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FtsK is an essential cell division protein that is localized to the septum and induced as part of the SOS response

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Summary

The role of *ftsK* in the growth of *Escherichia coli* was examined by turning off its expression. This resulted in smooth filaments without constrictions, indicating that FtsK was required at an early step in septation. Consistent with this, FtsK was found to localize to the septum in 70% of the cells, indicating that it was recruited relatively early in this process. FtsK localization required the function of FtsZ and FtsA but not FtsI and FtsQ. Consistent with this, Z rings were present in FtsK-depleted filaments. Subcellular localization of FtsK confirmed that it was a membrane protein. Only the first 202 amino acids of FtsK were essential for its role in membrane localization, cell division and viability. The expression of *ftsK* increased as part of the SOS response, and increased expression of *ftsK* conferred increased resistance to DNA damage.

Introduction

Cell division is a complex process involving the formation of a septum at the correct time and place within the cell (Lutkenhaus and Addinall, 1997; Rothfield and Justice, 1997). A number of genes, many designated *fts* for filamenting temperature sensitive, have been described that are essential for this process. Among these, *ftsZ* has been the most studied. It encodes a protein with limited sequence but significant structural and functional homology to eukaryotic tubulins (Mukherjee and Lutkenhaus, 1994; Erickson and Stoffler, 1996; Lowe and Amos, 1998). *In vitro* FtsZ undergoes polymerization into protofilaments and larger structures in the presence of various promoting agents (Bramhill and Thompson, 1994; Mukherjee and Lutkenhaus, 1994; Erickson and Stoffler, 1996; Yu and Margolin, 1997). In their absence, FtsZ polymerizes into protofilaments that, like microtubules, are dynamic as a result of GTP hydrolysis (Mukherjee and Lutkenhaus, 1998). *In vivo* FtsZ assembles into the Z ring at the division site that directs septation (Bi and Lutkenhaus, 1991; Addinall and Lutkenhaus, 1996a).

With the application of immunofluorescence microscopy to the cell biology of bacteria (Maddock and Shapiro, 1993; Arigoni *et al*., 1995), it has been possible to begin to examine the localization of additional cell division proteins, all of which are less abundant than FtsZ, and to address the order at which they appear at the division site. FtsA and ZipA appear at the division site about the same time as FtsZ (Addinall and Lutkenhaus, 1996b; Hale and de Boer, 1997; Ma *et al*., 1997). FtsA is a cytoplasmic protein that has sequence homology to the actin-DnaK-hexokinase superfamily and is predicted to be an ATPase (Bork *et al*., 1994). ZipA is a membrane protein with its carboxy-terminus in the cytoplasm (Hale and de Boer, 1997). Both of these proteins interact with FtsZ (Hale and de Boer, 1997; Ma *et al*., 1997; Wang *et al*., 1997), and it is clear that FtsA is recruited by FtsZ to the septum (Addinall and Lutkenhaus, 1996a; Ma *et al*., 1996).

The remaining cell division proteins are all membrane proteins and three of these, FtsN, FtsI and FtsW, have been localized to the septum (Addinall *et al*., 1997; Weiss *et al*., 1997; Wang and Lutkenhaus, 1998). The localization of FtsN has been examined in some detail (Addinall *et al*., 1997). Interestingly, FtsN localization depends upon the prior localization of FtsZ and FtsA, and requires functional FtsI and FtsQ as well. The localization of FtsI also depends upon FtsZ and FtsA. The order of FtsW addition to the septum has not been examined, but it is also likely to depend upon FtsZ (Wang and Lutkenhaus, 1998).

Recently, Begg *et al.* (1995) described a novel temperature-sensitive, cell division mutant *ftsK44* that appeared blocked late in the cell division pathway as the resulting filaments contained deep constrictions. Furthermore, the gene did not appear strictly essential as the temperature-sensitive phenotype was suppressed by deletion of *dacA*. This result was supported by the isolation of a transposon insertion in the *ftsK* gene, which was viable but showed sensitivity to high salt (Diez *et al*., 1997). Sequence of the *ftsK* gene revealed that it was a homologue of the *spoIIIE* gene of *Bacillus subtilis*, although it was larger because of the insertion of a long segment rich in proline and glutamine (Begg *et al*., 1995).

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SpoIIIE is required for translocation of the chromosome into the forespore during sporulation and is localized to the forespore septum but only as it nears completion (Wu and Errington, 1994; 1997). Although *spoIIIE* is not required for vegetative growth, a deletion mutant has increased sensitivity to DNA-damaging agents (Sharpe and Errington, 1995). SpoIIIE is localized to the vegetative septum at a very late stage as only 6% of cells have SpoIIIE localized (Wu and Errington, 1997).

In this study, we have further characterized the *ftsK* gene from *Escherichia coli* and find that it is essential, although the first 202 codons are sufficient. FtsK is localized to the septum and the localization is dependent upon FtsZ and FtsA. Furthermore, the *ftsK* gene is induced as part of the SOS response and increased expression of *ftsK* confers increased resistance to DNA damage.

Results

ftsK *is an essential cell division gene*

The *lac* promoter was placed upstream of *ftsK* on the chromosome, so that the effects of depleting FtsK on viability and cell division could be determined. This FtsK depletion strain (W3110::P*lac–ftsK*) with P*lac* upstream of *ftsK* was constructed as described in *Experimental procedures* and a diagram is shown in Fig. 1A. W3110::P*lac–ftsK* was dependent upon IPTG for growth, suggesting that *ftsK* was essential. To verify that the inserted *kan* cassette, tandem terminators and P*lac* were in the expected position on the chromosome, two primers were used to amplify a fragment that should be only present in the desired strain. The sequence for one primer is located within the *kan* cassette, whereas the sequence for the other primer is located immediately downstream of the 3′ end of *ftsK′*. The PCR was performed using pUCK37TK, W3110 chromosomal DNA and W3110::P*lac–ftsK* chromosomal DNA as templates. A PCR product of the expected size was obtained with W3110::P*lac–ftsK* but not with the controls, confirming that P*lac* was indeed inserted upstream of *ftsK* on the chromosome (Fig. 1B).

To determine whether *ftsK* is an essential cell division gene, we removed IPTG from the cells to see whether the depletion of FtsK would inhibit cell division. W3110::P*lac–ftsK* was grown for several generations in the presence of 0.1 mM IPTG and then incubated in LB containing 0.2% glucose. Microscopic examination of the culture indicated that the cells stopped dividing about 60 min after the removal of IPTG. At 180 min, the cells were filamentous (Fig. 2) with a smooth morphology, indicating that FtsK acts early in septation and that *ftsK* is an essential cell division gene. It was noticed, however, that the filaments at 180 min had occasional constrictions. These constrictions are due to residual expression of *ftsK* from the *lac* promoter because they were not present when plasmid pAM1,

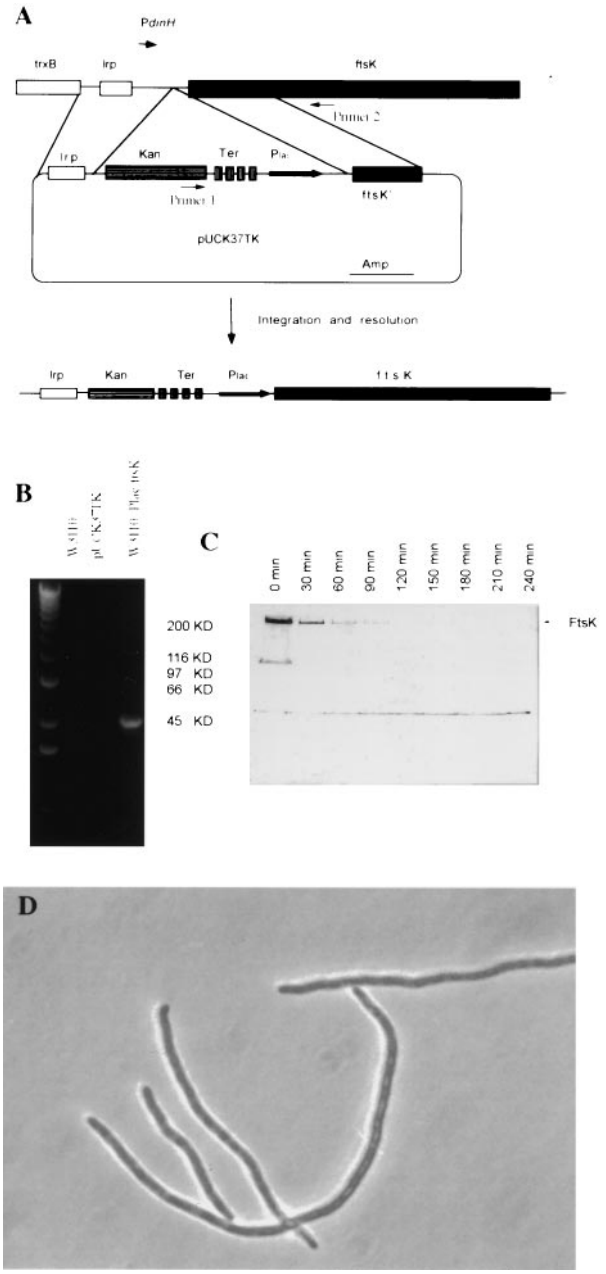

Fig. 1. Construction and analysis of a strain with *ftsK* downstream of the *lac* promoter.
A. Diagram of the construction of W3110::P*lac–ftsK*. pUCK37TK is a temperature-sensitive replicon containing a kan cassette, four *rrnB* terminators and the *lac* promoter inserted between *lrp* and a 5′ fragment of *ftsK*. Integration and resolution of this plasmid placed this insert on the chromosome.
B. PCR analysis of W3110::P*lac–ftsK*. DNA from W3110 (lane 1), pUCK37TK (lane 2) and W3110::P*lac–ftsK* (lane 3) were used for PCR amplification with a forward primer in the *kan* cassette and a backward primer within *ftsK* (see A).
C. Western Blot analysis of W3110::P*lac–ftsK*. An exponentially growing culture of W3110::P*lac–ftsK* in LB with 0.1 mM IPTG was washed and grown in the LB with 0.2% glucose. Samples were taken at various times after removal of IPTG. Lane 1, 0 min; lane 2 to lane 9, 30 min intervals after IPTG removal.
D. Depletion of FtsK leads to smooth filaments. Morphology of W3110::P*lac–ftsK* (pAM1) incubated in the absence of IPTG for 3 h.

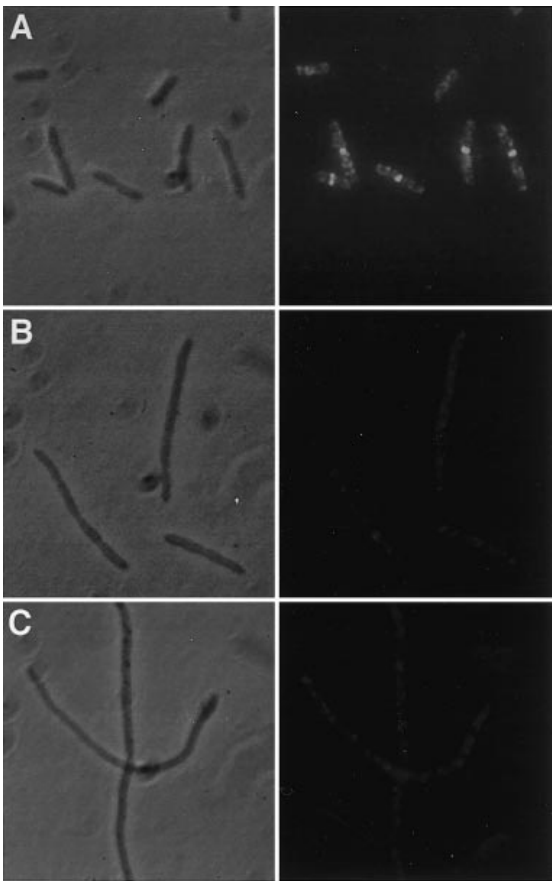

Fig. 2. Localization of FtsK to the septum. W3110::P*lac–ftsK* cells cultured in the absence of IPTG for 0 (A), 60 (B) and 180 (C) min after removal of IPTG were stained with anti-FtsK antibodies and observed by phase (left) and fluorescence (right) microscopy.

which increases the level of the Lac repressor, was introduced into the strain (Fig. 1D). The smooth filamentous phenotype of the FtsK depletion strain contrasts with the deep constrictions observed with a temperature-sensitive *ftsK44* mutation and suggests that *ftsK* is required at an earlier stage of cell division than previously thought. To examine the role of *ftsK* further, we transduced our construct into CS12-7, which is deleted for *dacA*. Although *dacA* suppresses *ftsK44* (Ts) (Begg *et al*., 1995), CS12-7::P*lac–ftsK* was still IPTG dependent and filamented upon removal of IPTG (data not shown).

A Western blot of lysates of W3110::P*lac–ftsK* with anti-FtsK antibody revealed two bands: one at 200 kDa and another at 100 kDa (Fig. 1C). After the removal of IPTG, the levels of both bands were reduced dramatically by 60 min. At 180 min, neither of the two bands was detected, although a faint non-specific band with molecular weight of around 50 kDa maintained constant intensity. If samples were not immediately boiled in SDS, the intensity of the 100 kDa band increased at the expense of the 200 kDa band. This strongly suggests that the faster migrating band is a proteolytic product of FtsK, most likely representing the N-terminal half of FtsK as it was present in the membrane fraction (see later). We conclude that the 200 kDa band is the product of the *ftsK* gene.

To determine if FtsK is localized to the division site, W3110::P*lac–ftsK* was grown in the presence and absence of IPTG, fixed and processed for immunofluorescent staining as described previously (Addinall *et al*., 1996). In the presence of IPTG, about half of the cells contained a bright band of fluorescence at mid-cell, indicating that FtsK is localized to the division site (Fig. 2). These cells also contained some non-localized fluorescence. Localized fluorescence was not detected in the FtsK-depleted filaments at 60 and 180 min after removal of IPTG. In addition, the non-localized fluorescence was decreased, indicating that both the localized and non-localized fluorescence was due to FtsK. The cells in the presence of IPTG have more non-localized fluorescence than wild-type cells because of an elevated level of FtsK (see later). These results confirm that the fluorescent band was due to the localization of FtsK. Further quantitative studies on FtsK localization were carried out with the wild-type strain (see below).

The N-terminal 202 amino acid residues of FtsK are sufficient to complement the depletion of FtsK

To determine if the filamentation of W3110::P*lac–ftsK* was due to the depletion of FtsK and not some downstream gene products, we performed complementation experiments with *ftsK* constructs contained on plasmids under arabinose promoter control (Fig. 3). Constructs with either the full-length *ftsK* or truncated *ftsK*s on the vector pBAD18 were transformed into W3110::P*lac–ftsK*. The transformants were tested for viability in the presence of 0.2% arabinose and absence of IPTG. The results showed that the full-length FtsK (pWL75) can complement the lethality caused by the removal of IPTG, confirming that this lethality was due to the depletion of FtsK from the cells and not downstream genes.

FtsK consists of several domains including an N-terminal hydrophobic domain, a P+Q-rich domain, and a large C-terminal domain homologous to SpoIIIE. To determine which domain is essential for cell viability, we checked complementation by various plasmids expressing different truncated *ftsK*s (Fig. 3). Plasmids that contained the N-terminal 202 codons comprising 15% of FtsK were sufficient to complement the depletion strain. Although viability was restored, the morphology was not wild type as cells were a little elongated (see later). pWL76, which expresses the C-terminal half of FtsK, including the region homologous to SpoIIIE, did not complement. This result indicated that only the N-terminal hydrophobic domain of FtsK is essential for cell division and that the SpoIIIE homologous domain is non-essential.

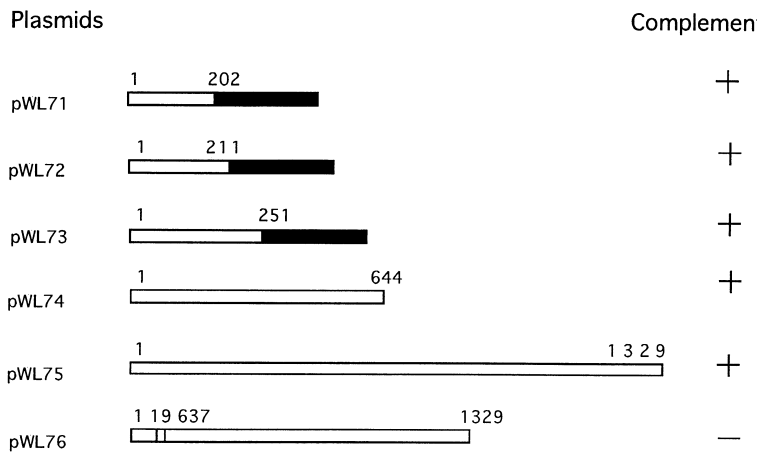

Fig. 3. Complementation analysis of W3110::P*lac–ftsK*. A schematic summary of different *ftsK* constructs expressed from the arabinose promoter in pBAD18. The length of FtsK fragments are indicated by the open rectangle and the number of amino acid residues of FtsK. The filled rectangle indicates in-frame fusions to GFP. pWL76 is a construct with the N-terminal 19 amino acid residues fused in phase to amino acid residue 637 of FtsK.

FtsK is localized to the division site and the localization domain overlaps with the domain essential for cell division

The affinity-purified FtsK antibodies were used to quantitatively examine the subcellular localization of FtsK in wild-type cells. A culture of MC4100 growing exponentially in LB medium at 37°C was fixed and processed for immunofluorescence microscopy, as described previously (Addinall *et al*., 1996). Figure 4A shows a typical photomicrograph of such cells. Many cells show a band of fluorescence at mid-cell, which decreases in diameter in constricted cells. This pattern of FtsK localization is similar to that seen with other cell division proteins. However, the frequency at which FtsK is clearly localized at mid-cell was 70% (302 out of 428 cells counted), which is less than observed for FtsZ (96%; 445 out of 462) and FtsA (85%, 324 out of 378) but more than the 50% observed for FtsI (Wang and Lutkenhaus, 1998) and FtsN (Addinall *et al*., 1997). Consistent with these frequencies, many cells without constrictions have FtsK localized to the septum, whereas localization of FtsI and FtsN is observed mostly in the cells with constrictions (Addinall *et al*., 1997; Weiss *et al*., 1997; Wang and Lutkenhaus, 1998). This suggests that localization of FtsK to the septum occurs later than FtsZ and FtsA but earlier than FtsI and FtsN.

To analyse the localization of FtsK further and its possible role in cell division, we used a construct with green fluorescent protein (GFP) fused to the N-terminal 251 amino acids of FtsK (pWL73). Complementation results showed that this plasmid can complement the depletion of FtsK in W3110::P*lac–ftsK* (Fig. 3). This GFP fusion protein was localized to the septum in W3110::P*lac–ftsK* (Fig. 5), which indicates that the domain of FtsK responsible for localization overlaps the domain that is essential for its function in cell division. However, further tests with constructs containing less N-terminal amino acid residues (1–202 and 1–211) did not show significant localization in either W3110::P*lac–ftsK* or W3110 cells. A Western blot with anti-GFP monoclonal antibody showed that these fusion proteins undergo rapid proteolytic cleavage at the fusion junction to generate free GFP, explaining why they are not localized (data not shown). Although all three of these N-terminal fusions complemented the depletion strain, the cells were elongated compared to the wild type (Fig. 5).

FtsZ localizes in the absence of detectable FtsK localization

Previously, we have shown that FtsZ is able to localize in several temperature-sensitive mutants (Addinall *et al*., 1996; Khattar *et al*., 1997). To determine whether FtsZ localizes in cells depleted for FtsK, we checked the localization of FtsZ in W3110::P*lac–ftsK* after IPTG was removed. We observed that 60 min after the removal of IPTG, the number of cells with FtsZ localized increased to almost 100%. By 180 min, multiple Z rings, regularly spaced, were observed along the filaments (Fig. 6). However, some filaments without FtsZ rings were also observed, perhaps indicating dead cells. In those cells displaying multiple Z rings, some appeared incomplete. These results suggested that the prior localization of FtsK was not needed for FtsZ ring formation, although the presence of FtsK may increase the stability of Z rings. Using antibodies to FtsA and ZipA, we observed that these proteins were localized in the same pattern as FtsZ (data not shown). This is consistent with previous observations that these proteins are localized to the division site coincident with FtsZ (Addinall and Lutkenhaus, 1996a; Ma *et al*., 1996; Hale and de Boer, 1997).

FtsK localization depends on the prior localization of FtsZ and FtsA but not of FtsI and FtsQ

As FtsK is localized to the septum, we wished to determine if other gene products were required for this localization. Previously, we had demonstrated that the localization of

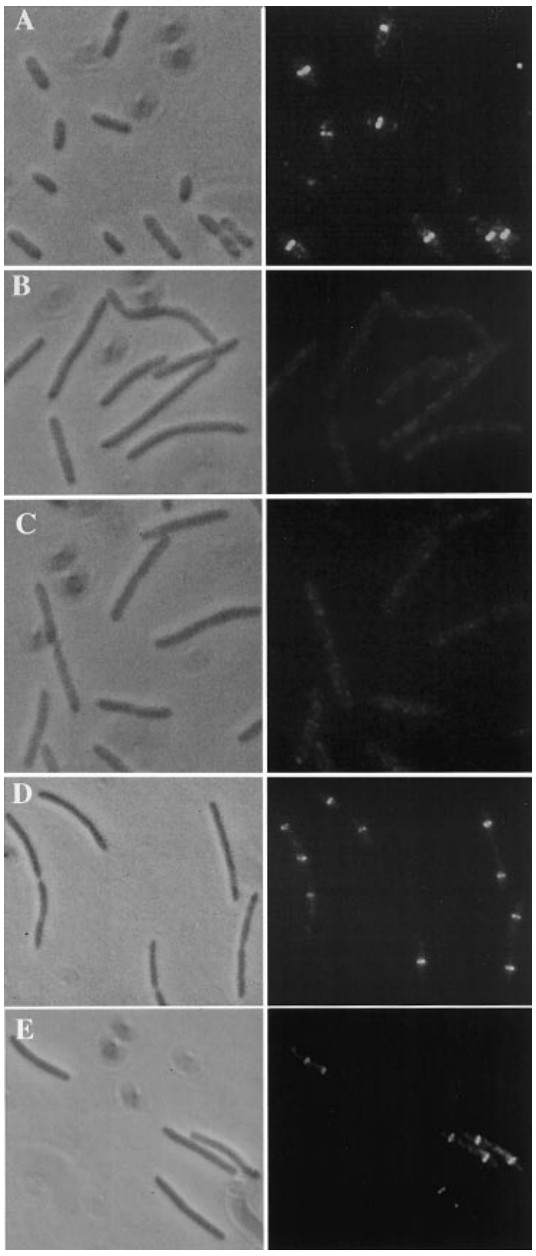

Fig. 4. FtsK localization in wild-type and various *fts* strains. The panels on the left are phase-contrast photomicrographs and those on the right are fluorescence photomicrographs of cells stained with anti-FtsK antibodies. (A) MC4100; (B) MCZ84 [*ftsZ84* (Ts)]; (C) MCA12 [*ftsA12* (Ts)]; (D) *ftsI23* (Ts); and (E) *ftsQ1* (Ts). B–E were shifted to 42°C for 30 min before microscopy.

FtsA is dependent upon FtsZ (Addinall and Lutkenhaus, 1996b). To assess the dependency of FtsK localization upon *ftsZ* and *ftsA*, strains with temperature-sensitive mutations in these genes were used. Exponentially growing cultures of MCZ84 [*ftsZ84* (Ts)] and MCA12 [*ftsA12* (Ts)] were shifted to the non-permissive temperature and samples were prepared for immunofluorescence microscopy. FtsK was localized efficiently in these strains at permissive temperature (data not shown). In contrast, filaments of both these strains lacked FtsK localization (Fig. 4B and C), indicating that it was dependent upon the prior localization of FtsZ and FtsA.

Other *fts* genes that have been studied in some detail, and *ftsQ* and *ftsI*, are thought to act after *ftsZ* and *ftsA* (Begg and Donachie, 1985). To determine whether they are required for FtsK localization, strains MCI23 and MCQ1, which contain temperature-sensitive mutations in *ftsI* and *ftsQ*, respectively, were examined. Cultures of these strains were shifted to non-permissive temperature for 30 min and processed for immunofluorescence microscopy. Figure 4D and E shows the results obtained with MCI23 and MCQ1 respectively. FtsK localization was observed in these filaments, although the intensity of the FtsK band was a little weaker than in the wild-type cells. These results suggest that FtsI and FtsQ proteins are not required for the localization of FtsK. As FtsZ and FtsA are localized in filaments of *ftsQ* and *ftsI* mutants but FtsN is not (Addinall *et al*., 1997), it suggests that FtsK localization is dependent upon FtsZ and FtsA but not upon FtsI, FtsQ and FtsN.

FtsK is a membrane-associated protein

The hydropathy profile of FtsK suggests that the N-terminus of FtsK contains four putative transmembrane domains similar to SpoIIIE (Begg *et al*., 1995). To determine whether FtsK is a membrane protein, we fractionated wild-type MC4100 cells. As shown in Fig. 7A, FtsK was predominately present in the membrane fraction, although three forms (200 kDa, 100 kDa and 90 kDa) were present. With a total cell lysate, the 200 kDa form is the predominant band, but after cell lysis more of the 100 kDa and 90 kDa forms were observed, suggesting that they arose by proteolysis of the 200 kDa form. The presence of the lower molecular weight forms in the membrane fraction indicates that they contain the N-terminus of FtsK.

Fig. 5. The FtsK (N-terminal 251 aa residues) GFP fusion is localized to the septum. W3110::P*lac*–*ftsK* containing pWL73 was observed by fluorescence microscopy.

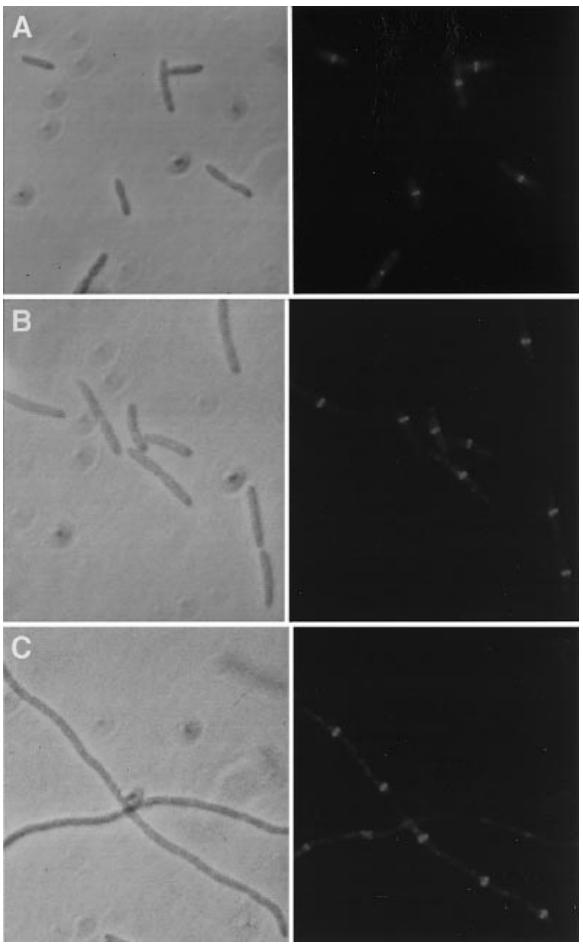

Fig. 6. Z rings are formed in cells depleted of FtsK. The panels on the left are phase-contrast photomicrographs, and those on the right are fluorescence photomicrographs of cells stained with anti-FtsZ. Cells of W3110::P*lac-ftsK* were examined 0, 60 and 180 min after removal of IPTG.

A Western blot was also performed on cell fractions of the transformants containing an FtsK–GFP fusion construct (pWL73). We used a monoclonal antibody against GFP. As shown in Fig. 7B, the band with slower mobility, which represents the GFP–FtsK fusion, was present in the membrane fraction, whereas the short form, which corresponds to GFP arising from proteolytic cleavage at the fusion junction, was present in the cytoplasmic fraction. As pWL73 can complement the depletion of FtsK and the expressed fusion can localize to the septum, it is likely that FtsK is localized to the septum through the multiple transmembrane domains.

FtsK expression is induced by DNA damaging agents in a recA–lexA*-dependent manner*

Just upstream from *ftsK* lies an SOS-inducible promoter *dinH*. Upon SOS induction, the *dinH* promoter activity is induced two- to fourfold (Lewis *et al*., 1992). To test whether FtsK expression can be induced by DNA-damaging agents, we treated wild-type MC4100 cells with nalidixic acid and examined samples before and after addition by Western blot. By 60 min after nalidixic acid, the FtsK level had increased, although some of it had degraded (Fig. 8), indicating that FtsK is induced by DNA-damaging agents. To confirm these results we used a *recA* mutant. As shown in Fig. 8, the level of FtsK was not increased after treatment with nalidixic acid. We also observed that FtsK levels increased after shifting a *lexA* (Ts) strain to 42°C (Fig. 8). These results confirm that the essential *ftsK* gene is induced as part of the SOS response.

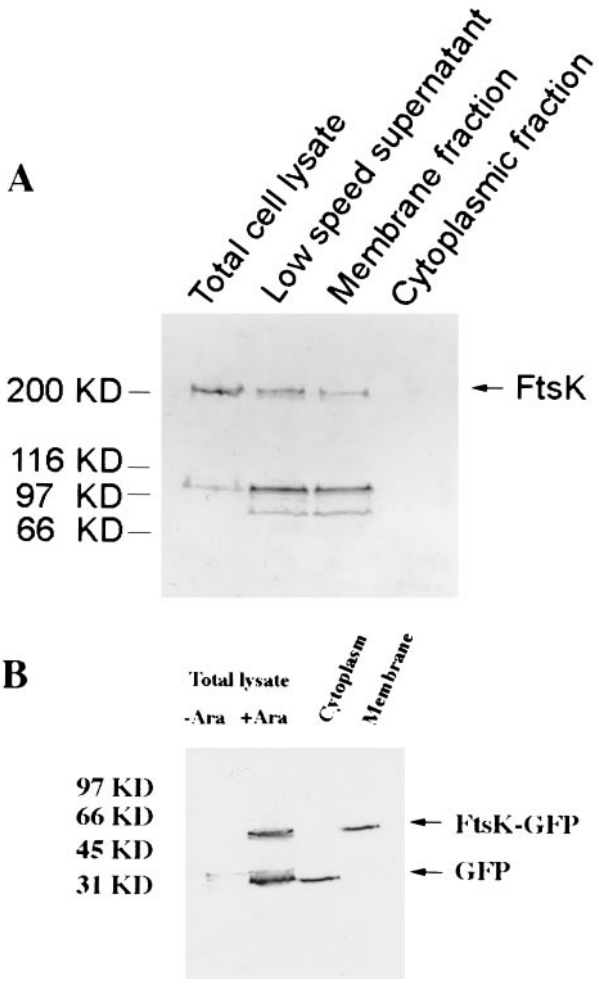

Fig. 7. FtsK is a membrane-associated protein.
A. Western blot of cell fractions of wild-type MC4100 using anti-FtsK.
B. Western blot of cell fractions of MC4100 harbouring pWL73, a fusion of FtsK(1–251) and GFP, using a monoclonal antibody against GFP. The molecular standards are 200 kDa, myosin; 116 kDa, β-galactosidase; 97 kDa, phosphorylase b; 66 kDa, bovine serum albumin; 45 kDa, ovalbumin; and 31 kDa, carbonic anhydrase.

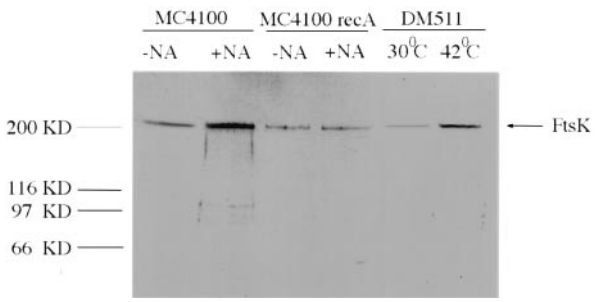

Fig. 8. FtsK is induced as part of the SOS response. A Western blot was used to examine the FtsK level 60 min after nalidixic acid treatment of MC4100 and MC4100 *recA*. The FtsK level was also examined before and 60 min after shifting a *lexA* (Ts) strain (DM511) to 42°C. The molecular weight markers are as in Fig. 7.

Overexpression of FtsK confers increased resistance to mitomycin C

To determine the biological significance of *ftsK* induction by DNA damaging agents, we tested the sensitivity of W3110::P*lac–ftsK* to the DNA-damaging agent mitomycin C (MC). We determined that 0.05 mM IPTG was the minimal concentration of IPTG that produced normal cellular morphology and growth of this strain on plates. At this concentration of IPTG, we found that 0.1 μg ml^{-1} MC reduced colony formation (Table 1). Therefore, colony formation was assessed in the presence of 0.1 μg ml^{-1} MC and different IPTG concentrations. The survival was 1% in the presence of 0.05 mM IPTG, but this increased to 71% in the presence of 1 mM IPTG (Table 1). To ensure that this effect was not due to permeability, we also examined the sensitivity to UV. W3110::P*lac–ftsK* was exposed to UV for 30 s, and survivors were determined on 0.05 mM IPTG and 1 mM IPTG. The survival rate was increased 10-fold at the higher IPTG concentration. These results indicate that overexpression of FtsK provides protection to DNA damage and is an additional SOS mechanism for mediating resistance to DNA-damaging agents such as mitomycin C and UV.

Discussion

The *ftsK* gene was thought to be a cell division gene that acted late in septation because a temperature-sensitive mutation in the gene produced filaments with deep constrictions (Begg *et al*., 1995). However, the gene did not appear to be absolutely essential because the temperature-sensitive mutation could be suppressed by deletion of *dacA*, and a transposon insertion had been isolated near the centre of the gene (Begg *et al*., 1995; Diez *et al*., 1997). Furthermore, the *B. subtilis* homologue *spoIIIE* is not essential (Sharpe and Errington, 1995). However, by constructing a strain in which we could restrict *ftsK* expression, we found that *ftsK* was essential and that depletion of FtsK led to formation of filaments lacking observable constrictions. Interestingly, the only region of this large gene that is essential encompasses the first 202 codons, which encode the hydrophobic domain.

The smooth filamentation morphology caused by depletion of FtsK, suggested that FtsK acts early in septation. This contrasts with the deeply constricted filaments observed with the temperature-sensitive *ftsK44* mutation. We assume that the mutant protein has partial activity at the non-permissive temperature. Another difference between that *ftsK44* (Ts) strain and the depletion strain we used, is that *ftsK44* (Ts) is suppressed by deletion of *dacA* whereas the lack of *ftsK* expression cannot be. Thus, the suppression mechanism is likely to involve restoration of activity of the mutant protein's activity and not a bypass of its function.

Like other cell division proteins that have been examined, we found that FtsK is localized to the septum. Consistent with the observed smooth morphology observed with the depletion of FtsK, indicating that FtsK acts early in septation, we observed that FtsK is localized in 70% of cells including cells lacking visible constrictions. Furthermore, we observed that the localization of FtsK was dependent upon FtsZ and FtsA but independent of FtsI, FtsQ and presumably FtsN. Consistent with this order of addition of proteins to the septum, we observed that FtsZ, FtsA and ZipA were localized in FtsK-depleted cells. From these results the model for assembly of proteins to the septum can be expanded. In this model FtsZ polymerizes into protofilaments that assemble into the Z ring. This leads to the recruitment of FtsA and possibly ZipA. The recruitment of FtsA in turn leads to the recruitment of FtsK, FtsN and FtsI (Addinall *et al*., 1997; Wang and Lutkenhaus, 1998). Once assembled, this septal ring complex brings about invagination of the cytoplasmic membrane and a coupled synthesis of the cell wall. After this work was submitted, Yu *et al*. (1998) reported localization results for FtsK similar to ours. They found that a FtsK–GFP fusion was localized to the septum and that it required FtsZ and FtsA but not FtsI.

Previous work indicated that *ftsK* was preceded by the *dinH* promoter, suggesting that *ftsK* was SOS inducible (Begg *et al*., 1995; Lewis *et al*., 1992). We have confirmed that *ftsK* is part of the SOS regulon requiring both *recA* and *lexA* to be induced by DNA damage. The SOS induction of FtsK appears to have a role in recovering from DNA damage. In our experimental system we used higher levels of IPTG to mimic SOS induction and found that this led to increased resistance to mitomycin C and UV. How does

Table 1. Survival of W3110::P*lac–ftsK* with mitomycin C.[a]

IPTG (mM)	0.05	0.07	0.1	1
Survival[b] (%)	0.92	2.5	4.5	71

a. The concentration of mitomycin C used was 0.1 μg ml^{-1}.
b. Survival is the ratio of the colony-forming units on media with IPTG and mitomycin C to those on media with IPTG only.

FtsK mediate this response? We have observed that addition of nalidixic acid or mitomycin C leads to rapid loss of FtsZ and FtsK localization. As cells recover, FtsK again localizes along with FtsZ so that it would be positioned at the septum. Here, it could function to move chromosomes, whose partition has been hindered by the DNA damage, out of the way of the septum as has been postulated for SpoIIIE in *B. subtilis* (Sharpe and Errington, 1995).

The sequence comparisons and functional studies of FtsK and SpoIIIE suggest similarities and differences between these two proteins. Both proteins have a similar organization with a hydrophobic N-terminal domain connected to a highly homologous C-terminal domain containing a nucleotide binding sequence. A difference is that FtsK contains a large spacer between these domains that is proline and glutamine rich (Begg *et al*., 1995). Both proteins are localized to the septum; however, FtsK is essential and localized early before constriction begins, whereas SpoIIIE is not essential and appears to be localized only when the septum is nearing completion. Thus, the N-terminal domain of FtsK has an additional function not present in SpoIIIE. Either this function is not required in *B. subtilis* or it is performed by another protein. Furthermore, the difference in the timing of the appearance of these two proteins at the septum, FtsK early and SpoIIIE late, implies that the mechanism for localization is different, although in both cases it appears to be the N-terminal hydrophobic domain that is required.

Experimental procedures

Bacterial strains

The strains used in this study are all derivatives of *E. coli* K-12 strains, including W3110, MC4100T(*leu*::Tn*10*), MCZ84 [*leu*::Tn*10 ftsZ84* (Ts)], MCA12 [*leu*::Tn*10 ftsA12* (Ts)], MCI23 [*leu*::Tn*10 ftsI23* (Ts)], and MCQ1 [*ftsQ1* (Ts)] and have been described previously (Addinall *et al*., 1996). CS12-7 (*dacA*) and its isogenic parent CS109 were obtained from K. Young (Henderson *et al*., 1997). DM511 [*lexA* (Ts)] was obtained from D. Mount (Mount *et al*., 1973).

General methods

The various DNA manipulations were carried out as described by Sambrook *et al*. (1989). Bacteria were grown in LB supplemented with arabinose and antibiotics, as described previously (Addinall *et al*., 1996).

Plasmid constructions

pWL71, pWL72, pWL73, pWL74 are plasmids that express various truncated N-terminal FtsKs under the control of the arabinose promoter. pWL75 expresses the full-length FtsK. To make these constructs, the sequences from nucleotide −14 upstream of *ftsK* to the corresponding codons within the *ftsK* open reading frame (ORF) were amplified and cloned into the pBAD18 vector (Guzman *et al*., 1995). pWL76 is a construct that expresses a fusion protein that has the N-terminal 19 amino acid residues of FtsK fused to the C-terminal 693 amino acid residues. To do this, a DNA fragment that corresponds to amino acid residues 639–1329 of FtsK was amplified and cloned into pGEM-T vector (Promega). Then, this C-terminal fragment was blunt-end ligated with the N-terminal 19 codons on pWL74 at the *Not*I site. The resultant plasmid, pWL76, was sequenced to confirm that the fusion junction was in-frame. The pWL71–3 constructs were made such that the truncated *ftsK* fragment was fused to the 5′ end of GFP. The expression of fusion protein was controlled by arabinose promoter. To do this, GFP (mut2) lacking the start codon was amplified (Cormack *et al*., 1996) and cloned into plasmid vector pBAD18. The resultant plasmid was named pWL70 and contained the polylinker at the 5′ end of GFP. Next, the DNA fragment from nucleotide −14 upstream of *ftsK* and the truncated *ftsK* ORF from codon 1–251 of *ftsK* were amplified from the W3110 chromosome and cloned into vector pWL70. The primers used for amplification contained restriction sites to facilitate cloning. All the GFP fusion constructs and *ftsK* expression constructs have the ribosome binding site (RBS) for *ftsK* but do not contain *dinH* promoter of *ftsK*. pAM1 is a pGB2 derivative containing *lacI*^{q} and is compatible with ColE1 vectors (Gil, 1990).

Construction of the FtsK depletion strain W3110::Plac–ftsK

The *lac* promoter was placed upstream of the *ftsK* gene by recombination. We constructed a temperature-sensitive plasmid pUCK37TK carrying *lrp* and a portion of the *ftsK* ORF with a kanamycin cassette, *rrnB* terminators and the *lac* promoter inserted between the *dinH* promoter and RBS site of *ftsK*. By integration and resolution of pUCK37TK from the chromosome, we obtained the desired strain. pUCK37TK was constructed by the following steps. First, a DNA fragment containing a small portion of *trxB*, *lrp* and the intervening sequence between *lrp* and *ftsK* was amplified by PCR and cloned into pGEM-T vector to give pGEMK34. Next, a 400 bp *ftsK* fragment containing the RBS site and 5′ end of *ftsK* obtained by PCR was cloned into pGEM-T to give pGEMK67. Then the fragments from pGEMK34 and pGEMK67 were subcloned into the pGEM-T vector in the same orientation to give pGEMK37. The kanamycin cassette from pUC-4K (Pharmacia) was inserted between *lrp* and *ftsK* on pGEMK37 at nucleotide −14 upstream of *ftsK* ORF in the opposite orientation of *ftsK*. Then, four copies of *rrnB* terminators from pRS551 (Simons *et al*., 1987) were subcloned between the kan cassette and *ftsK* in the same orientation as *ftsK*. The entire engineered DNA fragment was then subcloned into the temperature-sensitive replicon pEL3 (Armstrong *et al*., 1984). Finally, the *lac* promoter from pUC19 was inserted between the terminators and *ftsK* in the same orientation as *ftsK* to give plasmid pUCK37TK.

Plasmid pUCK37TK was transformed into W3110 and transformants selected on kan plates at 30°C. Ten colonies were pooled and grown overnight at 30°C and plated at 42°C to select co-integrates in the presence of various IPTG concentrations. The resultant colonies were grown (all steps in the presence of IPTG) at 30°C for 72 h to allow the resolution of the co-integrates and then plated at 30°C. These colonies

were replica-plated onto Amp and Kan plates containing IPTG to screen for Kan^R/Amp^S colonies. The Kan^R/Amp^S colonies were further tested for IPTG dependency and designated W3110::P*lac–ftsK*.

Antisera against FtsK

To raise anti-FtsK antiserum a DNA fragment corresponding to amino acid residues 447–729 of FtsK was PCR amplified from W3110 chromosomal DNA and fused to GST in pGEX-5X-1 (Pharmacia) to give plasmid pGST–FtsK. DH5α containing pGST–FtsK overexpressed the GST–FtsK fusion protein, which was affinity purified with glutathione sepharose 4B (Pharmacia) and sent to Cocalico Biologicals for preparation of rabbit antiserum. The FtsK antiserum obtained was purified by passing it over a column containing a cytoplasmic extract from a strain overproducing GST and further affinity purified by binding and elution to GST–FtsK bound to a nitrocellulose membrane as follows: (i) the antiserum was first passed over an Affi-Gel column containing a cytoplasmic extract prepared by high-speed centrifugation (to remove membranes containing FtsK) of an extract from a culture induced for expression of GST; and (ii) 4 ml of the flowthrough from this column was incubated for 4 h at room temperature on a rocking platform with a nitrocellulose membrane containing GST–FtsK (transferred from an SDS–PAGE) that had been incubated for 1 h at room temperature in PBS buffer containing 5% dried milk and 0.1% Tween 20. Bound antibodies were eluted with 1 ml of glycine–HCl buffer (pH 2.5) at room temperature for 15 min after the nitrocellulose filter had been washed three times with PBS for 15 min. The eluted antibody was dialysed overnight at 4°C against PBS and frozen at −70°C.

Cell fractionation and Western blot analysis

MC4100 was grown to exponential phase, harvested, suspended in buffer A (50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 1 mM DTT, 50 mM NaCl, 0.5 mM PMSF and 10% glycerol) and broken with a French press. The cell lysates were centrifuged at 10 000 r.p.m. for 10 min to remove unbroken cells and cell debris. The low-speed supernatant was centrifuged at 40 000 r.p.m. for 1 h to obtain a crude membrane pellet and cytoplasmic fraction. The membrane pellet was washed once with buffer A. The whole cell lysate, low-speed supernatant, membrane and cytoplasmic fractions were resolved on 8% SDS–PAGE and transferred onto a nitrocellulose membrane. The affinity-purified FtsK antibody was used at 1:250.

Induction of FtsK as part of the SOS response and measurement of mitomycin C resistance

To examine FtsK induction by nalidixic acid, exponentially growing cultures of MC4100 or MC4100 *recA* were incubated in LB with 25 μg ml^{-1} nalidixic acid for 1 h. FtsK induction was also monitored after shifting an exponentially growing culture of a *lexA* (Ts) strain (DM511) from 30°C to 42°C for 1 h. Samples from the cultures were mixed with an equal volume of ice-cold 10% TCA, centrifuged, the pellet washed with acetone, resuspended in 8 M urea, mixed with an equal volume of 2× SDS sample buffer and analysed by Western blotting. To measure viability in the presence of mitomycin C, overnight cultures of W3110::P*lac–ftsK* were diluted and plated on the LB plates with different concentrations of IPTG. It was determined that 0.05 mM IPTG was the minimal IPTG concentration for the depletion strain to grow. Next, a series of mitomycin C plates with 0.05 mM IPTG were prepared, containing twofold dilutions of the drug. It was observed that 0.1 μg ml^{-1} mitomycin C inhibited colony formation. Overnight cultures of W3110::P*lac–ftsK* were diluted and plated out on mitomycin C (0.1 μg ml^{-1}) plates with different concentrations of IPTG to determine the viability.

Immunofluorescent staining and microscopy

Cells were prepared for immunofluorescent staining as described previously (Addinall *et al*., 1996). Fixed cells were incubated with affinity-purified anti-FtsK antibody (1:1000). The affinity-purified FtsZ antibodies were described previously (Addinall *et al*., 1996). The secondary antibody was conjugated to the fluorophore Cy3 (Jackson Immunoresearch). Cells were photographed with a Nikon Optiphot fluorescence microscope equipped with a filter block containing a 517–552 nm excitation filter and a 590 nm barrier filter (XF34, Omega Optical).

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