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Fully efficient chromosome dimer resolution in *Escherichia coli* cells lacking the integral membrane domain of FtsK

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In bacteria, septum formation frequently initiates before the last steps of chromosome segregation. This is notably the case when chromosome dimers are formed by homologous recombination. Chromosome segregation then requires the activity of a double-stranded DNA transporter anchored at the septum by an integral membrane domain, FtsK. It was proposed that the transmembrane segments of proteins of the FtsK family form pores across lipid bilayers for the transport of DNA. Here, we show that truncated *Escherichia coli* FtsK proteins lacking all of the FtsK transmembrane segments allow for the efficient resolution of chromosome dimers if they are connected to a septal targeting peptide through a sufficiently long linker. These results indicate that FtsK does not need to transport DNA through a pore formed by its integral membrane domain. We propose therefore that FtsK transports DNA before membrane fusion, at a time when there is still an opening in the constricted septum.

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Introduction

During cell proliferation, the timing of DNA synthesis, chromosome segregation and cell division must be coordinated to ensure the stable inheritance of the genetic material. In eukaryotes, this is achieved by the temporal separation of these processes and the existence of checkpoint mechanisms that delay certain steps until others are completed. Likewise, nucleoid occlusion normally prevents the initiation of septum formation in the presence of un-segregated DNA at the site of division in bacteria (Figure 1A (i); Wu and Errington, 2004; Bernhardt and de Boer, 2005). Nevertheless, septum formation frequently initiates before the last stages of chromosome segregation. This is notably the case during sporulation in *Bacillus subtilis* and during chromosome dimer resolution in *Escherichia coli* (Figure 1A (ii) and (iii); see Errington *et al*, 2001; Barre, 2007 for a review). The correct distribution of the genetic material is then achieved by the respective activities of two related double-stranded DNA transporters, SpoIIIE and FtsK (Bath *et al*, 2000; Aussel *et al*, 2002).

The assembly of SpoIIIE at the sporulation septum of *B. subtilis* (Figure 1A (ii)) correlates with the establishment of a barrier to protein and membrane dye diffusion between the mother cell and the prespore compartments (Wu and Errington, 1994; Wu *et al*, 1995; Liu *et al*, 2006; Burton *et al*, 2007), which suggested that SpoIIIE transports DNA after septal membrane fusion (Liu *et al*, 2006; Becker and Pogliano, 2007; Burton *et al*, 2007). In *E. coli* and in most proteobacteria, chromosome dimers are resolved into monomers by two tyrosine recombinases, XerC and XerD, which add a crossover at a specific chromosomal site, *dif* (Bigot *et al*, 2007; Val *et al*, 2008). FtsK has two successive roles in this process: it mobilizes DNA to bring together the two *dif* sites carried by a dimer and it activates Xer recombination at *dif* by a direct contact with XerD (Figure 1A (iii)). Although FtsK is recruited at an early stage of septum formation, Xer recombination at *dif* occurs at a later stage, concomitantly with cell wall constriction (Figure 1A (iii); Kennedy *et al*, 2008). This observation suggested that FtsK might initiate DNA transport after membrane fusion like SpoIIIE.

The carboxy-terminal domains of SpoIIIE and FtsK include a RecA-type ATPase fold (Figure 1B, translocation module), which forms hexamers around double-stranded DNA (Massey *et al*, 2006) and uses the energy of ATP to translocate (Saleh *et al*, 2004, 2005). The same module is found in the transfer (Tra) proteins of various conjugative plasmids of *Streptomycetes* (Figure 1B; (Grohmann *et al*, 2003)). SpoIIIE, FtsK and the Tra proteins also share a common structural organization, which includes an integral membrane domain (Figure 1B). In view of this organization, it was proposed that their integral membrane domains form a pore in lipid bilayers across which DNA is transported in a process akin to conjugation (Liu *et al*, 2006; Becker and Pogliano, 2007; Burton *et al*, 2007).

Previous structural and functional analysis indicated that *E. coli* FtsK is composed of an amino-terminal domain with four transmembrane segments that is essential for cell division ($FtsK_N$, aa 1–210; Figure 1B; Begg *et al*, 1995; Diez *et al*, 1997; Draper *et al*, 1998; Liu *et al*, 1998; Wang and Lutkenhaus, 1998; Yu *et al*, 1998; Dorazi and Dewar, 2000), a long linker region of low complexity ($FtsK_L$; aa 211–817; Figure 1B; Bigot *et al*, 2004) and a carboxy-terminal domain that contains all the necessary determinants for DNA translocation and Xer recombination activation ($FtsK_C$; aa 818–1329; Figure 1B; Barre *et al*, 2000; Aussel *et al*, 2002; Yates *et al*, 2003; Bigot *et al*, 2004). It was observed that a truncation of FtsK lacking most of FsK_N and $FtsK_L$ did

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not support chromosome dimer resolution even if it could efficiently process plasmid dimers *in vivo* (Aussel *et al*, 2002), which fitted with the idea that $FtsK_N$ and $FtsK_L$ might be implicated in the formation of a pore for the transport of chromosomal DNA at cell division.

To gain further insights into the function of $FtsK_N$ and $FtsK_L$ in DNA transport, we decided to investigate the efficiency of chromosome dimer resolution of *E. coli* cells carrying new mutant *ftsK* alleles in which the coding regions of $FtsK_N$ and $FtsK_L$ were modified, but in which the coding region of $FtsK_C$ was left intact. We report that FtsK peptides lacking all of the transmembrane segments of $FtsK_N$ allow for the efficient resolution of chromosome dimers if they are connected to a septal targeting peptide through a sufficiently long linker. These results indicate that the transport of chromosomal DNA across lipid bilayers during the last stage of chromosome segregation in *E. coli* does not require the formation of a pore by $FtsK_N$ and $FtsK_L$. We propose therefore that *E. coli* FtsK most likely transports DNA before membrane fusion, at a time when there is still an opening in the constricted septum.

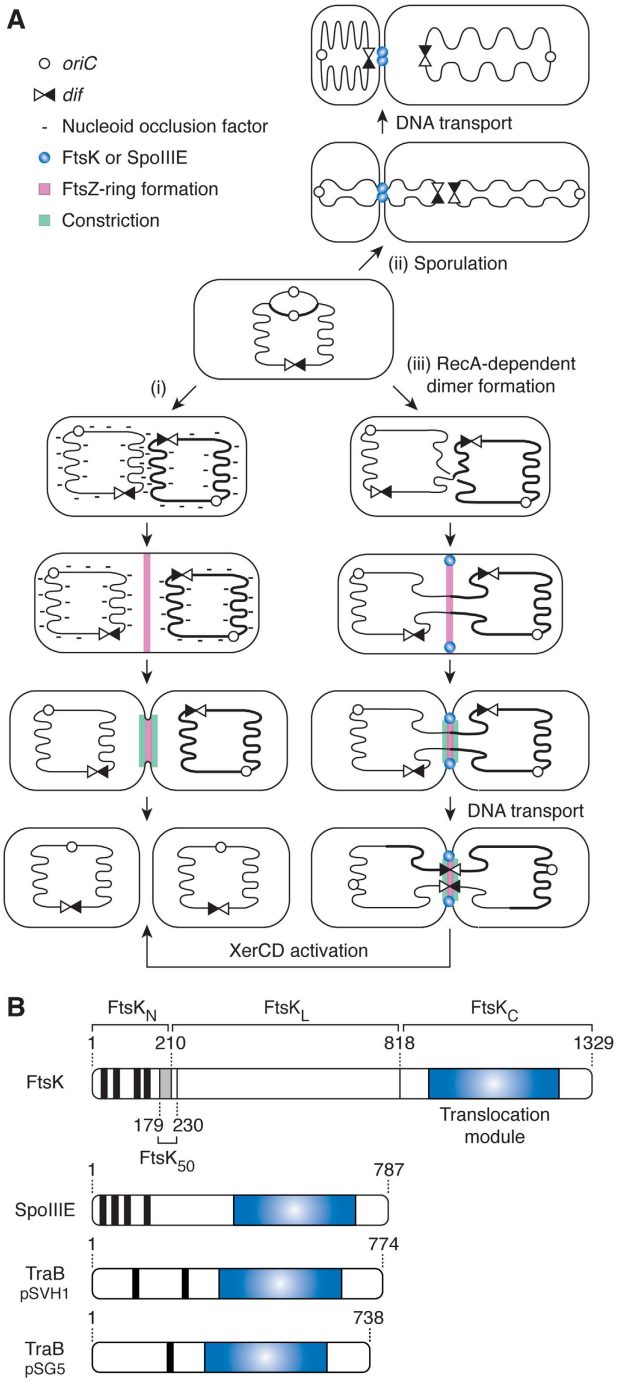

Figure 1 DNA traffic in bacteria. (**A**) Coordination between chromosome segregation and cell division in *B. subtilis* and *E. coli*. (i) Normal vegetative cell division in *E. coli* or *B. subtilis*. When the bulk of the sister chromosomes remains at mid-cell, nucleoid occlusion factors prevent FtsZ-ring formation. Segregation of the nucleoids away from mid-cell allows the recruitment of the division machinery. (ii) Sporulation in *B. subtilis*. Only a third of the chromosome is trapped in the prespore compartment during the asymmetric cell division of sporulation. SpoIIIE transports the remaining two-third of the chromosome. (iii) Chromosome dimer resolution in *E. coli*. RecA-mediated homologous recombination during replication can lead to the formation of dimers of the circular chromosome. Replication and segregation proceed until the bulk of the sister chromosomes are distributed in each daughter cell, but the two nucleoids remain linked by DNA passing through the division septum. FtsK-dependent DNA transport brings *dif* sites together. FtsK subsequently activates Xer recombination to resolve the dimer into monomers. (**B**) Schematic representation of FtsK, SpoIIIE and the Tra proteins of two *Streptomycetes* conjugative plasmids. Transmembrane domains are depicted as vertical black bars and translocation modules as blue rectangles. Numbers indicate the position of amino acid residues. A grey rectangle indicates the part of $FtsK_N$ that belongs to a ~50 amino acid region ($FtsK_{50}$; aa 179–230) that increases the efficiency with which FtsK peptides carrying an intact C-terminal domain activate Xer recombination under low expression levels.

Results

Experimental strategy

The role of $FtsK_N$ and $FtsK_L$ in DNA transport could not be directly addressed because both domains are implicated in cell division and because $FtsK_N$ is essential (Bigot *et al*, 2004). To circumvent this difficulty, we made use of a hyperactive mutant of FtsA, FtsA*, which can partially bypass the need for $FtsK_N$ in cell division, so that the entire *ftsK* gene can be deleted (Geissler and Margolin, 2005; Geissler *et al*, 2007). FtsK mutants were introduced in *ftsK*$^-$ *ftsA** cells on a low copy pSC101 vector and under the natural *ftsK* promoter to prevent overproduction or deregulation of their expression (Bigot *et al*, 2004; Kennedy *et al*, 2008). We could thus monitor, in the absence of any additional functional copy of $FtsK_N$, if FtsK peptides mutated in $FtsK_N$ and $FtsK_L$ supported DNA transport by measuring chromosome dimer resolution.

A good estimation of the efficaciousness of chromosome dimer resolution in a given strain is provided by the excess number of viable cells produced at each generation when compared with an isogenic strain totally deficient in chromosome dimer resolution. We therefore monitored the growth advantage that mutant *ftsK* alleles provide to *ftsA** *ftsK*$^-$ cells compared with *ftsA** *ftsK*$^-$*dif*$^-$ cells, in which the deletion of *dif* abolishes dimer resolution. Note that the difference in fitness of *ftsA** *ftsK*$^-$ and *ftsA** *ftsK*$^-$*dif*$^-$ cells producing a given FtsK peptide only reflects the ability of this peptide to sustain chromosome dimer resolution as any other effect that

its production might have on growth, such as by interfering with cell division, will be identical in the two cell lines.

The expression and the localization of the different *ftsK* alleles were checked using fusions with green and/or yellow fluorescent proteins (GFP and YFP) expressed from a low copy pSC101 vector under the natural *ftsK* promoter. Their ability to activate Xer recombination, which reflects the formation of stable and active $FtsK_C$ hexamers on DNA, was checked using a pseudo-dimer plasmid recombination assay (Barre *et al*, 2000). To this aim, the *ftsK* alleles were expressed from an arabinose promoter on a high copy vector and we measured the frequency of excision of a DNA segment placed between two direct tandem repeats of *dif* on a pSC101 plasmid.

Chimaeras between $FtsK_{LC}$ and ZipA or FtsW support chromosome dimer resolution

We reported earlier that a truncated protein lacking the whole of the N-terminal domain of FtsK, $FtsK_{LC}$ (Figure 2B), supports *dif*-recombination on plasmids (Barre *et al*, 2000). $FtsK_{LC}$ is efficiently produced from a pSC101 vector under the *ftsK* promoter and is stable (Supplementary Figure 1). However, it provided no growth advantage to *ftsA* ftsK*$^-$ cells compared with *ftsA* ftsK*$^-$*dif*$^-$ cells (Figure 2D), indicating that it is completely inactive in chromosome dimer resolution. Actually, $FtsK_{LC}$::YFP yielded a diffuse cytoplasmic signal (Figure 2C), indicating that $FtsK_L$ and $FtsK_C$ do not carry sufficient determinants for septum localization. We decided therefore to test the activity of FtsK chimaeras that are anchored at the site of division by a fusion to another integral membrane cell division protein. To this aim, we fused $FtsK_{LC}$ to FtsW and ZipA, the only two integral membrane cell division proteins that have a C-terminal cytoplasmic tail like FtsK (Figure 2A). FtsW is anchored to the membrane by 10 transmembrane helices and arrives at the septum after FtsK. ZipA is anchored to the membrane by a single transmembrane helix and arrives at the septum before FtsK (Harry *et al*, 2006). ZipA::$FtsK_{LC}$ and FtsW::$FtsK_{LC}$ (Figure 2B) displayed the characteristic localization pattern of cell division proteins, that is one band at the middle of the cell in pre-dividing cells and one focus at the middle of the cell in dividing cells (Figure 2C). In growth competition, they supported chromosome dimer resolution to a similar level than full-length FtsK, indicating that they allowed for the eventual resolution of all occuring dimers (Figure 2D). Similar results were obtained in $FtsK_{LC}^-$ cells, indicating that the activity of ZipA::$FtsK_{LC}$ and FtsW::$FtsK_{LC}$ was not limited to the particular *ftsA** genetic context (data not shown). We conclude that anchoring $FtsK_{LC}$ in the cytoplasmic membrane and targeting it to the septum is sufficient for the resolution of chromosome dimers in *E. coli*, which argues against the idea that formation of a pore by $FtsK_N$ is essential for the transport of DNA across lipid bilayers.

Chromosome dimer resolution by an FtsK truncation lacking transmembrane segments

To rule out the unlikely possibility that the transmembrane segments of ZipA and FtsW could substitute for those of $FtsK_N$ in the formation of a DNA channel across lipid bilayers, we searched for a way to target $FtsK_{LC}$ to the septum in the absence of any transmembrane segments (Figure 3). To this aim, we fused it to ZapA, a cell division protein that is fully

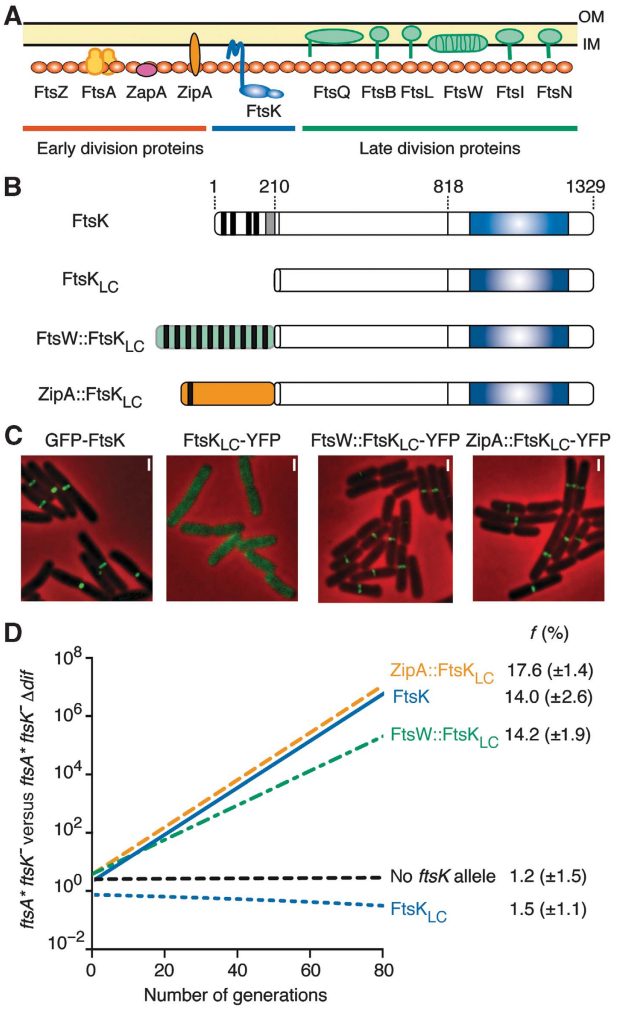

Figure 2 Localization and chromosome dimer resolution activity of $FtsK_{LC}$ chimaeras. (**A**) Schematic view of septum assembly. The protein icons are ordered from left to right according to the commonly accepted assembly sequence. OM, outer membrane; IM, inner membrane. (**B**) Schematic representation of the $FtsK_{LC}$ chimaeras as in Figure 1B. (**C**) Localization of $FtsK_{LC}$ chimaeras fused at their C-terminal ends to YFP and of full-length FtsK fused at its N-terminal end to GFP in *ftsK*$^+$ cells. A similar localization pattern was observed in *ftsA* ftsK*$^-$ and in *ftsK*$_{LC}^-$ cells (data not shown). (**C**) Pictures of *ftsK*$^+$ cells are shown because of their simpler cell division phenotype. Scale bars (white bars), 1 μm. (**D**) Growth competition between *ftsA* ftsK*$^-$ and *ftsA* ftsK*$^-$ Δ*dif* cells. The graph of a typical result is shown. f, mean and standard deviation of the growth advantage given by the ectopic production of each $FtsK_{LC}$ chimaera (result from three independent experiments).

cytoplasmic (Figure 2A; Harry *et al*, 2006). The *zapA*::*ftsK*$_{LC}$ allele (Figure 3A) is totally inactive in chromosome dimer resolution (Figure 3D). However, *zapA*::*ftsK*$_{LC}$ activates *dif*-recombination on plasmids less efficiently than full-length *ftsK*, *zipA*::*ftsK*$_{LC}$ or *ftsW*::*ftsK*$_{LC}$ under low expression levels (Figure 3B). In addition, it is imperfectly recruited to the septum, much of the fluorescent signal observed with its YFP derivative remaining diffuse and cytoplasmic (Figure 3C). Taken together, these results suggested that the fusion of $FtsK_{LC}$ to ZapA interfered either with the stability of the protein, its septum localization and/or its capacity to activate Xer recombination.

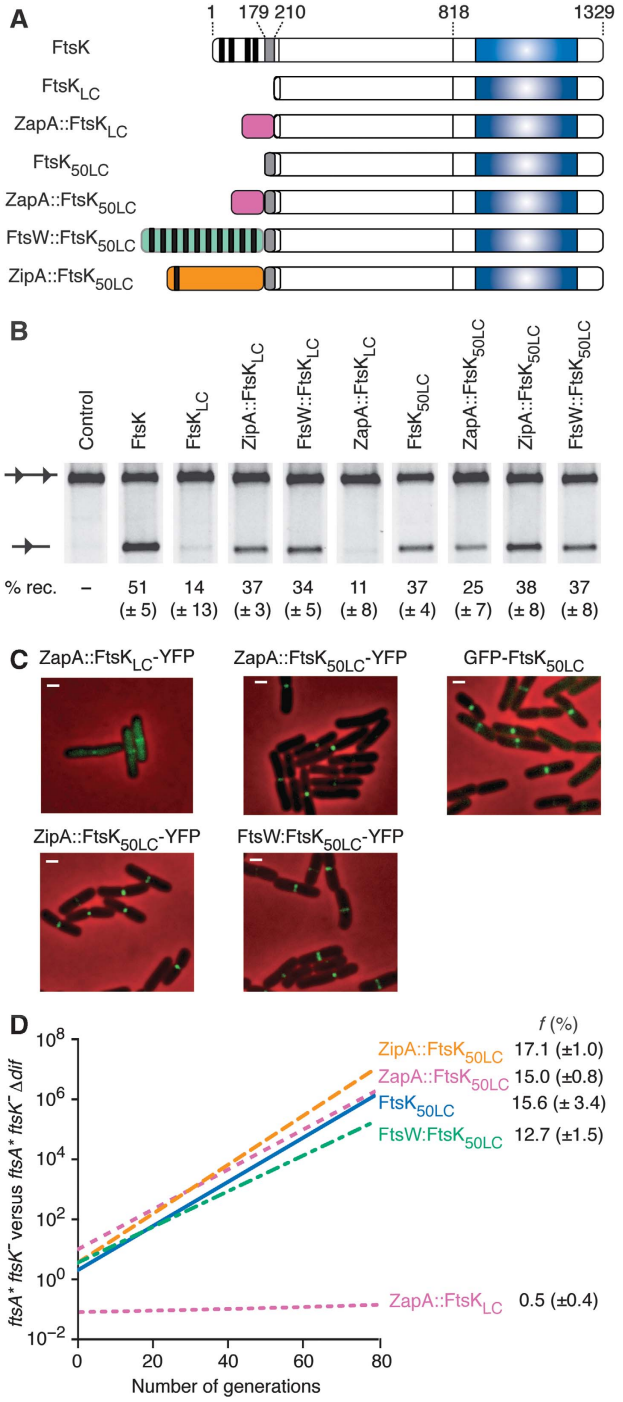

Figure 3 Localization and chromosome dimer resolution activity of FtsK$_{50LC}$ chimaeras. (**A**) Schematic representation of the FtsK$_{50LC}$ chimaeras as in Figure 1B. (**B**) *In vivo* pseudo-dimer resolution assay on plasmids. A scheme of the plasmid-borne substrate and product is shown on the left of the gel. *dif* sites are represented as black triangles. The efficiency of recombination was determined after 4 h of growth in 0.2% arabinose. (**C**) Localization of FtsK$_{50LC}$ chimaeras fused at their C-terminal ends to YFP as in Figure 2B. (**D**) Growth competition between *ftsA* ftsK*$^-$ and *ftsA* ftsK*$^-$ Δ*dif* cells ectopically producing the FtsK$_{50LC}$ chimaeras as in Figure 2C.

We characterized earlier a region of ~50 amino acids (FtsK$_{50}$; aa 179–230; Figure 1B) that increase the efficiency with which FtsK peptides carrying an intact C-terminal domain activate Xer recombination under low expression levels (Aussel *et al*, 2002). Only half of this region was present in our initial chimaeras (aa 211–230; Figure 3A). We decided therefore to include the rest of this region (aa 179–210) in our ZapA fusion to increase its capacity to activate Xer recombination. The *zapA*::*ftsK*$_{50LC}$ allele yielded 25% (±7) of cassette excision compared with 11% (±8) for *zapA*::*ftsK*$_{LC}$ in the plasmid Xer recombination assay (Figure 3B). We checked by SDS–PAGE that this was not linked to a major change in the stability and/or production of the chimaera (Supplementary Figure 2), which prompted us to test its capacity to resolve chromosome dimers. Indeed, the *zapA*::*ftsK*$_{50LC}$ allele fully supported chromosome dimer resolution (Figure 3D). In addition, we noticed that the presence of FtsK$_{50}$ also corrected the imperfect localization of ZapA::FtsK$_{LC}$ (Figure 3C), which suggested that FtsK$_{50}$ could be involved in the septal recruitment of FtsK. We decided therefore to investigate the localization and activity of FtsK$_{50LC}$. Surprisingly, FtsK$_{50LC}$ displayed a typical cell division localization pattern and supported chromosome dimer resolution (Figure 3C and D). It is very unlikely that FtsK$_{50}$ contains a full transmembrane segment as previous characterization of the membrane topology of FtsK indicated that all the amino acid residues after 146 are in the cytoplasm (Dorazi and Dewar, 2000). Further evidence for this can be taken from our observation that neither the presence of FtsK$_{50}$ alter the pattern of localization of chimaeras between FtsK$_{LC}$ and ZipA or FtsW nor their capacity to resolve chromosome dimers (Figure 3C and D), whereas the addition of an extra transmembrane segment would have either led to the re-location of FtsK$_{LC}$ in the periplasm or to the inversion of the membrane topology of ZipA or FtsW. Thus, we have identified an FtsK truncation, FtsK$_{50LC}$, which allows for the eventual resolution of all occuring dimers even if it lacks transmembrane segments.

Effective dimer resolution depends on the length of the linker

We next investigated the importance of FtsK$_L$ in chromosome dimer resolution. We reported earlier that the direct fusion of FtsK$_N$ to FtsK$_C$ (FtsK$_{NC}$) led to a 70% diminution in chromosome dimer resolution (Bigot *et al*, 2004), even if the chimaera supported *dif*-recombination on plasmids (Barre, unpublished results). However, *ftsK*$_{NC}$ cells displayed a filamentous phenotype that was independent from their deficiency in chromosome dimer resolution (Bigot *et al*, 2004), suggesting that this fusion interfered with the function of FtsK$_N$ in septum and/or pore formation. We therefore decided to introduce linker deletions in the coding region of the *ftsW*::*ftsK*$_{LC}$ allele and to test their activity in the *ftsA* ftsK*$^-$ background, that is in a genetic context in which the loss of fitness cannot be attributed to the role(s) played by FtsK$_N$ and FtsK$_L$ in cell division and/or pore formation (Figure 4A). A 500 aa deletion of the linker led to a 70% reduction of chromosome dimer resolution (Figure 4A), even if the truncated peptide supported *dif*-recombination on plasmids to a similar level than full-length *ftsK* (Figure 4B) and was efficiently recruited to the septum (Figure 4C). However, shorter deletions did not, or only slightly, affected chromosome dimer resolution, independently of their location in FtsK$_L$ (Figure 4; Δ100–200 aa and Δ300 aa deletions). Similar results were obtained with deletions in the linker domain of the FtsW::FtsK$_{50LC}$ chimaera (data not shown).

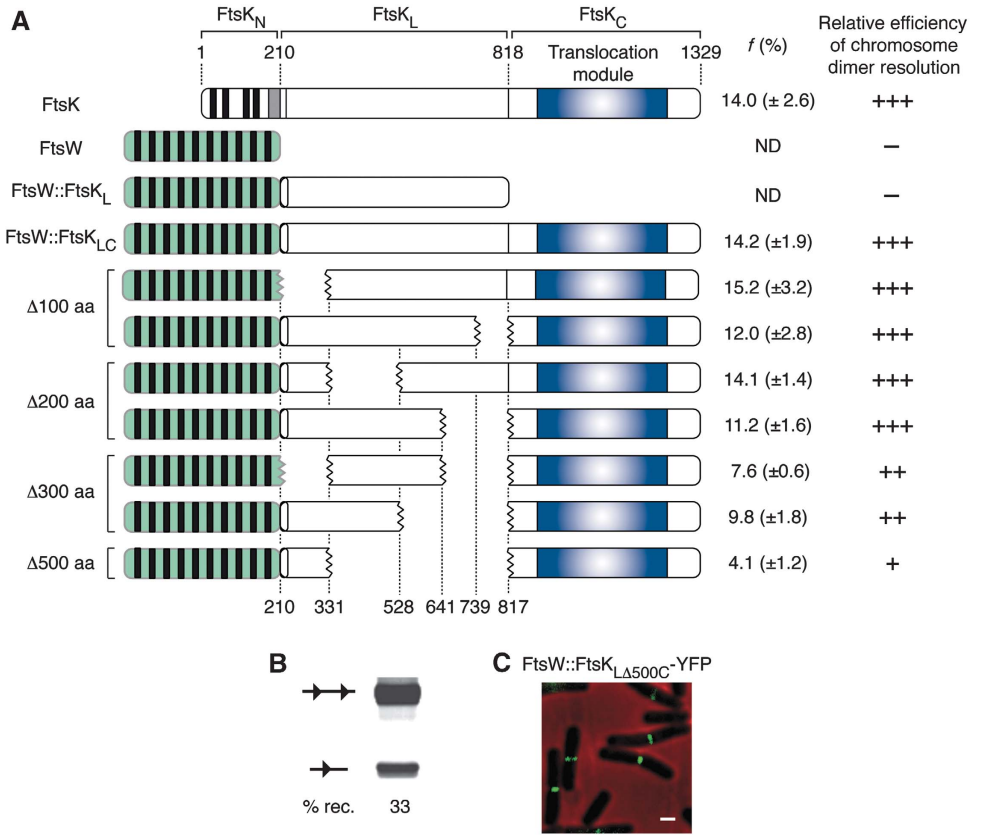

Figure 4 Role of linker in chromosome dimer resolution. (**A**) Scheme of the FtsK peptides tested for their chromosome dimer resolution ability. Numbers on the bottom refer to the amino acid position residues delimiting the different FtsK$_L$ deletions. The range of the deletion size is indicated on the left. f, mean and standard deviation of the growth advantage given by the ectopic production of each FtsK$_{LC}$ chimaera (result from three independent experiments). The relative efficiency of chromosome dimer resolution is indicated as follows: >70% (+++); 35–70% (++); <35% (+); 0% (–). ND, not determined. (**B**) *In vivo* pseudo-dimer resolution assay on plasmids for the FtsW::FtsK$_{LΔ500C}$ chimaera as in Figure 3B. (**C**) Localization of the YFP derivative of FtsW::FtsK$_{LΔ500C}$ as in Figure 2C.

We conclude that no specific region of FtsK$_L$ is implicated in chromosome dimer resolution, but that efficacious chromosome dimer resolution depends on the total length of the linker domain.

Fully efficient resolution of chromosome dimers by chimaeras lacking the FtsK transmembrane segments

The competition assay between *ftsA* ftsK*$^-$ cells and *ftsA* ftsK*$^-$*dif*$^-$ cells provides a good estimate of the ability of a protein to support dimeric chromosome resolution. However, it does not provide a direct estimate of the efficiency of the process as the latter requires an assessment of the time and energy that are spent to eventually achieve it. Nevertheless, we noticed in the course of our growth competition experiments that *dif*$^+$ *ftsA* ftsK*$^-$ cells expressing FstK chimaeras that were fully active in dimer resolution yielded a similar number of colony forming units after 4 days of growth than *dif*$^+$ *ftsA* ftsK*$^-$ cells expressing full-length FtsK, which suggested that these chimaeras efficiently resolved chromosome dimers (data not shown).

To further investigate the efficiency with which FtsK chimaeras resolved chromosome dimers, we decided to directly compare the growth of cells expressing them with the growth of cells expressing full-length FtsK. However, this must not be done in the *ftsA* ftsK*$^-$ background because these chimaeras do not necessarily compensate for the division defect of *ftsA** *ftsK*$^-$ cells in contrast to full-length FtsK. Consequently, we compared the growth of FtsK$^-_{LC}$ cells ectopically expressing FtsK chimaeras from a low copy pSC101 plasmid under the *ftsK* promoter to the growth of FtsK$^-_{LC}$ cells ectopically expressing full-length FtsK (Figure 5A). FtsK$^-_{LC}$ cells expressing FtsK$_{LC}$ had a growth disadvantage of 15.7% per cell per generation over cells expressing full-length FtsK (Figure 5A), which fits with the estimated rate of dimer formation (Steiner and Kuempel, 1998; Perals *et al*, 2001). In contrast, FtsK$^-_{LC}$ cells expressing FtsW::FtsK$_{LC}$, FtsW::FtsK$_{50LC}$ and FtsK$_{50LC}$ had the same fitness than cells expressing full-length FtsK, suggesting that these three chimaeras are fully efficient in chromosome dimer resolution (Figure 5B). Note that this result also indicates that the ectopic expression of these chimaeras did not interfere with other cellular processes than chromosome dimer resolution such as cell division.

The phenotype of FtsK$^-_{LC}$ cells ectopically expressing ZipA::FtsK$_{LC}$ or ZipA::FtsK$_{50LC}$ from a low copy pSC101 vector under the *ftsK* promoter suggested that the resulting overexpression of ZipA altered the process of division (data not shown), which would confound the growth competition result. Consequently, we had to use a second approach to assess the efficiency with which ZipA::FtsK$_{LC}$ processes chromosome dimers. We described earlier how Xer recombination can be monitored in real time using quantitative PCR

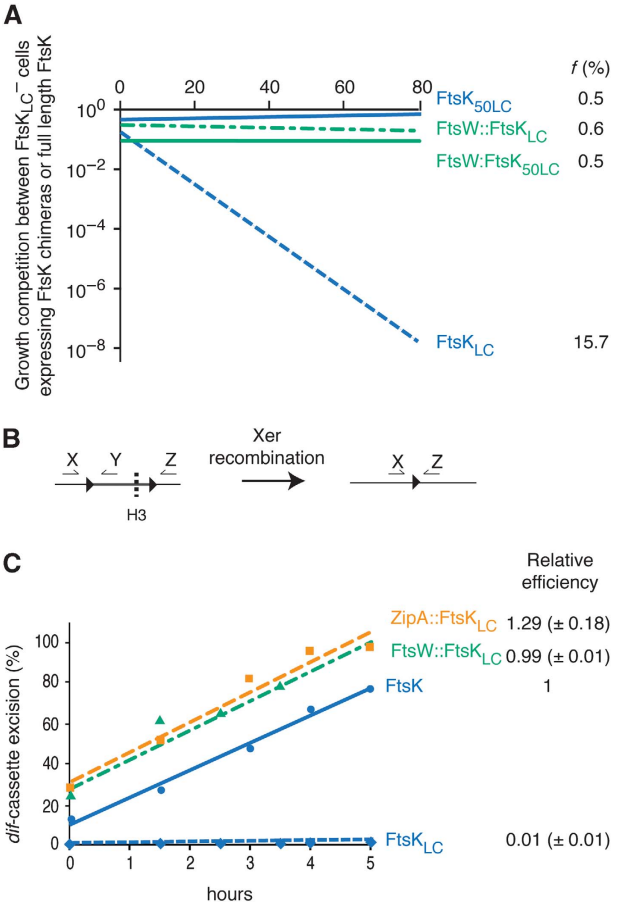

Figure 5 Fully efficient chromosome dimer resolution by FtsK chimaeras. (**A**) Growth competition between *ftsK*$_{LC}^-$ cells ectopically expressing wild-type FtsK or FtsK chimaeras from a low copy vector under the *ftsK* promoter. f represents the growth disadvantage of the strain producing the FtsK chimaera compared to the strain producing wild-type FtsK. (**B**) Scheme of the chromosomal *dif*-cassette (left drawing) and of the recombinant product (right drawing) that is used to monitor the efficiency with which FtsK chimaeras induce Xer recombination at the normal *dif* locus on the chromosome. *dif* sites are represented by black triangles. Arrows indicate the positions of the primers used in the qPCR assay. Primers X/Z serve to quantify the relative number of chromosomes in which the DNA cassette between the two repeated *dif* sites is excised (right drawing). To this aim, genomic DNA needs to be digested with *Hin*dIII (dashed line, H3) to prevent contamination by larger products encompassing the cassette. Primers X/Y serve to quantify the relative number of chromosomes that still harbour the *dif*-cassette. (**C**) Chromosomal *dif*-cassette excision on ectopic production of full-length FtsK or FtsK chimaeras. The % of cassette excision was monitored by qPCR at different times after the induction of the production of the *ftsK* alleles. The actual data points and best-fit lines from a typical experiment are shown on the left. The slopes of the best-fit lines provide a good estimate of the rate of cassette excision as a function of time, which allowed us to calculate the relative efficiency of cassette excision of each chimaera when compared with full-length FtsK. The mean relative efficiencies are indicated on the right (results from three independent experiments for ZipA::FtsK$_{LC}$ and FtsW::FtsK$_{LC}$ and from two independent experiments for FtsK$_{LC}$).

in FtsK$_{ATP}^-$ cells habouring two *dif* sites in direct tandem repeat at the normal *dif* locus (Kennedy *et al*, 2008). Briefly, activation of Xer recombination leads to the excision of the *dif*-cassette, which can be detected by PCR using two pairs of primers (Figure 5B). Previous work indicated that in the majority of cases, this only occurs in the presence of a chromosome dimer (Barre *et al*, 2000; Perals *et al*, 2001; Kennedy *et al*, 2008) and at the time of cell division (Kennedy *et al*, 2008). Therefore, this assay allows us to directly address the time needed for the alignment of *dif* sites carried on a chromosome dimer and the subsequent activation of Xer recombination. The assay is only reliable when the production of FtsK is precisely controlled (Kennedy *et al*, 2008). To this aim, full-length FtsK, FtsW::FtsK$_{LC}$, ZipA::FtsK$_{LC}$ and FtsK$_{LC}$ were produced from a low copy pSC101 plasmid under the arabinose promoter. Under these conditions, ZipA::FtsK$_{LC}$ proved to be as efficient as full-length FtsK and FtsW::FtsK$_{LC}$ (Figure 5C). In contrast, FtsK$_{LC}$ did not promote excision of the chromosomal *dif*-cassette (Figure 5C).

Taken together, these results suggest that anchoring FtsK$_{LC}$ in the cytoplasmic membrane and targeting it to the septum is sufficient for the fully efficient resolution of chromosome dimers in *E. coli*.

Discussion

E. coli FtsK transports DNA across a 'pre-existing opening' in the septum

Our *in vivo* analysis of the role(s) played by FtsK$_N$ and FtsK$_L$ in DNA transport in *E. coli* is based on the fact that during chromosome dimer resolution, FtsK needs to bring together the two *dif* sites carried by a chromosome dimer through oriented DNA transport, in addition to activating Xer recombination. Consequently, our observation that FtsW::FtsK$_{LC}$, ZipA::FtsK$_{LC}$, ZapA::FtsK$_{50LC}$ and FtsK$_{50LC}$ support chromosome dimer resolution indicates that these chimaeras are active in DNA transport, even if FtsW and ZipA share no homology with FtsK$_N$ and if ZapA::FtsK$_{50LC}$ and FtsK$_{50LC}$ lack transmembrane segments (Figures 2 and 3). Likewise, no specific region of FtsK$_L$ was essential for chromosome dimer resolution (Figure 4). We conclude that chimaeric FtsK can transport DNA through a 'pre-existing opening' between the two future daughter cells.

The nature of this 'pre-existing opening' is subject to speculations. We cannot exclude that chimaeric FtsK proteins use a pore created by another septal membrane protein than FtsK. However, the most likely explanation for the fully efficient chromosome dimer resolution activity of ZipA::FtsK$_{LC}$, FtsW::FtsK$_{LC}$, FtsW::FtsK$_{50LC}$ and FtsK$_{50LC}$ (Figure 5) is that FtsK transports DNA before the final closure of the septum, at a time when there is still a rather large opening between the two daughter cells. In this model, the length of FtsK$_L$ is important for DNA accessibility. Accordingly, we found that the efficiency of chromosome dimer resolution depended on the length of the linker, independently of its role in cell division (Figure 4). This model also explains our previous observation that Xer recombination activation depends on the initiation of constriction (Kennedy *et al*, 2008) as FtsK$_C$, which is connected to the integral membrane of FtsK by a 600 aa linker that can only span 200 nm, should have a limited access to chromosomal DNA when the protein is distributed along the 800 nm-wide pre-division ring. In addition, this model answers two questions raised by the previous hypothesis of FtsK-dependent DNA transport across a closed septum: (i) how the last segment of a circular double-stranded DNA molecule is

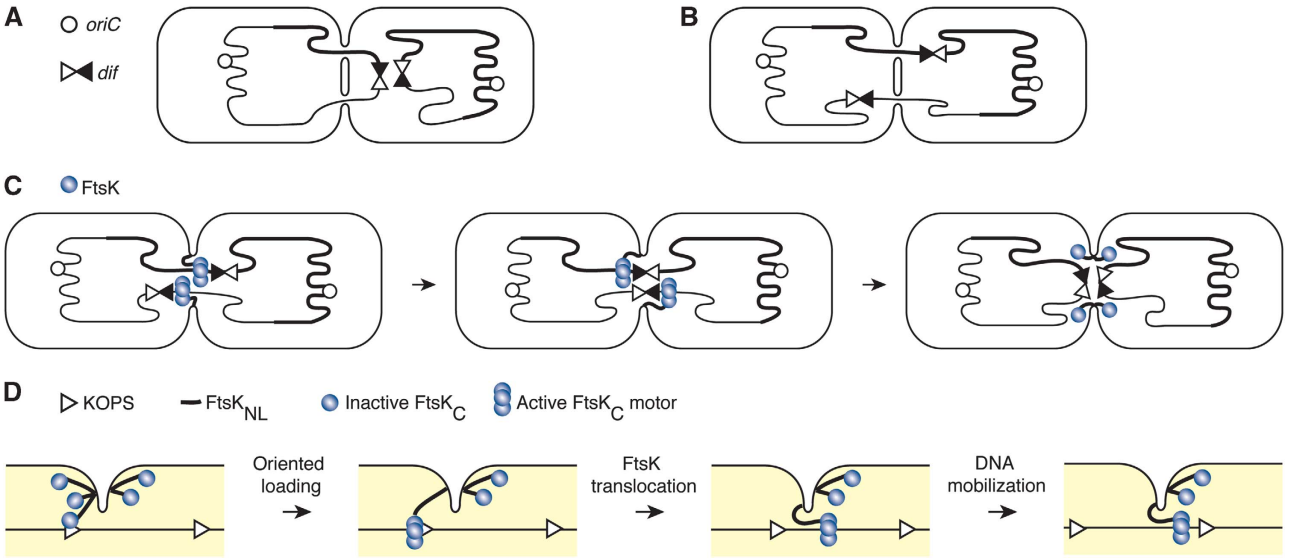

Figure 6 Schematic of DNA transport by FtsK. (**A**, **B**) Limiting steps for chromosome dimer resolution in the hypothesis in which FtsK transports DNA through a pore formed by its N-terminal domain: transfer of the last segment of the circular double-stranded chromosome (**A**) and formation of a recombination synapse between *dif* sites carried by a dimer when they are positioned on either side of a closed septum at cell division (**B**). (**C**) The assembly of several FtsK complexes in an incompletely formed septum ensures synapsis of *dif* sites carried by a dimer and the transfer of circular DNA molecules. (**D**) The inertia of chromosomes drives the C-terminal domains of active FtsK molecules towards the side of the division plan from which DNA is pumped until the maximal extension of the linker is reached. To avoid clutter, only one of the six linkers attaching FtsK hexamers to the internal membrane of the cell is drawn.

transferred (as depicted in Figure 6A and C) and (ii) how a recombination synapse between two *dif* sites is formed when they are segregated on either side of the septum rather than on the same side at the time of cell division (as depicted in Figure 6B and C).

Note that the idea that FtsK transports DNA across an incompletely formed septum does not exclude the possibility that a pore created by FstK$_N$ might be involved in another cellular process, such as membrane fusion at the end of cell division as suggested for SpoIIIE (Liu *et al*, 2006).

Generalization to other proteins of the FtsK family

In the generally admitted model for the transport of DNA by SpoIIIE during *B. subtilis* sporulation and by the Tra proteins during the conjugation of various *streptomycetes* plasmids, DNA transfer occurs across pores created by the transmembrane segments of these proteins (Grohmann *et al*, 2003; Liu *et al*, 2006; Burton *et al*, 2007). Our observation that a different mechanism has been adopted in *E. coli* for the transport of DNA during chromosome dimer resolution could be explained by the difference in the biological functions of FtsK, SpoIIIE and the Tra proteins and/or by the difference in the architecture of the cell walls of the Gram$^-$ *E. coli* bacterium and the Gram$^+$ *B. subtilis* and *streptomycetes* bacteria.

However, a model in which SpoIIIE transports DNA before membrane fusion, across an incompletely formed septum, is compatible with the observation that cytoplasmic proteins and membrane dyes do not diffuse freely between the mother cell and the prespore compartments during *B. subtilis* sporulation. Actually, reduction of the opening during septum constriction and molecular crowding at the site of division, which involves more than a dozen proteins, could diminish the rate of diffusion of membrane dyes between these compartments. In addition, formation of the SpoIIIE motors at the periphery of the incompletely formed sporulating septum might participate in blocking the diffusion of proteins between the mother cell and prespore compartments, which could partially explain why in its absence the two compartments are no more isolated (Liu *et al*, 2006).

Transport of chromosomes across an incompletely formed septum is also compatible with the observation that active SpoIIIE molecules are only found on the side of the septum from which DNA is exported (Sharp and Pogliano, 2002a, b; Becker and Pogliano, 2007): because of the expected inertia of chromosomes, the C-terminal domains forming the SpoIIIE engines will initially move away from the septum after loading (as depicted in Figure 6D). This movement should persist until the linker arms are fully extended. As DNA translocation by SpoIIIE is oriented by KOPS-like motifs (Ptacin *et al*, 2008), the C-terminal domains should end up on the side from which DNA is exported (Figure 6D).

Finally, the idea that the *Streptomycetes* Tra proteins transport plasmids across a pre-existing opening between contacting mycelial tips could explain how double-stranded DNA is transferred from both the donor to the recipient and *vice versa* without any requirement for a relaxase and a type IV secretion system (Possoz *et al*, 2001; Grohmann *et al*, 2003), in contrast to classical conjugation (Llosa *et al*, 2002).

Coordination between chromosome segregation and cell division

The notion that FtsK and SpoIIIE could transport DNA through an incompletely formed septum fits with the previously accumulated evidences implicating SpoIIIE in membrane fusion during sporulation (Sharp and Pogliano, 1999, 2003; Liu *et al*, 2006). Indeed, the role played by SpoIIIE in membrane fusion could serve as a mechanism to delay cell

fission until SpoIIIE has cleared the septum from DNA. In *E. coli*, the long FtsK linker might enable it to function at an early stage during septum constriction, which would leave more time for DNA transport before septum closure. However, FtsK could also be implicated in a mechanism that would delay membrane fusion in the presence of unsegregated DNA at the septum. In favour of this hypothesis, pre-division figures with fully constricted septa accumulate in cells harbouring large inversions of the *E. coli* chromosome (Lesterlin *et al*, 2008), in which the activity of FtsK is required to re-distribute large amounts of chromosomal DNA in the future daughter cells at each cell cycle. Furthermore, this late cell division arrest is lost when the activity of the C-terminal domain of FtsK is affected (Lesterlin *et al*, 2008). Thus, it will be important to determine the role played by the C-terminal and linker domains of FtsK in cell division, how this might lead to a delay in membrane fusion in the presence of DNA, and if such a mechanism is conserved in bacteria in which the linker of FtsK is shorter than in *E. coli*.

Materials and methods

Strains, growth conditions and plasmid constructions

All strains used in this study are AB1157 derivatives. They were obtained by P1 transduction and are described in Supplementary Table 1. Bacteria were grown at 37°C in Lennox-Lysogenic Broth. The following antibiotics (μg/ml) were added as appropriate: chloramphenicol (30), spectinomycin (50), kanamycin (50), ampicillin (100) and tetracyclin (15). The FtsK chimaeras were constructed by translational fusion of ZapA, ZipA or FtsW with derivatives of FtsK in which restriction sites had been introduced by PCR at the end of the cell division module (Spe1, aa 211), just before the 50 amino acid region implicated in hexamer stabilization (Spe1, aa 179) or the chromosome segregation module (*Xho*I, aa 818). This led to the addition of one (S) or two (LE) amino acid residues at positions 179, 210 or 818 of FtsK. Earlier studies indicated that these additions had no consequences on Xer recombination activation, septum localization and chromosome dimer resolution (Aussel *et al*, 2002; Yates *et al*, 2003; Bigot *et al*, 2004). The *ftsK* alleles were expressed from a pSC101 derivative carrying the resistance to spectinomycin under the native *ftsK* promoter, in translational fusion or not with fluorescent proteins. They were also placed on pBAD expression vectors carrying the resistance to ampicillin.

Microscopy

Cells were grown to $OD_{600} = 0.2–0.3$ at 30°C in LB, washed twice in M9 medium and spread on a microscope slide on a layer of M9 medium containing 1% agarose. A DM6000-B Microscope (Leica; Wetzlar, Germany) coupled to a Coolsnap HQ CCD camera (Photometrics; Tucson, AZ) was used to image cells. Images were analysed using the Metamorph software.

Growth competition assay

As described in Bigot *et al* (2004). Briefly, a 1:1 mixture of two strains was grown in serial culture in LB at 37°C and their relative frequencies determined by plating every 20 generations on appropriate media. The *ftsK* alleles were expressed from a pSC101 vector. The rates at which *ftsA* ftsK*$^{-}$ and *ftsK*$^{-}_{LC}$ strains overtake their Δ*dif* derivative correspond to the efficiencies with which the *ftsK* allele express sustain chromosome dimer resolution.

In vivo plasmid resolution assay

As described in Yates *et al* (2003). Briefly, cells were transformed with the pBAD FtsK chimaera expression vectors and a recombination substrate (pFX142; pSC101 derivative carrying the resistance to spectinomycin). At least 20 colonies were re-suspended, diluted a 100-fold in LB and grown for 4 h in 0.2% arabinose to induce FtsK chimaera production. Plasmid DNA was extracted by using a Qiagen miniprep kit and analysed by electrophoresis through a 1% agarose gel in 1 × TAE. Resolution efficiency was computed as the amount of replicative, supercoiled circle product over the amount of supercoiled substrate, which was quantitated using Sybgreen staining (Molecular probe) on a Typhoon fluorescence scanner (GE Healthcare).

qPCR analysis of chromosomal dif-cassette excision

As described in Kennedy *et al* (2008). Briefly, an AB1157 *ftsK*$^{-}_{ATP}$ *dif-km-dif* strain was transformed by pSC101 vectors expressing either *ftsK*, *ftsK*$_{LC}$, *ftsW*::*ftsK*$_{LC}$ or *zipA*::*ftsK*$_{LC}$ under an inductible promoter. The strains were grown overnight in LB supplemented with 0.2% of glucoce, diluted in fresh LB to an OD of 0.05. After one generation, 0.2% of arabinose was added. Cultures were continually diluted with pre-warmed fresh media to maintain logarithmic growth. Aliquots were taken at several time points to measure OD at 600 nm and to purify genomic DNA. Genomic DNA was restricted with *Hind*III. qPCR was then performed on a LigthCycler 2.0 (Roche Scientific, Nutley, NJ) as recommanded. The primers used for qPCR were as follows: forward X, 5-TGACCG CCAACGACTGGATTC; reverse Y, 5-TTAATCGCGGCCTCGAGCAAG; reverse Z, 5-GCGACAGACACTGCGCTCTTAG.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Aussel L, Barre FX, Aroyo M, Stasiak A, Stasiak AZ, Sherratt D (2002) FtsK is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. *Cell* **108:** 195–205

Barre FX (2007) FtsK and SpoIIIE: the tale of the conserved tails. *Mol Microbiol* **66:** 1051–1055

Barre FX, Aroyo M, Colloms SD, Helfrich A, Cornet F, Sherratt DJ (2000) FtsK functions in the processing of a Holliday junction intermediate during bacterial chromosome segregation. *Genes Dev* **14:** 2976–2988

Bath J, Wu LJ, Errington J, Wang JC (2000) Role of bacillus subtilis SpoIIIE in DNA transport across the mother cell-prespore division septum. *Science* **290:** 995–997

Becker EC, Pogliano K (2007) Cell-specific SpoIIIE assembly and DNA translocation polarity are dictated by chromosome orientation. *Mol Microbiol* **66:** 1066–1079

Begg KJ, Dewar SJ, Donachie WD (1995) A new *Escherichia coli* cell division gene, ftsK. *J Bacteriol* **177:** 6211–6222

Bernhardt TG, de Boer PA (2005) SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over Chromosomes in E. coli. *Mol Cell* **18:** 555–564

Bigot S, Corre J, Louarn J, Cornet F, Barre FX (2004) FtsK activities in Xer recombination, DNA mobilization and cell division involve overlapping and separate domains of the protein. *Mol Microbiol* **54:** 876–886

Bigot S, Sivanathan V, Possoz C, Barre FX, Cornet F (2007) FtsK, a literate chromosome segregation machine. *Mol Microbiol* **64:** 1434–1441

Burton BM, Marquis KA, Sullivan NL, Rapoport TA, Rudner DZ (2007) The ATPase SpoIIIE transports DNA across fused septal membranes during sporulation in Bacillus subtilis. *Cell* **131:** 1301–1312

Diez AA, Farewell A, Nannmark U, Nystrom T (1997) 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. *J Bacteriol* **179:** 5878–5883

Dorazi R, Dewar SJ (2000) Membrane topology of the N-terminus of the escherichia coli FtsK division protein. *FEBS Lett* **478:** 13–18

Draper GC, McLennan N, Begg K, Masters M, Donachie WD (1998) Only the N-terminal domain of FtsK functions in cell division. *J Bacteriol* **180:** 4621–4627

Errington J, Bath J, Wu LJ (2001) DNA transport in bacteria. *Nat Rev Mol Cell Biol* **2:** 538–545

Geissler B, Margolin W (2005) Evidence for functional overlap among multiple bacterial cell division proteins: compensating for the loss of FtsK. *Mol Microbiol* **58:** 596–612

Geissler B, Shiomi D, Margolin W (2007) The ftsA* gain-of-function allele of Escherichia coli and its effects on the stability and dynamics of the Z ring. *Microbiology* **153:** 814–825

Grohmann E, Muth G, Espinosa M (2003) Conjugative plasmid transfer in gram-positive bacteria. *Microbiol Mol Biol Rev* **67:** 277–301, table of contents

Harry E, Monahan L, Thompson L (2006) Bacterial cell division: the mechanism and its precison. *Int Rev Cytol* **253:** 27–94

Kennedy SP, Chevalier F, Barre FX (2008) Delayed activation of Xer recombination at dif by FtsK during septum assembly in Escherichia coli. *Mol Microbiol* **68:** 1018–1028

Lesterlin C, Pages C, Dubarry N, Dasgupta S, Cornet F (2008) Asymmetry of chromosome Replichores renders the DNA translocase activity of FtsK essential for cell division and cell shape maintenance in Escherichia coli. *PLoS Genet* **4:** e1000288

Liu G, Draper GC, Donachie WD (1998) FtsK is a bifunctional protein involved in cell division and chromosome localization in *Escherichia coli*. *Mol Microbiol* **29:** 893–903

Liu NJ, Dutton RJ, Pogliano K (2006) Evidence that the SpoIIIE DNA translocase participates in membrane fusion during cytokinesis and engulfment. *Mol Microbiol* **59:** 1097–1113

Llosa M, Gomis-Ruth FX, Coll M, de la Cruz Fd F (2002) Bacterial conjugation: a two-step mechanism for DNA transport. *Mol Microbiol* **45:** 1–8

Massey TH, Mercogliano CP, Yates J, Sherratt DJ, Lowe J (2006) Double-stranded DNA translocation: structure and mechanism of hexameric FtsK. *Mol Cell* **23:** 457–469

Perals K, Capiaux H, Vincourt JB, Louarn JM, Sherratt DJ, Cornet F (2001) Interplay between recombination, cell division and chromosome structure during chromosome dimer resolution in Escherichia coli. *Mol Microbiol* **39:** 904–913

Possoz C, Ribard C, Gagnat J, Pernodet JL, Guerineau M (2001) The integrative element pSAM2 from Streptomyces: kinetics and mode of conjugal transfer. *Mol Microbiol* **42:** 159–166

Ptacin JL, Nollmann M, Becker EC, Cozzarelli NR, Pogliano K, Bustamante C (2008) Sequence-directed DNA export guides chromosome translocation during sporulation in Bacillus subtilis. *Nat Struct Mol Biol* **15:** 485–493

Saleh OA, Bigot S, Barre FX, Allemand JF (2005) Analysis of DNA supercoil induction by FtsK indicates translocation without groove-tracking. *Nat Struct Mol Biol* **12:** 436–440

Saleh OA, Perals C, Barre FX, Allemand JF (2004) Fast, DNA-sequence independent translocation by FtsK in a single-molecule experiment. *EMBO J* **23:** 2430–2439

Sharp MD, Pogliano K (1999) An *in vivo* membrane fusion assay implicates SpoIIIE in the final stages of engulfment during Bacillus subtilis sporulation. *Proc Natl Acad Sci USA* **96:** 14553–14558

Sharp MD, Pogliano K (2002a) MinCD-dependent regulation of the polarity of SpoIIIE assembly and DNA transfer. *EMBO J* **21:** 6267–6274

Sharp MD, Pogliano K (2002b) Role of cell-specific SpoIIIE assembly in polarity of DNA transfer. *Science* **295:** 137–139

Sharp MD, Pogliano K (2003) The membrane domain of SpoIIIE is required for membrane fusion during Bacillus subtilis sporulation. *J Bacteriol* **185:** 2005–2008

Steiner WW, Kuempel PL (1998) Sister chromatid exchange frequencies in *Escherichia coli* analyzed by recombination at the *dif* resolvase site. *J Bacteriol* **180:** 6269–6275

Val M-E, Kennedy SP, El karoui M, Bonné L, Chevalier F, Barre F-X (2008) FtsK-dependent dimer resolution on multiple chromosomes in the pathogen Vibrio cholerae. *PLoS Genet* **4:** 1–11

Wang L, Lutkenhaus J (1998) FtsK is an essential cell division protein that is localized to the septum and induced as part of the SOS response. *Mol Microbiol* **29:** 731–740

Wu LJ, Errington J (1994) Bacillus subtilis spoIIIE protein required for DNA segregation during asymmetric cell division. *Science* **264:** 572–575

Wu LJ, Errington J (2004) Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in Bacillus subtilis. *Cell* **117:** 915–925

Wu LJ, Lewis PJ, Allmansberger R, Hauser PM, Errington J (1995) A conjugation-like mechanism for prespore chromosome partitioning during sporulation in *Bacillus subtilis*. *Genes Dev* **9:** 1316–1326

Yates J, Aroyo M, Sherratt DJ, Barre FX (2003) Species specificity in the activation of Xer recombination at dif by FtsK. *Mol Microbiol* **49:** 241–249

Yu XC, Tran AH, Sun Q, Margolin W (1998) Localization of cell division protein FtsK to the *Escherichia coli* septum and identification of a potential N-terminal targeting domain. *J Bacteriol* **180:** 1296–1304