

Interplay Between RecQ Mechanochemistry and Domain Architecture Supports Quality Control of Homologous Recombination

NIH DNA REPAIR INTEREST GROUP

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Keir Neuman

Laboratory of Single Molecule Biophysics

NHLBI, NIH

In collaboration with Mihály Kovács
Eötvös Loránd University, Hungary

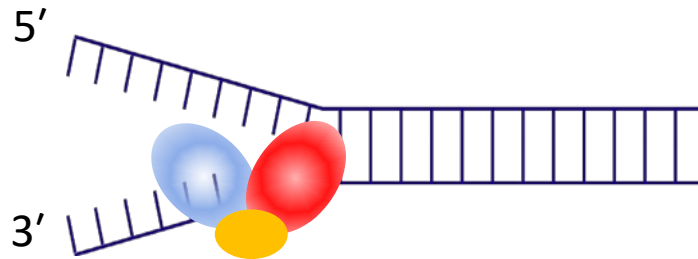
Harami, Seol, et al (2017) *PNAS* and unpublished results

Outline

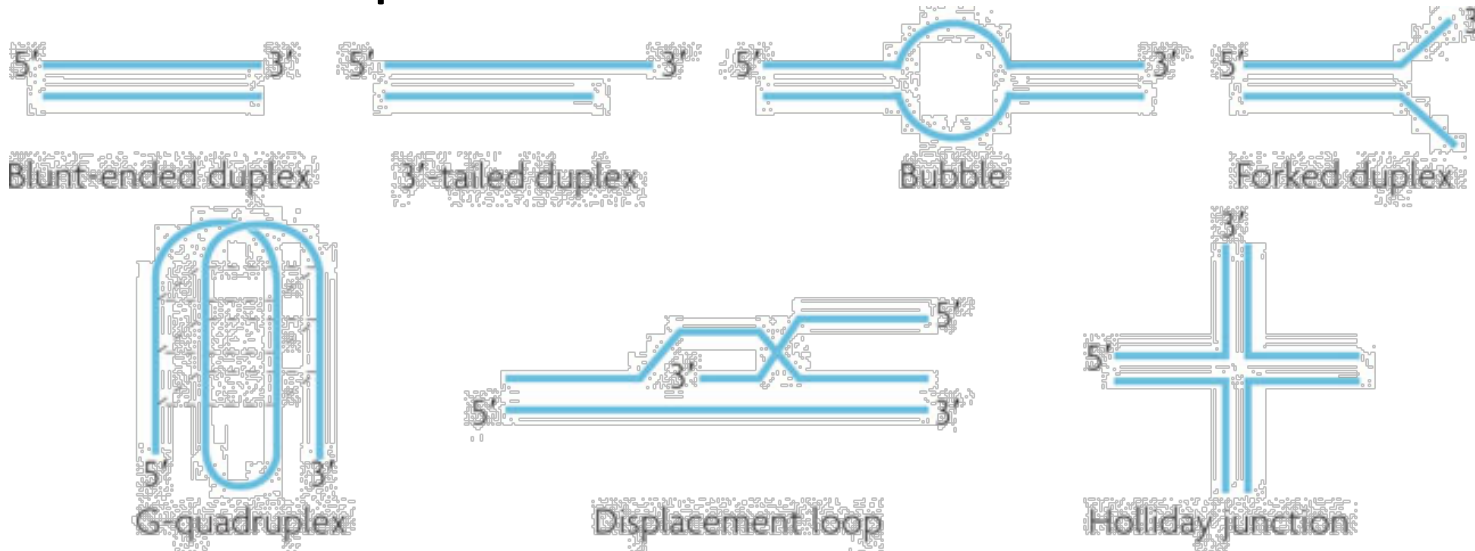
- RecQ helicases
 - Functional roles in genome maintenance
 - Conserved domain architecture
- Magnetic tweezers
- Single-molecule measurements of RecQ helicases
 - DNA sequence- and geometry-dependent pausing
 - Mechanochemistry of DNA unwinding
- Model of illegitimate recombination suppression
 - Structure- and homology-specific modulation of RecQ activity
 - Directional loading of RecQ on recombination intermediates
- Conclusions

RecQ DNA helicases

- Conserved from *E. coli* to human
- Unwind DNA duplex in the 3' to 5' direction



- Process complex DNA structures



RecQ mutations linked to genome instability

Genome instability

- Chromosome breaks
- Sister chromatid exchanges
- Illegitimate recombination



<http://humgen677s11.weebly.com>

RecQ helicase deficiencies in humans: BLM, WRN, and RecQ4

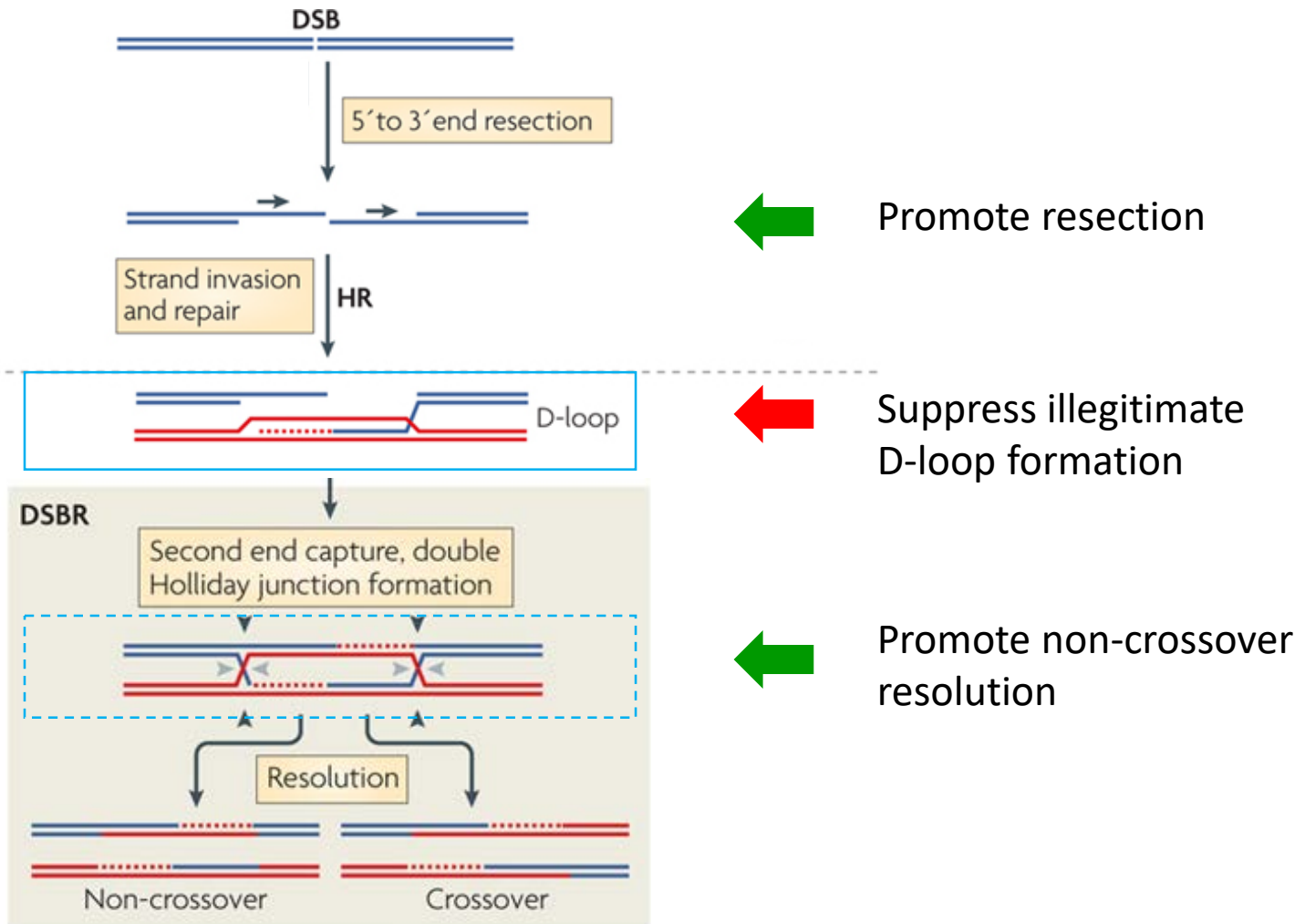
- Rare autosomal recessive diseases
- Chromosomal instability
- Cancer predisposition
- Premature aging and infertility



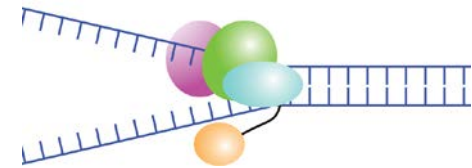
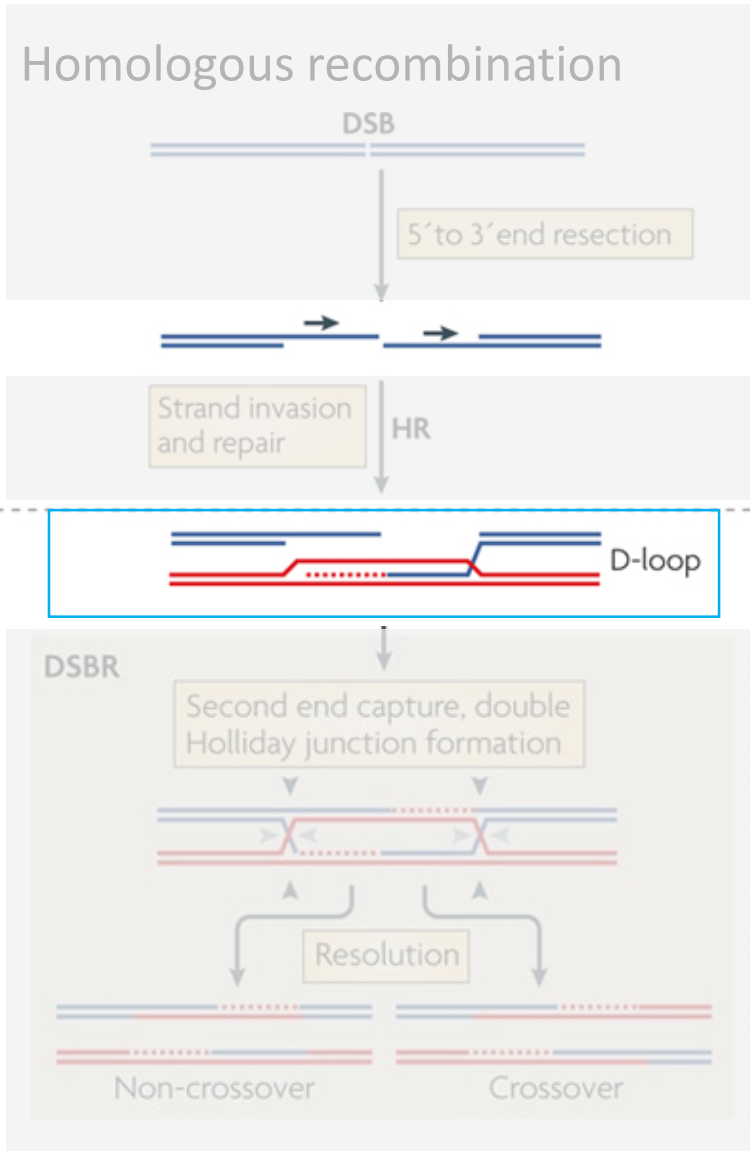
Blooms Syndrome

RecQ plays multiple roles in recombination

Homologous recombination



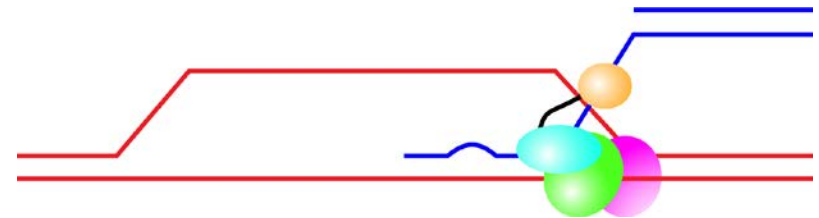
RecQ plays multiple roles in recombination



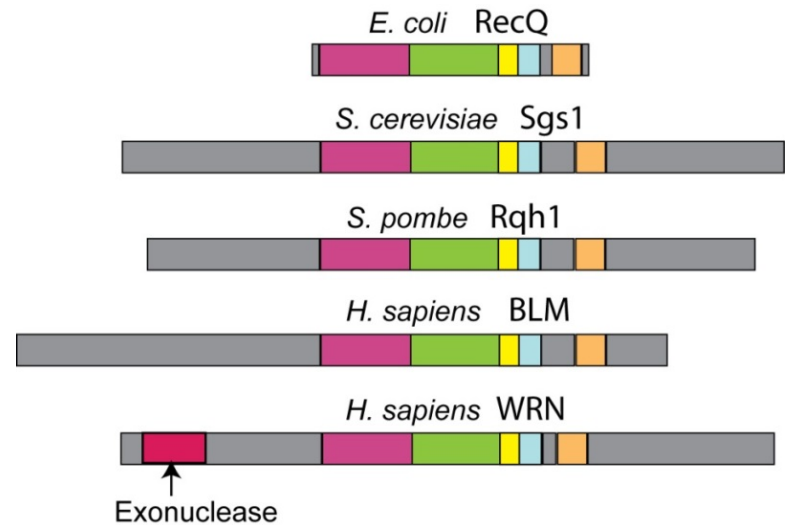
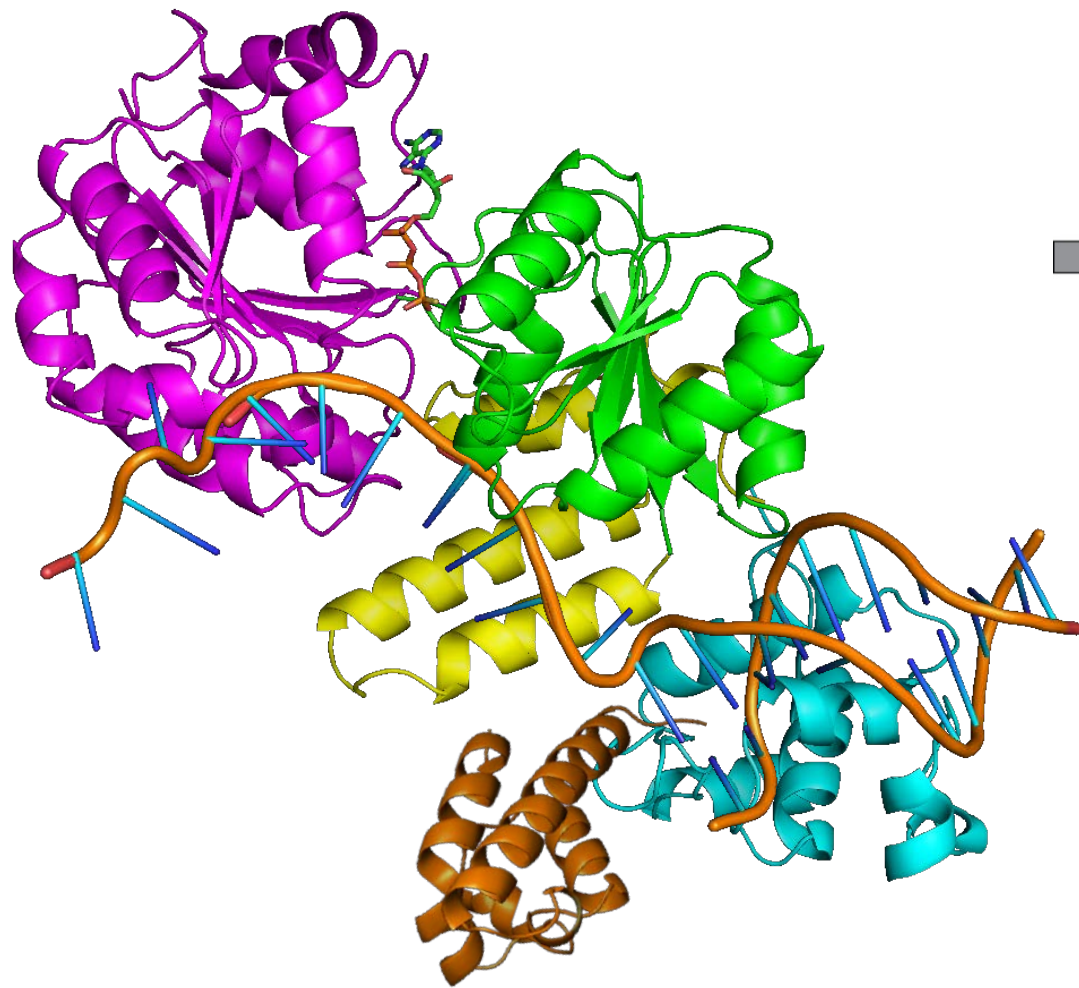
Unwinding 3' ends to promote resection



Suppress illegitimate D-loop formation

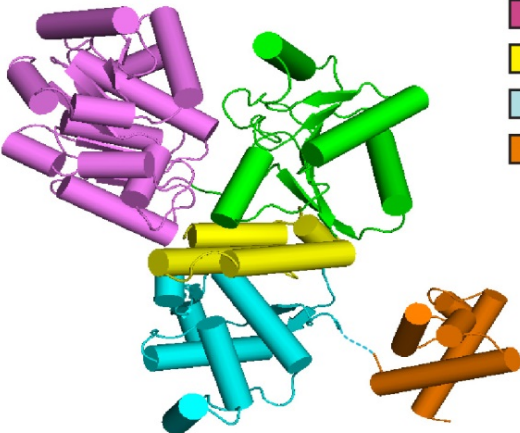


Conserved RecQ domain architecture

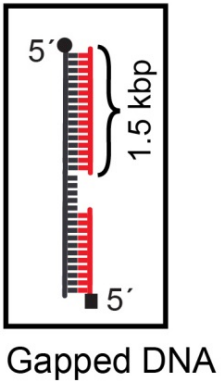
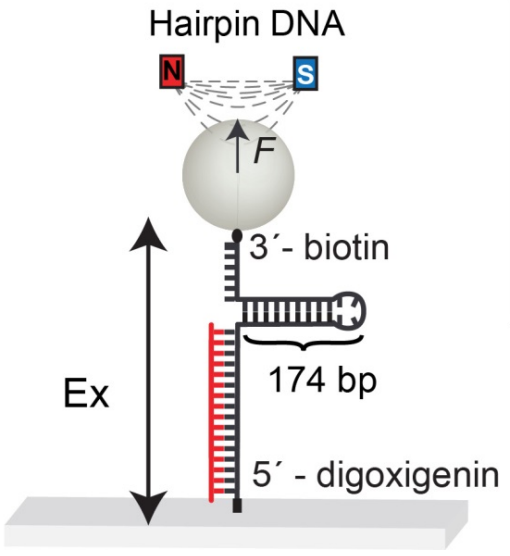
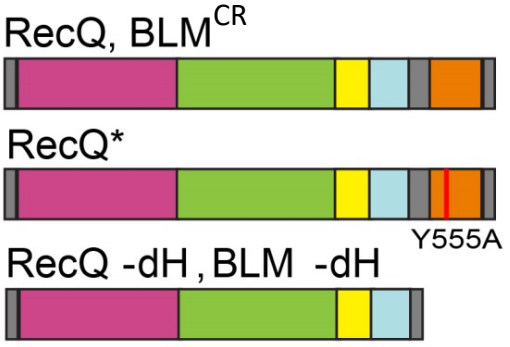


- Helicase domains (**H1** and **H2**): ATP dependent DNA translocation
- RecQ C- terminal (RQC) [**Zn binding** and **winged helix**]: protein stability and duplex DNA binding
- Helicase-and-RnaseD like C-terminal domain (**HRDC**): ssDNA binding and substrate specificity

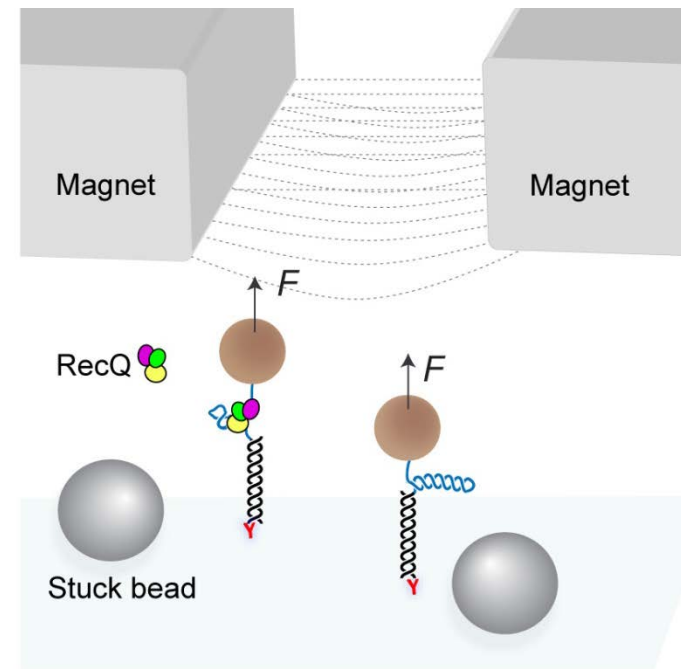
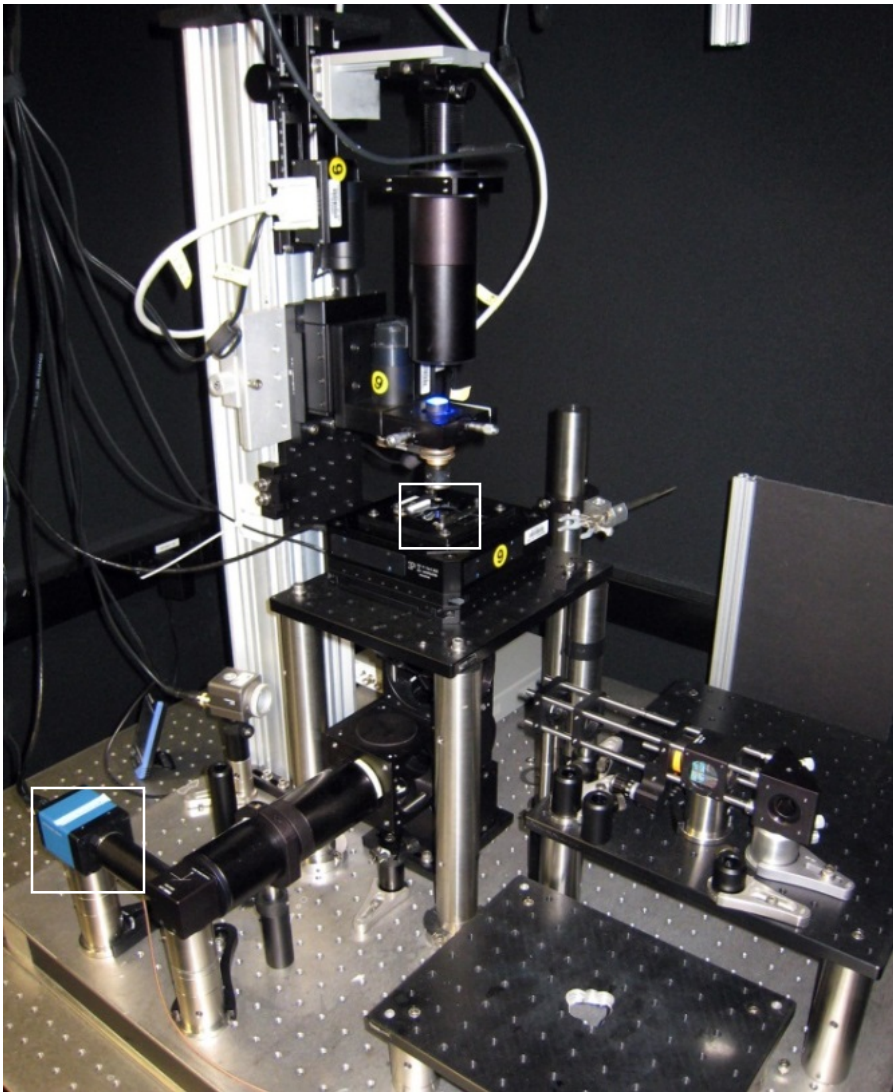
E. coli RecQ and BLM variants and DNA substrates



- RecA like
- ZBD
- WHD
- HRDC



Single molecule helicase measurements with magnetic tweezers

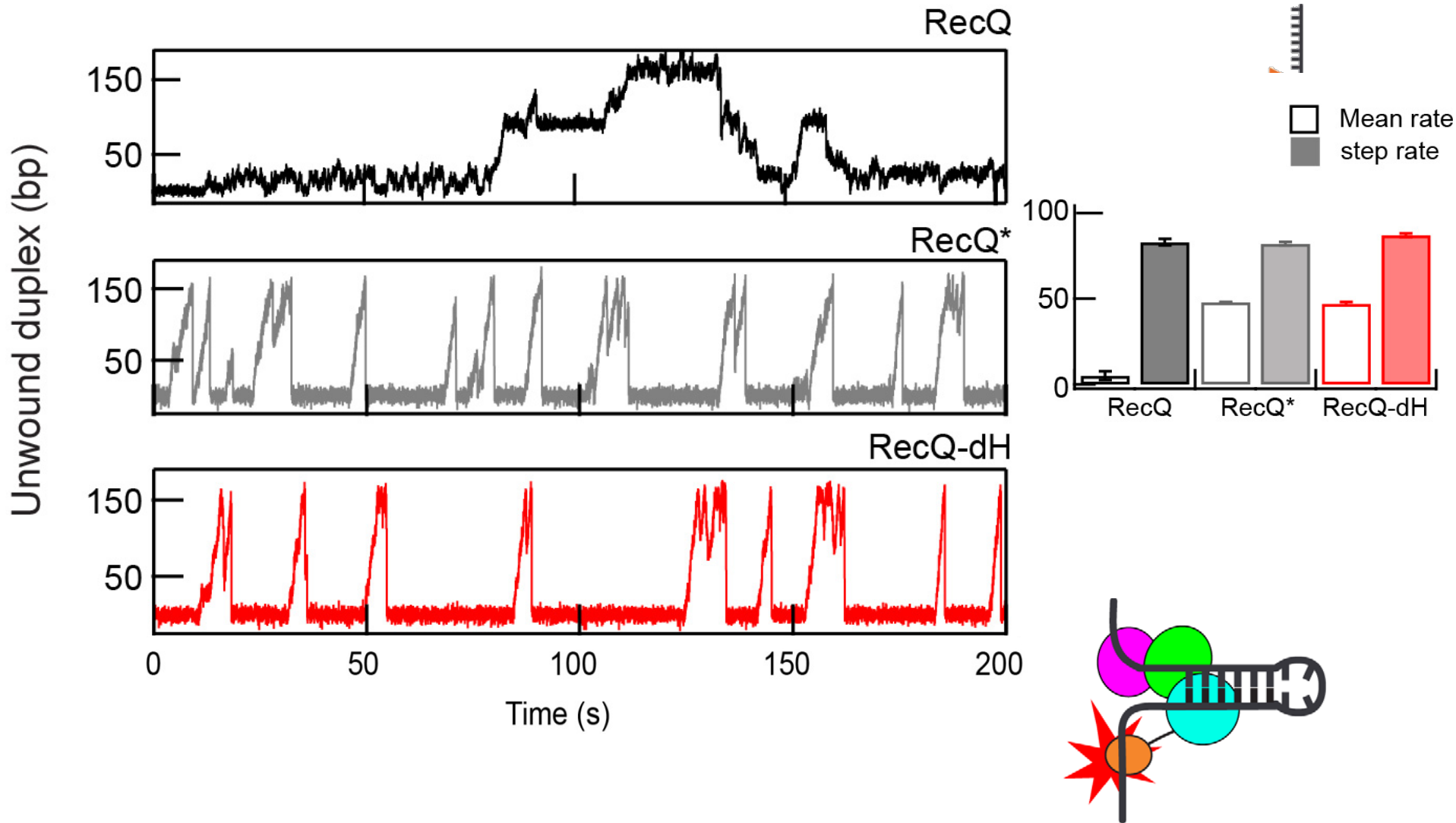


Seol, Strub and KCN (2016) *Methods*

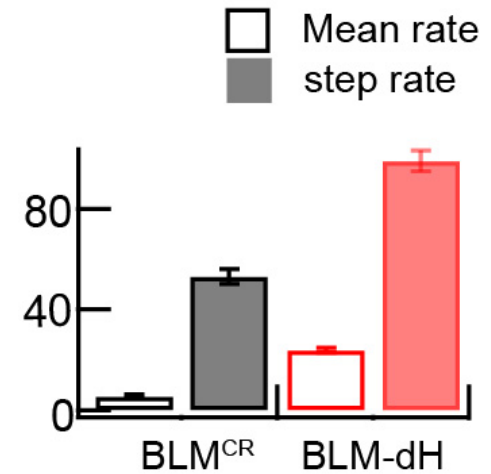
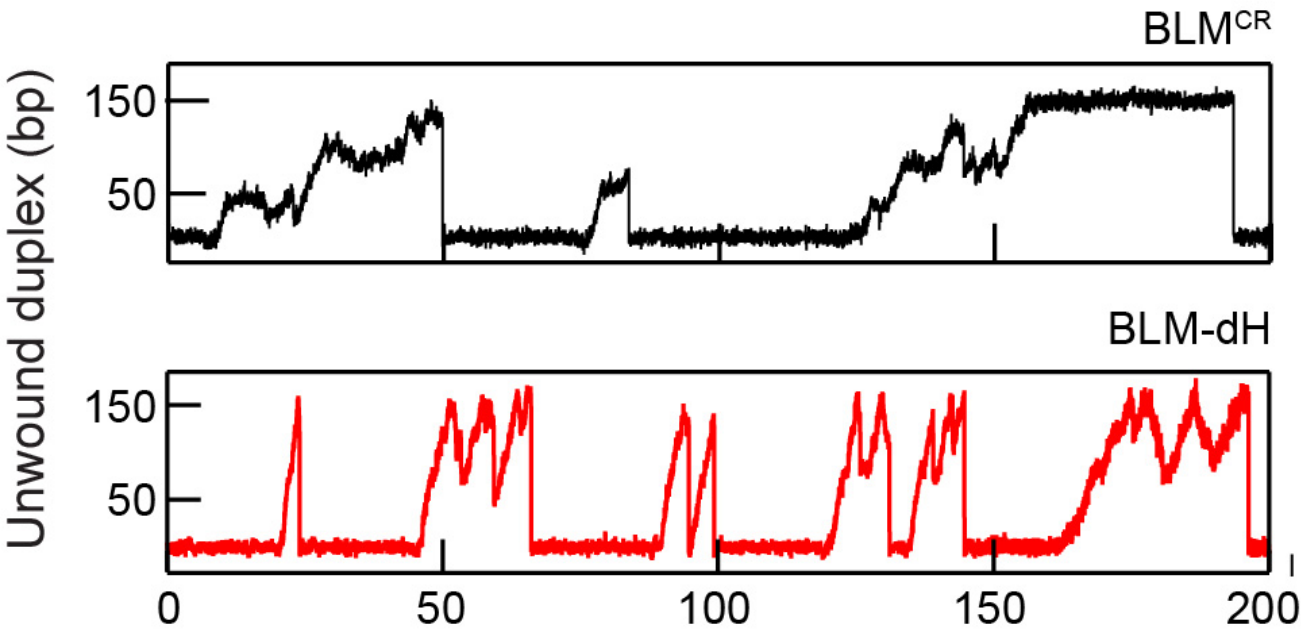
Seol and KCN (2011) *Single Molecule Analysis: Methods and Protocols*

Harami, Seol,...KCN (2017) *PNAS*

HRDC induces long and frequent pauses

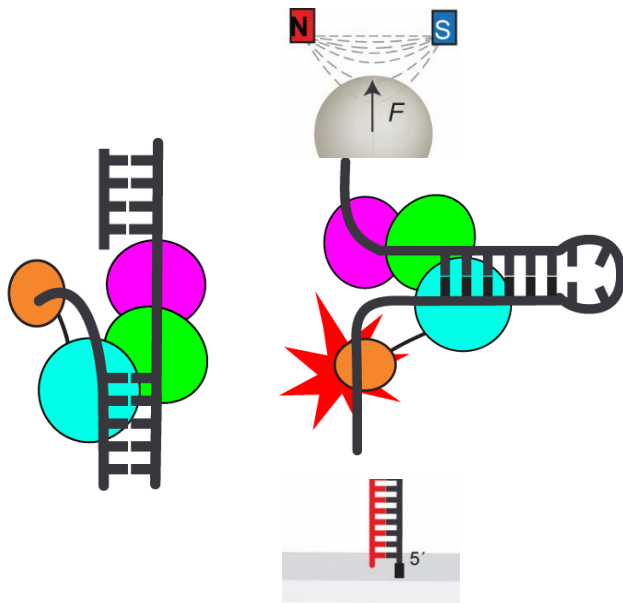


Bloom's syndrome helicase domains are functionally identical to RecQ domains

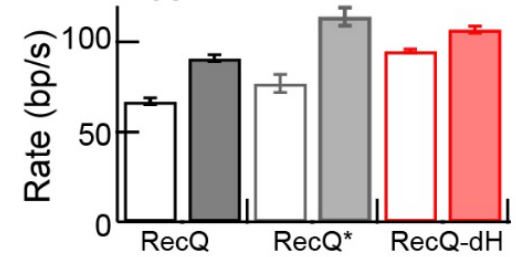


Pauses arise from HRDC binding displaced ssDNA

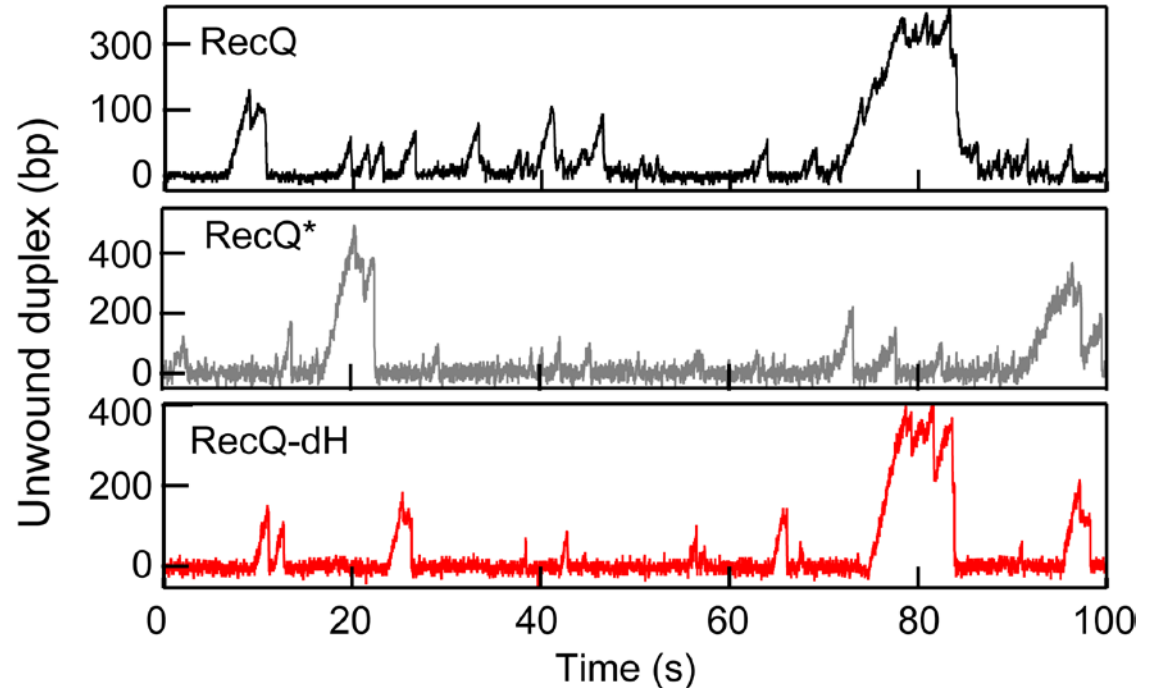
RecQ does not pause on gapped substrates



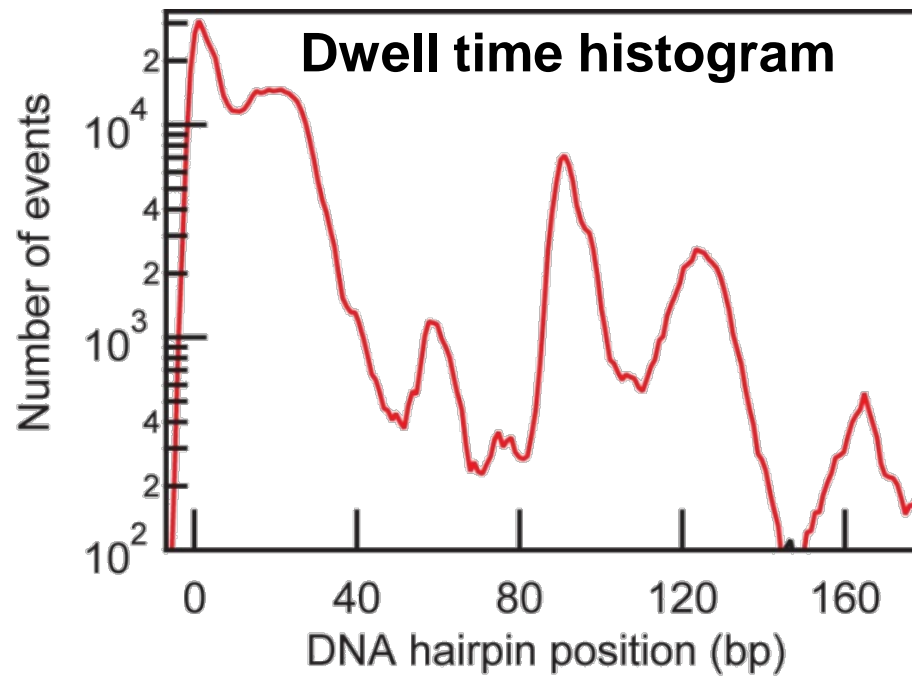
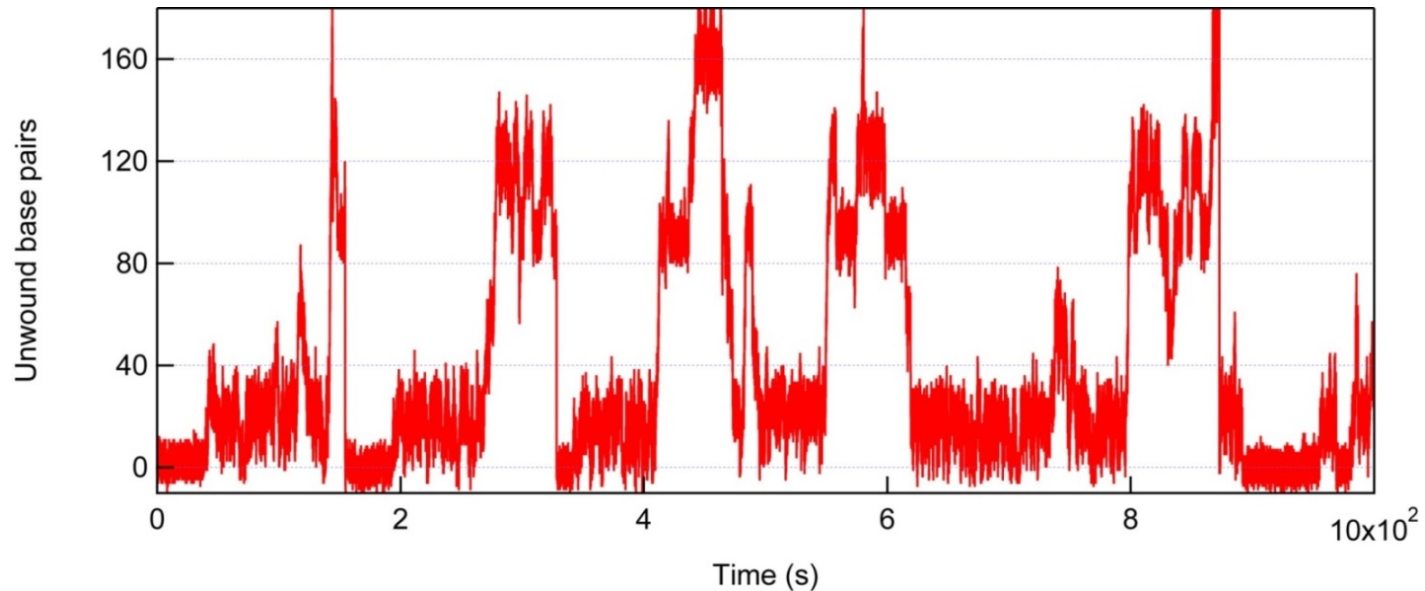
Gapped DNA
Substrate



Gapped DNA

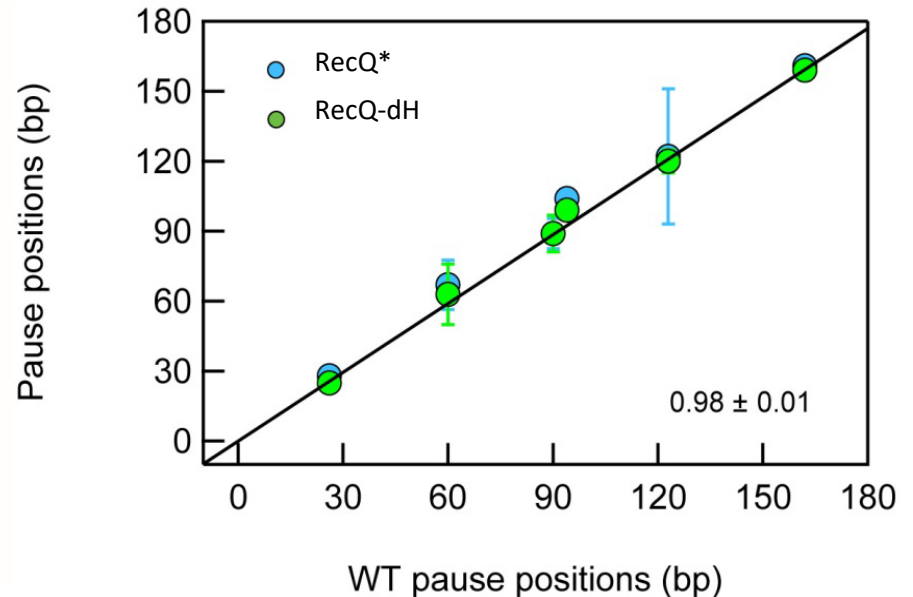
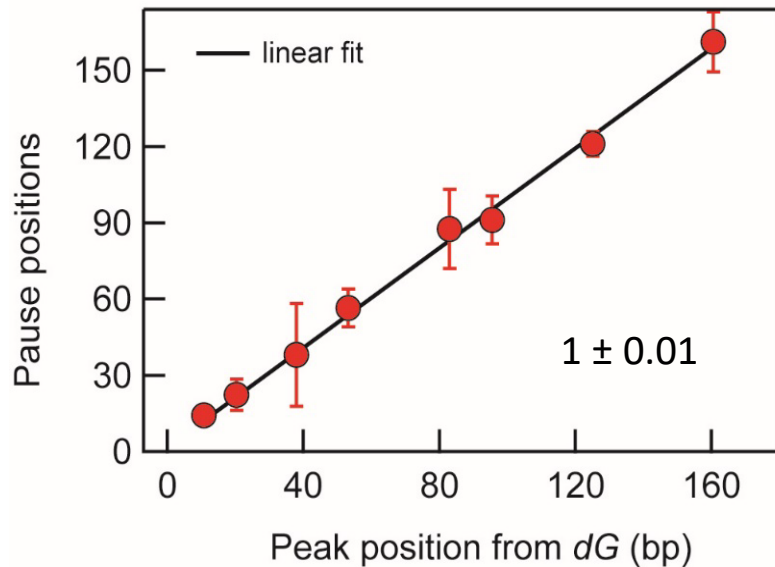
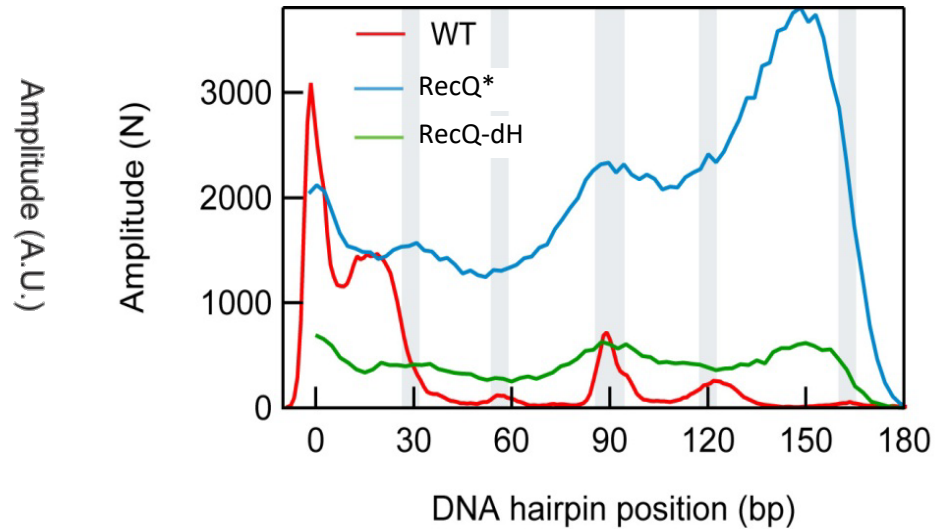
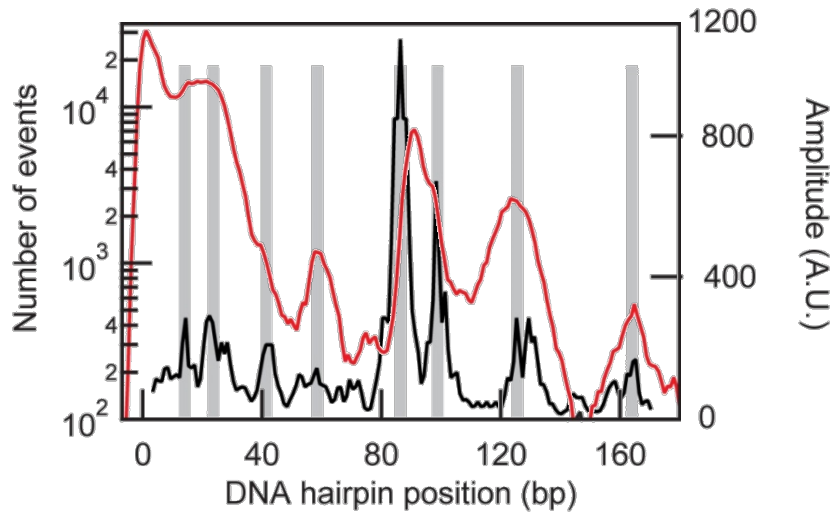


Pauses occur at specific locations on hairpin

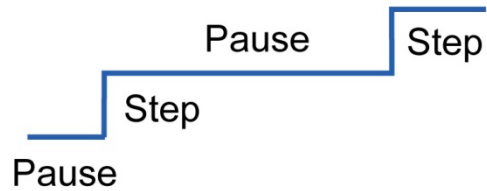


Pauses correlate with DNA duplex stability

HRDC stabilizes sequence-dependent pauses of core RecQ



Pause kinetics indicate 5 base-pair kinetic step



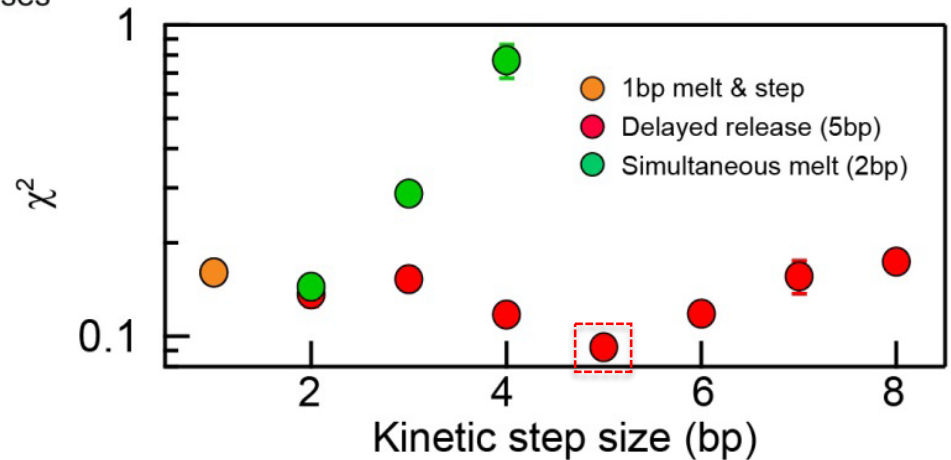
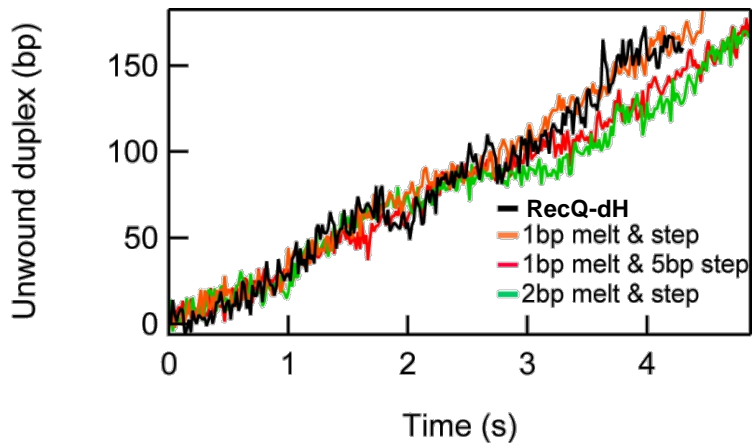
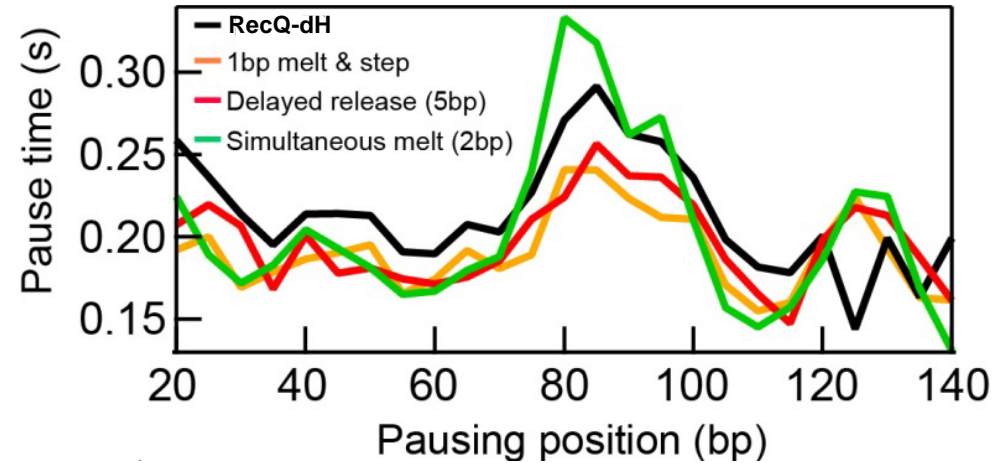
τ (Pause duration) $\sim \exp(\Delta G(n\text{-base-pair opening}))$



n bp melting and a fast translocation of n bases

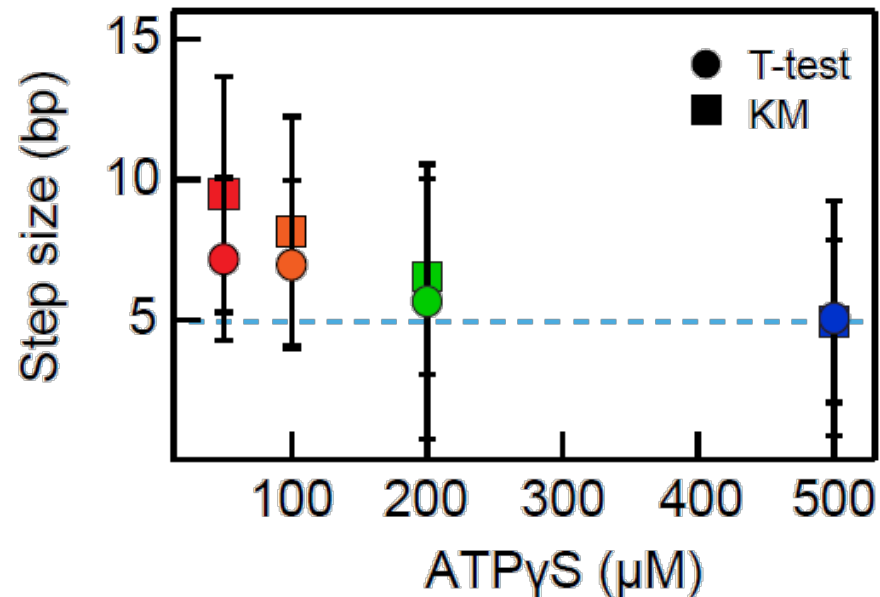
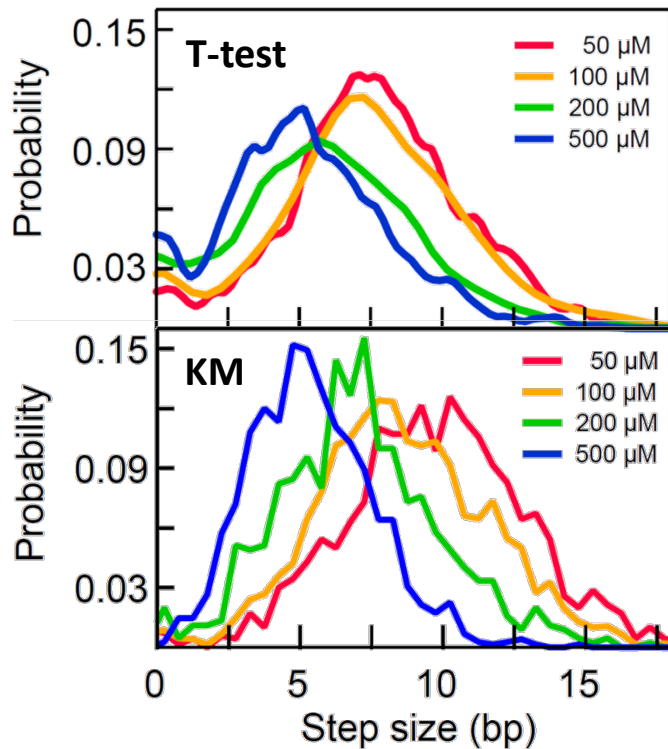
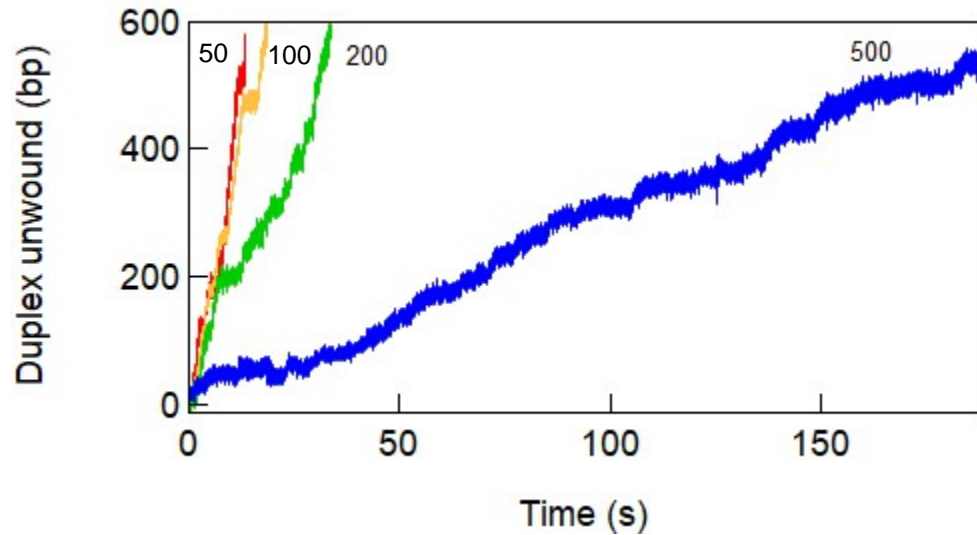
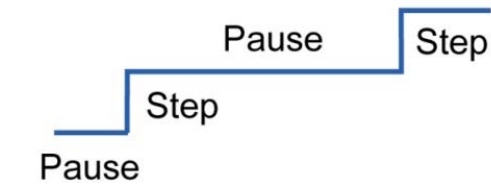


1bp melting and a delayed single-strand release of n bases

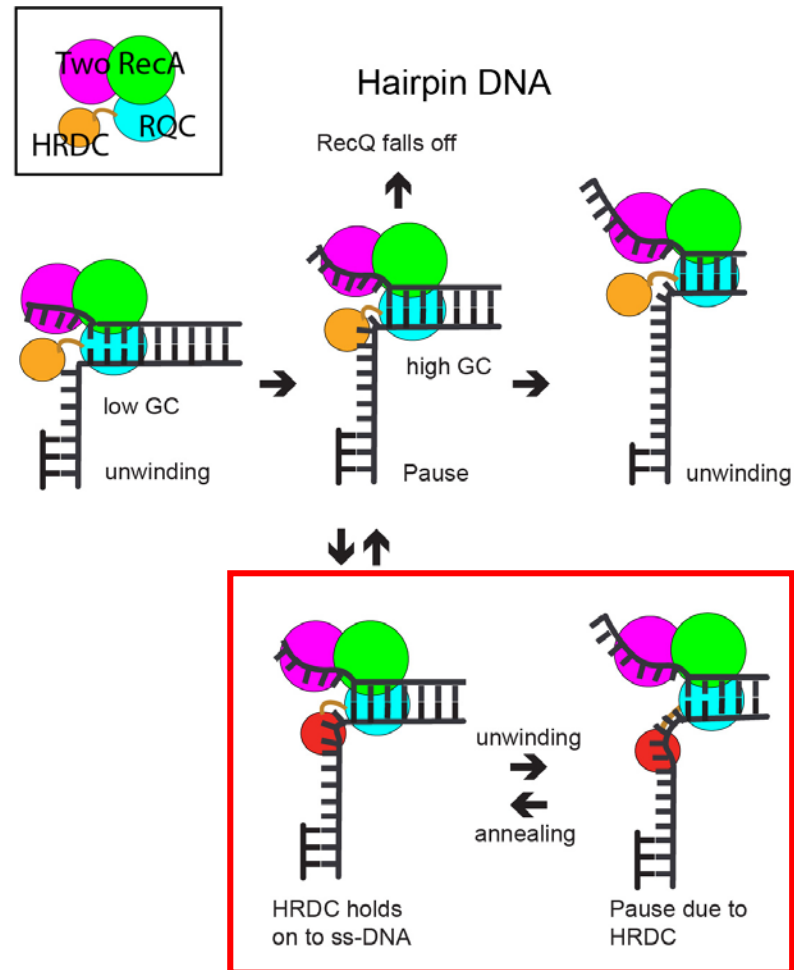
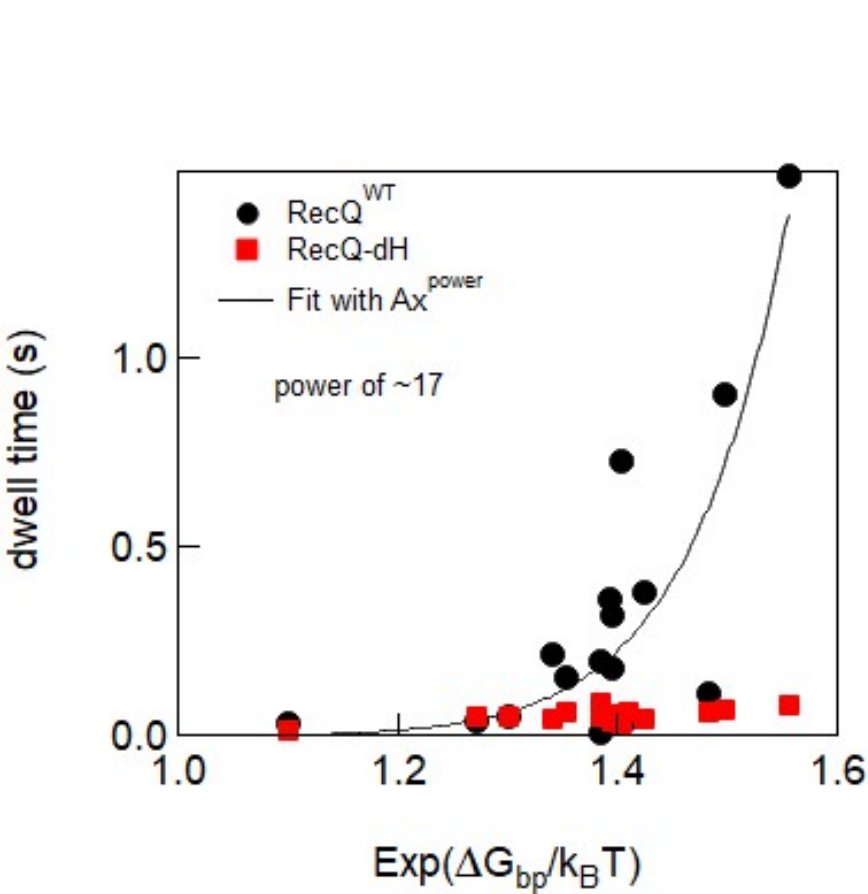


Direct observation of 5bp kinetic step

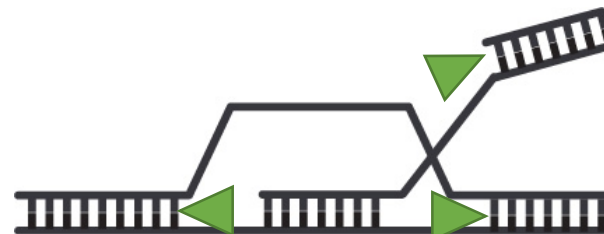
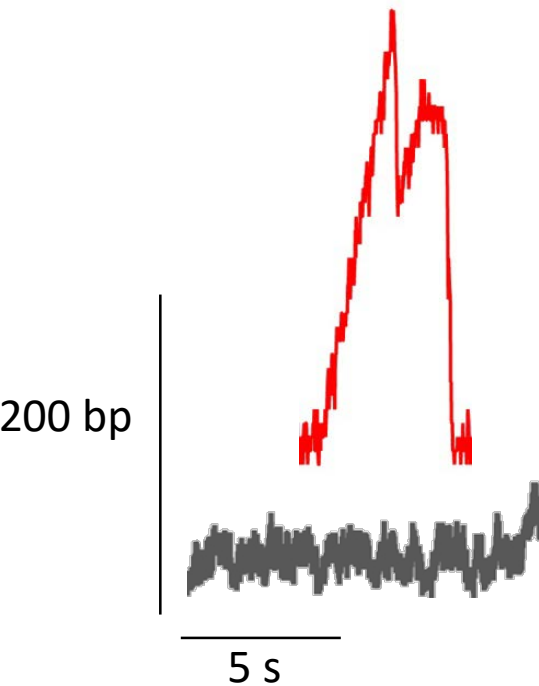
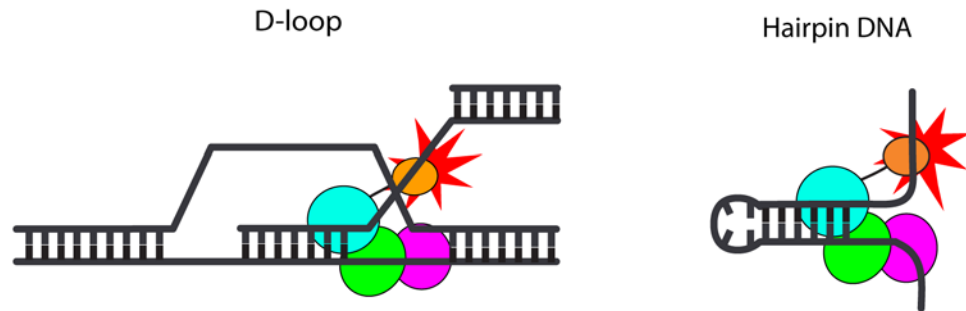
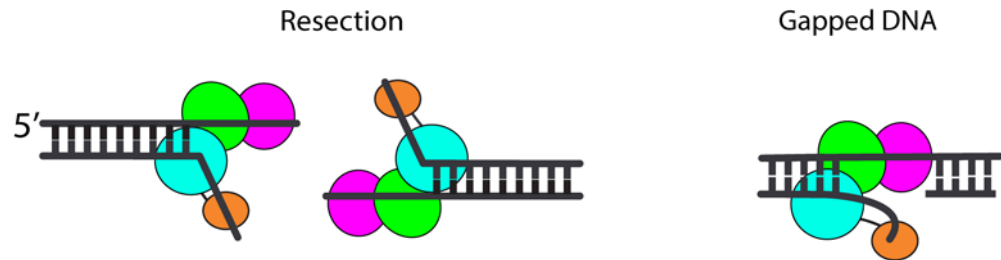
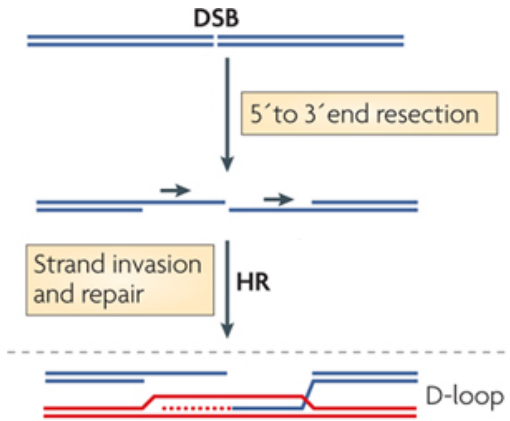
ATP γ S induced stalling of RecQ-dH



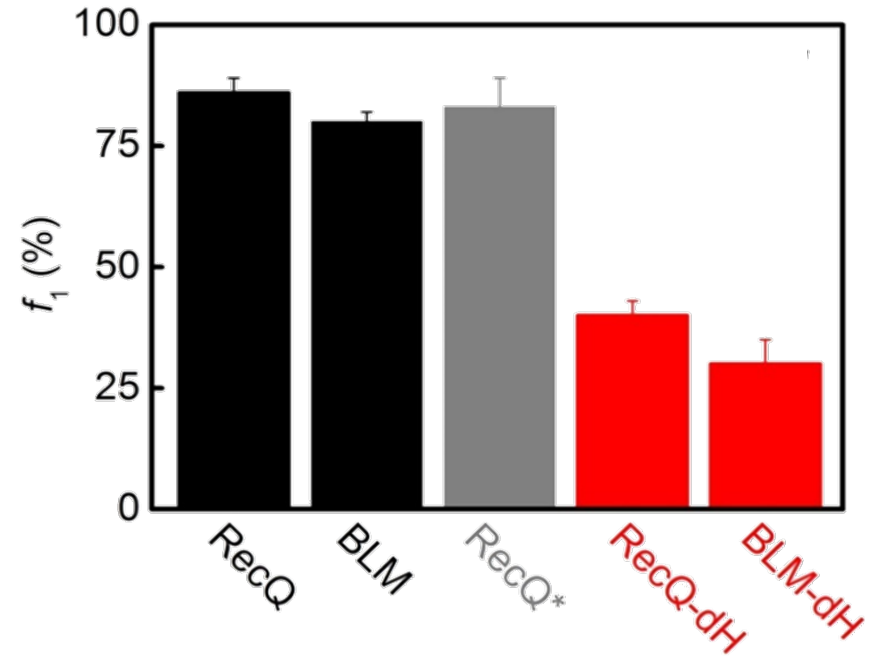
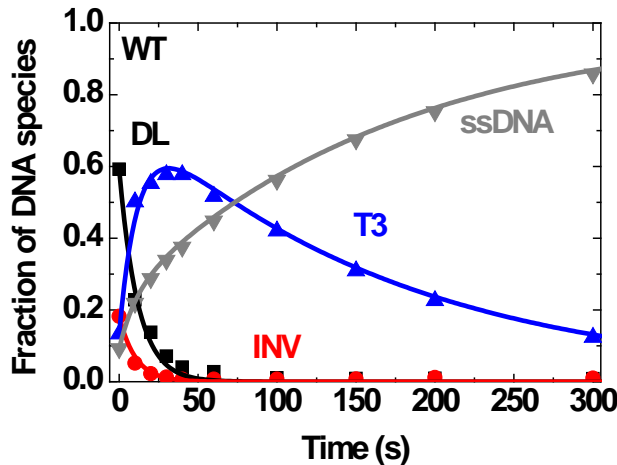
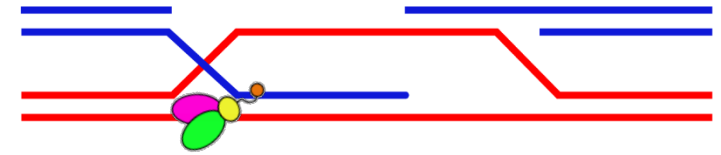
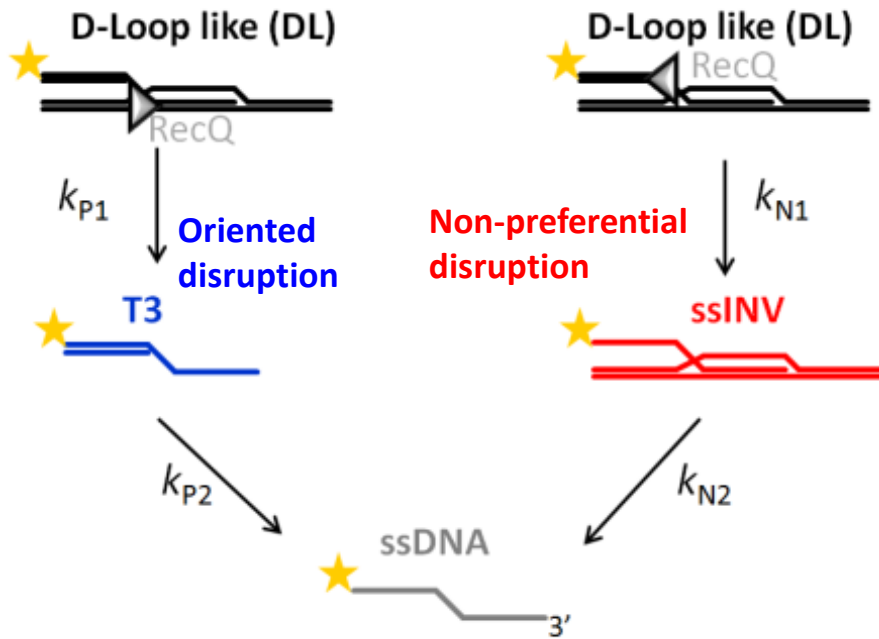
HRDC nonlinearly amplifies sequence dependent pausing of RecQ core



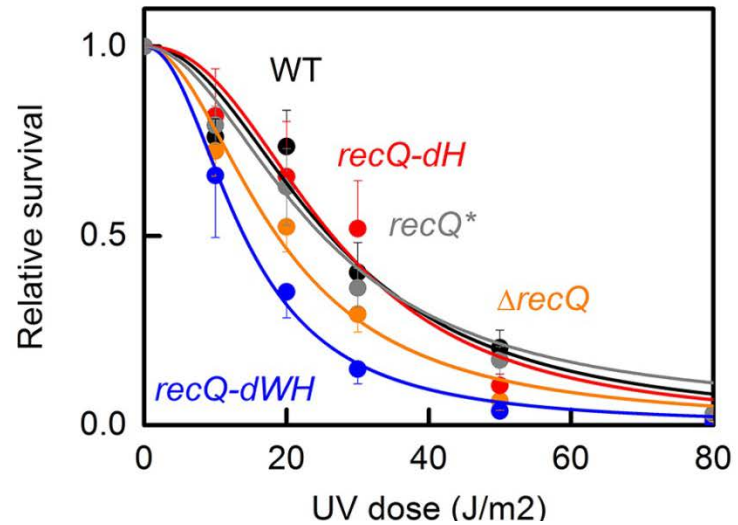
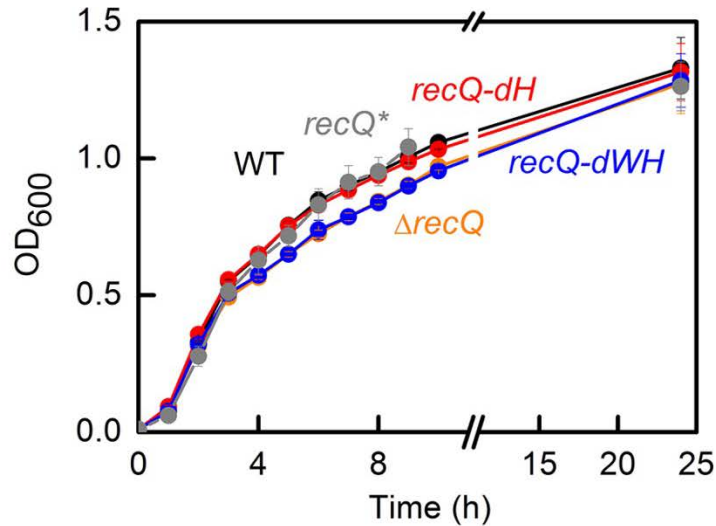
Physiological implications of geometry-dependent DNA processing by RecQ



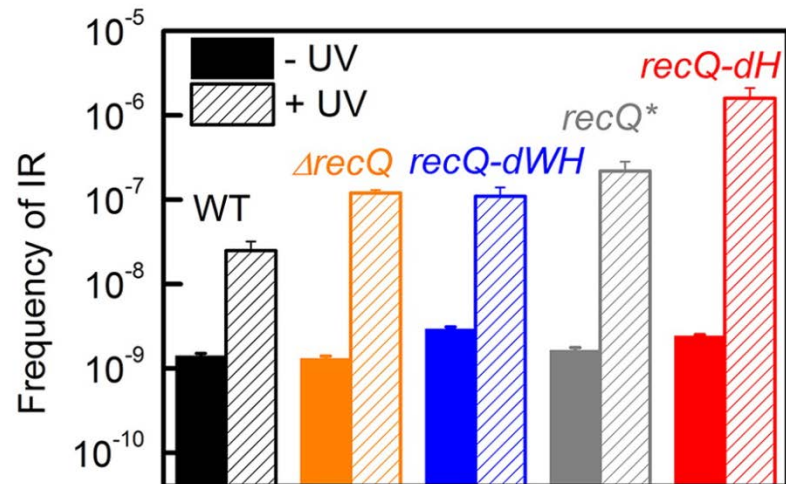
HRDC orients RecQ to preferentially disrupt invading DNA strand in D-loop



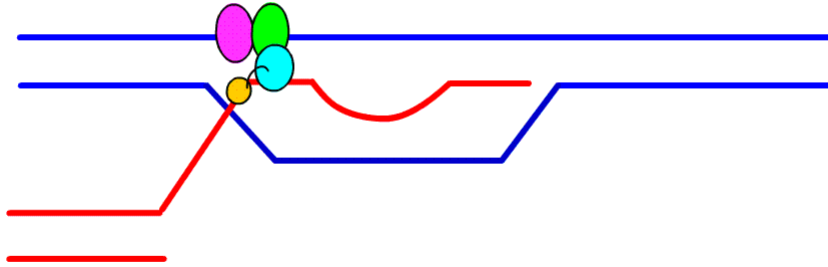
RecQ-dH helicase promotes illegitimate recombination (IR) *in vivo*



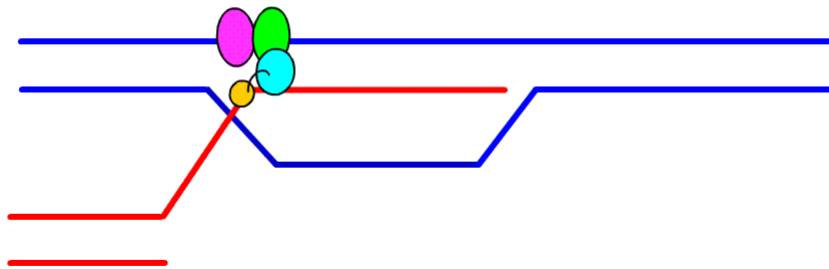
- UV-induced IR increases in absence of RecQ
 - **$\Delta HRDC$ enzyme further increases IR**
- λ Spi- bacteriophage assay
(Hanada et al. 1997 PNAS)



Model for suppression of illegitimate recombination by RecQ helicases



Illegitimate recombination
poor or short homology



Legitimate
recombination

Conclusions

Mechanistic details of RecQ unwinding and pausing

- RecQ takes a 5 bp kinetic step
 - 5 bp kinetic step results in sequence dependent unwinding and pausing
- DNA geometry- and sequence -dependent pausing of RecQ
 - Pausing on DNA hairpin but not gapped DNA substrates
 - Non-linear stabilization of sequence dependent pausing by HRDC domain binding to displaced strand
- HRDC orients RecQ to disrupt D-loop homologous recombination intermediates

***In vivo* implications**

- RecQ unwinding and pausing provide a mechanism to control the disruption of D-loops based on homology and extent of invasion
- This suggests a mechanism to promote genome stability by suppressing illegitimate recombination

Acknowledgements



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