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

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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

RecQ mutations linked to genome instability

Genome instability
- Chromosome breaks
- Sister chromatid exchanges
- Illegitimate recombination

RecQ helicase deficiencies in humans: BLM, WRN, and RecQ4
- Rare autosomal recessive diseases
- Chromosomal instability
- Cancer predisposition
- Premature aging and infertility

http://humgen677s11.weebly.com
RecQ plays multiple roles in recombination

Homologous recombination

Promote resection

Suppress illegitimate D-loop formation

Promote non-crossover resolution

RecQ plays multiple roles in recombination

Homologous recombination

DSB

5’ to 3’ end resection

Strand invasion and repair

HR

Unwinding 3’ ends to promote resection

Suppress illegitimate D-loop formation

DSBR

Second end capture, double Holliday junction formation

Resolution

Non-crossover

Crossover

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 binding and substrate specificity

4TMU; SWISS MODEL homology modeling
E. coli RecQ and BLM variants and DNA substrates
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

Harami, Seol,...KCN (2017) PNAS
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
Pauses occur at specific locations on hairpin
Pauses correlate with DNA duplex stability
HRDC stabilizes sequence-dependent pauses of core RecQ

Yeonee Seol unpublished
Pause kinetics indicate 5 base-pair kinetic step

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

\( n \) bp melting and a fast translocation of \( n \) bases

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

Yeonee Seol *unpublished*
Direct observation of 5bp kinetic step

ATPγS induced stalling of RecQ-dH

Yeonee Seol unpublished
HRDC nonlinearly amplifies sequence dependent pausing of RecQ core

- Graph showing dwell time vs. $\exp(\Delta G_{bp}/k_B T)$
- Points for RecQ WT and RecQ-dH
- Fit with $A^\text{power}$
- Power of $\sim 17$

- Diagram illustrating:
  - Hairpin DNA
  - RecQ falling off
  - Transition from low GC to high GC
  - Unwinding
  - Pause
  - HRDC holds onto ss-DNA
  - Pause due to HRDC
  - Annealing

- HRDC stabilizes and amplifies pausing.
Physiological implications of geometry-dependent DNA processing by RecQ

- DSB
- 5’ to 3’ end resection
- Strand invasion and repair
- HR
- D-loop
- Gapped DNA
- Hairpin DNA
- Resection
- D-loop
- 200 bp
- 5 s
HRDC orients RecQ to preferentially disrupt invading DNA strand in D-loop

Oriented disruption

Non-preferential disruption

D-Loop like (DL)

RecQ

k_{P1}

T3

ssDNA

k_{P2}

ssINV

k_{N1}

k_{N2}

Fraction of DNA species

Time (s)

WT

DL

ssDNA

T3

INV

0 50 100 150 200 250 300

0.0 0.2 0.4 0.6 0.8 1.0

f_1 (%)

RecQ  BLM  RecQ*  RecQ-dH  BLM-dH

Mihály Kovács
RecQ-dH helicase promotes illegitimate recombination (IR) \textit{in vivo}

- UV-induced IR increases in absence of RecQ
- ΔHRDC enzyme further increases IR

\(\lambda\) 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
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