Supplementary Data

Replicative helicase encircles double-stranded DNA during unwinding

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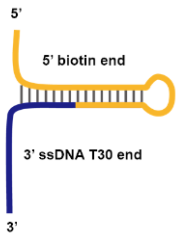
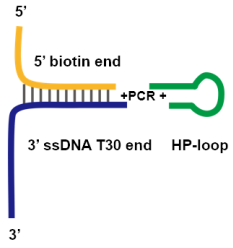
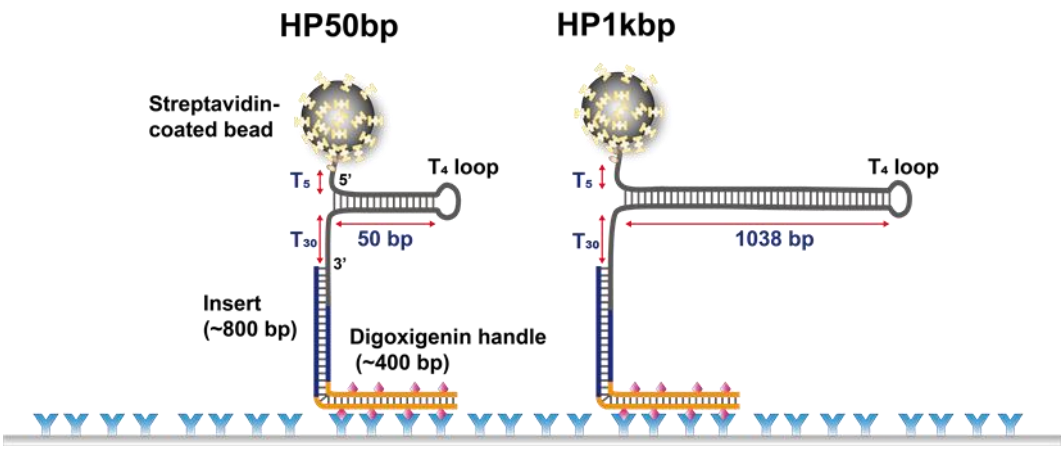
Supplementary Table S1. Oligonucleotide sequences for the core hairpin part of HP constructs

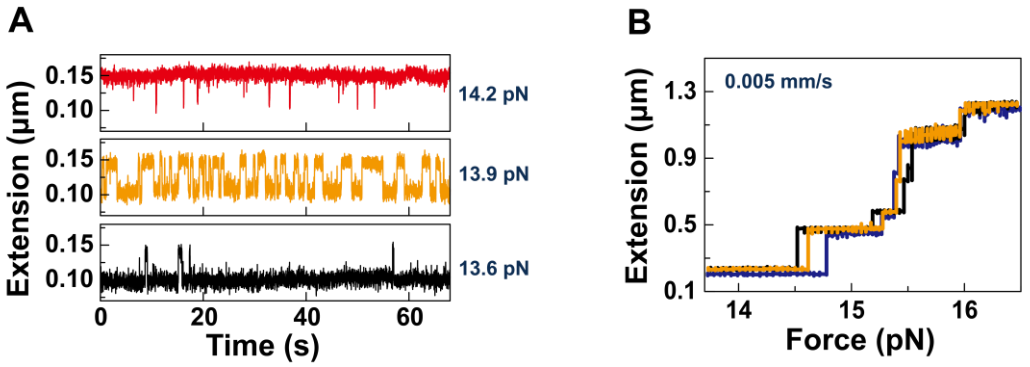
HP50bp		**5' biotin end** (bead connector)	5'-/5'Biosg/TT/iBiodT/T /iBiodT/ TTTTTAACTAACATGTCATATCAAGTTACTAGTAG ATCTGATCAGTATACTCTTCTTTTGAAGAGTATAC TG-3'
		3' ssDNA T30 end (insert connector)	5'-/5'Phos/ATCAGATCTACTAGTAACTTGATATGACAT GTTAGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTG GTCACCGGC-3'
HP1kbp		**5' biotin end** (bead connector)	5'-/5Biosg/TT/iBiodT/ T/iBiodT/TTTTTGGCAAACATGTCCTAGCAAGGCA CTGGTAGAATTCGG-3'
		3' ssDNA T30 end (insert connector)	5'-/5Phos/CCATCTCGCTCCGAATTCTACCAGTGCCT TGCTAGGACATGTTTGCCTTTTTTTTTTTTTTTTT TTTTTTTTTTTTGGTCACCGGC-3'
		HP-loop	5'-/5Phos/GGTGTGAAAGCAGCGTGCTTCTCTTTTGA GAAGCAC GCTG-3'

Supplementary Figure S1. HP constructs used in experiments



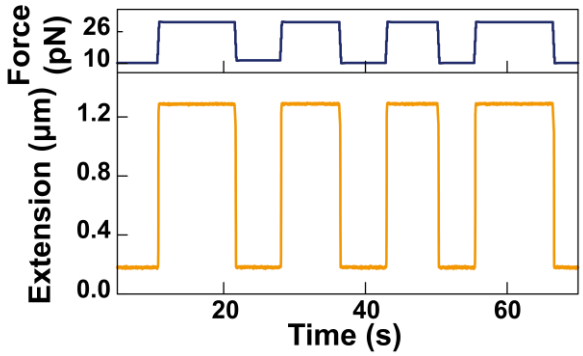
Two types of HP constructs (HP50bp and HP1kbp) were used in the experiments. The HP constructs were bound to streptavidin-coated paramagnetic beads through biotin-streptavidin interactions and to anti-digoxigenin-coated glass surfaces through digoxigenin-anti-digoxigenin interactions. The core hairpin part consists of a duplex stem, a tetrathymine (T_4) loop, and two single-stranded regions. The single-stranded region at the 5' end is a five thymine overhang with three biotin labels, and the other region at the 3' end contains a 30 base thymine sequence (T_{30}) for helicase loading and 10 bases complementary to the overhang of the insert.

Supplementary Figure S2. Mechanical unzipping of HP and determination of the unzipping force (F_{unzip})



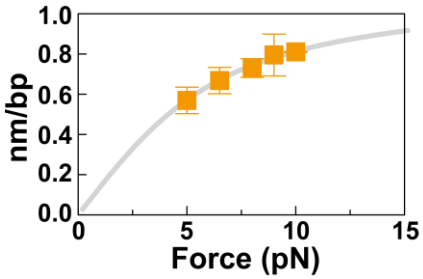
(A) Traces of an HP50bp molecule at three different forces near the unzipping force (F_{unzip}). The extension fluctuated between two levels corresponding to the unzipped and zipped states of HP. The populations in the unzipped and zipped states were sharply inverted near 13.9 pN. The exact F_{unzip} determined from the force values in which the probability in two states became equal, as in the center panel. The measurement was performed under the same buffer conditions used in the unwinding experiments. The F_{unzip} obtained for HP50bp was 14.3 ± 0.8 pN. (B) Traces of three different HP1kbp molecules recorded while moving the magnet at a very slow speed of 0.005 mm/s to minimize hysteresis. HP1kbp molecules started the unzipping transition near the F_{unzip} of HP50bp. However, HP1kbp presented several intermediate states because of pauses in the high GC region.

Supplementary Figure S3. Reversible unzipping and rezipping of HP1kbp



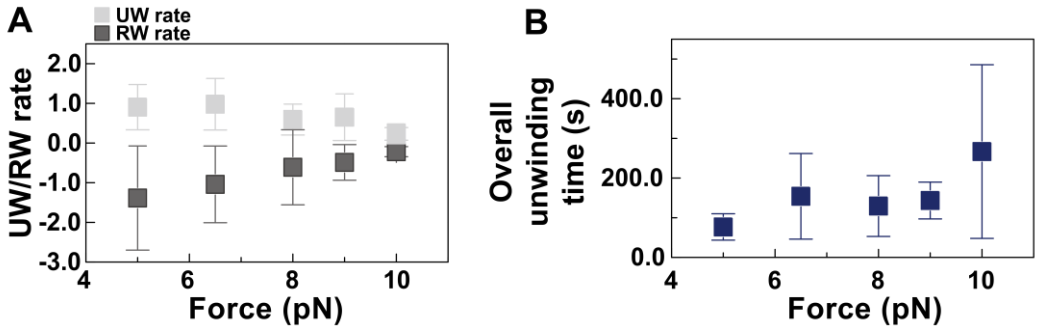
Bare HP1kbp was rapidly unzipped when the force was increased to a value greater than F_{unzip} and rezipped when the force was lowered in a reversible way.

Supplementary Figure S4. Conversion of the extension increase to the number of unwound base pairs



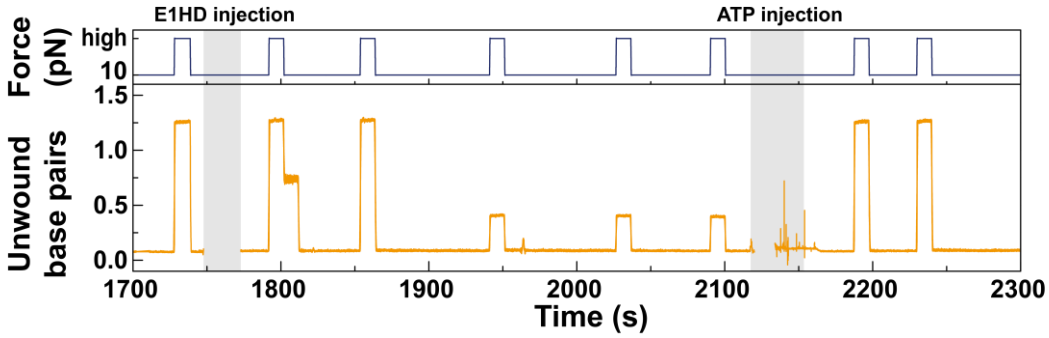
The extension increase per unwound base pair was obtained for HP50bp. The extension increase when HP50bp was fully unwound by E1 was normalized to the total number of base pairs in the HP50bp stem. The normalized constants were obtained at various forces (solid orange squares) and agreed well with the calculations from the extensible FJC model (gray line) (1). The constants of HP1kbp were almost the same as those of HP50bp. Then, the extension increase at a force was converted to the number of unwound base pairs using the normalized constant at the force.

Supplementary Figure S5. Activities of E1 on HP50bp at various forces



(A) UW and RW rates obtained with HP50bp at various forces. In contrast to the general consensus that rewinding is not sensitive to applied force, the RW rate was also affected by the force in E1. Like the UW rate, the RW rate tended to decrease as the force increased, which suggests that E1 interacts with both strands during rewinding and unwinding. Total 157 events were analyzed for UW and RW rates and more than 9 events were used for each data point. (B) To determine the effect of force on the overall unwinding activity, we collected the time required to fully unwind HP50bp. The overall unwinding time increased as the force increased. Total 39 full winding events were measured and at least 4 events were used per data point.

Supplementary Figure S6. Encircling dsDNA by E1HD without ATP



A similar trace to that shown in Figure 4A was recorded for E1HD. Judging from the trace before injecting ATP, E1HD seemed to encircle dsDNA without ATP as in E1. The dsDNA encircling occurred only at a high concentration (i.e., above 1.3 μM) because the binding affinity of E1HD on ssDNA is low without ATP (the observed K_d ~ 1.8 μM) (2). Contrary to E1, we failed to observe the active unwinding of E1HD after sequentially injecting ATP. One explanation for the failure may be that E1HD assembled without ATP is a wrongfully associated complex that is unable to unwind DNA. The second might be that E1HD was readily dissociated from DNA during the injection of buffer containing ATP because of low affinity. Active unwinding was observed only when E1HD and ATP were added at the same time. These observations suggest that ATP is required for the reorganization of E1HD to an unwinding-competent form. From another perspective, they might stress the importance of DBD domain (missing part of E1HD) for a working E1 helicase.

Supplementary References

1. Johnson, D.S., Bai, L., Smith, B.Y., Patel, S.S. and Wang, M.D. (2007) Single-molecule studies reveal dynamics of DNA unwinding by the ring-shaped T7 helicase. *Cell*, **129**, 1299-1309.

2. Castella, S., Burgin, D. and Sanders, C.M. (2006) Role of ATP hydrolysis in the DNA translocase activity of the bovine papillomavirus (BPV-1) E1 helicase. *Nucleic Acids Research*, **34**, 3731-3741.