

Identification of the FtsK sequence-recognition domain

Jerod L Ptacin¹, Marcelo Nöllmann¹, Carlos Bustamante^{1,2} & Nicholas R Cozzarelli^{1,3}

FtsK is a prokaryotic multidomain DNA translocase that coordinates chromosome segregation and cell division. FtsK is membrane anchored at the division septum and, guided by highly skewed DNA sequences, translocates the chromosome to bring the terminus of replication to the septum. Here, we use *in vitro* single-molecule and ensemble methods to unveil a mechanism of action in which the translocation and sequence-recognition activities are performed by different domains in FtsK.

The *Escherichia coli* DNA translocase FtsK is an oligomeric ATP-fueled motor that coordinates chromosome segregation and cell division^{1,2}. Homologous recombination during DNA replication can lead to the formation of a chromosome dimer that cannot be properly segregated to daughter cells³. The site-specific recombinases XerC and XerD act at *dif*, a 28-nucleotide sequence in the terminus of replication, to resolve chromosome dimers into separable monomers³. FtsK assembles at the division septum and translocates the DNA to bring the two *dif* sites in the dimer into close proximity⁴. Upon formation of the XerCD-*dif* synapse, FtsK stimulates recombination by direct interaction with XerD^{5–7}.

The FtsK monomer is composed of three domains: a 217-residue N-terminal transmembrane domain, a poorly conserved and putatively unstructured 600-residue linker and a 512-residue motor domain (FtsK_C) that has been classified as a member of the AAA+ family of ATPases and shown to translocate duplex DNA^{5,8,9}. A soluble variant of FtsK_C (FtsK_{50C}) has been used extensively to study FtsK translocation *in vitro*^{5,8–12}. Single-molecule experiments using FtsK_{50C} have shown that its translocation direction is dictated by DNA sequence⁹. The DNA sequence motif 5'-GNGNAGGG-3' (FtsK-recognition sequence, or FRS¹⁰, also called KOPS¹²) have been found to control translocation directionality by reversing FtsK movement when the translocase encounters the sequence from the

3' end of the G-rich strand, termed the nonpermissive orientation. When approaching from the 5' end (permissive orientation), FtsK passes the sequence unobstructed^{10,12}. FRS motifs are over-represented on the chromosome's leading strand and switch strand at *dif*, thus guiding FtsK toward *dif* from any location on the chromosome.

Despite characterization of the FRS, little is known about the mechanism of FRS recognition by FtsK. Secondary and tertiary structure predictions indicate that the C-terminal 64 residues of FtsK (γ domain) folds as a DNA-binding domain joined to the rest of FtsK_C by a short unstructured linker (J.L.P. and M.N., unpublished data). This is confirmed in the accompanying high-resolution structures of the γ domain, which show that it folds as a winged helix¹³. We hypothesized that the γ domain mediates FtsK interaction with the FRS. To test this hypothesis, we constructed and purified a truncated version of FtsK_{50C} that lacks the γ domain and linker (FtsK_{50C} $\Delta\gamma$).

To assay for FRS recognition, we monitored the FtsK-mediated displacement of a DNA triplex on a substrate containing FRS motifs in permissive or nonpermissive orientations¹⁰ (**Supplementary Methods** and **Supplementary Fig. 1** online). FtsK_{50C} triplex displacement rates were considerably faster on the permissive than on the nonpermissive substrate (**Supplementary Fig. 1**), in agreement with our previous results¹⁰. In contrast, FtsK_{50C} $\Delta\gamma$ displacement rates were similar on permissive and nonpermissive substrates (**Supplementary Fig. 1**), consistent with FtsK_{50C} $\Delta\gamma$ being unable to respond to FRS.

We directly examined FtsK_{50C} $\Delta\gamma$ translocation and directionality by tracking FtsK particles in real time on single λ DNA molecules using

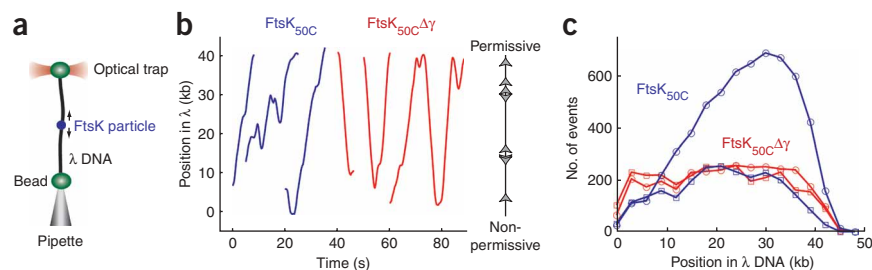


Figure 1 FtsK_{50C} $\Delta\gamma$ overall translocation is not unidirectional on λ DNA. **(a)** An FtsK particle (blue) translocates on a 41-kb fragment of λ DNA (black line) stretched between two beads (green spheres) held between a micropipette (gray) and an optical trap (red). **(b)** Left, selected traces of FtsK_{50C} (blue) and FtsK_{50C} $\Delta\gamma$ (red) particle positions during translocation events, plotted against time. Right, distribution of FRS motifs (arrows), on λ DNA, pointing in the permissive (upward) and nonpermissive (downward) orientations. **(c)** Direction of translocation of FtsK_{50C} and FtsK_{50C} $\Delta\gamma$ particles was determined at positions along λ DNA (bin size \sim 2.5 kb). Number of times the enzyme passed each position while moving in the permissive (circles) or the nonpermissive (squares) direction is plotted.

¹Department of Molecular and Cell Biology and ²Department of Physics, University of California, Berkeley, California 94720-3204, USA. ³In memoriam. Correspondence should be addressed to C.B. (carlos@alice.berkeley.edu).

Received 1 June; accepted 26 September; published online 15 October 2006; doi:10.1038/nsmb1157

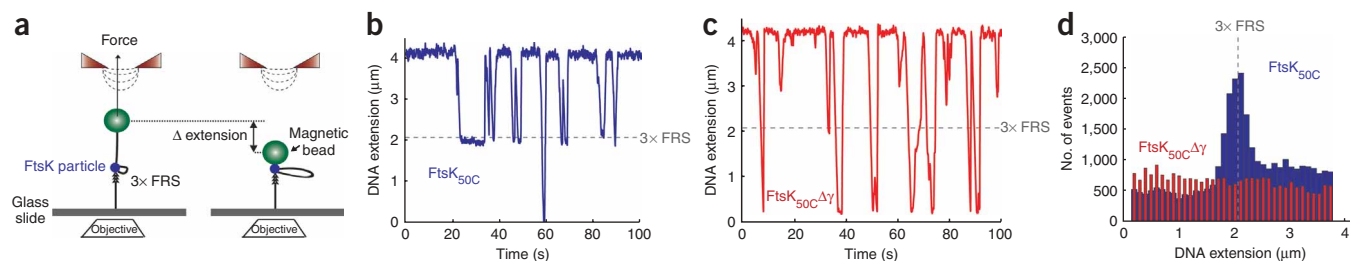


Figure 2 FtsK_{50C}Δγ does not interact with FRS motifs. **(a)** A DNA molecule (black line) is tethered between a glass surface and a magnetic particle (green sphere), and tension in the DNA is introduced using magnets (red triangles). FtsK (blue sphere) binds DNA and forms a loop, shortening the DNA extension. Translocation-induced looping from FRSs (arrowheads) leads to FtsK pauses and reversals at characteristic extensions. **(b,c)** Representative traces of the change in DNA extension versus time owing to FtsK_{50C} **(b)** or FtsK_{50C}Δγ **(c)** translocation on DNA_{FRS,1/2}. **(d)** Occupancy-time distribution as a function of DNA extension, for FtsK_{50C} (blue) and FtsK_{50C}Δγ (red). Regions corresponding to pauses at the bead and surface were omitted for clarity.

optical tweezers⁹ (Fig. 1a and Supplementary Methods). The distribution of FRS in λ DNA promotes FtsK translocation preferentially toward one end of the DNA¹⁰ (in the permissive direction), which allowed us to quantify the directional movement of FtsK. We characterized the movements of 81 FtsK_{50C} particles on 16 λ DNA tethers and of 65 FtsK_{50C}Δγ particles on 18 tethers. The mean translocation velocities were 4.0 ± 1.5 kilobases (kb) s⁻¹ for FtsK_{50C} particles and 4.1 ± 1.5 kb s⁻¹ for FtsK_{50C}Δγ, comparable to the 4.9 ± 0.9 kb s⁻¹ previously reported for FtsK_{50C}⁹. As previously observed⁹, FtsK_{50C} translocated in either direction (Fig. 1b, blue traces), but its net movement was in the permissive direction in ~90% of events. At each position on the DNA, FtsK_{50C} translocated more frequently in the permissive than in the nonpermissive direction (Fig. 1c), and ~72% of the total distance traveled by FtsK_{50C} was in the permissive direction. We define burst size as the distance an FtsK particle traveled in one direction without pausing or turning around. For FtsK_{50C}, the distribution of burst sizes was asymmetrically biased toward bursts in the permissive direction (Supplementary Fig. 2 online), illustrating quantitatively that FtsK_{50C} is more likely to move without reversals in the permissive than in the nonpermissive direction.

In contrast, FtsK_{50C}Δγ particles translocated on λ DNA in both directions with equal frequency (Fig. 1b,c). The net distance translocated by FtsK_{50C}Δγ was in the permissive direction in only ~50% of events, and the burst size distribution is symmetric. Long bursts in the nonpermissive direction were more frequent than for FtsK_{50C} (Supplementary Fig. 2). Monte Carlo simulations assuming that FtsK_{50C}Δγ does not recognize FRS predicted translocation behaviors of FtsK_{50C} and FtsK_{50C}Δγ that were congruent with our experimental results (Supplementary Data online), suggesting FtsK_{50C}Δγ does not recognize FRS.

Finally, we used magnetic tweezers to examine the ability of FtsK_{50C} and FtsK_{50C}Δγ to directly recognize FRS¹⁴ (Supplementary Methods). In this assay, a single nicked DNA molecule is stretched between a glass surface and a paramagnetic bead. Translocation events were measured by monitoring changes in DNA extension induced by FtsK looping^{9,14,15} (Fig. 2a and Supplementary Data). On a DNA lacking FRS (DNA_{no-FRS}), FtsK_{50C} frequently looped the full length of the DNA without pausing at 4.5 ± 1 kb s⁻¹ (Supplementary Fig. 3 online). We constructed a second DNA (DNA_{FRS,1/2}) by inserting three overlapping FRS motifs at the center of DNA_{no-FRS}¹². With DNA_{FRS,1/2}, we observed frequent reversals and pauses at extensions corresponding to the locations of FRS repeats (Fig. 2b). We shortened one end of DNA_{FRS,1/2}, and the pause position shifted in agreement with the change in the location of the FRS repeat (Supplementary Fig. 3).

FtsK_{50C}Δγ translocated DNA_{no-FRS} with translocation rates (4.7 ± 1 kb s⁻¹) and loop sizes similar to those of FtsK_{50C} (Supplementary Fig. 3). On DNA_{FRS,1/2}, however, FtsK_{50C}Δγ did not specifically pause or reverse at the location of FRS (Fig. 2c). The ability of FtsK_{50C} and FtsK_{50C}Δγ to interact with FRS was quantified by compiling the distribution of occupancy times as a function of DNA extension for all experiments (Fig. 2d). The FtsK_{50C} occupancy-time distribution peaks at the position of the FRS repeat, whereas that of FtsK_{50C}Δγ is flat, consistent with FtsK_{50C}Δγ having equal probability of interacting with any DNA sequence on DNA_{FRS,1/2}.

The γ domain of FtsK specifically recognizes FRS, but it may also interact nonspecifically with other DNA sequences, affecting the processivity of FtsK even in the absence of FRS⁸. In this study, we used the experimental FtsK_{50C}Δγ distribution of burst sizes to estimate the inherent processivity of the FtsK motor to be ~9 kb (Supplementary Data), considerably shorter than distances FtsK translocates during chromosome dimer resolution¹⁶ (see below).

In summary, we show that deletion of the γ domain disrupts the ability of FtsK to respond to FRS sequences without affecting other properties of its motor domain. Point mutations in the γ domain have recently been found to abrogate FtsK interactions with FRS¹³, in support of our results. FRS recognition could be communicated between the γ and motor domains in two ways. FRS-γ domain interactions behind a translocating FtsK could communicate mechanical strain to the complex that could switch the motor into reverse translocation. Alternatively, FRS binding in front of a translocating FtsK may result in a specific interaction that could lead to reversal of the translocation direction allosterically. The latter mechanism may also be used to stop the movement of FtsK upon an encounter with DNA-bound XerD. In either model, the interaction between the γ domain-FRS complex and the motor could directly influence the mechanochemical cycle of the motor.

FtsK does not track the DNA helix while translocating¹⁵, yet it retains the ability to recognize specific DNA sequences via major groove interactions^{10,12}. The flexible linkage between the sequence-recognition and motor domains could allow major groove interactions without obligate groove tracking.

We found that the FtsK motor domain reverses direction stochastically over distances considerably smaller than those FtsK must travel *in vivo* (~250 kb¹⁶). This reversal behavior may have prompted the evolution of a separate DNA-recognition module to exploit the natural sequence polarity of the chromosome and ensure the

directional movement of FtsK. The limited mean burst size of the FtsK motor (~ 9 kb) may explain the high density of FRS motifs along the chromosome (every ~ 3.5 kb¹⁰), as frequent FRS motifs may minimize nonproductive translocations after stochastic reversals. Finally, the conservation of γ domains in the FtsK/SpoIIIE family of translocases and the stochastic reversibility of the FtsK motor domain suggest a sequence-directed translocation mechanism for other related translocases.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We dedicate this work to our friend and colleague Nick Cozzarelli, who passed away during preparation of this paper. We thank J. Moffitt, J. Gore, P. Pease and N. Crisona for critiques. US National Institutes of Health Grant GM31655 (J.L.P. and N.R.C.) and the Human Frontiers Science Organization (M.N.) supported this work.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at <http://www.nature.com/nsmb/>

Reprints and permissions information is available online at <http://npg.nature.com/reprintsandpermissions/>

1. Liu, G., Draper, G.C. & Donachie, W.D. *Mol. Microbiol.* **29**, 893–903 (1998).
2. Iyer, L.M., Makarova, K.S., Koonin, E.V. & Aravind, L. *Nucleic Acids Res.* **32**, 5260–5279 (2004).
3. Blakely, G., Colloms, S., May, G., Burke, M. & Sherratt, D. *New Biol.* **3**, 789–798 (1991).
4. Begg, K.J., Dewar, S.J. & Donachie, W.D. *J. Bacteriol.* **177**, 6211–6222 (1995).
5. Aussel, L. *et al. Cell* **108**, 195–205 (2002).
6. Yates, J. *et al. Mol. Microbiol.* **59**, 1754–1766 (2006).
7. Yates, J., Aroyo, M., Sherratt, D.J. & Barre, F.X. *Mol. Microbiol.* **49**, 241–249 (2003).
8. Saleh, O.A., Perals, C., Barre, F.X. & Allemand, J.F. *EMBO J.* **23**, 2430–2439 (2004).
9. Pease, P.J. *et al. Science* **307**, 586–590 (2005).
10. Levy, O. *et al. Proc. Natl. Acad. Sci. USA* **102**, 17618–17623 (2005).
11. Massey, T.H., Aussel, L., Barre, F.X. & Sherratt, D.J. *EMBO Rep.* **5**, 399–404 (2004).
12. Bigot, S. *et al. EMBO J.* **24**, 3770–3780 (2005).
13. Sivanathan, V. *et al. Nat. Struct. Mol. Biol.* advance online publication 22 October 2006 (doi:10.1038/nsmb1158).
14. Bigot, S., Corre, J., Louarn, J.M., Cornet, F. & Barre, F.X. *Mol. Microbiol.* **54**, 876–886 (2004).
15. Saleh, O.A., Bigot, S., Barre, F.X. & Allemand, J.F. *Nat. Struct. Mol. Biol.* **12**, 436–440 (2005).
16. Corre, J. & Louarn, J.M. *Mol. Microbiol.* **56**, 1539–1548 (2005).