Supporting Information

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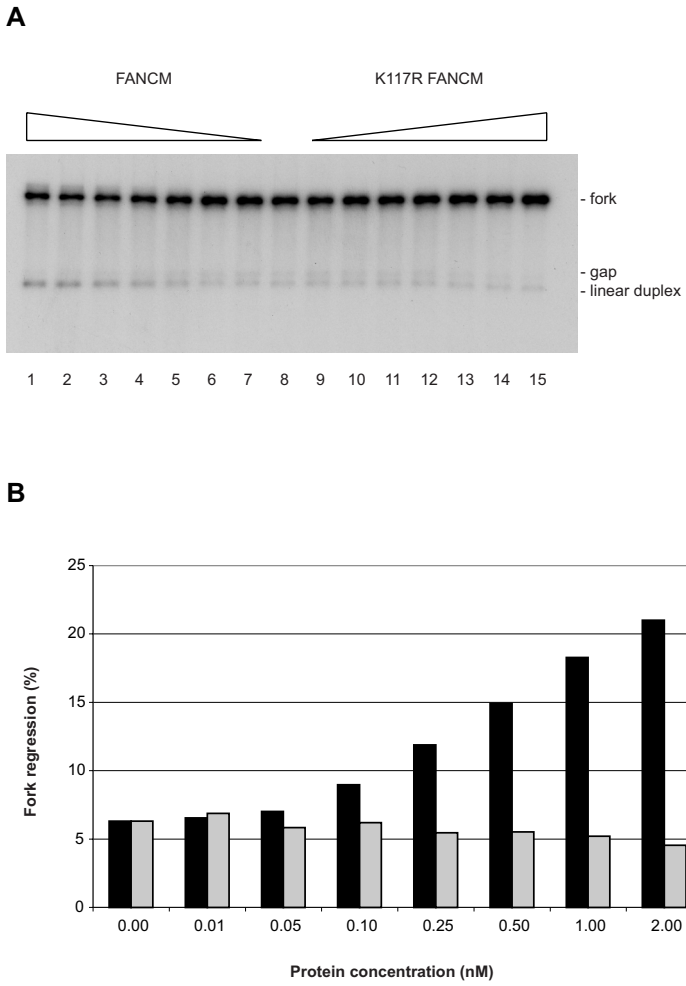

Fig. S1. Fork reversal mediated by FANCM is concentration-dependent. (*A*) Analysis of fork regression products by agarose gel electrophoresis. Increasing concentrations (0.01, 0.05, 0.1, 0.25, 0.5, 1, 2 nM) of FANCM (lanes 7–1) and K117R FANCM (lanes 9–15) were incubated with the replication fork substrate. In lane 8, no protein was added (mock control). (*B*) Quantification of the percentage of fork regression as a function of protein concentration (FANCM, black bars; K117R FANCM, gray bars). Fork regression was defined as linear duplex signal per total DNA signal.

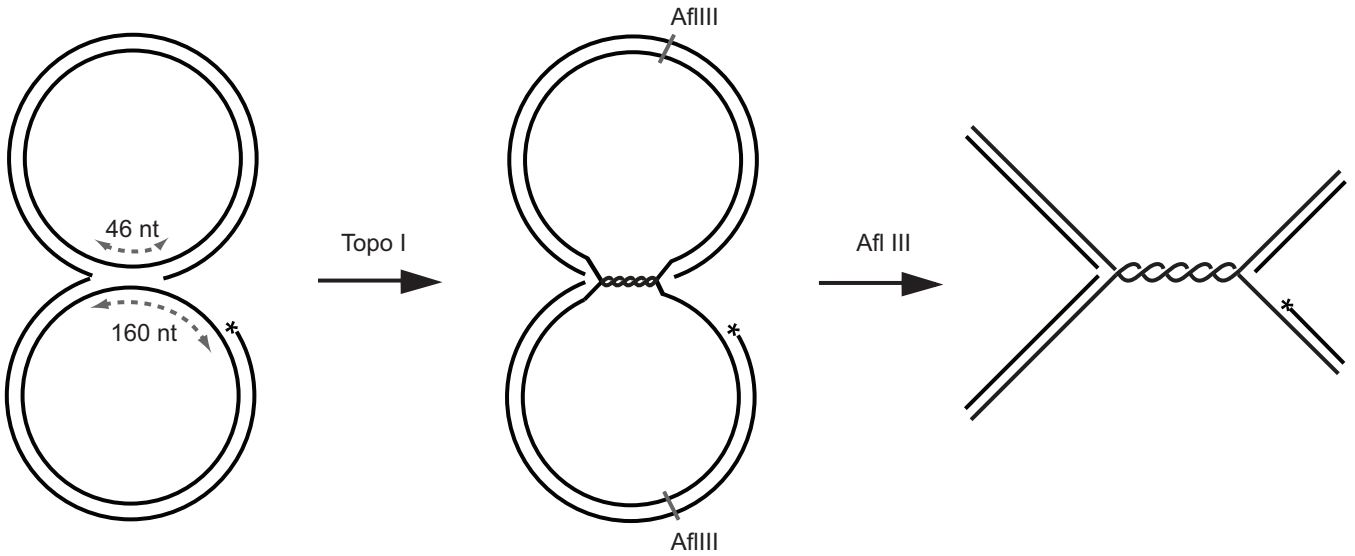

Fig. S2. Generation of replication fork with lagging strand gap. This substrate was created in a similar way as described [Ralf, *et al.* (2006) The Bloom's syndrome helicase can promote the regression of a model replication fork. *J Biol Chem* 281:22839–22846]. A 247 bp cassette (RF-lag100) was cloned into pUC18 sites to generate pRF-lag. The RF-lag100 cassette contains multiple recognition sites for the restriction enzymes BbvCI and BsmI (Table S1). Two differentially sized single-stranded gaps of 46 nt and 160 nt were generated on complementary strands by incubating pRF-lag with either Nt.BbvCI alone or Nb.BbvCI and Nb.BsmI, followed by heating to 80°C to denature and remove the short oligonucleotides that are created through nicking of the molecule. Gapped plasmids were control-digested and gel-purified. The plasmid with the 160 nt gap was end-labeled with T4 polynucleotide kinase, and annealed to the 46 nt gap plasmid by lowering the temperature gradually from 65°C to room temperature. The joint structure was treated with Topoisomerase I. After gel-purification, the structure was digested by AflIII and purified. The RF-lag100 insert was generated using four oligonucleotides (A-D) that contain complementary ends and thus, when annealed together, form a 259 nt partial duplex (Table S2). An intact fully contiguous duplex 259 bp molecule was generated by incubating oligonucleotides A-D together with oligonucleotides E and F (which are identical to the first 21 nt of oligonucleotides A and D, respectively) in a standard PCR mix containing *Pfu* polymerase, which was subjected to 30 cycles of 94°C, 30s; 55°C, 30s; 72°C, 30s. The resulting 259 bp product was cloned into pUC18 via EcoRI and HindIII sites.

Table S1. Sequence of RF-lag100

Sequence (5′-3′)
*GAATT*CGCTCCATCTCTGGCTTCCCGCTAGCCATTATGCGCAGGCAGCCGCCTAGGGTGACAGGCTCATGGATAT**GAATGC**ACTCGAG**GAATGC**ACGGTA**GA ATGC**AAA**GAATGC**AGGTT**GAATGC**ATTA**GAATGC**CCCATGG**GAATGC**ACAGA**GAATGC**AGTATC**GAATGC**AAATC**GAATGC**ACGTACCTCAGCGATC CCTCAGCACTAGTCCTCAGCTGTACCTCAGCACGTCCTCAGC*AAGCTT*

The sequence of RF-lag100 insert that was cloned into pUC18 via EcoRI/HindIII sites is italicized. Recognition sequences for BbvCI and BsmI are underlined and marked in bold, respectively.

Table S2. Oligonucleotides used for the construction of RF-lag100

Oligo	Sequence (5′-3′)
A	GAGAAT*GAATTC*GCTCCATCTCTGGCTTCCCGCTAGCCATTATGCGCAGGCAGCCGCCTAGGGTGACAGGctcatggatat**gaatgc**actcgag
B	tctgt**gcattc**ccatggg**gcattc**taaT**GCATTC**AACCT**GCATTC**TTT**GCATTC**TACCGT**GCATTC**ctcgagt**gcattc**atatccatgag
C	tta**gaatgc**cccatgg**gaatgc**acaga**GAATGC**AGTATC**GAATGC**Aaatc**gaatgc**acgtacctcagcgatc
D	TCTGTC*AAGCTT*GCTGAGGACGTGCTGAGGTACAGCTGAGGACTAGTGCTGAGGgatcgctgaggtacgt**gcattc**gatt
E	GAGAAT*GAATTC*GCTCCATCT
F	TCTGTC*AAGCTT*GCTGAGGAC

RF-lag100 oligonucleotide sequences used in the construction of pRF-lag. Recognition sequences for the nicking endonucleases Nb.BsmI and Nb.BbvCI/Nt.BbvCI are in bold and underlined, respectively. EcoRI and HindIII sites have been italicized. Complementary sequences at the ends of oligonucleotides A–D that allow the formation of a 259-bp partial duplex are shown in lower case.