# Supporting Information

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### SI Materials and Methods

**Proteins.** Wild-type helicase-like transcription factor (HLTF) and ATPase mutant DE557,558AA HLTF were purified to apparent homogeneity after being overexpressed as GST-FLAG-fusion proteins in yeast using plasmids PIL1520 and PIL1734, respectively, as described (1, 2). Human Bloom helicase (BLM) was overexpressed as a GST-FLAG-fusion protein in yeast using plasmid pIL1863. BLM purification was carried out in a buffer containing 50 mM KPO4, 500 mM KCl, 10% Glycerol, 1 mM EDTA, 1 mM DTT, and 0.01% NP40 on glutathione-Sepharose 4B beads. After elution by PreScission protease cleavage, apparently 95% homogeneous FLAG-human BLM was obtained. *Escherichia coli* E111Q EcoRI endonuclease mutant protein was purified from an overproducing bacterial strain by a two-column procedure as described in ref. 3. Human replication protein A (RPA), human proliferating cell nuclear antigen (PCNA) and yeast replication factor C (RFC) were purified as previously described (4, 5). *E. coli* ssDNA-binding protein (SSB) was purchased from USB Corporation.

**DNA Substrates.** Oligonucleotide-based DNA substrates were generated by annealing highly purified oligonucleotides in various combinations, followed by purification on polyacrylamide gels as described previously (2). The term homologous fork (HomF) indicates forks with complementary leading and lagging arms, whereas gap substrate (GapHomF) indicates a homologous fork with a 15-nt gap in the leading arm toward the junction. Oligonucleotides were used in the following combinations to generate respective substrates, in which underlined oligonucleotide(s) are $^{32}$P-labeled at the 5′-end:

HomF: <u>O1054</u>/ O1056/ <u>O1058</u>/ O1118
GapHomF: <u>O1055</u>/ O1244/ <u>O1058</u>/ O1118
HomF-Biotin: O1054/ O1056/ <u>O1058</u>/ O1118-Biotin
EcoRI site on base: O2809/ <u>O2810</u>/ O1058/ <u>O1118</u>
Trap duplex: <u>O1118</u>/ O1058
Oligonucleotide sequences were as follows:

O1054:
AgCTACCATgCCTgCCTCAAgAATTCgTAA
O1056:
TTACgAATTCTTgAggCAggCATggTAgCT
O1058:
AgCTACCATgCCTgCCTCAAgAATTCgTAATATgCCTAC-ACTggAgTACCggAgCATCgTCgTgACTgggAAAAC
O1118: gTTTTCCCAgTCACgACgATgCTCCggTACTC-CAgTgTAggCATATTACgAATTCTTgAggCAggCATggTAgCT
O1055:
AgCTACCATgCCTgCCTCAAgAATT
O1244:
TgTAggCATATTACgAATTCTTgAggCAggCATggTAgCT
O2809
AATATgCCTACACTggAgTACCggAgCATCgTCgTgACTgg-gAAAAC
O2810
gTTTTCCCAgTCACgACgATgCTCCggTACTCCAgTgTAgg-CATATT

**Fork Reversal Assay on Plasmid-Sized Replication Fork Model Substrate.** The plasmid-sized replication fork model substrate was created essentially as described (2, 6). Briefly, pG46 and pG68 plasmids were gapped by digestion with nicking endonucleases Nt.BbvCI and Nb.BbvCI yielding pG46B and pG68A, respectively. pG46B was then treated with shrimp alkaline phosphatase and subsequently labeled with T4 polynucleotide kinase to yield the plasmid pG46B'. pG68A was linearized with XhoI digestion to yield pG68A Xh. The resulting plasmids containing complementary single stranded gaps were then annealed together at 53 °C in a buffer containing 50 mM Tris·HCl pH 7.5, 10 mM $MgCl_2$, and 5 mM DTT to form a joint molecule (Fig S7*A*). Note, that the joint molecule contains a structural mimic of a stalled replication fork in which the labeled lagging strand is longer by 14 nucleotides. Assays with the plasmid-sized forks were carried out essentially as described in *Materials and Methods* but using 5 nM substrate DNA, which was either naked or preincubated with PCNA (640 nM) and RFC (640 nM) at 37 °C for 15 min followed by incubation with HLTF (80 nM) for 15 min at 37 °C. Reaction mixtures were quenched after incubation and analyzed by restriction enzyme digestion as described previously (2, 6).

1. Unk I, et al. (2008) Human HLTF functions as a ubiquitin ligase for proliferating cell nuclear antigen polyubiquitination. *Proc Natl Acad Sci USA* 105:3768–3773.
2. Blastyak A, Hajdu I, Unk I, Haracska L (2010) Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA. *Mol Cell Biol* 30:684–693.
3. Wright DJ, King K, Modrich P (1989) The negative charge of Glu-111 is required to activate the cleavage center of EcoRI endonuclease. *J Biol Chem* 264:11816–11821.
4. Unk I, et al. (2006) Human SHPRH is a ubiquitin ligase for Mms2-Ubc13- 15 dependent polyubiquitylation of proliferating cell nuclear antigen. *Proc Natl Acad Sci USA* 103:18107–18112.
5. Henricksen LA, Umbricht CB, Wold MS (1994) Recombinant replication protein A: Expression, complex formation, and functional characterization. *J Biol Chem* 269:11121–11132.
6. Ralf C, Hickson ID, Wu L (2006) The Bloom's syndrome helicase can promote the regression of a model replication fork. *J Biol Chem* 281:22839–22846.

Fig. S1. Quantitative comparison of HLTF and BLM fork regression activity on a modeled replication fork bound by E111Q EcoRI protein. As shown in Fig. 1C, fork regression assays by HLTF and BLM in the presence or absence of E111Q EcoRI were carried out and measured from three independent experiments by using PhosphorImager and ImageQuant software. Standard deviation is then calculated and plotted.

Fig. S2. Comparison of HLTF fork reversal activities on homologous forks bound by E111Q EcoRI protein on one or both of the arms. Before initialization of the fork reversal assays by HLTF (10 nM), homologous fork (1 nM) containing an EcoRI binding site on both the arms (II), only on the lagging arm (III), or only on the leading arm (IV) of the fork was preincubated with E111Q EcoRI (350 nM) protein. The control experiment is shown in I in which the homologous fork containing an EcoRI binding site on both the arms was not preincubated with E111Q EcoRI protein.

Fig. S3. Fork reversal activity of HLTF and BLM on homologous fork bound by E111Q EcoRI on the base of the fork. (*A*) Gel retardation assay showing sequence-specific binding and formation of stable DNA-protein complex by E111Q EcoRI and oligo-based fork-like structures. Increasing amount of E111Q EcoRI was incubated with homologous fork containing an EcoRI binding site on the base of the fork as shown schematically. (*B*) Comparison of HLTF on protein-free and base-bound E111Q EcoRI protein containing homologous forks. In I, activity of HLTF on naked fork; II, activity of HLTF on E111Q EcoRI-bound fork. Each lane within the panel represents time points at which samples were collected and are noted at the bottom of the gel. On the left side of the gel, the appropriate markers with expected fragment sizes are shown in nucleotides.

**Fig. S4.** Gel retardation assays for confirming the binding of RPA and SSB to a homologous fork containing a 15-nt gap on its leading arm and activity of BLM on RPA or SSB-bound fork DNA. (*A*) Binding of RPA to the single-stranded region of the fork. In I, homologous fork without any single-stranded region; II, homologous fork with a 15-nt long single-stranded region. (*B*) Binding of *E. coli* SSB to the single-stranded region of the fork. In I, homologous fork without any single-stranded region; II, homologous fork with a 15-nt long single-stranded region. (*C*) Fork reversal activity of BLM on RPA or SSB-bound substrate. In I, control: RPA-bound gapped fork without BLM; II, BLM activity on gapped fork without RPA; III, BLM activity on RPA-bound gapped fork; IV, control: SSB-bound gapped fork without BLM; V, BLM activity on gapped fork without SSB; VI, BLM activity on SSB-bound gapped fork.

**Fig. S5.** Schematic representation of a possible mechanism through which HLTF can coordinately remodel gapped replication fork-like structures bound by ssDNA-binding protein like RPA or SSB.

**Fig. S6.** Comparison of HLTF and its yeast homologue Rad5 fork regression activity on a homologous fork bound by PCNA, RFC, and RPA. Activity of HLTF (10 nM) and Rad5 (10 nM) is compared on a homologous fork containing a 15-nt gap on the leading arm of the fork. In I and III, controls without PCNA, RFC, and RPA, whereas II and IV were incubated with 80 nM each of RFC, PCNA, and 160 nM RPA prior to fork regression assay.

**Fig. S7.** Fork regression activity of HLTF on a plasmid-based fork bound by PCNA and RFC. Schematic representation of the joint DNA substrate (pG46B/pG68AXh) and the outcome of its HLTF-mediated regression. Letters B, E, and P refer to restriction endonuclease sites BamHI, EcoRI, and PvuII, respectively. The positions of 5′ $^{32}$P labels on the "lagging strand" are marked with an asterisk. Fork regression activity of HLTF on a plasmid-based fork bound by RFC and PCNA. In I and III, controls without PCNA and RFC, whereas DNA for II and IV were preincubated by PCNA and RFC (640 nM each). Lane 1, without any restriction enzymes, whereas samples in lanes 2, 3, and 4 were digested with BamHI (B), EcoRI (E), and PvuII (P) before loading onto the gel. Note that plasmid has two cleavage sites for PvuII.