The *Escherichia coli* DNA translocase FtsK

David J. Sherratt¹, Lidia K. Arciszewska, Estelle Crozat, James E. Graham and Ian Grainge
Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.

Abstract

*Escherichia coli* FtsK is a septum-located DNA translocase that co-ordinates the late stages of cytokinesis and chromosome segregation. Relatives of FtsK are present in most bacteria; in *Bacillus subtilis*, the FtsK orthologue, SpoIIIE, transfers the majority of a chromosome into the forespore during sporulation. DNA translocase activity is contained within a ~512-amino-acid C-terminal domain, which is divided into three subdomains: α, β and γ. α and β comprise the translocation motor, and γ is a regulatory domain that interacts with DNA and with the XerD recombinase. *In vitro* rates of translocation of ~5 kb · s⁻¹ have been measured for both FtsK and SpoIIIE, whereas, *in vivo*, SpoIIIE has a comparable rate of translocation. Translocation by both of these proteins is not only rapid, but also directed by DNA sequence. This directionality requires interaction of the γ subdomain with specific 8 bp DNA asymmetric sequences that are oriented co-directionally with replication direction of the bacterial chromosome. The γ subdomain also interacts with the XerCD site-specific recombinase to activate chromosome unlinking by recombination at the chromosomal *dif* site. In the present paper, the properties *in vivo* and *in vitro* of FtsK and its relatives are discussed in relation to the biological functions of these remarkable enzymes.

In vivo functions of FtsK and its orthologues

*Escherichia coli* FtsK is a 1329-amino-acid protein that has three domains (Figures 1A and 1B). The ~200-amino-acid N-terminal domain is an integral membrane protein with four membrane-spanning segments that functions at the septum in the late stages of cytokinesis [1,2]. In otherwise wild-type strains, this N-terminal domain is essential for cell viability. The ~512-amino-acid C-terminal domain is the DNA translocase, which is a member of the RecA-fold ATPases [3–5]. The N- and C-terminal domains are connected by a ~600-amino-acid PQ (Pro-Gln)-rich linker. The linker is of highly variable size in FtsK proteins derived from different bacteria and is important for normal FtsK function. Although the organization of the N-terminal domain is highly conserved within bacteria, its primary sequence has diverged. In contrast, the primary sequence of the C-terminal translocase domain is highly conserved.

Our own work has focused on the C-terminal FtsK translocase domain, which forms an active homohexamer [5]. A major function of FtsK translocase is in the resolution of dimeric chromosomes that form by homologous recombination about once every six generations [6–10]. The two sister chromosomes that constitute a dimer cannot be segregated to daughter cells unless the dimers are converted into monomers. The failure to resolve dimeric chromosomes into monomers leads to a failure of chromosome segregation and subsequent cell death. Dimers are converted into monomers by the XerCD-*dif* site-specific recombinase reaction, which is highly conserved within bacteria [11–13]. The XerCD recombinase belongs to the tyrosine recombinase family [12] and acts in the stable inheritance of multicopy plasmids as well as in chromosome dimer resolution (Figures 1C and 1D) [14–16]. FtsK is not required for the conversion of plasmid dimers into monomers when they contain the natural plasmid recombination sites psi (pSC101) and cer (ColE1), but is required for recombination at the chromosomal *dif* site irrespective of whether it is present in the chromosome or has been transferred to plasmids [17]. The evidence available indicates that FtsK activates the XerD recombinase through a specific interaction of the γ subdomain with the C-terminus of XerD [18].

The 28 bp chromosomal *dif* site is located in the replication termination region (ter) of the chromosome. By the time *dif* is replicated, ter is localized close to midcell, the position at which active FtsK translocase is assembled in the late stages of cytokinesis [19,20]. It seems likely that, when DNA replication has been completed normally to give monomeric sister chromosomes, and that these have been decatenated in a timely manner, segregation of sister chromosomes will have been completed before FtsK translocase is active [20]. In contrast, if the final steps of replication, or decatenation, are delayed, or if chromosome dimers have formed, DNA will remain in the region of the septum when functional FtsK becomes available. The translocase can then assemble directionally on DNA to facilitate translocation-mediated synapsis of sister *dif* sites and subsequent dimer resolution–decatenation (Figures 1C and 1D). There are no reasons to believe that FtsK-directed XerCD recombination at *dif* can discriminate between intermolecular or intramolecular recombination; therefore, in principle, this recombination can form chromosomal dimers as well as resolve them to...
Figure 1 | FtsK structure and action

(A) Schematic diagram of *E. coli* FtsK. The 179-amino-acid N-terminal domain has four transmembrane segments (vertical black bars). The C-terminal translocase has three subdomains: α, β, and γ. α and β comprise the motor, and γ is the regulatory subdomain. The N- and C-terminal domains of FtsK are separated by a 660-amino-acid linker (horizontal black line). (B) Structures of homohexameric FtsK translocase [5,30]. Three of the six γ subdomains bound to KOPS are shown modelled on αβ. The various shades of grey distinguish the different subunits. (C) Schematic diagram of the chromosome dimer resolution reaction. The dif recombination site is indicated as a triangle on the chromosomal DNA (line). The single origin of replication is denoted by a filled circle. The action of FtsK at the septum to facilitate synopsis of dif sites and activate XerCD resolution occurs in the grey ovoid encompassing the sister dif sites. (D) FtsK-facilitated dimer resolution by XerCD-dif site-specific recombination. Coupled translocation and activation of XerCD recombination leads exclusively to free circles, arising from recombination on simple synapses (solid arrows). Complex synapses resulting from random three-dimensional collisions would generate catenanes and knots (dotted arrow). These complex products do not occur.

Genetic experiments first indicated that FtsK translocation on the chromosome might be directional and guided by polarized DNA sequences present on the leading strand template for DNA replication [10,23]. Furthermore, it was shown that chromosome dimer resolution, mediated by monomers. Indeed, XerCD-dif–FtsK recombination can lead to decatenation of newly replicated chromosomes in the absence of TopoIV (topoisomerase IV), in reactions that interconvert monomers and dimers at each round of recombination [21,22].
XerCD recombination at dif, required that dif be positioned where these polarized DNA sequences converge within ter [10]. Subsequently, an 8 bp asymmetric sequence consensus, KOPS (FtsK orienting/polarizing sequence), was identified as guiding FtsK directional translocation [24,25]. The initial ideas and experiments suggested that KOPSs act in a way akin to replication terminator sites: i.e. in the ‘permissive’ orientation they allow translocation of FtsK through the sequence, whereas in the ‘non-permissive’ orientation they cause FtsK to stop translocation and sometimes to then reverse translocation direction [24–26]. Nevertheless, more recent data support the view that KOPS act only as directional loading sites for FtsK, and the apparent stalling and reversal of translocation at a non-permissive KOPS is the consequence of the translocating FtsK encountering a second FtsK bound at the non-permissive site [27,28].

In vitro properties of FtsK translocase

The great majority of biochemical studies of FtsK translocase have utilized a derivative, FtsK<sub>50C</sub>, that contains the C-terminal translocase domain fused to 50 amino acids derived from the region at the boundary of the N-terminal and linker domains (Figure 1A) [3]. The additional 50 amino acids appear to facilitate multimerization, and even aggregation. In the absence of these 50 amino acids, the translocase forms hexamers on DNA and exhibits biochemical activity only at very high protein concentrations, or when monomers are covalently linked through a flexible linker (E. Crozat and D.J. Sherratt, unpublished work).

A number of complementary ensemble and single-molecule assays have been used to assess translocase function in vitro. For example, FtsK<sub>50C</sub> exhibits a robust DNA-dependent ATPase activity in solution, whereas its translocation can be assessed directly by its ability to displace triplex-dependent ATPase activity in solution, whereas its translocation rates and processivity, and will be even more powerful analytical tools when the translocating protein can be visualized directly.

Ongoing experiments are aimed at understanding the molecular mechanism of DNA translocation, and in particular to test the sequential rotary motor hypothesis [5] by using covalently linked FtsK monomers, thereby allowing the formation of hexamers with defined mutant subunits at fixed positions (E. Crozat and D.J. Sherratt, unpublished work). In addition, we also aim to understand how FtsK activates XerCD–dif recombination, while at the same time avoiding stripping these recombinases from DNA during translocation.

Perspective

FtsK and its orthologues are remarkable proteins. They are some of the largest bacterial proteins and their action is confined to the closing septum in the late stages of cytokinesis. This ensures that DNA translocation activity on the bacterial chromosome is only available when and where it is required, thereby facilitating the final stages of chromosome unlinking and segregation to daughter cells. Consistent with this, inappropriate spatial and temporal overexpression of the FtsK translocase domain is highly toxic and leads quickly to cessation of protein synthesis. This may be because extensive DNA translocation leads to changes in chromosome organization and/or because extensive translocation strips proteins and transcription complexes from DNA.

Is the activation of XerCD–dif recombination the main function of the FtsK translocase and why has XerCD–dif evolved to have a stringent requirement for FtsK, when this and other recombinases can act in the absence of FtsK when the appropriate recombination sites are present?

Genetic experiments point to the most obvious function for FtsK translocation being in XerCD–dif recombination during chromosome dimer resolution, since the absence of FtsK translocase has little or no phenotype in cells that are unable to form chromosome dimers because they lack RecA [17,25]. Nevertheless, we cannot yet eliminate the possibility that FtsK accesses the ter region before newly replicated ter segregate away from the septum in most cell cycles and that this translocation facilitates segregation in ways other than through activating unlinking, and in ways that do not impart an obvious mutant phenotype in cells that lack
the translocase. The ability of FtsK to strip proteins from DNA during translocation may be important for function in new born cells; certainly the wirestripping activity of SpoIIIIE during sporulation would appear to be important for resetting the transcriptional programme in the *Bacillus subtilis* forespore [33].

The stringent requirement for FtsK translocase in activation of chromosome unlinking by XerCD recombination at *dif* seems to be intimately associated with translocation, and the ability of translocation to facilitate synthesis of *dif* sites in a way that avoids introduction of further catenation-knotting that would compromise the unlinking process. This and the fact that functional FtsK is only available at the septum immediately before the completion of cell division ensures that the final stages of chromosome unlinking and segregation are efficiently accomplished by enzymes that could cause genetic and epigenetic havoc if they acted inappropriately.

Acknowledgements

We thank our many colleagues for stimulating discussions.

Funding

Our research is funded by the Wellcome Trust.

References


Received 19 May 2009
doi:10.1042/BST0380395