected a strong accumulation of FtsH-GFP within the midcell septum (Fig. 2C). Inspection of many hundred cells revealed that about 15% of them exhibited accumulation of FtsH-GFP within the midcell septum. These observations indicate that FtsH concentrates in the midcell most probably within the membranes growing from the outside toward the interior of the dividing cells. What might be the function of FtsH during cell division? Three possibilities, not mutually exclusive, can be envisaged. Since FtsH belongs to the group of metalloproteases, it might be involved in the degradation of one or more proteins acting as inhibitors of septal biogenesis. In addition, there are indications that FtsH might also act as a molecular chaperone (3). Therefore, FtsH might aid insertion of one or more proteins into the cytoplasmic membrane, being part of the cell division complex. A third possibility might be that FtsH acts as a quality control system involved in the integration of integral membrane proteins and degrading those which fail to insert properly. Experiments are in progress to distinguish between these possibilities and to identify the target proteins of FtsH.

The FtsH-GFP protein accumulates in asymmetrically located septa in sporulating *B. subtilis* cells. Sporulation involves a complex series of intracellular morphological events occurring in a temporal sequence and resulting in the formation of a heat-resistant endospore in *B. subtilis*. At the onset of sporulation, two polar ring-like structures can be seen, but only one is used for septation, which separates the forespore from the mother cell (15). As can be seen in Fig. 2D, there is strong fluorescence at one of these two polar ring-like structures and some labeling at the opposite pole. This picture is reminiscent of what Pogliano and coworkers have reported (18). Using time-lapse deconvolution microscopy, they showed that partial septa first appear and then disappear near the cell poles during sporulation. Later during sporulation, the fluorescence concentrates in one-third of the cell, most probably representing the forespore (Fig. 2E). In later stages, an intense fluorescence can be observed along the forespore, while that marking the second septum has disappeared (Fig. 2F and G). These sequences of pictures, not taken from one and the same cell, demonstrate, too, that FtsH accumulates in newly synthesized membranes. The mechanism by which this occurs is completely unknown. Newly synthesized FtsH might directly be channeled to the forespore. Alternatively, FtsH present in the forespore might be protected from degradation by deposition of the cortex, and those present in the mother cell might be degraded. Again, it can be assumed that the FtsH protein is involved in the degradation of one or more target proteins, in chaperoning integral membrane protein(s), or in both, thereby acting as a protein quality control system. It was recently published that the integral membrane protein SpoIVFA is substantially stabilized in the absence of FtsH, indicating that the metalloprotease might be involved in the degradation of this protein (20).

Conclusions. The principal contribution of this study is the finding that FtsH accumulates in the midcell septum during vegetative cell division and at the onset of sporulation at positions near the cell poles that appear to coincide with future division sites. Then, FtsH becomes concentrated at the sporulation septum and disappears from the distal pole. This behavior is reminiscent of SpoIIE, which also first localizes near the two poles and later becomes concentrated at the sporulation septum (5). Later during the sporulation process, most of FtsH is found within the membrane engulfing the forespore. The function of FtsH during these two processes is not known but might involve its proven protease activity and/or its postulated chaperone function. What signal triggers accumulation of FtsH at these sites? Is only newly synthesized FtsH preferentially inserted at these sites, or does preformed FtsH migrate into these sites? In both cases, what causes either preferential insertion or migration of FtsH to its new location? These are some of the questions that remain to be answered.

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REFERENCES

1. **Addinall, S. G., E. Bi, and J. Lutkenhaus.** 1996. FtsZ ring formation in *fts* mutants. J. Bacteriol. **78:**3877–3884.
2. **Addinall, S. G., and J. Lutkenhaus.** 1996. FtsZ is localized to theseptum in an FtsZ-dependent manner. J. Bacteriol. **178:**7167–7172.
3. **Akiyama, Y., M. Ehrmann, A. Kihara, and K. Ito.** 1998. Polypeptide binding of *Escherichia coli* FtsH (HflB). Mol. Microbiol. **28:**803–812.
4. **Akiyama, Y., A. Kihara, and K. Ito.** 1996. Subunit a of proton ATPase F_0 sector is a substrate of the FtsH protease in *Escherichia coli*. FEBS Lett. **399:** 26–28.
5. **Arigoni, F., K. Pogliano, C. D. Webb, P. Stragier, and R. Losick.** 1995.

Localization of protein implicated in establishment of cell type to sites of asymmetric division. Science **270:**637–640.
6. **Bi, E., and J. Lutkenhaus.** 1991. FtsZ ring structure associated with division in *Escherichia coli*. Nature **354:**161–164.
7. **Bouché, J.-P., and E. Pick.** 1998. On the birth and fate of bacterial division sites. Mol. Microbiol. **29:**19–26.
8. **Cormack, B. P., R. H. Valdivia, and S. Falkow.** 1996. FACS-optimized mutants of the green fluorescent protein (GFP). Gene **173:**33–38.
9. **Crameri, A., E. A. Whitehorn, E. Tate, and W. P. C. Stemmer.** 1996. Improved green fluorescent protein by molecular evolution using DNA shuffling. Nat. Biotechnol. **14:**315–319.
10. **Deuerling, E., A. Mogk, C. Richter, M. Purucker, and W. Schumann.** 1997. The *ftsH* gene of *Bacillus subtilis* is involved in major cellular processes such as sporulation, stress adaptation and secretion. Mol. Microbiol. **23:**921–933.
11. **Geisler, U., and W. Schumann.** 1993. Isolation of stress mutants of *Bacillus subtilis* by a novel genetic method. FEMS Microbiol. Lett. **108:**251–254.
12. **Kihara, A., Y. Akiyama, and K. Ito.** 1995. FtsH is required for proteolytic elimination of uncomplexed forms of SecY, an essential protein translocase subunit. Proc. Natl. Acad. Sci. USA **92:**4532–4536.
13. **Kihara, A., Y. Akiyama, and K. Ito.** 1997. Host regulation of lysogenic decision in bacteriophage lambda: transmembrane modulation of FtsH (HflB), the cII degrading protease, by HflKC (HflA). Proc. Natl. Acad. Sci. USA **94:**5544–5549.
14. **Kim, L., A. Mogk, and W. Schumann.** 1996. A xylose-inducible *Bacillus subtilis* integration vector and its application. Gene **181:**71–76.
15. **Levin, P. A., and R. Losick.** 1996. Transcription factor Spo0A switches the localization of the cell division protein FtsZ from a medial to a bipolar pattern in *Bacillus subtilis*. Genes Dev. **10:**478–488.
16. **Ma, X. L., D. W. Ehrhardt, and W. Margolin.** 1996. Colocalization of cell division proteins FtsZ and FtsA to cytoskeletal structures in living *Escherichia coli* cells by using green fluorescent protein. Proc. Natl. Acad. Sci. USA **93:**12998–13003.
17. **Nanninga, N.** 1998. Morphogenesis of *Escherichia coli*. Microbiol. Mol. Biol. Rev. **62:**110–129.
18. **Pogliano, J., N. Orborne, M. Sharp, A. Abanes-De Mello, A. Perez, Y.-L. Sun, and K. Pogliano.** 1999. A vital stain for studying membrane dynamics in bacteria: a novel mechanism controlling septation during *Bacillus subtilis* sporulation. Mol. Microbiol. **31:**1149–1159.
19. **Pogliano, J., K. Pogliano, D. S. Weiss, R. Losick, and J. Beckwith.** 1997. Inactivation of FtsI inhibits constriction of the FtsZ cytokinetic ring and delays the assembly of FtsZ rings at potential division sites. Proc. Natl. Acad. Sci. USA **94:**559–564.
20. **Resnekov, O.** 1999. Role of the sporulation protein BofA, in regulating activation of the *Bacillus subtilis* developmental transcription factor σ^K. J. Bacteriol. **181:**5384–5388.
21. **Santos, D., and D. F. Almeida.** 1975. Isolation and characterization of a new temperature-sensitive cell division mutant of *Escherichia coli* K-12. J. Bacteriol. **124:**1502–1507.
22. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolic repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54:**704–711.
23. **Schumann, W.** 1999. FtsH—a single-chain charonin? FEMS Microbiol. Rev. **23:**1–11.
24. **Shotland, Y., S. Koby, D. Teff, N. Mansur, D. A. Oren, K. Tatematsu, T. Tomoyasu, M. Kessel, B. Bukau, T. Ogura, and A. B. Oppenheim.** 1997. Proteolysis of the phage lambda CII regulatory protein by FtsH (HflB) of *Escherichia coli*. Mol. Microbiol. **24:**1303–1310.
25. **Stragier, P., and R. Losick.** 1996. Molecular genetics of sporulation in *Bacillus subtilis*. Annu. Rev. Genet. **30:**297–341.
26. **Tomoyasu, T., J. Gamer, B. Bukau, M. Kanemori, H. Mori, A. J. Rutman, A. B. Oppenheim, T. Yura, K. Yamanaka, H. Niki, S. Hiraga, and T. Ogura.** 1995. *Escherichia coli* FtsH is a membrane-bound, ATP-dependent protease which degrades the heat-shock transcription factor σ^{32}. EMBO J. **14:**2551–2560.
27. **Tomoyasu, T., K. Yamanaka, K. Murata, T. Suzaki, P. Bouloc, A. Kato, H. Niki, S. Hiraga, and T. Ogura.** 1993. Topology and subcellular localization of FtsH protein in *Escherichia coli*. J. Bacteriol. **175:**1352–1357.
28. **Tsien, R. Y.** 1998. The green fluorescent protein. Annu. Rev. Biochem. **67:**509–544.
29. **Wang, X., and J. Lutkenhaus.** 1996. FtsZ-ring: the eubacterial division apparatus conserved in archaebacteria. Mol. Microbiol. **21:**313–319.
30. **Weiss, D. S., K. Pogliano, M. Carson, L.-M. Guzman, C. Fraipont, M. Nguyen-Distèche, R. Losick, and J. Beckwith.** 1997. Localization of the *Escherichia coli* cell division protein FtsI (PBP3) to the division site and cell pole. Mol. Microbiol. **25:**671–681.