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FtsK: a groovy helicase.

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Recent additions to the helicase family include motor proteins that do not actually unwind DNA, but rather translocate it. By sensing short polar sequences that orient the bacterial chromosome, the FtsK helicase translocates DNA so as to align the termini of replicated chromosomes with each other, facilitating the late stages of chromosome segregation.

Contributions by three biophysics groups on pages 965, 1023 and 1026 of this issue¹⁻³ provide compelling *in vivo*, *in vitro* and single-molecule experiments that elucidate the mechanism of sequence recognition by FtsK. The septum-localized FtsK motor protein reels in the chromosome terminus *dif* by sensing the orientation of short, repeated 'FtsK-orienting polar sequences' (KOPS), which point from the chromosome origin of replication (*ori*) to the terminus (*dif*). The α and β domains of FtsK provide motor functionality, and the γ domain recognizes KOPS. From these data, combined with recent structural investigations of FtsK⁴ and single-molecule analysis of SF2 helicases^{5,6}, a fascinating model for the action of this SF3 hexameric helicase is beginning to emerge.

The FtsK protein was first identified in 1995 in a screen for temperature-sensitive *Escherichia coli* mutants having a filamentation phenotype during cell division⁷. Bacteria with the mutant phenotype grow without fully dividing, forming a long, concatamer-like filament of conjoined bacteria with mislocalized nucleoids. Fluorescence microscopy shows DNA bridges between cells unable to separate after division⁸. Bound to the septum⁹, wild-type FtsK activates the emergency resolution of chromosome dimers by aligning the two *dif* termini of daughter chromosomes at the septum. The XerCD system then recombines the aligned *dif* termini, resolving the chromosome dimer into two separate chromosomes and allowing septation to proceed¹⁰. For this strategy to work, FtsK must translocate DNA from each daughter chromosome in the correct direction, bringing *dif* sites close to the septum. How does FtsK know which way to translocate DNA?

Genetic experiments and sequence analysis have identified interspersed stretches of purine-rich DNA known as KOPS which orient the chromosome from *ori* to *dif*^{11,12}. In *E. coli*, the KOPS consensus 5' – GGGNAGGG – 3' is overrepresented on the top strand of the chromosome on the 3' side of *ori*, and also on the bottom strand of the

chromosome on the 5' side to *ori*. How FtsK interacts with these oriented sequences is becoming clearer, thanks to the structural studies presented in the current issue.

FtsK is a multidomain protein with an N-terminal transmembrane domain responsible for directing FtsK to the septum, a proline/glutamine-rich (PQ) domain and a tripartite, C-terminal helicase domain (FtsK_C, comprised of α , β , and γ domains) responsible for DNA translocation⁷. Most mechanistic studies have focused on this functional FtsK_C domain and its ATPase activity. The crystal structure of the FtsK- α and - β domains has recently been obtained⁴. It shows a ring-like assembly reminiscent of that observed in replicative SF3 helicases such as the SV40 large T-antigen¹³ and the E1 protein from bovine papillomavirus¹⁴. In FtsK- $\alpha\beta$ the central cavity is just large enough to snugly accommodate double-stranded DNA. There are most probably at least two paired rings in a functional complex. Using solution NMR studies, recombination assays, and analysis of DNA binding to FtsK- γ , Sivanathan *et al.*¹ demonstrate that this domain folds as a winged helix and can recognize and bind KOPS. Bulk biochemistry and single-molecule experiments presented page by Bigot *et al.*² further show that the KOPS recruits FtsK, presumably in the so-called 'permissive' orientation. In the so-called 'non-permissive' orientation, KOPS may transiently retain FtsK.

FtsK_C translocation along DNA has been explored using both standard biochemical assays¹⁰ and single-molecule DNA manipulation experiments^{15,16}. The single-molecule methodology is particularly well-suited for measuring, in real-time, the chemomechanical properties of molecular motors that transiently track along the double helix. Direct visualization of large (micron-scale) aggregates of FtsK_C moving along an extended DNA molecule, done by Ptacin *et al.*³, shows that FtsK- $\alpha\beta$ is a functional motor protein capable of translocating along DNA but that it does not interact with the KOPS. In these visualization experiments, the complete FtsK_C system will translocate through permissively-oriented KOPS but stop and eventually reverse course when it encounters KOPS in the nonpermissive orientation. Single-molecule measurements thus show that FtsK_C can translocate DNA at a rate of up to ~7 kbp/second, or more than two microns per second, making it the fastest molecular motor currently known^{15,16}.

Single-molecule assays are also useful for observing the formation by FtsK_C of a KOPS-delimited DNA loop² (**Fig. 1**). The results of this experiment can be interpreted by consider the following. The first of two paired FtsK rings binds the DNA, preferentially at the KOPS site; upon binding it will rapidly translocate along the double helix until it abruptly stops (either by abutting one of the surfaces to which the DNA is anchored, or by encountering the KOPS in a nonpermissive orientation). At this point, the second FtsK ring completes assembly on the DNA and begin translocating in the other direction. Because it is joined to the first ring which is immobilized, the second ring also remains immobile and it is the DNA that is now translocated through the ring, forming a large loop between the two rings. Translocation ends when another nonpermissive KOPS is detected or when the motor reaches the surface. Such a large loop reduces the DNA end-to-end extension and is easily detected by tracking, in real-time, the position of the tethered bead above the surface (**Fig. 1c**).

In all of the single-molecule experiments presented in this issue^{2,3}, the change in DNA extension owing to FtsK_C-dependent loop extrusion matches the distance engineered between two convergent KOPS or between a KOPS and the solid surface it points toward. Comparable results were obtained in triplex displacement and XerCD-mediated recombination assays under control of permissively or nonpermissively oriented KOPS sequences^{1,3}.

Single-molecule experiments have also demonstrated that FtsK_C translocation is exquisitely tuned so as to not perturb chromosome topology¹⁷. Indeed according to the model proposed by Liu and Wang¹⁸, a protein that tracks the DNA groove is expected to generate a negative supercoil in its wake and a positive supercoil ahead of itself for every ~10 base pairs (bp) translocated. Instead, FtsK induces positive supercoiling ahead of itself at a rate of only one supercoil for every ~150 base-pairs translocated – just enough to avoid perturbing the native topology of the bacterial chromosome. Thus FtsK does not simply track along the DNA but also subtly manipulates its topology. Yet how can this protein closely monitor the DNA for any KOPS if it does not track the DNA groove?

The rotary inchworm model of DNA translocation by FtsK^{1,17} provides the basis for a mechanistic explanation of this phenomenon (**Fig. 2**). Because DNA is a double helix, simple translocation along its length results in a ‘pseudo-rotation’, θ , of the double helix in a fixed plane perpendicular to the molecule’s axis. To give an example, translocation of FtsK along DNA by a single base-pair will cause a 35° pseudo-rotation in the counterclockwise direction, if the observer sees the protein approaching head-on. The discrepancy between this pseudo-rotation and the hexameric ring’s structural periodicity, $\phi = 60^\circ$, will cause the DNA to ‘slip’ a small angle ($\phi - \theta$) at every translation step, so as to maintain register with the ring’s active monomer (see **Fig. 2** for a schematic). If FtsK advances along the DNA by 1.6 base-pairs for each catalytic turnover, one can estimate that a positive supercoil will be formed ahead of the protein for every ~140 bp translocated, in good agreement with the crystal structure and single-molecule observations^{4,17}. This rectified rotary inchworm model could also help explain the observation of large-scale rotational slippage of DNA during FtsK translocation, in which the positive torque built up ahead of the complex is sometimes abruptly released¹⁷. Also, the small step size is sufficient to allow FtsK- γ to sense the DNA sequence beneath it without tight groove tracking.

This loose coupling between DNA translocation and rotation stands in stark contrast to the tight coupling observed in the SF2 helicases EcoR124I^{5,19} and RSC⁶. In the case of EcoR124I, groove tracking occurs without slipping and one positive supercoil is formed ahead of the complex for every ~10 bp translocated. In the case of RSC the coupling constant has not yet been quantified, but it is almost certainly stronger than that observed for FtsK. This may reflect the positive role of torsional DNA deformations in chromatin remodelling.

It has been just over ten years since FtsK was identified through genetics. In that span of time, positive feedback between genetics, bioinformatics, biochemistry, structural analysis and single-molecule observations has greatly potentiated research on this

bacterial system and related helicases. Helicases do not just unwind DNA, and understanding the complex topological tricks they can play will be fundamental to explaining their role in chromatin and chromosome dynamics.

REFERENCES

1. Sivanathan, V. *et al.* *Nat. Struct. Mol. Biol.* **13**, 965—972 (2006).
2. Bigot, S., Saleh, O.A., Cornet, F., Allemand, J.-F. & Barre, F.-X. *Nat. Struct. Mol. Biol.* **13**, 1026—1028 (2006).
3. Ptacin, J.L., Marcelo, N., Bustamante, C. & Cozzarelli, N.R. *Nat. Struct. Mol. Biol.* **13**, 1023—1025 (2006).
4. Massey, T.H., Mercogliano, C.P., Yates, J., Sherratt, D.J. & Lowe, J. *Mol. Cell.* **23**, 457—69 (2006).
5. Stanley, L.K., Seidel, R., van der Scheer, C., Dekker, N.H., Szczelkun, M.D. & Dekker, C. *EMBO J.* **25**, 2230—2239 (2006).
6. Lia, G., Praly, E., Ferreira, H., Stockdale, C., Tse-Dinh, Y.C., Dunlap, D., Croquette, V., Bensimon, D. & Owen-Hughes, T. *Mol. Cell.* **21**, 417—425 (2006).
7. Begg, K.J., Dewar, S.J. & Donachie, W.D. *J. Bacteriol.* **177**, 6211—6222 (1995).
8. Goksor, M., Diez, A., Enger, J., Hanstorp, D. & Nystrom, T. *EMBO Rep.* **4**, 867—871 (2003).
9. Wang, L. & Lutkenhaus, J. *Mol. Microbiol.* **29**, 731—740 (1998).
10. Aussel, L., Barre, F.-X., Aroyo, M., Stasiak, A., Stasiak, A.Z. & Sherratt, D. *Cell.* **108**, 195—205 (2002).
11. Bigot, S., Saleh, O.A., Lesterlin, C., Pages, C., El Karoui, M., Dennis, C., Grigoriev, M., Allemand, J.-F., Barre, F.-X. & Cornet, F. *EMBO J.* **24**, 3770—3780 (2005).
12. Lobry, J.R. & Louarn, J.M. *Curr. Opin. Microbiol.* **6**, 101—108 (2003).
13. Li, D., Zhao, R., Lilyestrom, W., Gai, D., Zhang, R., DeCaprio, J.A., Fanning, E., Jochimiak, A., Szakonyi, G. & Chen, X.S. *Nature.* **423**, 512—518 (2003).
14. Enemark, E.J. & Joshua-Tor, L. *Nature.* **442**, 270—275 (2006).
15. Saleh, O.A., Peral, C., Barre, F.-X. & Allemand, J.-F. *EMBO J.* **23**, 2430—2439 (2004).
16. Pease, P.J., Levy, O., Cost, G.J., Gore, J., Ptacin, J.L., Sherratt, D., Bustamante, C. & Cozzarelli, N.R. *Science.* **307**, 586—590 (2005).
17. Saleh, O.A., Bigot, S., Barre, F.-X. & Allemand, J.-F. *Nat. Struct. Mol. Biol.* **12**, 436—440 (2005).
18. Liu, L.F. & Wang, J.C. *Proc. Natl. Acad. Sci. USA.* **84**, 7024—7027 (1987).
19. Seidel, R., van Noort, J., van der Scheer, C., Bloom, J.G., Dekker, N.H., Dutta, C.F., Blundell, A., Robinson, T., Firman, K. & Dekker, C. *Nat. Struct. Mol. Biol.* **11**, 838—843 (2004).

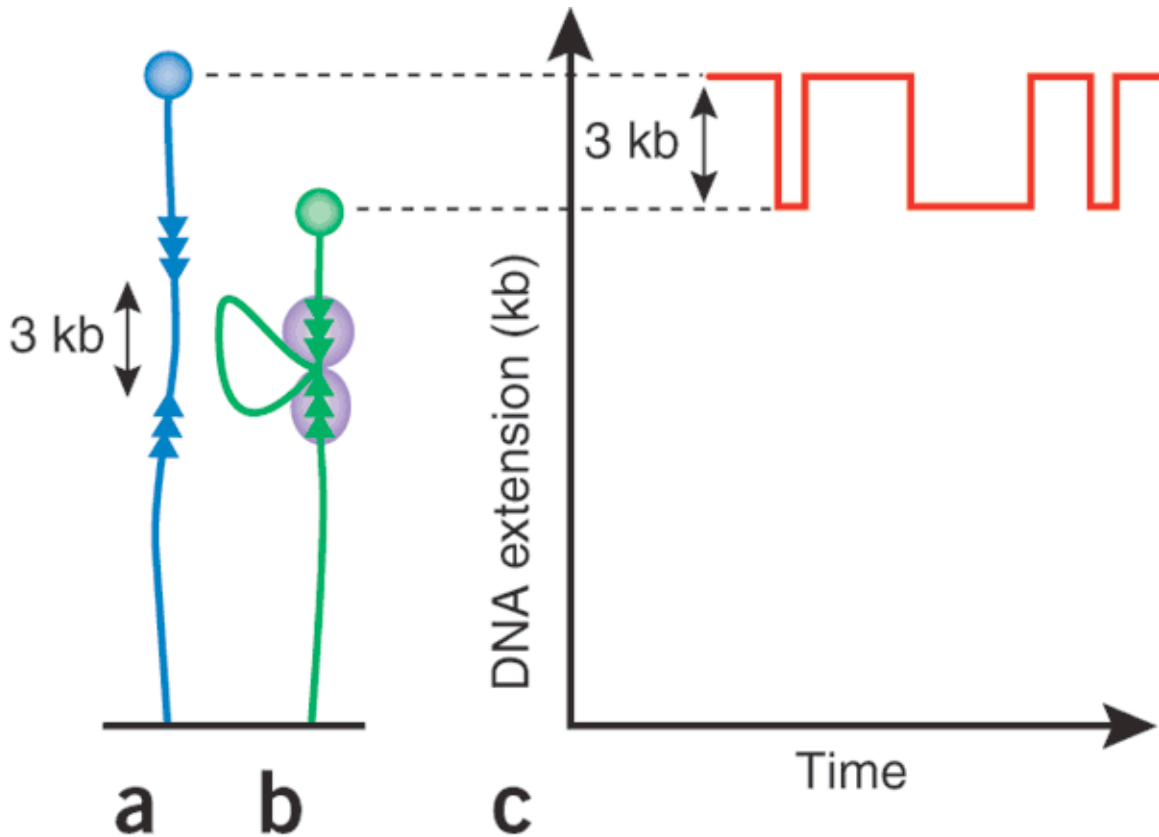


Figure 1. Single-molecule FtsK_C assay.

(a) An individual DNA molecule with two converging KOPS is tethered between a glass surface and a small bead. By manipulating the bead using an optical or a magnetic trap, the DNA can be quantitatively extended and supercoiled so as to reproduce the mechanical constraints experienced by the double helix *in vivo*. The readout in such an assay is the end-to-end extension of the DNA, determined by measuring, in real-time, the position of the tethered bead above the glass surface.

(b) FtsK_C binds to one KOPS and translocates DNA, extruding a loop. Upon encountering the second KOPS in the non-permissive orientation, FtsK_C stops before eventually reversing direction.

(c) Real-time measurement of the DNA extension gives the size of the extruded loop, which is equal to the distance engineered between the converging KOPS.

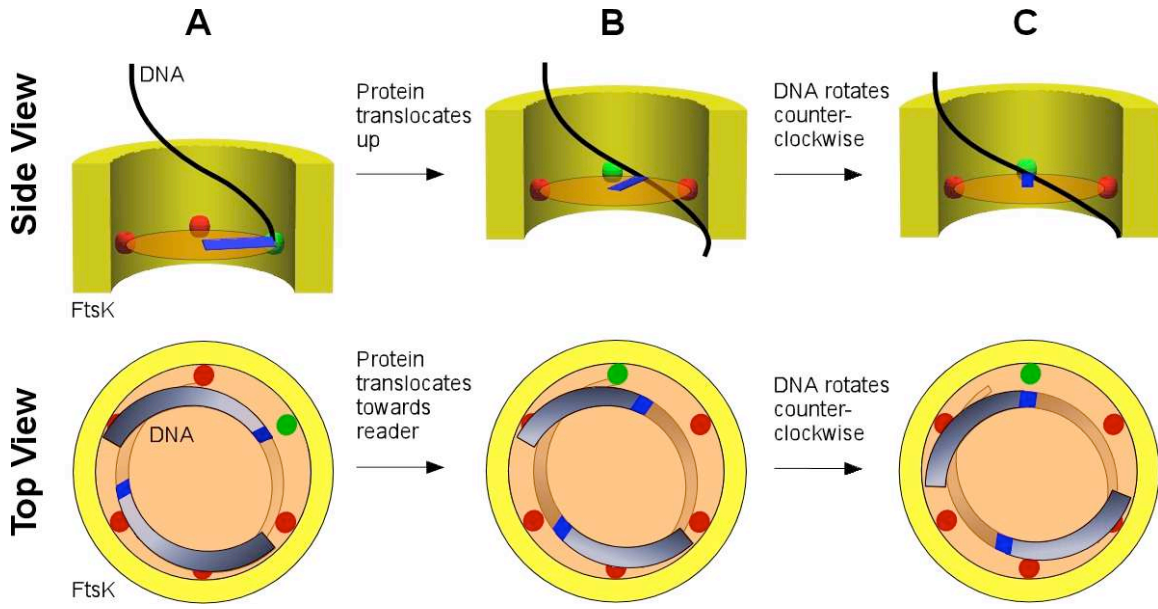


Figure 2. Rectified rotary inchworm model of DNA translocation by FtsK_C. In this schematic the catalytic sites of the hexamer are depicted as active (green) or inactive (red) and define a ‘catalytic plane’ which intersects (blue) the DNA phosphate backbones. Monomers in the ring are sequentially activated and the active catalytic site ‘rotates’ by 60° for every catalytic turnover. In side view, we present only the DNA strand tracked by FtsK_C, and blue line points to the intersection between the catalytic plane and the phosphate backbone. (a) At the active monomer, an ATP-dependent conformational change causes FtsK to translocate, without rotating, a distance on the order of 5.5 Angstroms, or about 1.6 bp. (b) The helical pitch of DNA (~35° per bp) is such that the position of the phosphate backbone in the catalytic plane has now rotated only 56° and therefore falls short of the neighboring catalytic site. (c) The DNA rotates by +4° to catch up with and bind the newly active site of the neighboring monomer. No translocation occurs at this step. Thus over the entire catalytic cycle, translocation and rotation are weakly coupled. If the step size is slightly greater than 1.75 bp, the DNA will have to counter-rotate to maintain register with the active site, generating negative supercoils ahead of the complex.