

Supplementary Data

Computer simulations

A Monte Carlo approach was employed to simulate the movement of FtsK particles on λ DNA. Each run encompassed the following steps: (1) an FtsK particle binds randomly to λ DNA; (2) FtsK starts moving in either direction with equal probability; (3) Upon encounter of an FRS in the non-permissive orientation, FtsK reverses translocation direction with a probability p_{FRS} ; (4) The sequence-independent stochastic reversibility of the FtsK motor was simulated by assuming that FtsK had a probability $p_0 = 0.001$ (see below) of turning around at each translocation step; (5) Upon encounter of any of the ends of λ DNA, FtsK pauses with a probability of 95% or reverses translocation direction with a probability of 5% per time step; (6) the event ends when the pause on one end of λ DNA was longer than 10 time steps. The distribution and orientation of FRS correspond to those naturally occurring on λ DNA. Following these rules, a trace of the position of FtsK versus time was obtained. This process was repeated 2500 times, and mean burst sizes were calculated as for the experimental translocation traces.

For $p_{FRS} = 0.4$ (as determined by Levy *et al.*¹), the burst sizes distribution was asymmetric (Supplementary Fig. 2B), with bursts in the permissive direction being more likely than those in the non-permissive one. The total distance traveled by FtsK particles in the permissive direction was $\sim 70\%$ of the total distance translocated. The net distance translocated was in the permissive direction in 88% of events. By repeating this simulation for other values of p_{FRS} we found that values lower or higher than $p_{FRS} = 0.4$ did not reproduce well the experimental burst size distribution for FtsK_{50C}.

The lack of sequence recognition by FtsK_{50C}Δγ particles was simulated by assuming that FtsK had a probability p_0 of turning around at each translocation step. The burst size distribution that best represented the experimental data was obtained for $p_0 = 0.001$. In this case, the burst size distribution was symmetric and thus the probability of a given burst size was independent of the translocation direction (Supplementary Fig. 2B). Here, long bursts spanning the whole length of λ DNA were as frequent as in the experimental size distribution. The distance traveled by FtsK particles in the permissive direction was ~50% of the total and net distances translocated. By repeating this simulation for other values of p_0 we found that values lower or higher than $p_0 = 0.001$ did not reproduce the experimental burst size distribution for FtsK_{50C}Δγ.

Thus, our experimental results can be computationally predicted by assuming that the probability of turn around by FtsK_{50C} when encountering an FRS is ~40% and that FtsK_{50C}Δγ turns around stochastically without sequence recognition.

The FtsK motor turns around without sequence recognition

FtsK reverses stochastically in the absence of its FRS recognition domain (Fig. 1B and 2C). The burst size distribution of FtsK_{50C}Δγ allows us to measure the FtsK motor domain's intrinsic processivity, defined as the mean distance translocated in a burst. Since the translocation of FtsK_{50C}Δγ is unaffected by FRS, the burst size distribution is expected to decay following a Poissonian distribution. The best fit to the FtsK_{50C}Δγ experimental burst size distributions gave a mean processivity of ~9 kbp (Supplementary Fig. 2C, solid black lines). This mean processivity of FtsK_{50C}Δγ is consistent with our

magnetic tweezers experiments (data not shown). Previous processivity measurements for FtsK_{50C}² (~ 6.5 kbp) are consistent with our results, but may be lower due to non-specific γ -domain/DNA interactions. Our measured processivity is significantly shorter than distances FtsK translocates during chromosome dimer resolution (~ 250 kbp³). This propensity of the motor to turnaround every ~9 kbp may have necessitated the evolution of a mechanism that recognizes the natural distribution of FRS (every ~ 3.5 kbp). This mechanism ensures FtsK directional movement and may provide a rationale for the evolution and/or maintenance of the high density of FRS along the chromosome.

Interpretation of DNA looping in the magnetic tweezers assay

To loop DNA, FtsK must establish two points of contact. While translocating one DNA fragment, FtsK needs to be anchored to either another region of the same DNA molecule or a static surface. In this paper, we show that the binding of the γ -domain to DNA is not required for anchoring to DNA, as looping is still observed for FtsK_{50C} $\Delta\gamma$.

Looping of DNA by FtsK could be interpreted in terms of an FtsK complex that translocates DNA while remaining anchored to the surface of the capillary or the bead. Our optical tweezers experiments⁴ showed that FtsK particles loop from any location on DNA, in disagreement with this model.

Here, we favor a model (with some similar elements to that previously proposed²) in which an FtsK complex is formed by two coupled motors. Initially, the complex binds to DNA and one active motor translocates on DNA without looping. At some position on the DNA tether, one motor binds DNA and remains fixed to it; translocation by the other motor thus generates a DNA loop. In the absence of FRS, FtsK binds to any DNA sequence with equal probability. Because of the physical constraint imposed by the

surface of the capillary and bead, the FtsK complex is often localized to the extremity of the DNA, consistent with the frequently observed full-length translocation events. In the presence of FRS, however, one of the motors preferentially anchors close to the position of FRS due to γ -domain/FRS interactions. In this model, pauses at the location of the FRS repeat are related to simultaneous specific interactions of FtsK with FRS and non-specific interactions with the bead or the surface.

In our experiments, we measure pauses and reversals at specific extensions that correlate to the position of the FRS repeat. Both proposed models for DNA looping imply that those pauses and translocation direction reversals result from direct interactions between FRS and FtsK that are γ -domain specific.

Translocation of FtsK_{50C} and FtsK_{50C} $\Delta\gamma$ on DNA_{no-FRS}

The DNA tether DNA_{no-FRS} did not contain any FRS sequence. Other DNA tethers (DNA_{FRS,1/2} and DNA_{FRS,1/3}) contained only a repeat of three FRS sequences in positions corresponding to 1/2 or 1/3 of the tether length, respectively.

To test the specificity of the FtsK/FRS interaction, we shortened one end of DNA_{FRS,1/2} to create DNA_{FRS,1/3}. In this case, the position of the pauses and translocation direction reversals representing interactions between FRS and FtsK shifted from $\sim 2 \mu\text{m}$ (position of the FRS repeat in DNA_{FRS,1/2}) to $\sim 0.9 \mu\text{m}$, the position of the FRS repeat in the new DNA tether (Supplementary Fig. 3A).

On a substrate containing no FRS repeat (DNA_{no-FRS}), FtsK_{50C} and FtsK_{50C} $\Delta\gamma$ behave similarly: frequent loops the size of the full length of the DNA tether are observed

and pausing behavior is infrequent and non-sequence specific (Supplementary Fig. 3B and C, respectively).

Supplementary References

1. Levy, O. et al. Identification of oligonucleotide sequences that direct the movement of the Escherichia coli FtsK translocase. *Proc Natl Acad Sci U S A* **102**, 17618-23 (2005).
2. Saleh, O.A., Perals, C., Barre, F.X. & Allemand, J.F. Fast, DNA-sequence independent translocation by FtsK in a single-molecule experiment. *Embo J* **23**, 2430-9 (2004).
3. Corre, J. & Louarn, J.M. Extent of the activity domain and possible roles of FtsK in the Escherichia coli chromosome terminus. *Mol Microbiol* **56**, 1539-48 (2005).
4. Pease, P.J. et al. Sequence-directed DNA translocation by purified FtsK. *Science* **307**, 586-90 (2005).