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A Mutation in the *ftsK* Gene of *Escherichia coli* Affects Cell-Cell Separation, Stationary-Phase Survival, Stress Adaptation, and Expression of the Gene Encoding the Stress Protein UspA

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An insertional mutation in *ftsK*, encoding an *Escherichia coli* product similar to the sporulation protein SpoIIIE of *Bacillus subtilis*, results in *uspA* overexpression in stationary phase and impairs cell division. The *ftsK1*::*cat* insertion mutant forms chains which are the result of inhibited cell-cell separation, while chromosome synthesis and partitioning appear to be normal as judged by flow cytometry and electron and light microscopy in combination with DNA staining. The cells of the chains are attached to each other by a small envelope structure, and unlike in a *spoIIIE* mutant of *B. subtilis*, there is no DNA trapped in the division plane. In addition, plasmids harboring a truncated *ftsK* allele lacking the last 195 bp of the gene cause chain formation in wild-type cells. While the mutant cells grow at essentially the same rate as the parent in complex and defined minimal media, they are sensitive to stresses. Specifically, the mutant failed to grow at elevated salt concentrations and survived stationary phase poorly. The phenotypes of the *ftsK1*::*cat* mutant are complemented by the 3′ end (*spoIIIE*-like half) of the *ftsK* locus. In contrast, the 5′ end of the *ftsK* locus reported to complement *ftsK44*(Ts) phenotypes does not complement the phenotypes of the *ftsK1*::*cat* mutant.

Recent work in several laboratories has identified networks and individual genes expressed early in stationary phase that are important for the longevity of starving *Escherichia coli* populations (10, 12, 13, 15, 17, 18, 21–27, 29, 31, 33). One such gene, *uspA*, which is required for survival during growth arrest, is unique in its almost universal responsiveness to diverse stresses (25–27). Production of UspA is induced during growth inhibition caused by exhaustion of any of a variety of nutrients or by the addition of various toxic agents (25–27). UspA production is related to growth phase rather than growth rate, and transcription of *uspA* is rapidly shut off during nutritional upshifts (25). Differential expression of the gene is the result of altered transcription initiation from a promoter with the characteristics of a σ^{70}-dependent promoter (25, 27). The gene is part of the FadR regulon, suggesting that UspA may have a role in fatty acid/membrane lipid metabolism (10), and *uspA* insertion mutants excrete large amounts of acetate during growth on catabolite-repressing carbon sources, such as glucose and gluconate (26). In addition, *uspA* mutants fail to grow on poorly buffered media, while overproduction of the UspA protein locks the cells in a growth-arrested state (28). However, the exact physiological and molecular assignments of UspA have not been determined.

Here we demonstrate that an insertional mutation in the *ftsK* gene, encoding a product similar to SpoIIIE of *Bacillus subtilis*, significantly affects *uspA* promoter activity in stationary phase and that this new *ftsK* mutation impairs cell-cell separation, the survival of growth-arrested cells, and salt stress adaptation.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains used in this work are W3110 [IN(*rrnD-rrnE*)], MC4100 (F$^-$ *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*), TN4100 (MC4100 *uspA*::*lacZ-kan*), AF634 (MC4100 λ*uspA-lacZ*), AD10 (TN4100 *ftsK1*::*cat*), AD11 (W3110 *ftsK1*::*cat*), and AD12 (MC4100 *ftsK1*::*cat*). Cultures were grown aerobically in liquid Luria broth (LB) or M9 as described previously (30). When required, the media were supplemented with glucose (0.4%), thiamine (10 mM), kanamycin (50 μg/ml), carbenicillin (50 μg/ml), tetracycline (20 μg/ml), chloramphenicol (30 μg/ml), spectinomycin (100 μg/ml), and/or streptomycin (200 μg/ml).

Transposon mutagenesis. Mutations in the *E. coli* chromosome were generated with the Tn*10*d-*cat* transposon as described previously (5, 9). The generated Tn*10*d-*cat* mutations were subsequently introduced into the *uspA-lacZ* reporter strain (TN4100) by using general transduction mediated by bacteriophage P1. Cells were plated on LB, M9, or MacConkey plates containing chloramphenicol, kanamycin, and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (LB and M9) to select for transductants and screen for altered expression of *lacZ*.

Mapping the insertion mutation by Hfr matings. The insertion mutation of AD10 was approximately mapped by using the Hfr strains described by Wanner (35).

Cloning of the transposon insertion. The chromosome of the transposon mutant was digested with various restriction enzymes (*Xho*I, *Kpn*I, *Pst*I, and *Hin*dIII) that do not cut in Tn*10*d-*cat*. The digested chromosomal DNA was then ligated to pBluescript SK (Promega). DNA of interest was purified, and primers matching the ends of the polylinker were used to sequence into the insert. The sequences obtained were used to search databases.

Cloning the gene complementing mutant AD10 phenotypes. Sequence data obtained with the cloned mutation showed that the gene being disrupted by the Tn*10*d-*cat* insertion is located on the overlapping regions of the miniset Kohara clones 214 and 215. DNA from clone 215 (E6H3) was subcloned into the high-copy-number plasmids pUC18 and pUC19 and the low-copy-number plasmid pGB2 (6). The resulting plasmids' abilities to complement the mutant phenotypes were determined.

Microscopic examination and DNA staining. Cells were fixed in formaldehyde (2%), prepared for 4′,6-diamidino-2-phenylindole (DAPI) staining, and analyzed microscopically as described by Møller et al. (20). Nucleoids in cells were visualized by combined phase-contrast and fluorescence microscopy, and the images were digitized with a charge-coupled device camera connected to a computerized image analysis system as described previously (20).

Flow cytometry. Cells were fixed in 70% ethanol–10 mM Tris (pH 7.4) and stored at 4°C until staining. All samples were stained just before being applied to the flow cytometer. Stored cells were centrifuged and resuspended in 10 mM Tris (pH 7.4) containing 10 mM $MgCl_2$. DNA staining was performed by mixing equal parts of the resuspended cells with plicamycin (200 μg/ml) and ethidium bromide (40 μg/ml) in the same buffer.

Transmission electron microscopy. *E. coli* cells were pelleted by centrifugation, fixed (2.5% glutaraldehyde and 0.02% sodium azide in 0.05 M sodium cacodylate) for 1 h, and postfixed with 0.5% OsO_4 for 30 min. Specimens were then dehydrated and infiltrated with plastic resin (Agar 100). After specimens

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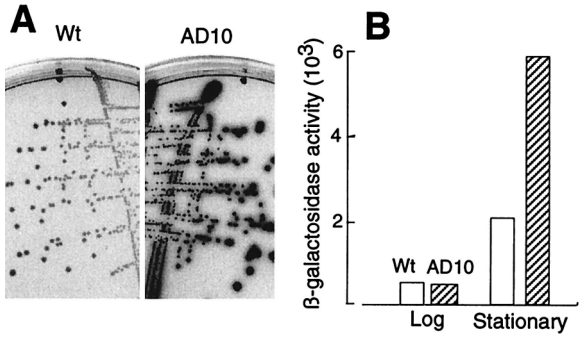

FIG. 1. Activity of the *uspA* promoter in the wild type (wt) and the AD10 mutant strain. (A) Cells were plated on LB–X-Gal plates with antibiotics as described in Materials and Methods. (B) β-Galactosidase levels determined for strain AF634 (λ*uspA-lacZ*) and the isogenic *usoM10* mutant strain. Cells were grown in LB, and β-galactosidase activity was determined during exponential growth and stationary phases (12 h after growth of the cells ceased).

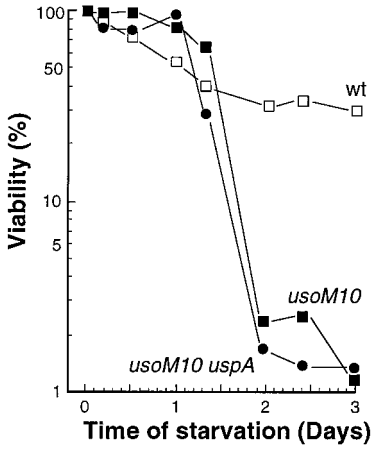

FIG. 2. Stationary-phase survival of wild-type (MC4100) (wt), *usoM10* mutant, and *usoM10 uspA*::*kan* cells. Strains were grown aerobically in LB medium at 37°C. After growth ceased, incubation was monitored for 7 days under the same conditions. Viable cells were counted as colonies plated on LB after appropriate dilutions. One hundred percent viability corresponds to the number of viable cells counted 5 h after growth (optical density) was arrested. At this time there was no further increase in cell number due to reductive division.

were cured, ultrathin sections (≈70Å) were cut with a Reichert Ultracut E ultramicrotome fitted with a diamond knife. Sections were contrasted with uranyl acetate and lead citrate before examination in a Zeiss CEM 902 transmission electron microscope.

General methods. Plasmid and λ phage DNA was purified with Qiagen columns according to the protocol provided by the manufacturer. P1 transductions and plasmid transformations were performed as described previously (19, 30). DNA sequencing was carried out with Sequenase (United States Biochemicals). In vitro transcription-translation analysis of cloned DNA was performed by using the Promega *E. coli* S30 coupled transcription-translation system according to the protocol provided by the manufacturer. β-Galactosidase levels were measured as described by Miller (19), with modifications (1). All measurements were repeated at least three times to confirm reproducibility. Typical results are depicted in the figures.

RESULTS

Isolation of mutants with altered *uspA* transcription levels. A strain (TN4100) in which the *uspA* promoter region is fused to the promoterless *lacZ* gene was used as the recipient for transducing insertion mutations from our Tn*10*d-*cat* mutant library constructed as described previously (9, 14). We screened for mutations that resulted in higher expression from the *uspA* promoter, and one mutant (AD10) was further characterized. This mutant appeared dark blue on LB–X-Gal plates (Fig. 1A) and dark red on MacConkey lactose plates (data not shown), while the isogenic parent is light blue (Fig. 1A) and pink on these plates (data not shown). The mutation (*usoM10* [*uspA* overexpression mutation]) was transduced to a wild-type *lacZ*$^+$ strain (W3110) to confirm that the mutation specifically affected P_{uspA}. Indeed, the P_{lac} promoter, or any component of the reporter system, such as β-galactosidase stability, was not affected by the mutation (data not shown). Because the AD10 strain carries the *uspA* mutation, we transduced the *usoM10* mutation to strain AF634 (UspA$^+$ λφ[P_{uspA}-*lacZ*]) to determine whether the effect of *usoM10* required the lack of UspA. This was not the case; the *usoM10* mutation resulted in overexpression from the *uspA* promoter regardless of whether the cells were UspA$^-$ or UspA$^+$.

The *usoM10* mutation increases P_{uspA} activity in stationary phase. When β-galactosidase activity was measured during growth of TN4100 and AF634 and the isogenic *usoM10* mutant strains in LB batch medium, we found that the *usoM10* mutation specifically affected growth phase-dependent expression from the *uspA* promoter. The AD10 mutant exhibited significantly higher levels of β-galactosidase in stationary phase than the isogenic parent, while the levels of β-galactosidase were indistinguishable during exponential growth (Fig. 1B).

The *usoM10* mutation results in salt sensitivity. We tested the growth of the AD10 mutant on a variety of different media to score for growth defects. The mutant was found to grow at the same rate as the parent on complex medium (LB) but somewhat slower on minimal media with single carbon sources. The mutant was severely impaired in its ability to grow on minimal media supplemented with 0.3 M NaCl. While the wild-type parent formed colonies after 2 days on 0.3 M NaCl–M9–glucose plates, the AD10 strain failed to form colonies even after incubation for a week. The same effects of NaCl were observed when tested in suspensions (data not shown).

The *usoM10* mutation impairs stationary-phase survival. Old colonies or cultures of the AD10 strain lagged for extended periods of time when inoculated into fresh medium. Therefore, we examined whether this could be explained by a poor ability of the *usoM10* cells to survive stationary phase. Indeed, the *usoM10* mutant lost viability at an elevated rate after the first few days of starvation in an *uspA*-independent manner (Fig. 2).

The *usoM10* mutation blocks cell-cell separation. The yield of AD10 (measured in CFU per milliliter) was always found to be lower (about fourfold) than that of the isogenic parent at the end of the growth phase. This phenomenon appeared to be due to the failure of AD10 cells to separate. Microscopic examinations revealed that the *usoM10* mutants were blocked at a late stage in cell division; the cells formed chains that appeared to be fully septated (Fig. 3). Chromosome partitioning appeared to be normal, and we could not detect DNA trapped in the division plane (Fig. 3). Electron microscopy demonstrated that the cells of the chains did not appear to share a cytoplasm and were attached to each other by a small surface structure (Fig. 4). Treatment with 0.1% sodium dodecyl sulfate did not break up the chains (data not shown), but incubation for extended periods in stationary phase appeared to cause cell separation of most chains.

Flow cytometer analysis demonstrated that the wild-type cells contained either one or two chromosomes when cultured in minimal medium (Fig. 5), while AD10 cells contained four chromosomes or more (Fig. 5). The data is explained by the fact that chains of four cell equivalents were the most abundant

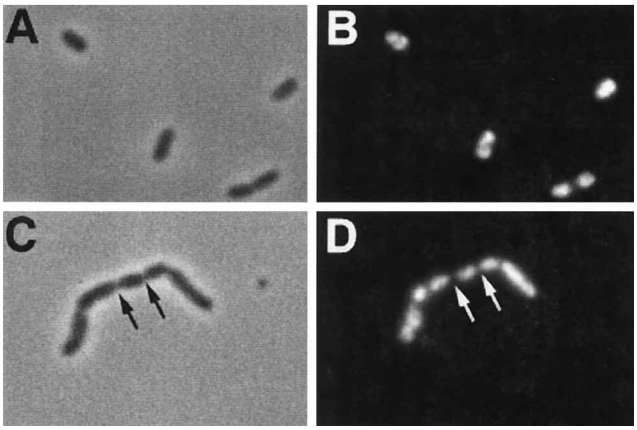

FIG. 3. Morphology of AD10 mutant cells. (A and C) Phase-contrast images of wild-type (A) and AD10 mutant (C) cells glucose starved for 5 h; (B and D) fluorescence micrographs of the same cells stained with DAPI. Arrows point at the constrictions between cells.

in the AD10 culture. However, the DNA contents per unit of biomass (optical density at 410 nm) and the individual cell volumes were very similar in the parent and AD10 mutant, indicating that the timing and processivity of DNA replication are normal in the mutant and that the mutation specifically affects a late stage in the division cycle.

The *usoM10* mutation maps at 20 min in the *ftsK* gene. The mutation was roughly mapped to 20 min on the chromosome by using the Hfr mapping strains (35) by scoring for acquisition of tetracycline resistance with the simultaneous loss of β-galactosidase overproduction and chloramphenicol resistance. Subsequently, the *usoM10* mutation was cloned, and the DNA flanking the mutation was sequenced. A BLAST search demonstrated that the mutation maps within the *ftsK* gene just upstream of the sequence showing significant homology to the *B. subtilis spoIIIE* gene (51.4% identity to SpoIIIE over the C-terminal 413 amino acids), and from here on we will call the *usoM10* mutation *ftsK1*::*cat*. The SpoIIIE-like region is part of a 1,329-amino-acid protein, FtsK, whose N-terminal region is involved in a late stage of cell division (4). We subsequently subcloned DNA from Kohara clone 215, which harbors DNA overlapping the *ftsK* region, for complementation analysis. The results from this analysis, summarized in Fig. 6A, demonstrated that DNA spanning the 3′-terminal half (from *Pst*I and downstream) (Fig. 6A) of the *ftsK* locus was sufficient to complement *uspA* overexpression (Fig. 6B), chain formation, and salt sensitivity (Fig. 7) in AD10. This DNA complemented regardless of whether it was carried on the high-copy-number plasmids (pAD14 and pAD12 are pUC19 derivatives) (Fig. 6A) or a low-copy-number plasmid (pGB2) (data not shown). However, DNA cloned in pAD15, which is a pGB2 derivative (Fig. 6A), and reported to complement the *ftsK44*(Ts) phenotype (filamentation at high temperatures and low salt concentrations [4]) did not complement chain formation or *uspA* overexpression, nor did DNA encoding the downstream *lplA* gene (Fig. 6A). Thus, only DNA harboring the *spoIIIE*-homologous sequence complemented the phenotypes of AD10. A complementing plasmid, pAD8, harboring the 3′ end of the *ftsK* locus was subjected to in vitro transcription-translation analysis, and we found that this region produced a polypeptide migrating between the 41.5- and 67-kDa markers (Fig. 8). While this data indicates the presence of two gene products expressed from the *ftsK* locus, this polypeptide may result from an aberrant translational start site within either the plasmid or the *ftsK* locus.

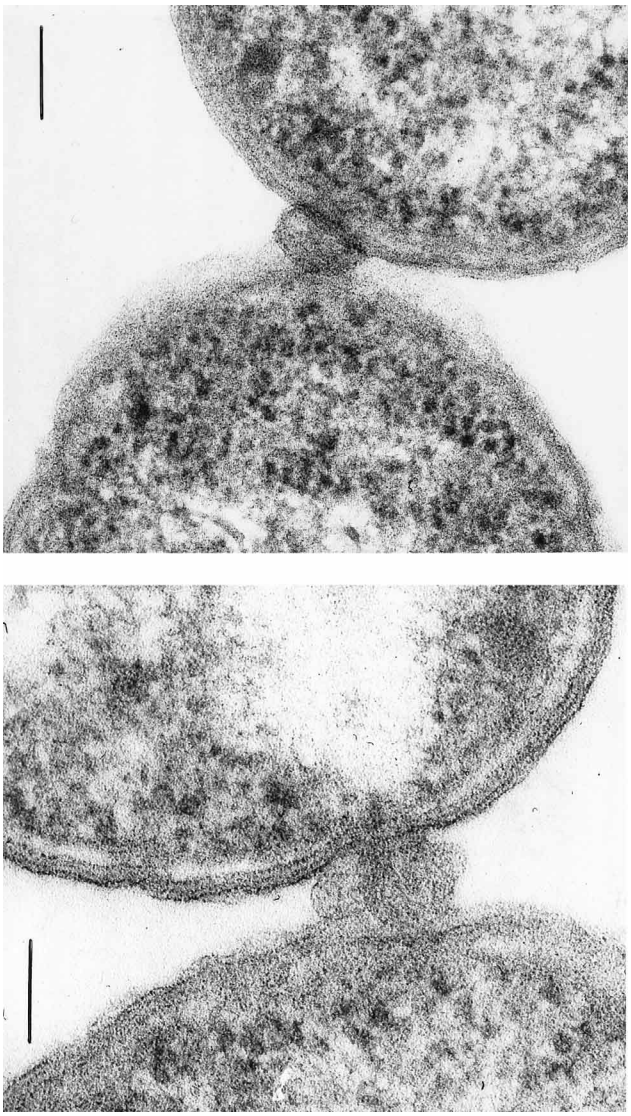

FIG. 4. Transmission electron microscopy images of the structure holding the AD10 mutant cells together. Bars, 0.1 μm.

The low-copy-number plasmid pAD15 harboring a truncated *ftsK* allele (this plasmid encodes a truncated product, FtsK*, lacking the last 65 amino acids of the predicted FtsK sequence) (Fig. 6A) did not complement but rather accentuated the *ftsK1* phenotypes. Specifically, both the wild-type strain and the *uspA* mutant formed long chains when transformed with pAD15 (Fig. 9). Chain formation of wild-type, *ftsK1*, and *uspA* strains carrying pAD15 could be abolished by transforming these cells with a high-copy-number plasmid (pAD12) (Fig. 6) carrying the 3′ end of the *ftsK* locus (data not shown).

DISCUSSION

Division of growing *E. coli* and *B. subtilis* cells involves similar rapid separation, decatenation, and segregation of daughter chromosomes followed by equatorial septum formation. A special form of division is seen during starvation of *Bacillus*

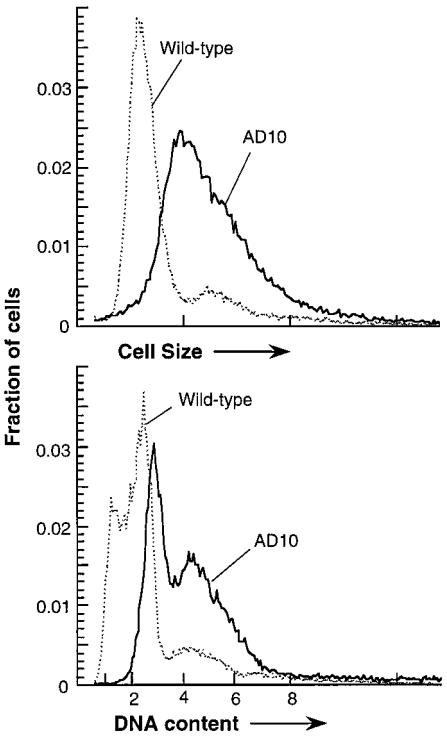

FIG. 5. Flow cytometry profiles of wild-type and AD10 cultures. Cells were cultured in glucose (0.05%)-limited M9 medium at 37°C. The relative cell sizes and the numbers of chromosome equivalents are depicted.

cells, a modified, asymmetric division which initiates sporulation. The processes of asymmetric division and vegetative division are related and required the same products (2, 3, 16). However, it has been shown recently that DNA partitioning during asymmetric division requires at least one unique product, SpoIIIE (36). Point mutations in the carboxy-terminal domain of *B. subtilis* SpoIIIE specifically block prespore chromosome partitioning (37), and the chromosome becomes trapped in the division plane. Thus, the sporulating cells fail to exit stage II of sporulation. The *spoIIIE*-like gene of *E. coli*, *ftsK*, has previously been shown to be involved in cell division, because a conditional mutation in the 5′ end of the gene forms filaments at nonpermissive temperatures (4). However, the *ftsK* locus of *E. coli* is predicted to encode a much larger polypeptide (1,329 amino acids) than the *spoIIIE* gene (787 amino acids). Like SpoIIIE, the N-terminal region of FtsK is predicted to contain several membrane-spanning regions, but the amino acid sequences in these regions are very different (4). The C-terminal half of FtsK shows great similarity at the amino acid level to SpoIIIE.

Unlike in a *spoIIIE* mutant of *B. subtilis*, there is no DNA trapped in the division plane of dividing *E. coli ftsK1*::*cat* cells. Rather, the *ftsK1*::*cat* insertional mutation of *E. coli* blocks cell-cell separation after completion of chromosome segregation, and the mutant forms chains. The cells of these chains appear to be attached to each other by a small envelope structure. Is FtsK involved in cleaving the peptidoglycan to form the new poles of the daughter cells? The FtsK protein was suggested to be required for a late stage of septum formation and involved in peptidoglycan synthesis because *ftsK44*(Ts) phenotypes are specifically suppressed by deletion of *dacA*, encoding the peptidoglycan D-alanine:D-alanine carboxypeptidase, PBP5

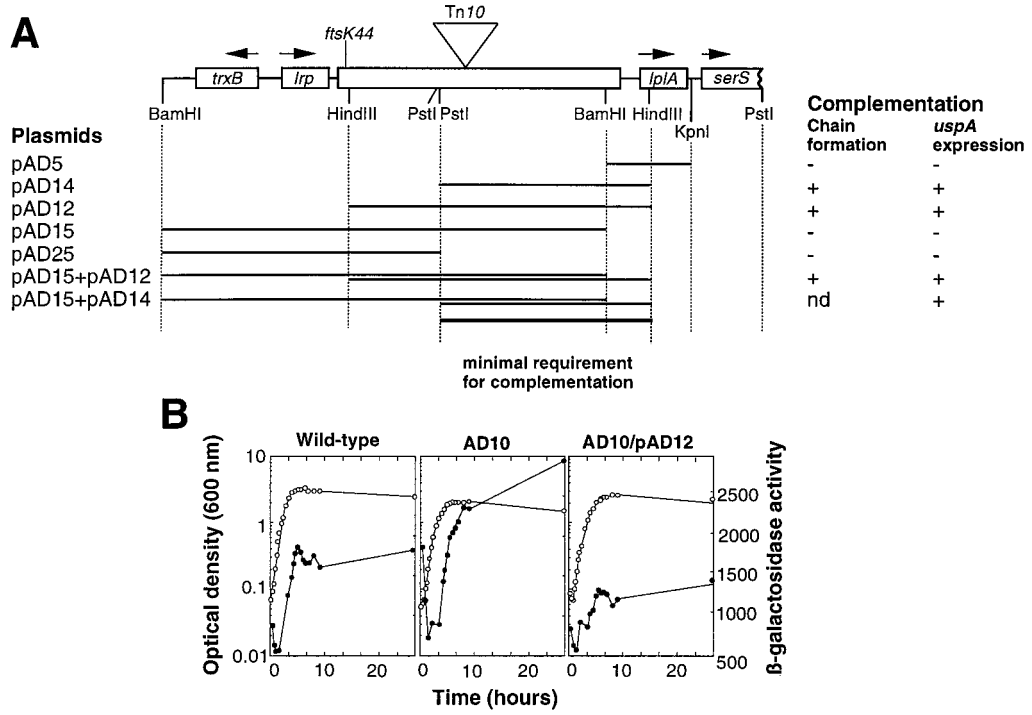

FIG. 6. Complementation analysis of the *usoM10* (*ftsK1*) mutation and effects of different DNA regions on *uspA* promoter activity and chain formation in the *usoM10* mutant. (A) Schematic summary of complementation analysis. Selected restriction sites are shown. Arrows show the direction of transcription, and the location of the *usoM10*::Tn*10* insertion is indicated. The location of the *ftsK44* mutation described by Begg et al. (4) is also shown. −, no complementation; +, complementation; nd, not determined. (B) Growth (open circles) and β-galactosidase levels (closed circles) of the wild-type/pGB2, *ftsK1*/pGB2, and *ftsK1*/pAD12 strains during growth in LB medium.

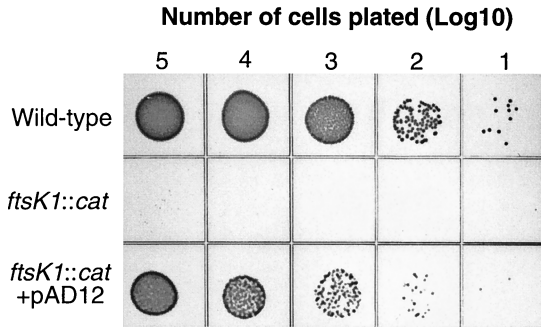

FIG. 7. Salt sensitivity of the *ftsK1* mutant and complementation with the pAD12 plasmid carrying the 3′-proximal part of the *ftsK* locus (Fig. 6A). Different dilutions of cells were plated on M9-glucose plates containing 0.3 M NaCl.

(4). A putative involvement of FtsK and other SpoIIIE-like proteins in peptidoglycan hydrolysis is not necessarily at odds with the observation that the SpoIIIE protein of *B. subtilis* appears to have a specific assignment in a conjugation-like chromosome transfer mechanism of the sporulating cell and that SpoIIIE shows significant similarity with Tra proteins required for intercellular DNA transfer (36, 37). It has been shown recently (8) that several proteins of cross-envelope Tra systems exhibit significant homology with peptidoglycan hydrolases. Thus, the SpoIIIE-like protein of *E. coli* could have specific assignments in peptidoglycan hydrolysis during vegetative cell-cell separation, while the *B. subtilis* version has evolved to specifically accommodate assembly of the chromosomal transfer system of the sporulating cell by localized peptidoglycan rearrangements.

Alternatively, FtsK may be involved in membrane lipid and/or lipoprotein metabolism of importance in the process of cell separation. The phenotype corresponding to the *ftsK1*::*cat* mutation is somewhat similar to the *envA* phenotype with respect to chain formation (38). The *envA* mutation of *E. coli* results in a defect in the synthesis of the acylated lipid A portion of the outer membrane lipopolysaccharide (38). In addition, a mutant of *Salmonella typhimurium* (*lkyD*) affected in lipoprotein biosynthesis and defective in outer membrane

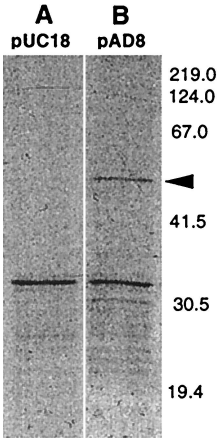

FIG. 8. In vitro transcription-translation analysis of purified DNA (pAD8) containing the 3′-proximal part of the *ftsK* locus carried on pUC18 (*Pst*I to *Kpn*I; [Fig. 6A]). Products of the control plasmid (pUC18) are depicted next to pAD8. The *lac* promoter of the pUC18 plasmid is located 3′ of the inserted *ftsK* sequence. Plasmid pAD8 also carries the *lplA* gene. However, the product of this gene was visible only after overexposure of the autoradiogram. The arrowhead shows the product of the cloned fragment in pAD8.

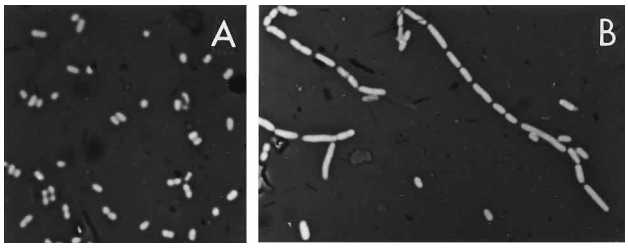

FIG. 9. Effect of the truncated *ftsK** allele carried on pAD15 on chain formation. Shown are phase-contrast images of nigrosin negative-stained TN4100 cells carrying the pGB2 vector plasmid (A) or pAD15 (B).

invagination has been isolated, and the mutant forms chains of unseparated cells held together by cell envelope bridges (11). In this context, it is interesting that the deduced amino acid sequence of the *ftsK* locus contains an unusual stretch of proline- and glutamine-rich repeats which are similar, but not identical, to the proline- and glutamine-rich repeats of the *E. coli* lipoprotein NlpD (12). Similar to a mutation in *ftsK* (this paper), insertional mutagenesis of *nlpD* results in decreased stationary-phase survival (12).

Cells carrying the truncated *ftsK* allele (*ftsK**) on the low-copy-number plasmid pAD15 show extensive chain formation even in a wild-type background, indicating that FtsK* can inhibit the process of vegetative cell separation. Possibly, the FtsK* product, which we assume is nonfunctional (at least the *ftsK** allele does not complement the *ftsK1*::*cat* mutant phenotypes), forms an inactive complex with the wild-type FtsK or other proteins with assignments in the separation process.

Besides affecting the process of cell-cell separation, the *ftsK1*::*cat* mutation also impairs stationary-phase survival and salt stress adaptation and causes an increased activity of the *uspA* promoter in stationary phase. Interestingly, the phenotypes of the *E. coli ftsK1*::*cat* mutant are very similar to those of a *B. subtilis ftsH* mutant. Specifically, the *B. subtilis ftsH*::*cat* mutant, besides being impaired in cell division, dies rapidly in stationary phase and grows poorly at high concentrations of NaCl (7). Most of the *B. subtilis ftsH* phenotypes were explained by the failure of the mutant to express appropriate amounts of Spo0A, and *ftsH* was suggested to be a developmental checkpoint (7). The *E. coli ftsH* mutation up-regulates the heat shock stress response because σ^{32} is not readily degraded at elevated temperatures in the *ftsH* mutant (32). Similarly, the *ftsK* mutation appears to up-regulate the expression of the *E. coli* universal stress response, which is normally induced by stresses causing growth arrest (25). We have reported that expression of *uspA* is, in part, dependent on the status of the *fadR* gene and that FadR binds to two regions downstream of the promoter in the noncoding *uspA* sequence (10). As demonstrated here, the activity of the *uspA* promoter is also affected by the status of the *ftsK* gene but only at times when the *uspA* gene is normally induced (derepressed). The basal levels of *uspA* expression during balanced growth were not affected. The effect of *fadR* mutations on *uspA* expression suggests that UspA may have a role in fatty acid and membrane lipid metabolism; all genes so far demonstrated to be regulated by FadR have assignments in fatty acid uptake, activation, or metabolism. In addition, it appears that FadR is important for normal cell-cell separation because *fadR* mutants often fail to separate properly (34). The putative role of UspA in cell separation may be linked to FadR-dependent stationary-phase-induced alterations in protein lipidation and the membrane structure supporting its role as the scaffold for

specific peptidoglycan-synthesizing or -modulating enzymes. Future work concerning *uspA* will be directed towards elucidating the signals and sensors responsible for *uspA* up-regulation in the *ftsK1*::*cat* mutant and investigating whether the phenotypes of this mutant are due to altered membrane composition and structure.

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