

Expanded View Figures

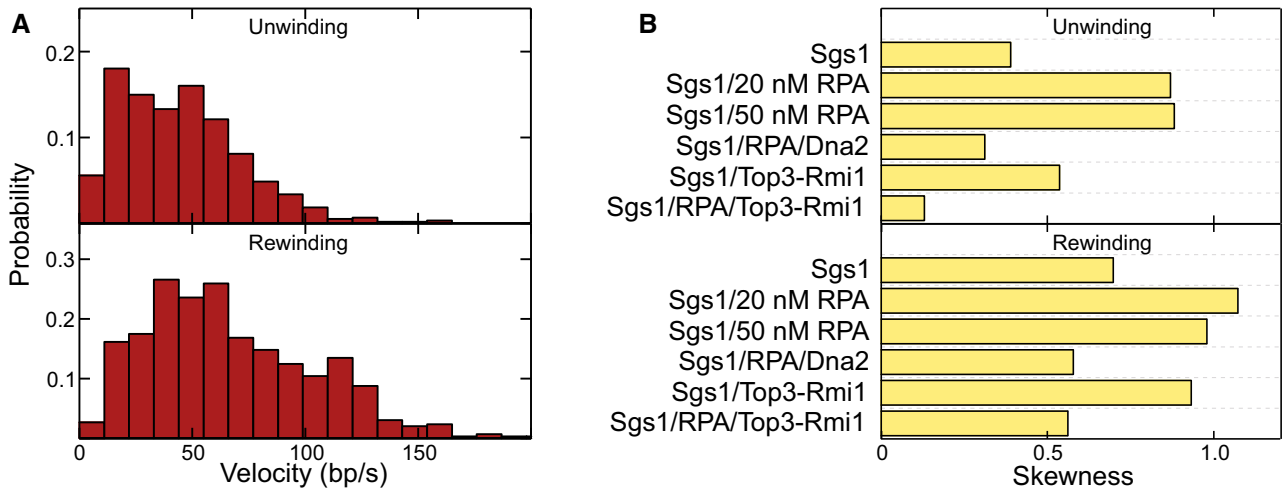


Figure EV1. Skewness of the velocity distributions for unwinding and rewinding for the different protein combinations.

A Histograms of the observed unwinding and rewinding velocities for Sgs1 in the presence of 50 nM RPA. The mean unwinding rate was 46 ± 3 bp/s ($N = 597$). The mean rewinding rate was 68 ± 3 bp/s ($N = 594$).
 B The skewness was calculated for the data shown in the velocity histograms in the main text.

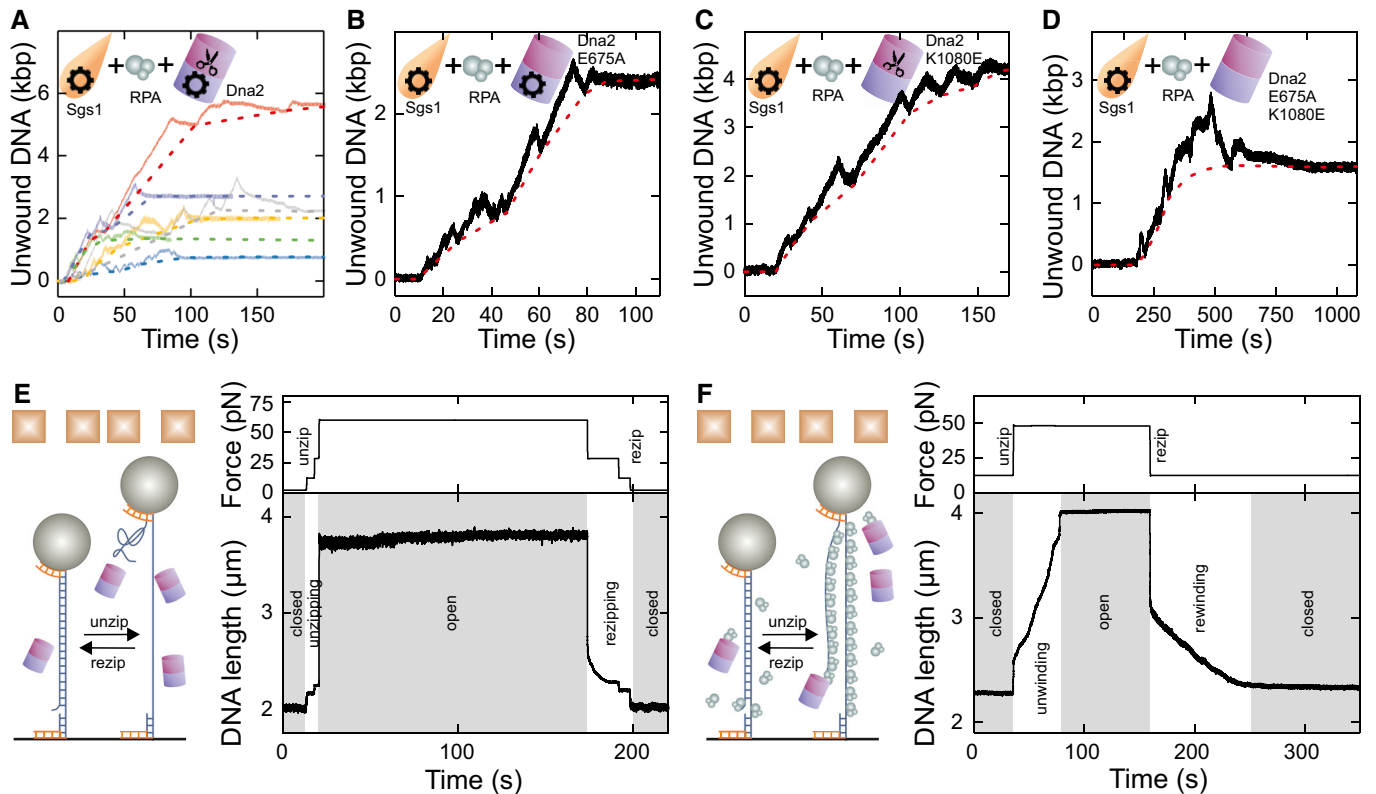


Figure EV2. DNA duplex processing by the DNA resection complex containing Sgs1, RPA, and mutated Dna2.

- A Different dsDNA processing events in the presence of Sgs1, Dna2, and RPA. Solid lines show the measured DNA unwinding, while dashed lines reveal the estimated Dna2 position.
- B dsDNA processing event in the presence of Sgs1, RPA, and the nuclease-dead mutant Dna2 E675A.
- C dsDNA processing event in the presence of Sgs1, RPA, and the helicase-dead mutant Dna2 K1080E.
- D dsDNA processing event in the presence of Sgs1, RPA, and the double helicase- and nuclease-dead mutant Dna2 E675A K1080E.
- E Mechanical unzipping of dsDNA in the presence of Dna2 E675A K1080E does not lead to inhibited DNA re-zipping. dsDNA was mechanically unzipped in the presence of Dna2 E675A K1080E and kept open for > 100 s. Subsequently re-zipping was induced by stepwise reduction of the applied force. The DNA length resumed to the original level, indicating re-zipping over the entire molecule length.
- F Mechanical unzipping of dsDNA in the presence of RPA and Dna2 E675A K1080E does not lead to inhibited DNA re-zipping. dsDNA was mechanically unwound in the presence of RPA and Dna2 E675A K1080E. After keeping it in the unwound form for ~70 s, the force was lowered to allow DNA rewinding against the bound RPA. The DNA length resumed to the original level, indicating that the rewinding occurred over the entire molecule length.

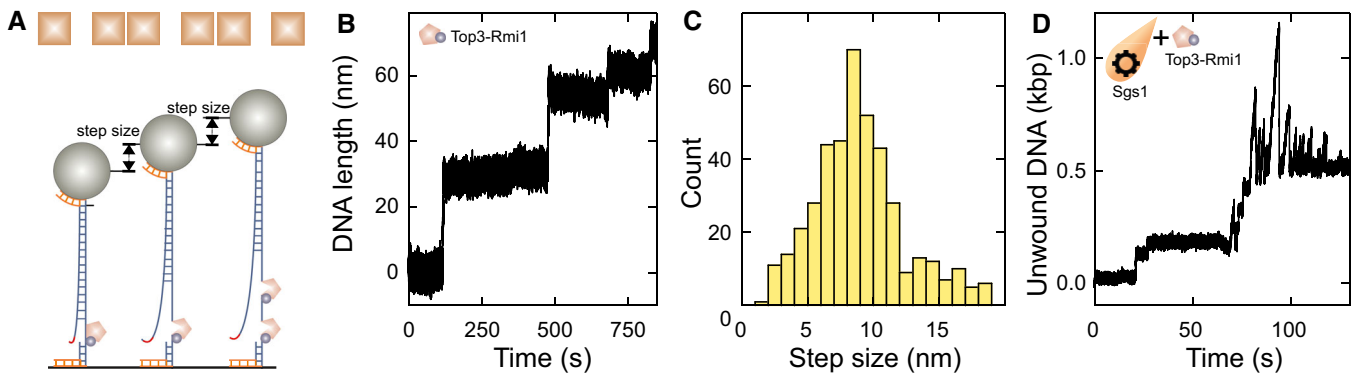


Figure EV3. Top3-Rmi1 cleavage activity of ssDNA.

- A Scheme illustrating the Top3-Rmi1 ssDNA cleavage activity observed in the magnetic tweezers assay.
- B Stepwise DNA length increase seen in the presence of Top3-Rmi1.
- C Histogram of the size of the stepwise length increase having a mean of 8.6 ± 0.2 nm ($N = 433$).
- D dsDNA processing events observed for Sgs1 in the presence of Top3-Rmi1. Stepwise DNA elongation due to ssDNA cleavage by Top3-Rmi is observed together with dsDNA unwinding–re-zipping bursts by Sgs1.

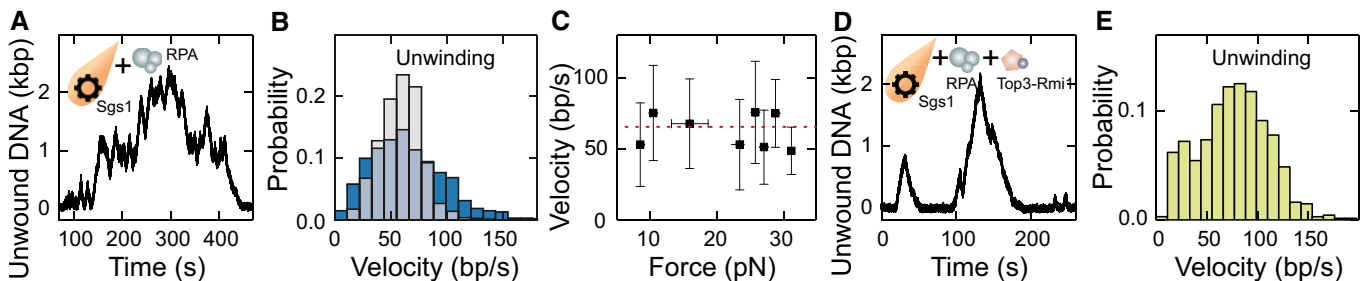


Figure EV4. DNA duplex processing by Sgs1 together with RPA and Top3-Rmi1 at high-salt conditions.

- A dsDNA processing events observed for Sgs1 in the presence of RPA at high-salt conditions.
- B Histograms of unwinding velocity of Sgs1 in the presence of RPA at high-salt conditions. Shown are the velocities in the presence of 20 nM RPA (gray) and 200 nM RPA (dark blue). Mean unwinding velocities are $v = 59 \pm 2$ bp/s ($N = 860$) and $v = 65 \pm 3$ bp/s ($N = 1,095$) for 20 and 200 nM RPA, respectively.
- C Force dependence of the Sgs1 unwinding (black squares) ($N = 1,095$) velocities in the presence of 200 nM RPA. Red dashed lines represent mean values of the velocities, and error bars represent the standard deviation of the mean.
- D dsDNA processing events observed for Sgs1 in the presence of RPA and Top3-Rmi1 at high-salt conditions.
- E Histogram of the unwinding velocity of Sgs1 in the presence of RPA and Top3-Rmi1. The mean unwinding velocity was $v = 77 \pm 4$ bp/s ($N = 678$).

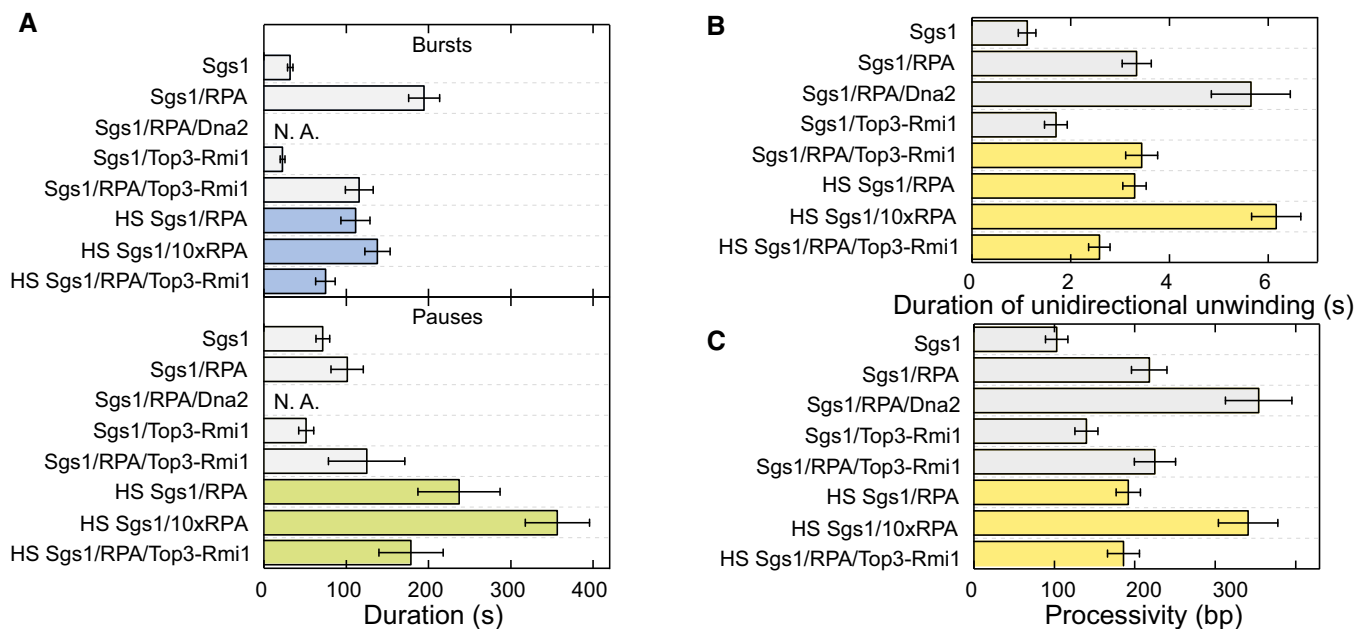


Figure EV5. Directionality, processivity, and initiation of DNA processing events for the different protein combinations at high-salt conditions.

A Mean duration of bursts (upper panel, violet bars) ($N > 40$) and pauses (lower panel, green bars) ($N > 40$) for the different enzyme combinations at high-salt conditions. Error bars represent standard error of the mean.
 B Mean duration of unidirectional unwinding ($N > 650$). Error bars represent standard error of the mean.
 C Mean processivity of unidirectional unwinding by the enzyme(s) ($N > 650$). Error bars represent standard error of the mean.