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A New *Escherichia coli* Cell Division Gene, *ftsK*

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A mutation in a newly discovered *Escherichia coli* cell division gene, *ftsK*, causes a temperature-sensitive late-stage block in division but does not affect chromosome replication or segregation. This defect is specifically suppressed by deletion of *dacA*, coding for the peptidoglycan DD-carboxypeptidase, PBP 5. FtsK is a large polypeptide (147 kDa) consisting of an N-terminal domain with several predicted membrane-spanning regions, a proline-glutamine-rich domain, and a C-terminal domain with a nucleotide-binding consensus sequence. FtsK has extensive sequence identity with a family of proteins from a wide variety of prokaryotes and plasmids. The plasmid proteins are required for intercellular DNA transfer, and one of the bacterial proteins (the SpoIIIE protein of *Bacillus subtilis*) has also been implicated in intracellular chromosomal DNA transfer.

Cell division is a much-studied process in *Escherichia coli*, and a number of division genes and proteins are now well known (17). The cell division-specific proteins include FtsZ, which forms a ring around the inside of the cell membrane during division (9, 11, 16, 39, 40, 45), and a set of membrane-associated proteins (the products of *ftsL*, *-I*, *-W*, *-Q*, *-A*, and possibly others) that include the septum-specific peptidoglycan transpeptidase and transglycosylase, PBP 3 (the *ftsI* gene product). The action of these proteins results in the formation of a covalently cross-linked double-layered septum across the cell center (43). Splitting of the bonds between the two completed layers (accompanied by invagination of the outer membrane) completes the process of cell division (3, 63).

A mutant, TOE44, described for the first time in this paper, was originally isolated in a search for conditional mutants that are blocked in cell division but in which the temperature-sensitive period is only a short fraction of the cell cycle (6). The same search produced the first mutations in *ftsQ*, as well as new *ftsI* and *ftsA* alleles (6). This set of mutants has now been rescreened to look for any that have a block at a late stage in division.

Many mutations cause an indirect block to cell division because they elicit the SOS response (by interference with DNA replication) and thus induce the synthesis of the FtsZ inhibitor, SfiA (26). Mutations (e.g., in *dnaK* or *groE*) that elicit the heat shock response also cause a block in division that can be reversed by increased amounts of FtsZ in some cases (13). Mutants of these kinds appear to be blocked in a very early stage in division, the stage at which FtsZ acts. The remaining mutants in this collection have now been screened for those that are blocked later in division. The present investigation of TOE44 was prompted by the finding that, at 42°C, this mutant appears to be blocked at a very late stage. The mutation responsible has been mapped and shown to be in a previously unidentified gene, *ftsK*, adjacent to *lrp*, the gene for the leucine-responsive protein (55, 59–62). We find that the mutant phenotype is specifically suppressed by the deletion of *dacA*, coding for the peptidoglycan-modifying D-alanine:D-alanine carboxypeptidase, PBP 5 (36). This suggests that the *ftsK* product is involved in peptidoglycan synthesis during septation. Interestingly, the deduced sequence of the FtsK polypeptide has extensive similarity to that of the SpoIIIE protein of *Bacillus subtilis*, which may be required to complete final closure of the septum between the prespore and the mother compartments of the sporulating cell and also to complete transfer of a chromosome from the mother to the prespore compartment (64, 65). Like other members of this family, FtsK has probable membrane-spanning regions at the N-terminal end and a consensus ATP/GTP-binding sequence in the putative cytoplasmic domain. FtsK, however, is much larger (ca. 147 kDa) than the other members of the SpoIIIE family, although it contains within it sequences that together closely match the total sequences of the others. The remainder of the FtsK sequence, intercalated into the SpoIIIE-like sequence, shows close similarities to the sequences of γ-gliadins (41) and some other proline-glutamine-rich proteins.

MATERIALS AND METHODS

Strains. The following *E. coli* K-12 strains were used: C600 (F⁻ *leuB6 thr-1 lacA lacY1 thi-1 supE44 fhuA21* T1^r ϕ80^r [λ⁻]), AB1157 [F⁻ Δ(*gpt-proA62*) *argE3 his-4 leu-6 thr-1 ara-14 galK2 lacY1 xyl-5 mtl-1 thi-1 supE44 rpsL31* Str^r *tsx-33* T6^r (λ⁻)], AB2497 (AB1157 *thyA*), TOE44 (AB2497 *ftsK44*), TOE23 (AB2497 *ftsI23*), SP5211 [F⁻ *his proA purB thi mtl xyl galK lacY supE rpsL rodA*(Ts)] (20), SP1070 (*his supF dacA*::Km^r Δ*dacC*), and AB4 (AB2497 Δ*dacB*) (20). Appropriate Hfr strains from the collection of Singer et al. (48) were used as donors to transfer the *ftsK*$^+$ allele to TOE44. The *ftsK44* and Δ*pcnB* (35) mutations were transduced into C600 to give strain C600 *ftsK44 pcnB*.

Chromosomal DNA for PCR analysis was obtained from TOE44, NM306 (F⁻ *thi-1 ilv-192 argH1 metB1 xyl-7 hisG1 lacY1 tnaA1 strA8,9 tsx-7 tonA2 supE44 trp uhp pyrE purA*), and MM28-2 (F⁻ *argG6 asnA31 asnB32 his-1 leuB6 metB1 pyrE gal6 lacI lacY1 xyl-7 supE44 fhuA2 gyrA rpsL104 tsx-1 uhp pcnB recA56 tonA*) (35).

The *ftsK44* allele was also transduced into the *minB* strain P678-54 (1).

Media and culture conditions. Cells were grown with shaking in Oxoid nutrient broth no. 2 (NB) or L broth (LB) or on NB or LB agar plates at the temperatures indicated in Results. LB without NaCl was used in some experiments (as indicated in Results); normal LB contains 1% NaCl.

Plasmids and transformation. pBS59 (*dacA*$^+$) (8), pLG346 (*rodA*$^+$) (7), and pSU44 (constructed by cloning a 6.3-kb *Bam*HI restriction fragment from λ215 into pUC19) were transformed into appropriate strains.

Bacteriophages. λ phages from the Kohara bank (30) were spotted onto lawns of test cells, as follows. Log-phase cultures (~2×10^7 cells per ml) were used to flood NB or LB (without NaCl) plates and then drained immediately, and the remaining liquid was allowed to dry. Aliquots (5 μl) of λ lysates were spotted onto marked positions, and the plates were incubated at 42°C. A positive result was growth of colonies on the test spot at the restrictive temperature. (Note that because these phages are defective and cannot lysogenize without helper, this is a test for recombination between the cloned chromosomal fragment and the mutation in the test strain [30].)

Transduction. P1 transduction was used to transfer markers from one strain to

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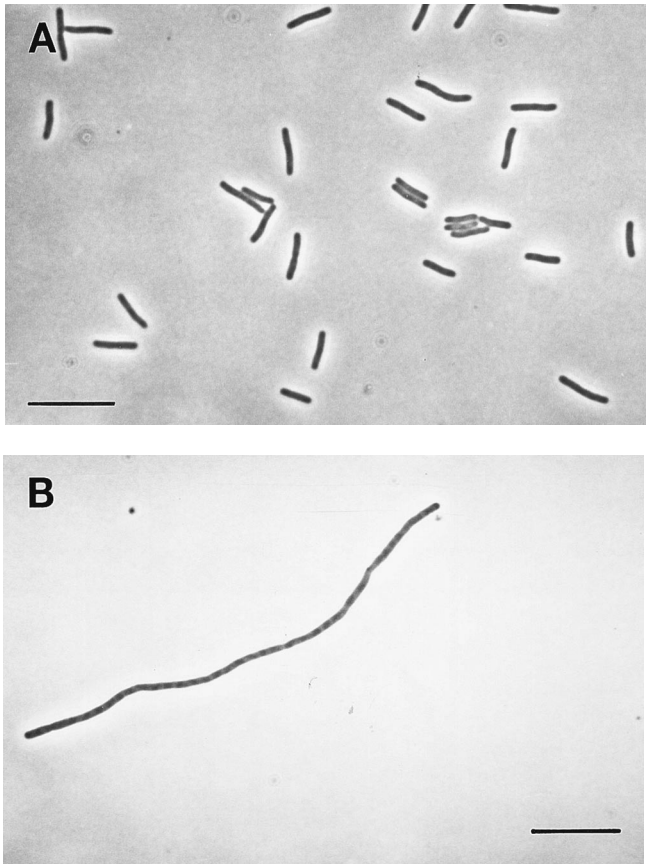

FIG. 1. The *ftsK44* phenotype. TOE44 in NB at 30°C (A) and after 180 min at 42°C (B) is shown. Bars, 10 μm.

another. Cotransduction of the wild-type *ftsK* allele with linked Tn*10* markers was done with lysates grown on suitably marked strains (Tn*10*-, Tn*5*-, and Tn*9*-marked strains kindly donated by the Genetic Stock Center, National Institute of Genetics, Mishima, Japan).

Visualization of chromosomal DNA. Cells were treated with chloramphenicol (200 μg/ml) for 5 min to condense the DNA into discrete nucleoids and then fixed and stained with DAPI (4′,6-diamidino-2-phenylindole) as previously described (18, 25). The cells were photographed under mixed phase-contrast–fluorescence illumination (25).

Nucleotide sequence accession number. The sequence for *ftsK* was deposited in the EMBL database under accession number Z49932.

RESULTS

The phenotype of a new cell division mutant, TOE44. Strain TOE44 was isolated after UV mutagenesis of *E. coli* K-12 strain AB2497 (a *thyA* derivative of AB1157) following an enrichment procedure (temperature-oscillatory enrichment, or TOE) designed to find mutants that required a temperature-sensitive protein for cell division only during a discrete interval in the cell cycle (6). The TOE44 mutant divides at 30°C but forms long, filamentous cells at 42°C. The thermosensitive phenotype is salt reversible, so that colonies will form at 42°C on LB agar containing 1% NaCl but not on NB agar with 0.5% NaCl. The conditional mutant phenotype is most marked in LB with no NaCl. Such salt reversibility is common among *fts* and other conditional mutants of *E. coli* (e.g., *ftsQ1* [6], *ftsZ84* [33], and about half of the known *ftsA* [46] mutants).

Figure 1 shows TOE44 cells (in NB) at 30 and 42°C. The cells are able to divide at 30°C, although they are longer than normal, but they cannot divide at 42°C. Figure 2 shows that the nucleoid numbers and distribution in TOE44 filaments are normal, demonstrating that cell morphology, growth, DNA synthesis, and partition are normal and that TOE44 is blocked only in cell division. After the *fts* mutation in TOE44 had been mapped (see below), it was transduced into another strain (C600), which then exhibited a phenotype identical to that of TOE44 itself.

We have shown earlier (4, 29) that mutations blocking division can be classified as either early or late by transfer to a *rodA* or *pbpA* mutant background. *rodA*(Ts) and *pbpA*(Ts) strains grow and divide as rod-shaped cells at 30 but as spheres at 42°C. If such strains also have a temperature-sensitive block in cell division, then cells grown at 42°C either are large, unconstricted spheres or show regularly spaced, incomplete constrictions, depending on the particular stage at which division is blocked. Inactivation of FtsZ or FtsW produces unconstricted spheres (4, 29), whereas inactivation of FtsA, FtsQ or FtsI (PBP 3) gives rise to swollen cells with regularly spaced constrictions (4). We have therefore proposed that FtsZ and FtsW are required to initiate constriction, whereas FtsA, FtsQ, and FtsI are required for postinitiation stages of septation (4, 29).

Many mutations cause damage to DNA or inhibition of its synthesis, leading to induction of the SOS response and indirect inhibition of cell division by induction of SfiA, a specific inhibitor of FtsZ (26). Such mutants are therefore blocked at a very early stage in cell division and form unconstricted, swollen cells when they also have *rodA* or *pbpA* mutations. The aim

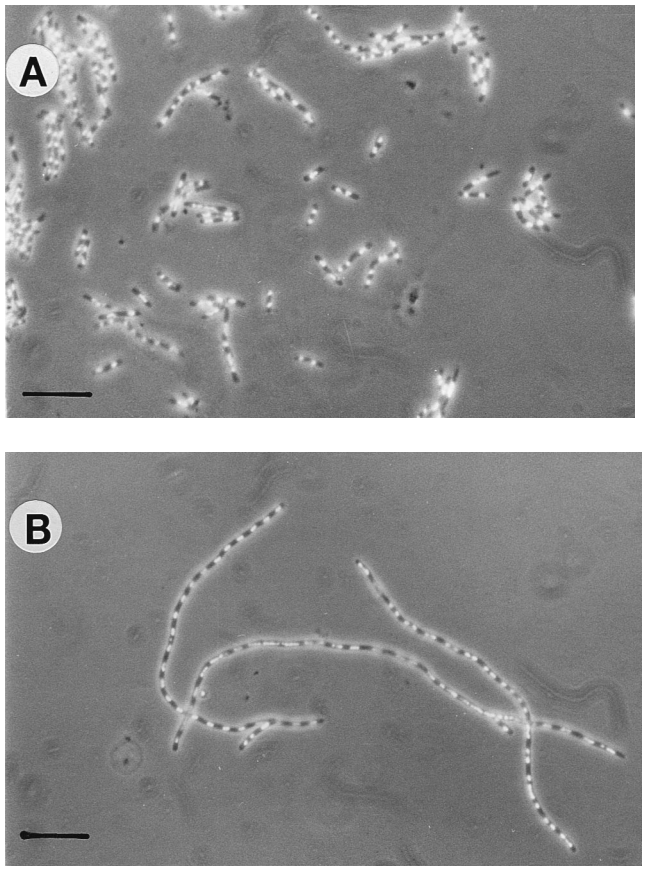

FIG. 2. DNA segregation in *ftsK44* cells. TOE44 cells were grown at 30°C (A) or for 180 min at 42°C (B) before chromosome condensation and fixing and staining with DAPI as described in Materials and Methods. Bars, 10 μm.

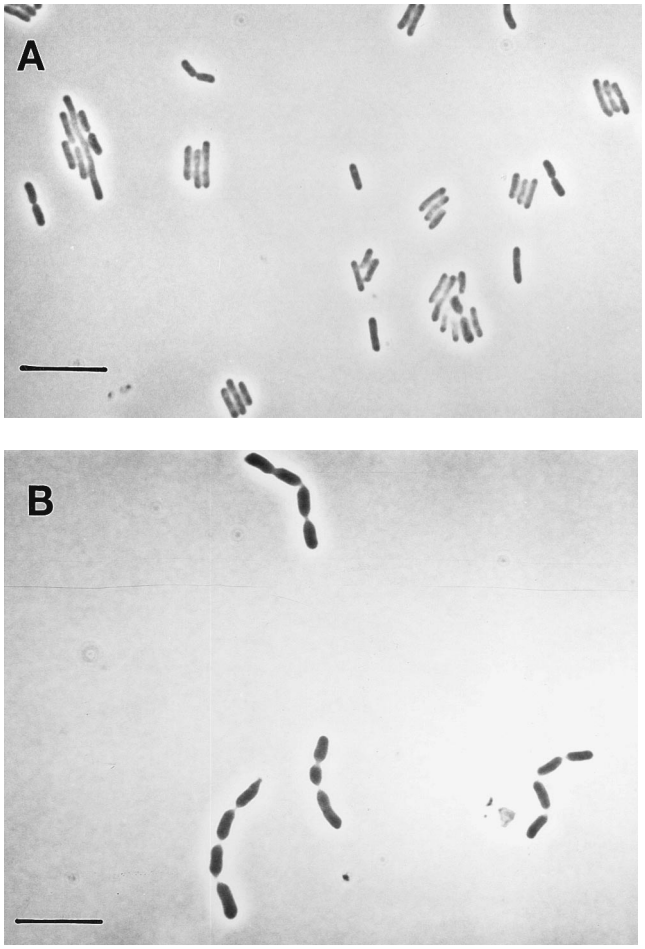

FIG. 3. Phenotype of the *ftsK44 rodA*(Ts) double mutant. TOE44 *rodA* at 30°C (A) and after 250 min at 42°C (B) is shown. Bars, 10 μm.

of the present study was to find mutants that were blocked at later stages in cell division. The *rodA5211* allele was therefore transduced into 15 TOE mutants, and their phenotypes were examined at 30 and 42°C. Thirteen of the mutants proved to be blocked at the earliest stage in division (three of these proved to have mutations in *dnaE*, and two proved to have mutations in *ftsE*), but one mutant, TOE44, behaved as if it was blocked at a late stage. Figure 3 shows the appearance of TOE44 *rodA5211* cells at 42°C. The constrictions seen in this strain are much more complete than those we have seen in any other *rodA fts* double-mutant strain, suggesting that TOE44 is blocked at a later stage in division than other mutants.

Suppression of the cell division block. In the course of genetic manipulations involving TOE44, it was found, serendipitously, that the temperature-sensitive phenotype is suppressed by inactivation of the gene *dacA*. TOE44 *dacA*::Km^r (49) is able to make normal colonies at 42°C, even on media without NaCl, and Fig. 4B shows that these cells are able to divide well.

The *dacA* gene codes for PBP 5, a major peptidoglycan DD-carboxypeptidase that removes the terminal D-alanine from peptidoglycan side chains. There is a second major DD-carboxypeptidase in *E. coli*, PBP 6, encoded by *dacC*, and a third protein with DD-carboxypeptidase activity, PBP 4, encoded by *dacB*. We therefore also constructed TOE44 Δ*dacC* and TOE44 Δ*dacB* strains, but both of these retained the *fts* phenotype of TOE44. Deletion of each or all of the *dac* genes has little or no detectable effect on the growth or division of wild-type cells (12, 20, 49).

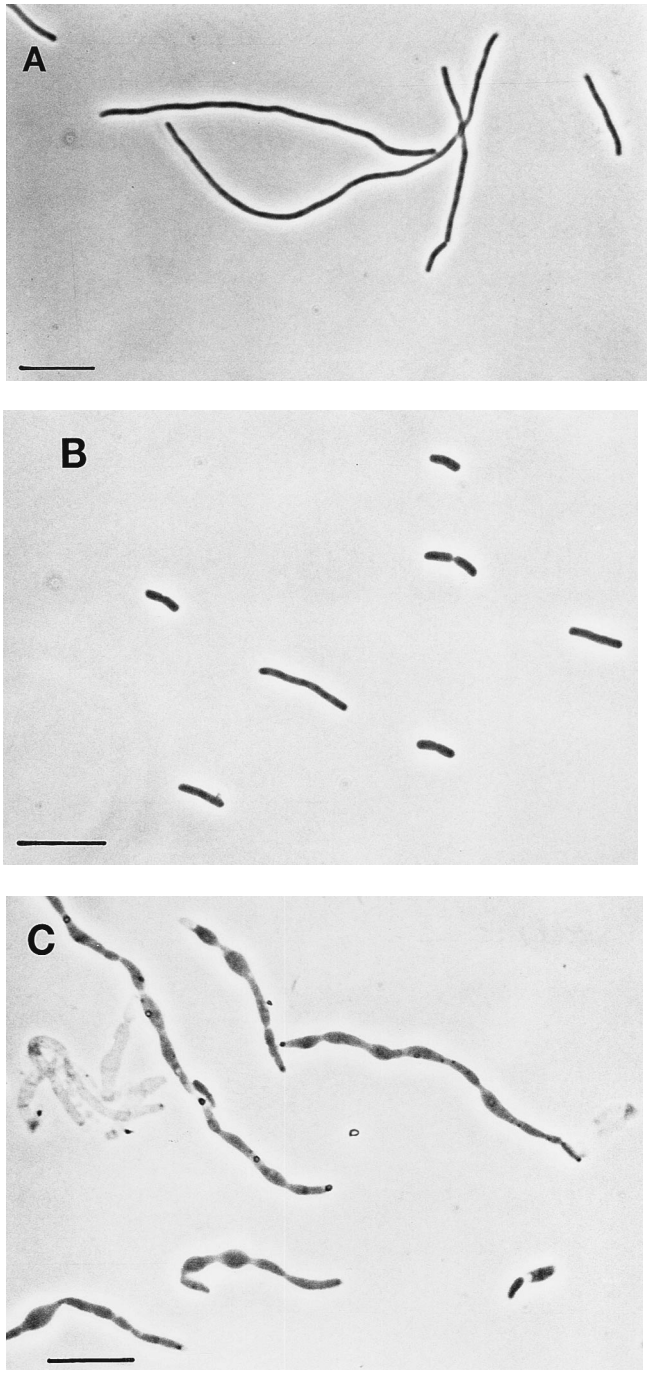

FIG. 4. Effect of the *dacA* gene on the *ftsK44* phenotype. TOE44 (A), TOE44 *dacA*::Km^r (B), and TOE44/pBS59 *dacA*$^+$ (C) after 180 min at 42°C in LB without NaCl are shown. Bars, 10 μm.

Because of the highly specific suppression of the division block in TOE44 by *dacA*::Km^r, TOE44 was next transformed with pBS59, which carries the *dacA*$^+$ allele (8). At 30°C this strain grew normally, but at 42°C cells grew into swollen, deeply constricted filaments that eventually lysed (Fig. 4C). (Overproduction of PBP 5 causes otherwise wild-type cells to

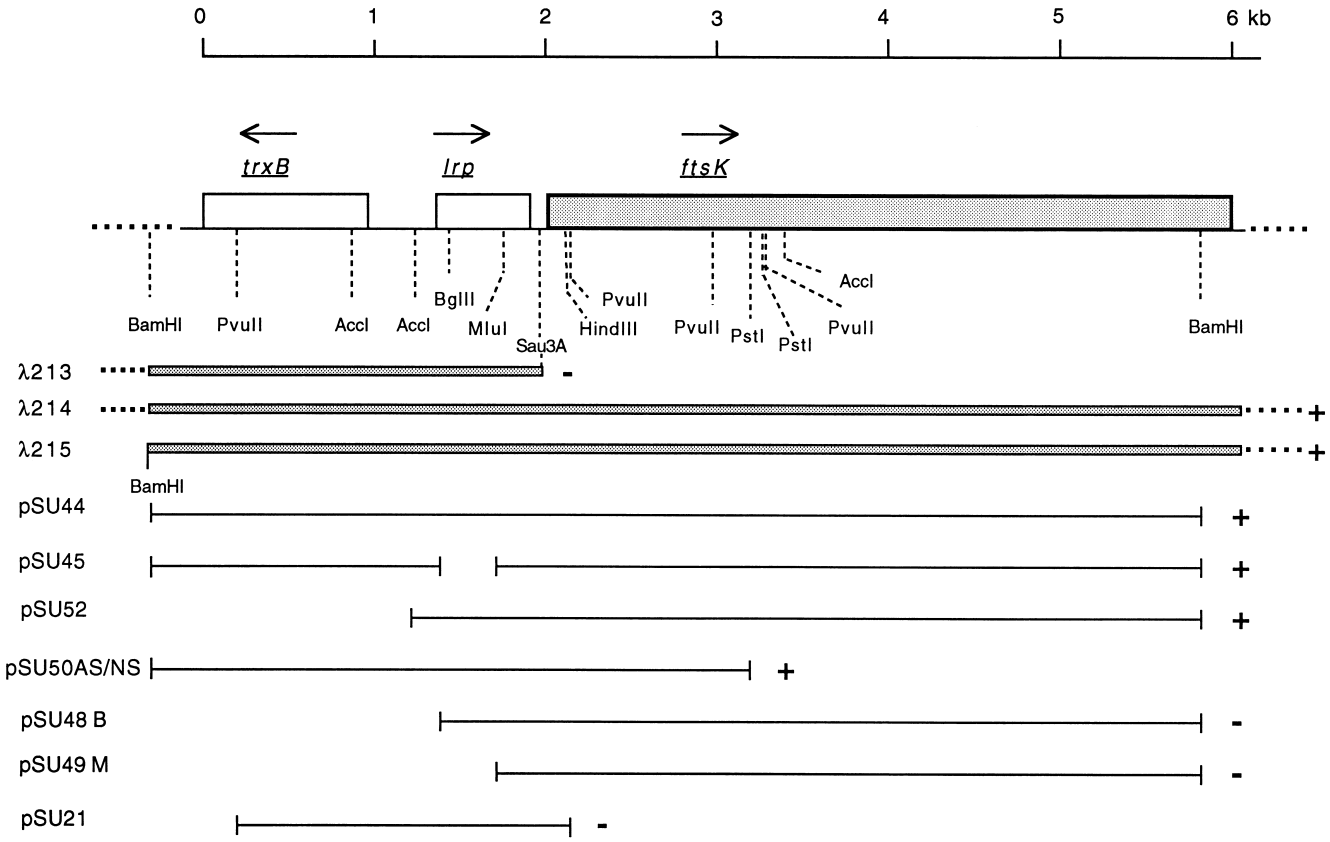

FIG. 5. Complementation and recombination analysis of the *ftsK44* mutation. ORFs (boxes) and selected restriction sites in the *trxB-lrp-ftsK* region are shown. The arrows show the direction of transcription. The parts of the cloned chromosomal DNA in λ213, λ214, and λ215 that overlap this region are shown as shaded bars; + and − indicate whether these phages gave temperature-resistant recombinants with *ftsK44*. A 6.3-kb *Bam*HI fragment was cloned from λ215 into pUC19 to give pSU44. Deletions of the cloned insert were made by using the indicated restriction sites to give the plasmids shown. (pSU50AS and pSU50NS contain the same *Bam*HI-*Pst*I fragment in opposite orientations.) + and − indicate whether the plasmid complements TOE44 at 42°C.

become spherical [34], but pBS59 is a low-copy-number vector, derived from pSC105, and has no morphological effects on normal cells.)

Because the *ftsI23* allele shows specific suppression by increased levels of PBP 5 (8), the *ftsI23* allele was also introduced into TOE44 (by cotransduction with *leu*::Tn*10*). Cells of this double-mutant strain stop dividing at 42°C but also soon lyse, so as to form short, lysed filaments rather than long filaments as in the single mutants. (The presence of the *ftsI23* allele in this strain was confirmed by cotransduction of *leu*::Tn*10 ftsI23* from it into a *leu*$^+$ *ftsI*$^+$ recipient. The presence of the *leu*::Tn*10* marker alone had no effect on the phenotype of TOE44.)

Mapping of the TOE44 mutation. Preliminary mapping of the mutation in TOE44 was done with a set of Hfr donor strains (48). The TOE44 mutation was then mapped more precisely by P1 cotransduction with various transposons at known locations (Genetic Stock Center, National Institute of Genetics) and finally to between 20.1 and 20.3 min (on the collated map of K. E. Rudd, contributed to reference 37) by recombination with λ phages carrying overlapping chromosomal fragments (λ214 and λ215 [30]). DNA from this region proved difficult to maintain in a plasmid vector except in a *pcnB* mutant host (35), in which the copy number is greatly reduced, but a single clone, containing part of the region common to both λ214 and λ215, was obtained by subcloning a 6.3-kbp *Bam*HI fragment from λ215 into pUC19. The TOE44 *pcnB* strain (C600 *ftsK44 pcnB*) is complemented by this plasmid (pSU44), so that colonies are formed on plates at 42°C, although cells growing at this temperature are somewhat longer than normal cells. The cloned region starts with a 337-bp noncoding sequence; this is followed by *trxB* (965 bp) (47), a 544-bp noncoding gap, *lrp* (495 bp), and a noncoding region of 134 bp (Fig. 5). The 134-bp noncoding gap has been reported to contain an SOS-inducible promoter (*dinH*) and a sequence conforming to the consensus sequence for a LexA box (31). Complementation of TOE44 does not require *trxB* (pSU52 [Fig. 5]). A 2.1-kb *Pvu*II fragment (′*trxB lrp ftsK*′) does not complement TOE44 (pSU21 [Fig. 5]). Complementation takes place with plasmids that carry an approximately 2-kb *Acc*I-*Pst*I fragment including *lrp* (pSU44, pSU50AS, pSU50NS, and pSU52), but deletion of most of *lrp* (*Bgl*II-*Mlu*I) does not prevent complementation (pSU45 [Fig. 5]). Deletion of the *Acc*I-*Bgl*II fragment within this region, however, abolished complementation (pSU48B [Fig. 5]). These results therefore suggest that a new cell division gene, *ftsK*, lies within this fragment and that DNA immediately upstream of *lrp* is required for its transcription (Fig. 5).

Sequencing of *ftsK* and *ftsK44*. The remainder of the 6.3-kbp fragment was then sequenced. No termination triplets were found before the *Pst*I sites, and the region downstream of the *dinH* region was found to consist of a single open reading frame (ORF). The sequence of this ORF was completed by sequencing the adjacent *Bam*HI fragment subcloned from

λ215 and was found to be 3,987 bp in length. We therefore must conclude that, surprisingly, the TOE44 defect can be corrected by the synthesis of the N-terminal 390-amino-acid fragment of the presumed FtsK polypeptide. (Note that none of the subcloned chromosomal DNA contains the entire *ftsK* reading frame [Fig. 5]. In all cases, the "complemented" cells are longer than normal at 42°C, which may be because only incomplete wild-type FtsK polypeptides are made.)

In sequencing an ORF of this size, it is always possible that a mistake is made in identifying the reading frame from the translated sequence alone, even when, as in this case, each section has been sequenced from both strands and rechecked several times. We therefore used the GeneMark program to identify probable protein-coding regions, as advocated by Borodovsky et al. (10). This analysis showed that the entire identical, whereas the *B. subtilis* SpoIIIE and *C. jejuni* protein hydrophobicity profiles are less similar.

The remainder of the FtsK sequence, without similarity to SpoIIIE-like proteins, contains six consecutive repeats of a 10-amino-acid sequence (PQQPV[A/P]PQ[P/Q]Q) separated by YQQ. Two similar sequences (LQQPVQPQQPYY and YQQ PQPVEQQPV) lie, respectively, 334 and 266 amino acids upstream. The function of these proline- and glutamine-rich sequences is unknown, but they show strong sequence similarities to γ-gliadins and C hordein, which are prolamin proteins from cereal seeds (and also to a variety of other PQ-rich protein sequences). For example, a comparison between a γ-gliadin precursor from wheat (41) (GDB2_WHEAT, positions 60 to 158) (top sequence) and FtsK (positions 727 to 822) (bottom sequence) gives

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     ***•    • •* ****** • *** • ****** *    * ***  ***  * ** ****
60   PHQPQ-QQVPQP-QQPQQPFLQPQQPFPQQPQQPFPQTQQPQQPFPQQPQQPFPQTQQPQ 117
727  PHEPLFTPIVEPVQQPQQP-VAPQQQY-QQPQQPVPPQPQYQQP--QQPVAPQPQYQQPQ 782
     ** •  *** • * ***  *  * ******  ****• * •*
118  QPFPQQPQQPFPQTQQPQQPFPQLQQPQQPF-PQPQQQLPQP 158
783  QPVA--PQQQYQQPQQPVAPQQQYQQPQQPVAPQPQDTLLHP 822
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3,987-bp sequence is indeed predicted, with a very high probability, to consist of *E. coli* coding DNA in a single reading frame (results not shown).

Because the TOE44 mutant can be complemented by a restriction fragment that contains only the first 1,170 bp of the *ftsK* ORF, we have sequenced this part of the chromosomal DNA from the TOE44 mutant. We find that there is a single transversion, G · C→C · G, at bp 239 of the deduced *ftsK* ORF, which will lead to glycine 80 being replaced by alanine in the mutant FtsK44 protein. This is a conservative change in sequence, but this is consistent with the fact that the TOE44 mutation causes loss of function only under extreme conditions (42°C in the absence of NaCl) and that its effects can be readily reversed (by low temperature, by NaCl, or by reduced PBP 5 activity).

Analysis of DNA sequence. The sequence of the deduced FtsK protein was analyzed. The result of a BLAST search (2) for similar known peptides was remarkable. Both the N-terminal 200 amino acids and the C-terminal 478 amino acids of the deduced FtsK polypeptide show highly significant sequence similarity to a family of three smaller proteins from *Coxiella burnetii*, *B. subtilis*, and *Campylobacter jejuni*. These blocks of similarity, adding up to 51% of the FtsK sequence, between them correspond to the entire sequences of the other three proteins. An alignment of these sequences is shown in Fig. 6 and 7.

Within the C-terminal block of similar sequence there is a clear ATP/GTP-binding site motif (K[7]G[3]SGKS[6]I) (motif A) (58) (Fig. 6) that is conserved in all four proteins. There is also a possible second nucleotide-binding site motif, motif B (FIMID) (58) (Fig. 7). (These sequences are also well conserved among the plasmid- and transposon-encoded proteins [see Fig. 6 and 7 and below].)

A hydrophobicity profile (TopPredII) (14) shows that all of the proteins in this family, including FtsK, are hydrophobic at the N-terminal region, with several probable membrane-spanning sequences (Fig. 8). The overall profiles of the N-terminal regions of FtsK and the *C. burnetii* SpoIIIE proteins are almost

where an asterisk indicates identity and a dot indicates a conservative substitution. Because this region is absent from other members of the SpoIIIE family of proteins, we considered the possibility that it was a cloning artifact, e.g., by intercalation of unrelated sequences that reduced the lethality of the native *ftsK* gene in multicopy. We therefore amplified chromosomal DNA by PCR (21) with different pairs of primers as shown in Fig. 9. These primers, lying within *lrp* or previously known sequences and in SpoIIIE-homologous regions, were chosen so as to amplify non-SpoIIIE-like regions. In this way four different fragments were amplified. The sizes of these fragments correspond to those predicted by our sequence (Fig. 9). In addition, there are unique *Pst*I and *Bsu*36I restriction sites within the non-SpoIIIE regions which were shown to be present at the correct locations in the amplified chromosomal DNA (Fig. 9). DNAs from three different strains of *E. coli* K-12 (TOE44, NM306, and MM28-2) gave identical results. These tests therefore show that the non-SpoIIIE-like blocks of sequence are indeed present within *ftsK* in uncloned chromosomal DNA.

The Gly-80-to-Ala-80 substitution in FtsK44 is in the hydrophobic N-terminal region, within a possible membrane-spanning α-helix (residues 75 to 97).

The C-terminal part of FtsK, the region that shows strong similarities to the three other bacterial proteins, also shares the nucleotide-binding motif and has other sequence similarity with a set of plasmid- and transposon-encoded proteins, some of which are shown in Fig. 6 and 7. (These proteins, not all of which are shown in Fig. 6 and 7, come from an extremely wide range of species, including gram-positive and gram-negative bacilli and cocci, *Streptomyces* species, *Agrobacterium* spp., and archaebacteria.)

DISCUSSION

The *ftsK44* phenotype. The *ftsK44* allele makes cell division temperature sensitive in any strain to which it is transferred. At

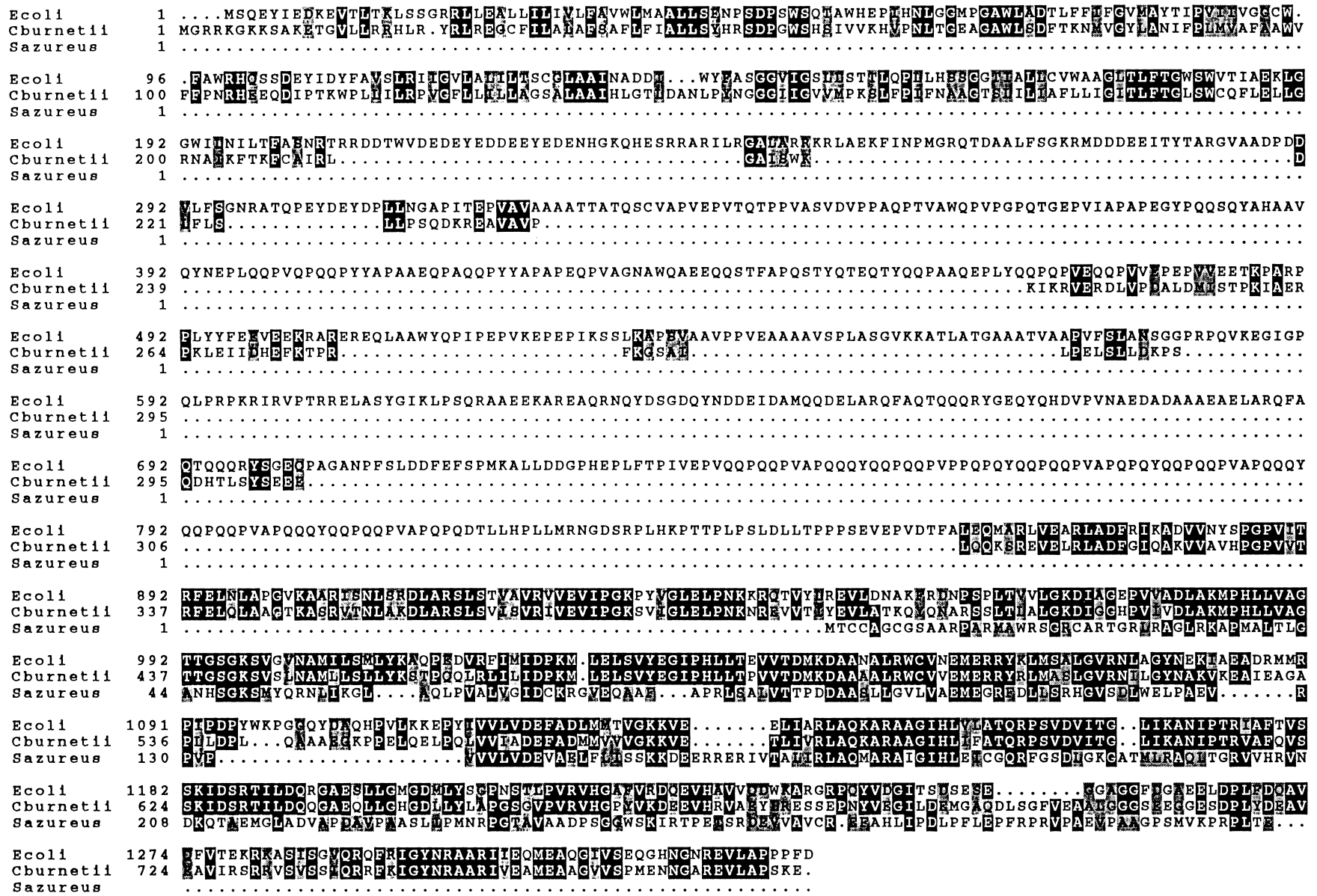

FIG. 6. Sequence alignment of *E. coli* FtsK predicted polypeptide with polypeptides from *C. burnetii* (SpoIIIE) (42) and *Streptomyces azureus* (Spi) (54). Identical amino acids are in black boxes and similar amino acids are in grey boxes.

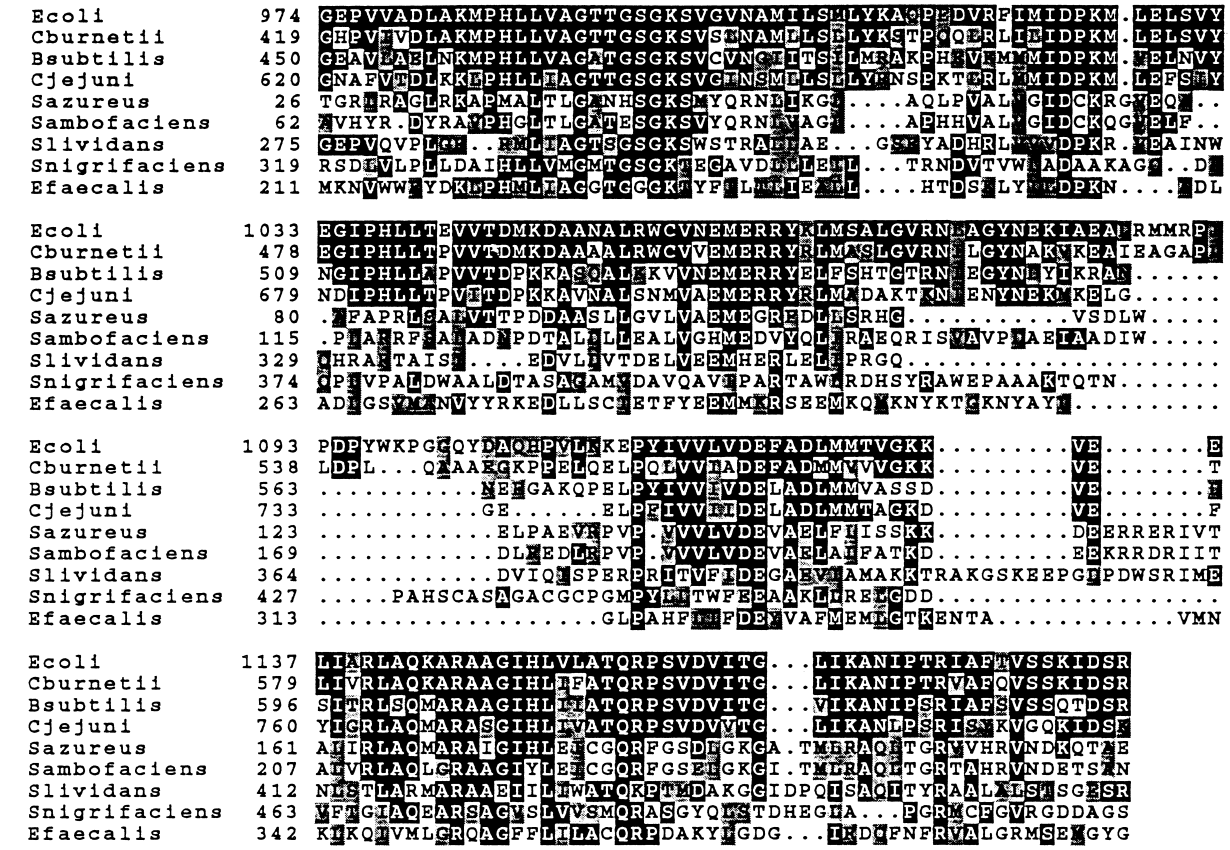

FIG. 7. Sequence alignment of the following polypeptides with amino acids 974 to 1157 of *E. coli* FtsK: *C. burnetii* SpoIIIE (42), *B. subtilis* SpoIIIE (64), *C. jejunii* (38), *S. azureus* (Spi) (54), *Streptomyces ambofaciens* (TraSA) (24), *Streptomyces lividans* (28), *Streptomyces nigrifaciens* (27), and *E. faecalis* (Psn) (22, 50). Amino acids identical to those in FtsK are shown in black, and conserved changes are shown in grey.

42°C *ftsK44* cells stop dividing but continue to grow in length and to replicate and segregate their DNA, as in classical *fts* mutants. The ability to divide and form colonies at 42°C can be restored by increasing the level of NaCl in the medium, again as in many previously described *fts* mutant strains (6, 33, 46). It is clear, therefore, that the *ftsK44* mutation makes the FtsK protein temperature sensitive but does not fully inactivate it.

Inactivation of the *dacA* gene in *ftsK44* strains almost completely restores the ability to divide and completely restores the ability to form colonies at 42°C. The *dacA* gene encodes PBP 5, a major periplasmic peptidoglycan D-alanine:D-alanine carboxypeptidase. Inactivation of PBP 5 should lead to an increase in the proportion of peptidoglycan chains with complete, pentapeptide side chains. These pentapeptide side chains are therefore available as donors in further cross-linking reactions between the glycan chains. The fact that such a change effectively restores the ability of *ftsK44* mutant cells to complete septa suggests that the FtsK protein is directly involved in the completion of septa and that it may itself be a peptidoglycan-modifying enzyme.

PBP 5 has previously been implicated in septation, because an increase in the PBP 5 level suppresses the cell division block in *ftsI23* mutants, which have a temperature-sensitive PBP 3 protein (8). PBP 3 is a specific septum-forming peptidoglycan transpeptidase. It has been proposed (8) that the suppression of *ftsI23* mutants by increased PBP 5 results from a decrease in pentapeptide side chains and a consequent increase in tripeptide side chains (resulting from the successive actions of the D-alanine:D-alanine carboxypeptidase PBP 5 and a DL-carboxypeptidase, carboxypeptidase II), which are used preferentially as acceptors in transpeptidation reactions carried out by PBP 3 (44). The increased amount of tripeptide substrate is therefore able to increase the residual PBP 3 activity in the *ftsI23* mutant (8). Our present results suggest the possibility of two different stages in septum formation, one requiring PBP 3 and tripeptide substrates and one requiring FtsK and pentapeptide side chains.

The *ftsK* gene. The *ftsK44* mutation lies in the *trxB lrp* region of the chromosome, as shown by Hfr mapping, P1 transduction, and recombination with λ transducing phages. Recombination with λ phages carrying overlapping fragments of this region suggests that the mutation lies downstream of *lrp* (Fig. 5).

Complementation studies with DNA subcloned from λ215 show that *ftsK44* lies within a 6.3-kb *Bam*HI fragment that also contains *trxB* and *lrp* (Fig. 5). Subcloned DNA from this fragment shows that neither *trxB* nor *lrp* is required for complementation but that DNA from both upstream and downstream of *lrp* is required. This suggests that the *ftsK44* mutation lies in a gene downstream of *lrp* and that the two genes are probably cotranscribed.

This deduction is put in doubt by two previous observations: that the short required region upstream of *lrp* does not include the previously assigned promoter for *lrp* (61) and that a large insertion into *lrp*, which might be expected to be strongly polar, has no obvious phenotypic effects (32). Thus, if *lrp* and *ftsK* were cotranscribed, FtsK would be a nonessential protein. The lethality of the *ftsK44* mutation and its ability to be comple-

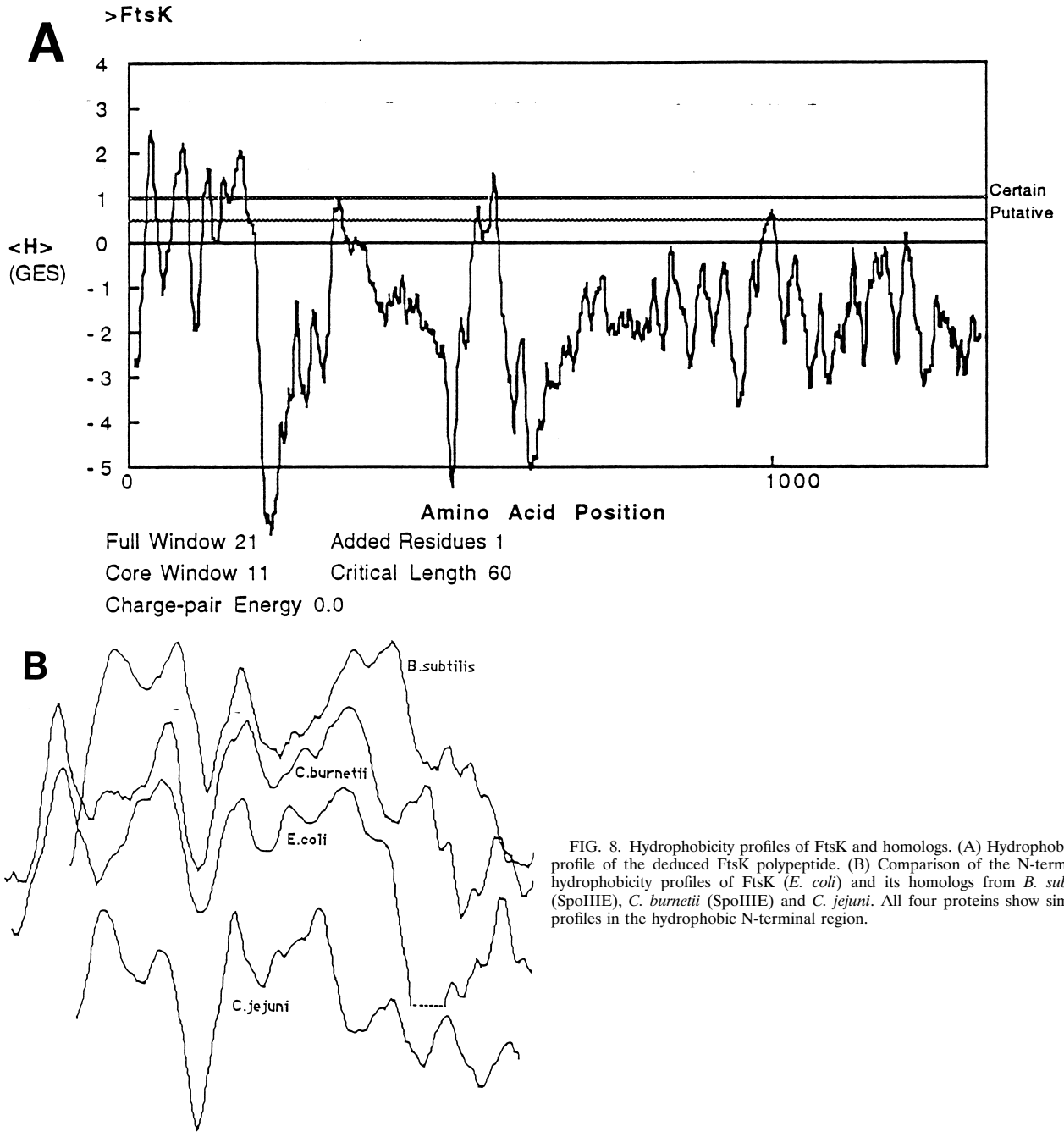

FIG. 8. Hydrophobicity profiles of FtsK and homologs. (A) Hydrophobicity profile of the deduced FtsK polypeptide. (B) Comparison of the N-terminal hydrophobicity profiles of FtsK (*E. coli*) and its homologs from *B. subtilis* (SpoIIIE), *C. burnetii* (SpoIIIE) and *C. jejuni*. All four proteins show similar profiles in the hydrophobic N-terminal region.

mented, however, are difficult to explain with this hypothesis. A further complication is the report of an SOS-inducible promoter, *dinH*, located immediately downstream of *lrp* (31), although this promoter would be repressed under the conditions that we have used to test for complementation. Further analysis of the transcription of these genes is therefore required. The role of FtsK in the SOS response also remains unknown.

FtsK is a member of a family of proteins involved in DNA transfer. The DNA downstream of *lrp* contains a large ORF. This sequence has several possible start codons. The first of these is the uncommon TTG triplet (66), but we favor this as the most probable start codon because a search for polypeptides with sequence similarities revealed a family of closely related proteins to which FtsK appears to belong and because the regions of similarity to one of these proteins (SpoIIIE from *C. burnetii*) includes the initial 200 amino acids that would be read from the first TTG codon (Fig. 6). In addition, this TTG codon is preceded by a good consensus ribosome-binding sequence in an appropriate location (23). This sequence is GGAG(8)TTG.

The alignments shown in Fig. 6 and 7 demonstrate that FtsK has blocks of very high sequence similarity to the SpoIIIE protein of *B. subtilis* (64) and to polypeptides of unknown function from *C. burnetii* (42) and *C. jejuni* (38). The closest

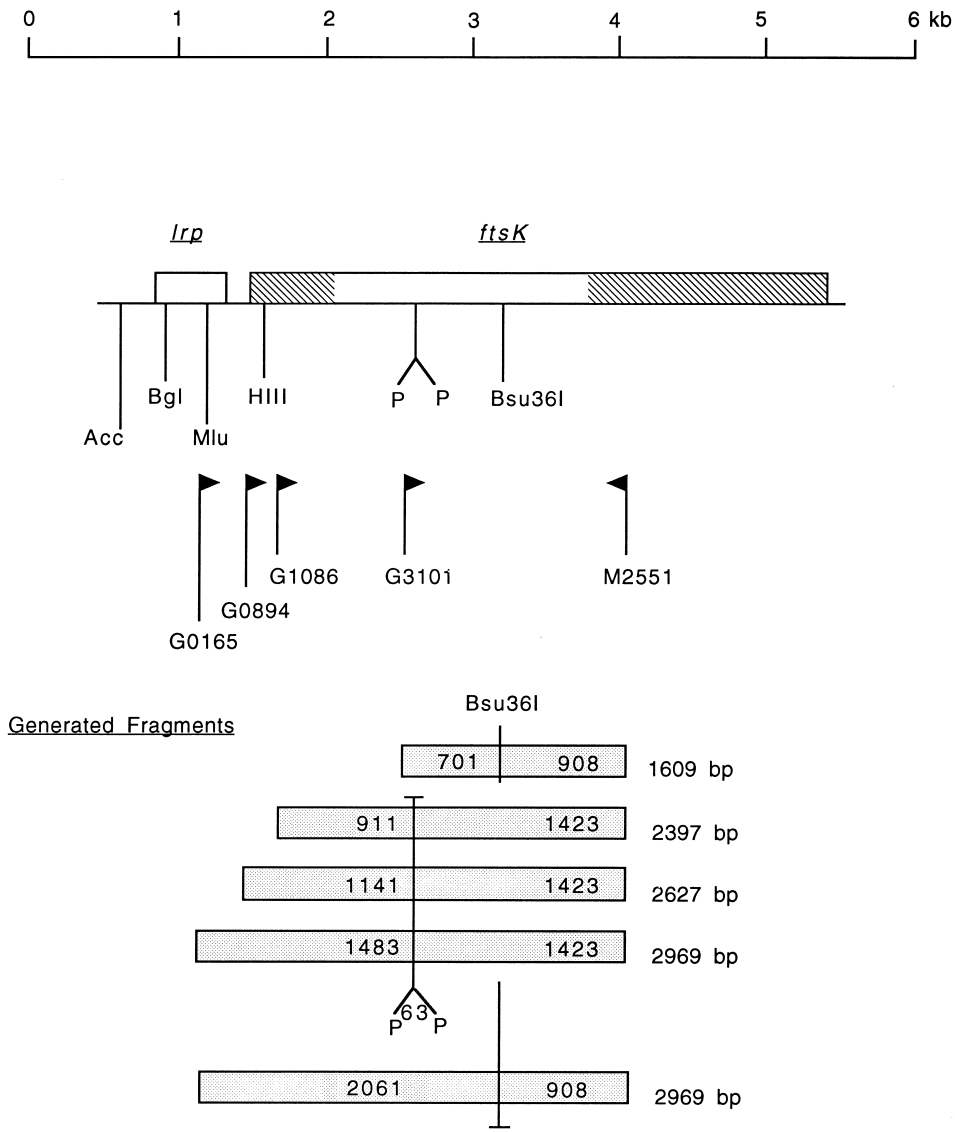

FIG. 9. PCR amplification of chromosomal DNA. A comparison of amplified segments with sequenced *ftsK* DNA, subcloned from λ215, is shown. The following primers were used: GO165 (TGTCATTTAGTATCCGG), GO894 (AGTCACATTGACAAAGT), G1086 (CCGTCATTATTGTCGGC), and the reverse primer M2551 (GAGAGTAATTGACGACAT). These primers lie in the SpoIIIE-like parts of *ftsK*, in the DNA upstream of the gene (e.g., in *lrp*), and in the non-SpoIIIE-like internal region. The amplified fragments therefore span all or part of the non-SpoIIIE-like internal segment of *ftsK*. The intervening sequences amplified from the given primers are shown. The numbers to the right are the sizes of the products; the numbers in the boxes refer to the restriction fragments generated by either *Pst*I or *Bsu*36I digestion of the PCR product. The estimated sizes (not shown) of the amplified DNA fragments were close to those predicted from the sequence (shown), and the unique restriction sites were in the predicted positions. The same fragments were obtained from PCR-amplified DNAs from three different strains of *E. coli* (see Materials and Methods).

similarity, especially in the N-terminal region, is with the *C. burnetii* SpoIIIE protein, which is in accord with the closer taxonomic relationship between these two species, but the similarity to each of them is very high. An equally striking finding is that whereas the other three members of this SpoIIIE family have similar sizes and are similar throughout, FtsK (1,329 amino acids) is much larger than the others (*C. burnetii*, 685 amino acids; *B. subtilis*, 787 amino acids; and *C. jejuni*, 866 amino acids). FtsK shares with the others the same general hydrophobicity profile (Fig. 8), with about four or five possible transmembrane domains at the N-terminal end and a large hydrophilic C-terminal domain. All of the proteins have a highly conserved putative nucleotide-binding sequence in this hydrophilic domain, which we therefore assume is cytoplasmic. The similarity of primary sequence in this domain is so high that FtsK seems likely to have many of the same properties as the other members of the family. This region, including the sequence around the conserved nucleotide-binding site, of the four bacterial proteins also shows similarity to the sequences of conjugative plasmid- or conjugative transposon-encoded proteins in *Streptomyces* species and *Enterococcus faecalis* (Fig. 7) and in a number of other eubacterial and archaebacterial species (data not shown). In cases in which a function is known, the protein is required for intercellular DNA transfer. It is most often the only protein with this function encoded by a given plasmid.

Wu et al. (64, 65) and Sharpe and Errington (47a) have shown that *B. subtilis spoIIIE* mutants are apparently blocked

after the completion (or near completion) of the prespore septum but that even deletion of *spoIIIE* appears to have no effect on septation during vegetative growth. Therefore, *spoIIIE* mutations, in contrast to *ftsK44*, are nonlethal, although they all prevent completion of sporulation. This lack of effect on vegetative-cell septation is unexpected, in view of the apparent requirement for FtsK for division of *E. coli* cells, but perhaps the cell wall structure in gram-positive organisms obviates the requirement for an FtsK-mediated stage, except during the formation of the rather different spore septum. Alternatively, *B. subtilis* may prove to have a second FtsK homolog dedicated to vegetative-cell division, in a manner similar to its possession of two PBP 3 proteins, one of which is dedicated to septation and the other to sporulation (15).

The most remarkable aspect of the *spoIIIE* mutant phenotype, however, is that transfer of a chromosome from the mother compartment to the prespore compartment is blocked partway (64, 65). The phenotype of sporulating *spoIIIE* mutant cells is therefore that they have two chromosomes, one of which is entirely within the mother-cell compartment and the other of which is apparently trapped in the prespore septum, with about two-thirds in the mother-cell compartment and one-third in the prespore compartment. It has therefore been concluded that SpoIIIE acts in an internal, postseptation, chromosome transfer step (64, 65). According to this idea, such a segregation mechanism is needed after the formation of an asymmetric septum (as in sporulation) but not after symmetric septation (between sister chromosomes, as in the vegetative-cell cycle). The SpoIIIE protein might therefore be a protein required either for completion of prespore septa and for transfer of DNA before completion or simply for transfer of chromosomal DNA through a completed septum. The similarities between all four bacterial proteins and the set of plasmid- and transposon-encoded proteins may also be relevant here. The plasmid- and conjugative transposon-encoded proteins are apparently the sole proteins required for intercellular transfer of their DNAs (24). We have therefore considered the possibility that the homologous FtsK protein has a similar function or functions. Since *E. coli* does not form spores and since segregation of sister chromosomes takes place before septation (19) or in the complete absence of septa (5), a postseptation chromosome transfer mechanism would seem to be unnecessary in this organism. There might, nevertheless, still be a role for a "rescue" segregation mechanism that could correct the occasional mislocation of chromosomes relative to growing septa by moving them out of the way of the closing septum, and this might even be an accessory function of an actual septum-completing protein, such as FtsK may be. The sequence similarity to DNA transfer proteins might reflect this, as might the presence of the ATP/GTP-binding site. Very little is yet known about the mechanism of chromosome partition in prokaryotes, and it is conceivable that FtsK plays a major part in this process, perhaps even being responsible for preseptation chromosome partition.

The involvement of FtsK in septation seems clear from our observations, and its involvement in peptidoglycan synthesis or modification is clearly indicated by the suppression of the *ftsK44* mutant phenotype by deletion of *dacA*. DNA replication and segregation, however, appear to be perfectly normal in this mutant (Fig. 2). It might nevertheless be possible to argue that chromosome segregation is in fact defective in our mutant but that our method of visualizing the DNA location (chloramphenicol treatment to condense the DNA, followed by staining with DAPI [18]) might either fail to show connecting DNA strands between sister nucleoids or even itself cause the completion of sister chromosome separation by causing DNA to contract around already separated organizational centers (5, 18, 19). In such a case, a failure to complete septa could be a secondary consequence of a failure to properly separate chromosomal DNA. If this were the case, however, the formation of aberrantly placed septa, in locations distant from the location of the chromosomes, should not be blocked in an *ftsK44* mutant. To test this prediction (47b), we made an *ftsK44 minB* double mutant, in which polar septa form at 30°C (giving rise to minicells without DNA [1]), and shifted it to 42°C. In this strain all septation, including polar septation, then stopped. We conclude that FtsK is directly required for septum formation, independent of chromosome segregation. This is in accord with the fact that the *ftsK44* mutation, which affects only septation, is due to a single amino acid substitution in the hydrophobic N-terminal region, where it might be expected to affect the configuration and function of a periplasmic loop in contact with the peptidoglycan sacculus. The existence of the nucleotide-binding motif in the large cytoplasmic domain that shares so much sequence similarity with SpoIIIE and with the plasmid-encoded DNA transfer proteins nevertheless suggests that the FtsK protein has additional roles, perhaps in chromosome partition. To find this out will require the analysis of new *ftsK* mutations, especially in the conserved cytoplasmic domain.

The large non-SpoIIIE-like domain within FtsK also requires explanation. The closest similarities in this region are with γ-gliadins from wheat (41); these serve as storage proteins, but the PQ-rich regions, which constitute most of these proteins and which are so similar to those seen in the central region of FtsK, are thought to form either extended helical structures or more compact helices, depending on the temperature and the hydrophobicity of the solvent (51–53). Thus, the gliadins may expand and cause swelling of the seed when water is present. Similar structures are found in elastins, proteins that take an extended form (β-spiral) because of a "sheath" of water molecules but can respond to disruption of this sheath by contracting to a more compact helical form (56, 57). In this way, elastin molecules are capable of performing work and can act as contractile proteins. This region of FtsK might therefore be a rod-shaped domain connecting the transmembrane and periplasmic domains to the SpoIIIE-Tra-like cytoplasmic domain and, perhaps by adopting extended or contracted configurations under different conditions, might act in moving something, e.g., DNA. Only the analysis of further mutants will determine whether such speculations have any justification.

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