Roles for RecQ Helicases in Telomere Preservation

Patricia L. Opresko

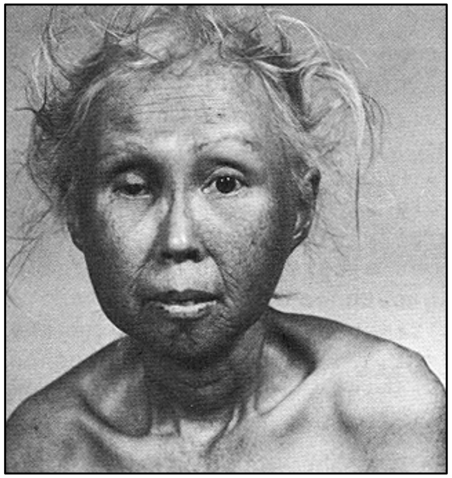
University of Pittsburgh
Department of Environmental and Occupational Health
Bridgeside Point
100 Technology Drive, Suite 350
Pittsburgh, PA 15219-3130
plo4@pitt.edu

Werner Syndrome

Symptoms	Average Age of Onset (yrs)
Greying of hair	20
Wrinkling of the skin	25.3
Loss of hair	25.8
Cataracts	30
Skin Ulcers	30
Diabetes (type II)	34.2
Death	**47**

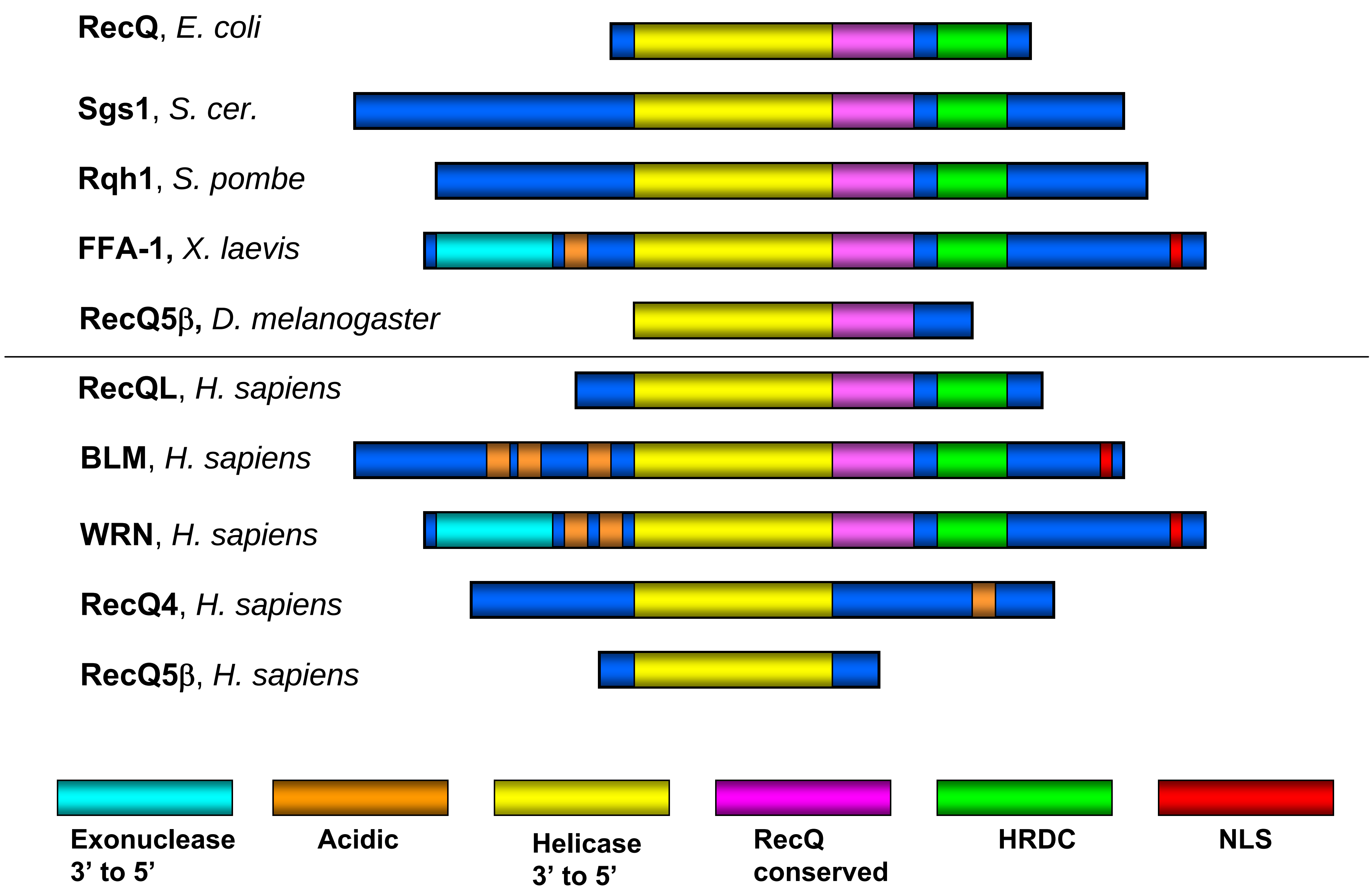
Osteoporosis
Atherosclerosis
Cancer

14 Years Old



48 Years Old

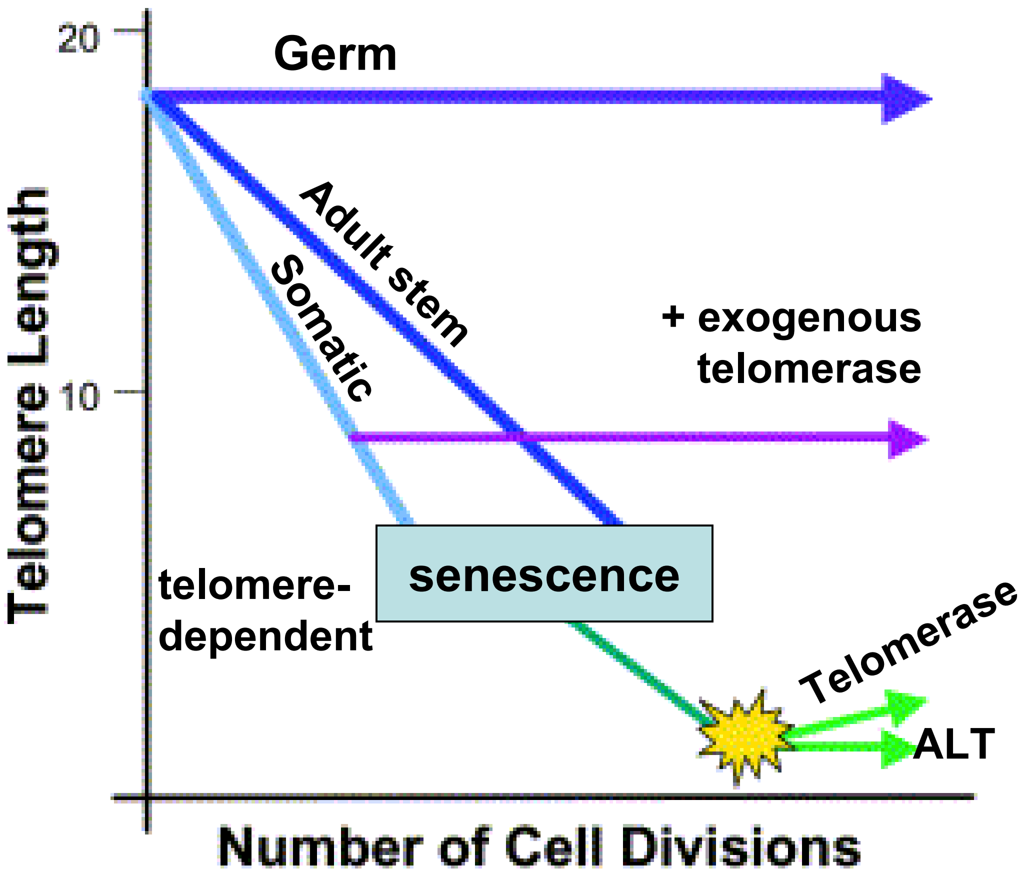
RecQ Family "Care Takers" of the Genome



Cellular defects in WS cell lines

- **Genomic Instability**
  - **Chromosomal rearrangements, translocations, dicentrics**
  - **Large deletions**
- **Replication**
  - **Reduced replicative lifespan**
  - **Extended S-phase**
- **Mitotic Homologous DNA Recombination**
  - **Defect in resolving intermediates**
- **DNA Repair**
  - **Hypersensitivity to**
    4-NQO
    DNA crosslinking agents
    topoisomerase inhibitors
    methyl methanesulfonate
- **Telomere instability**

Telomere-Associated Replicative Senescence



Modified from Campisi 2001 Exp. Geron.

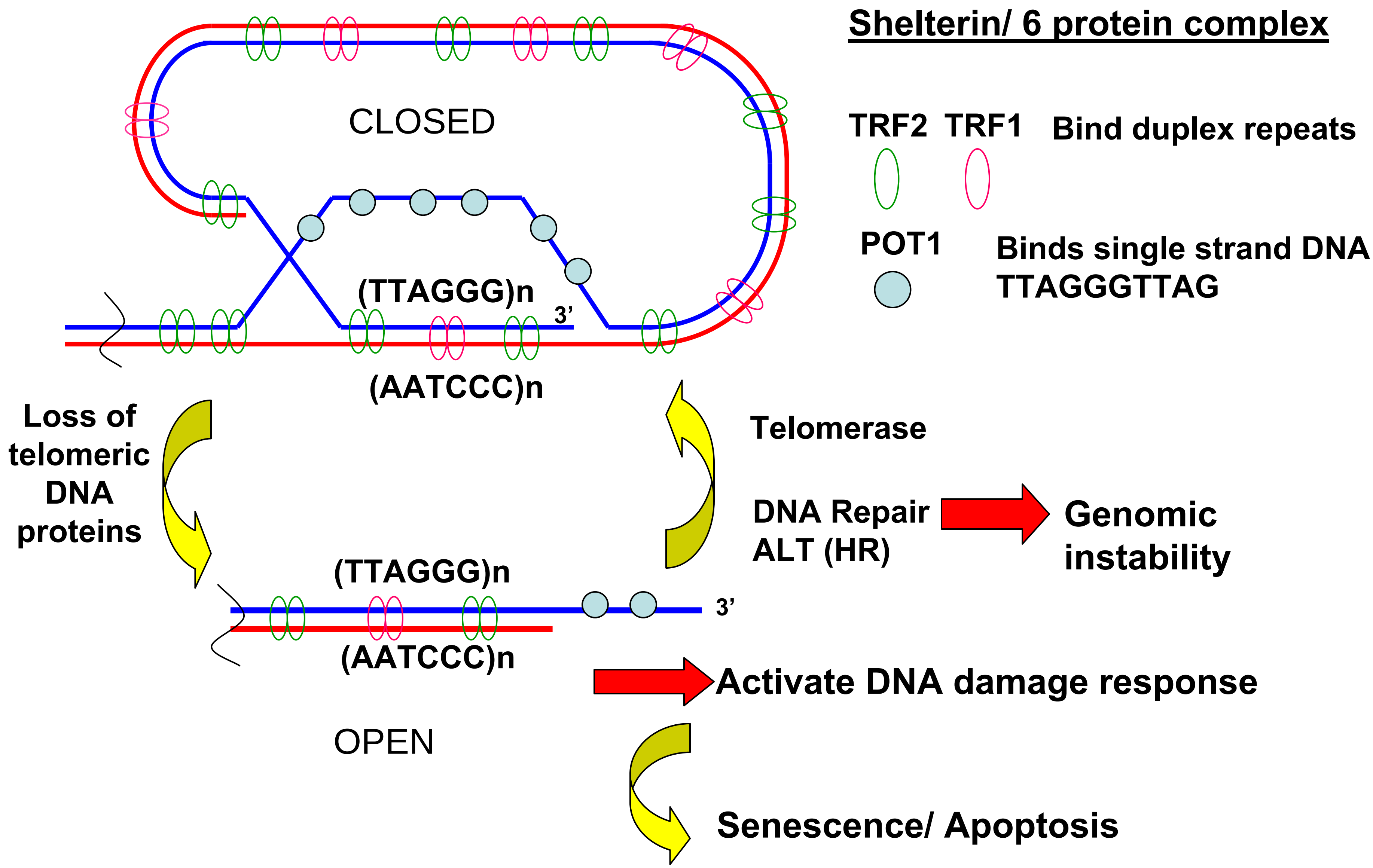
Germ cells:
sufficient telomerase activity
- no shortening

Adult stem cells:
variable levels of telomerase activity
- slow shortening

Somatic cells:
most have no telomerase activity
- exhibit faster rates of shortening

Cancer cells:
90% show high telomerase activity
10% use an alternative pathway
- no telomere loss

Telomeres Protect Chromosome Ends
Complex of Protein and DNA



Telomere Dysfunction Contributes to WS Pathology

WS primary fibroblasts

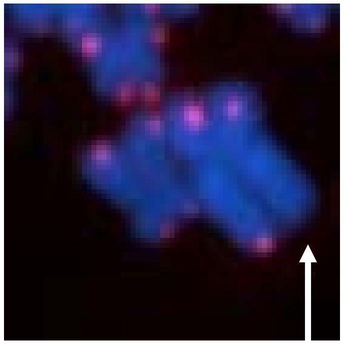


Exhibit telomere loss

1. **Accelerated decrease of <u>mean</u> telomere lengths (Shulz 1996)**
2. **Increased loss of telomeres from sister chromatids (Crabbe 2004)**

Expression of either WRN or telomerase can prevent

1. **Premature senescence (Wyllie 2000)**
2. **Sister telomere loss (Crabbe 2004)**
3. **Accumulation of aberrant chromosomes (fusions, breaks, translocations) (Crabbe 2007)**

Mouse models

***Wrn*$^{-/-}$ mice appear normal**

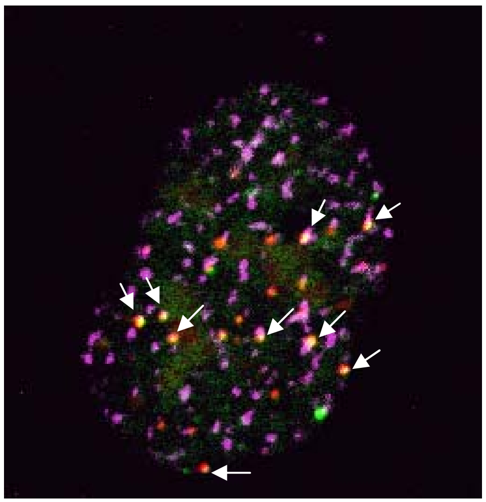
late generation *Wrn*$^{-/-}$*Terc*$^{-/-}$ mice with shortened telomeres exhibit WS phenotypes (Chang 2004, Du 2004)

Evidence For WRN Activity at Telomeres

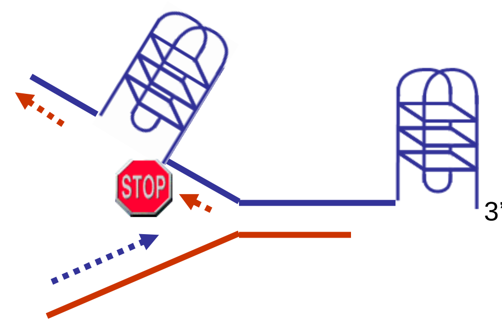
Telomere Replication

WRN localizes to telomeres in S-phase telomerase deficient cells

- **In telomerase-negative ALT cells (Opresko 2004)**



HcRed-PCNA
ECFP-TRF1
EYFP-WRN



- **In primary fibroblasts**
  - **WRN helicase prevents the loss of telomeres replicated from the <u>G-rich lagging</u> strand; by CO-FISH (Crabbe 2004).**
  - **Pot1a and FEN1 defects also cause preferential loss of lagging strand telomeres (Wu 2006; Saharia 2008)**

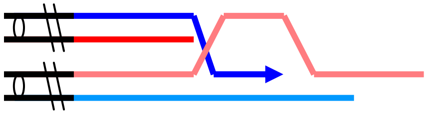
Evidence For WRN Activity at Telomeres

Telomere Recombination

WRN and POT1 suppress aberrant telomere recombination and exchanges (Laud 2005, Wu 2006, He 2006, Li 2008)

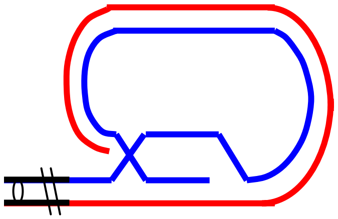
Late generation *Wrn*$^{-/-}$*Terc*$^{-/-}$ mice and *Pot1a*$^{-/-}$ mice exhibit:

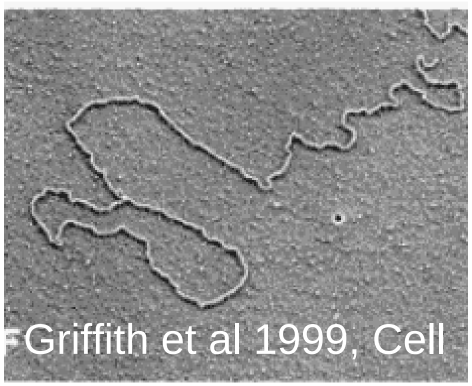
increased telomeric sister chromatid exchanges
intra-telomere recombination



WS human and *Pot1a*$^{-/-}$ mouse cells exhibit

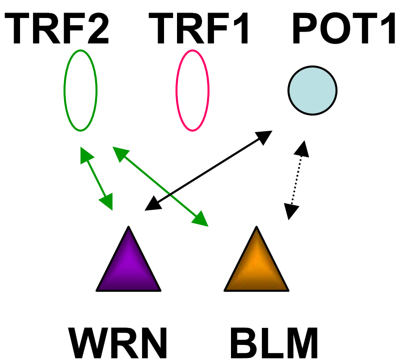
increased telomere circles
HJ cleavage of telomere T-loop



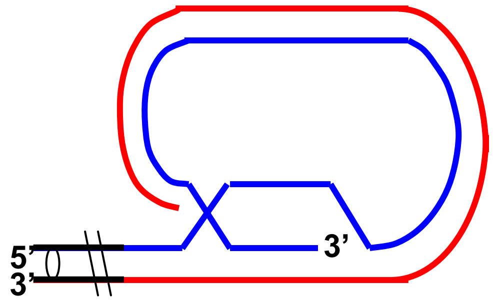

Griffith et al 1999, Cell

WRN and BLM Roles at Telomeres

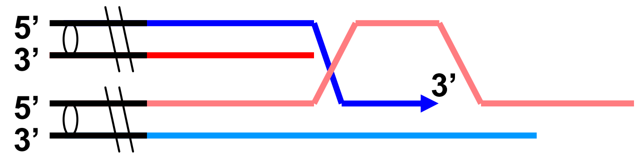
Telomere Binding Proteins

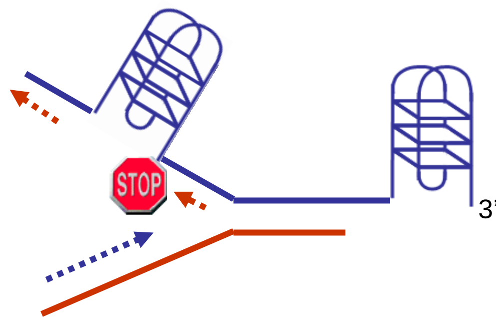


Intra-telomeric D-loop



Inter-telomeric D-loop



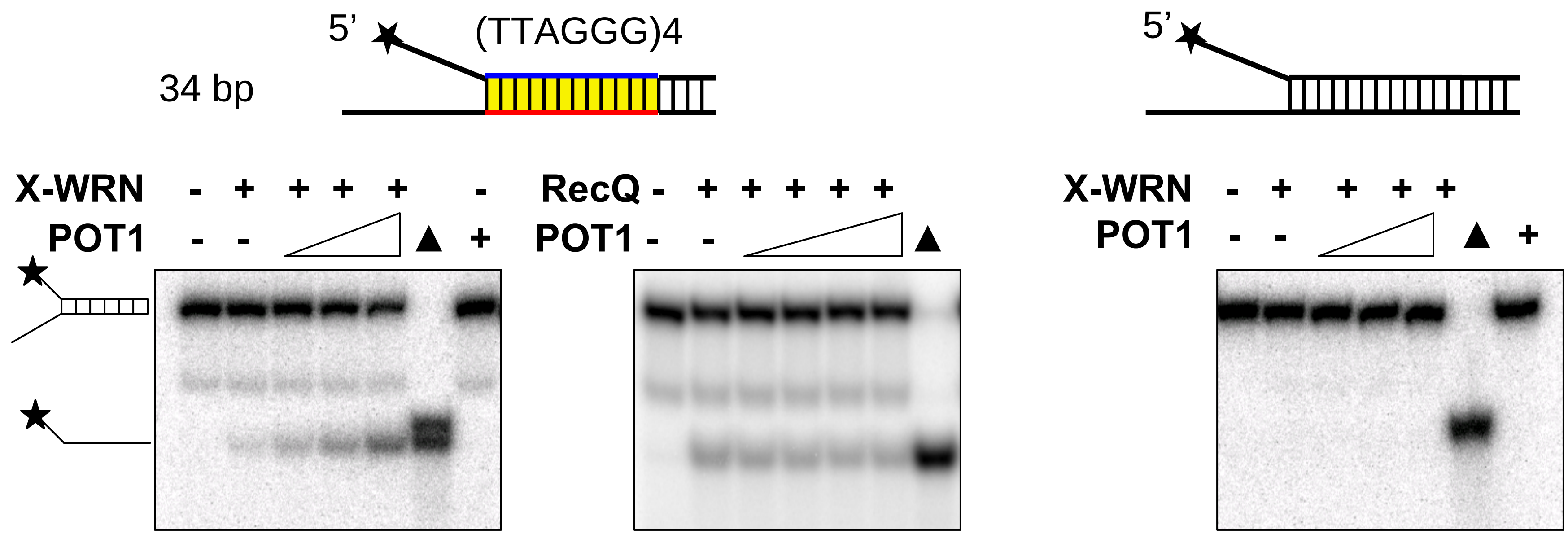


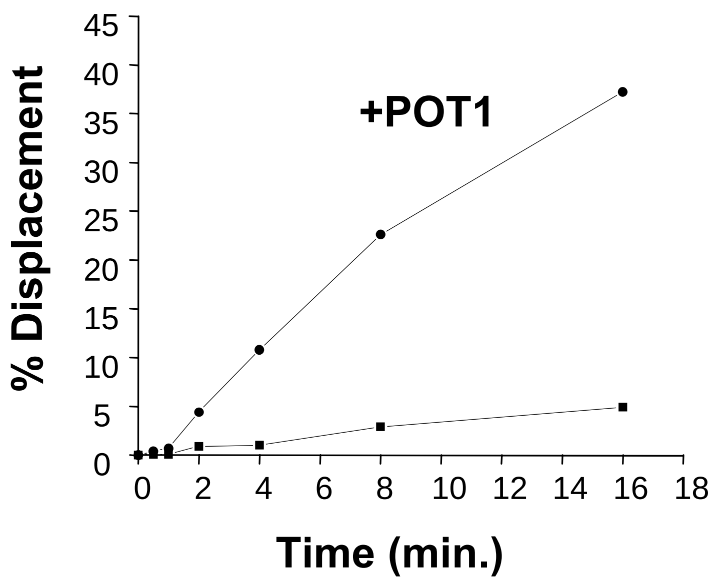
intra- or inter-telomeric G-quadruplex

Hypothesis: WRN and BLM protein cooperate with telomeric proteins to dissociate alternate DNA structures at telomeres during replication and repair

- **TRF2 recruits WRN and BLM to telomeric DNA (Opresko 2002; Machwe 2004)**
- **POT1 physically binds WRN in HeLa cells (BLM interaction is weaker) (Opresko 2005)**

POT1 stimulates the WRN and BLM helicase activity



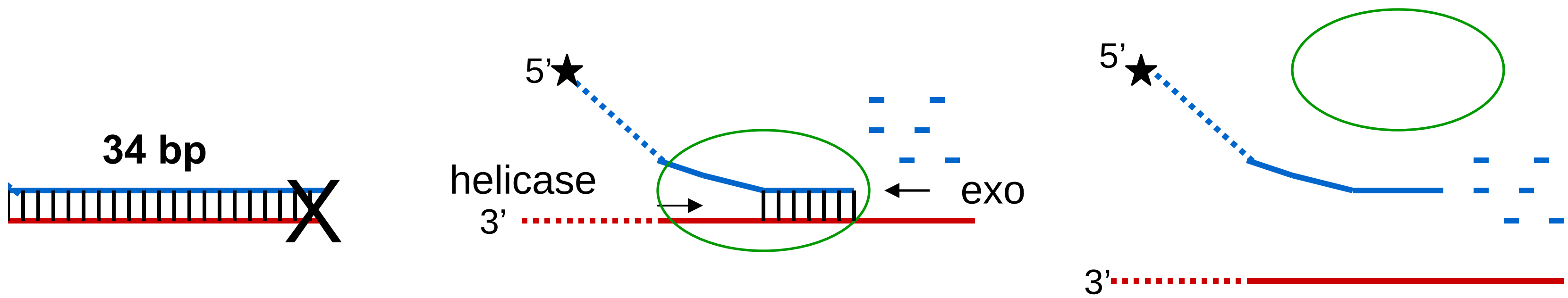


Opresko 2005

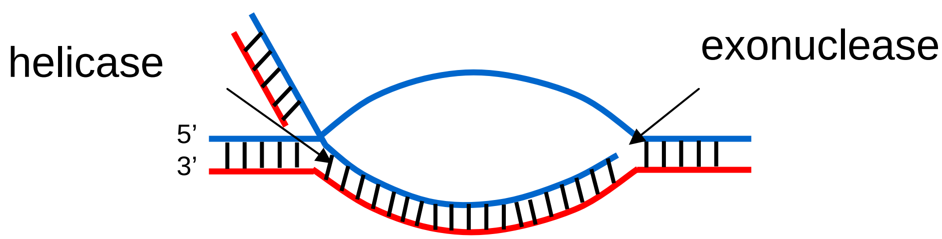
POT1

- Increases the amount and rate of WRN strand displacement; also BLM helicase
- Does not alter WRN or BLM unwinding of a non-telomeric fork
- Does not alter unwinding by bacterial helicases UvrD and RecQ

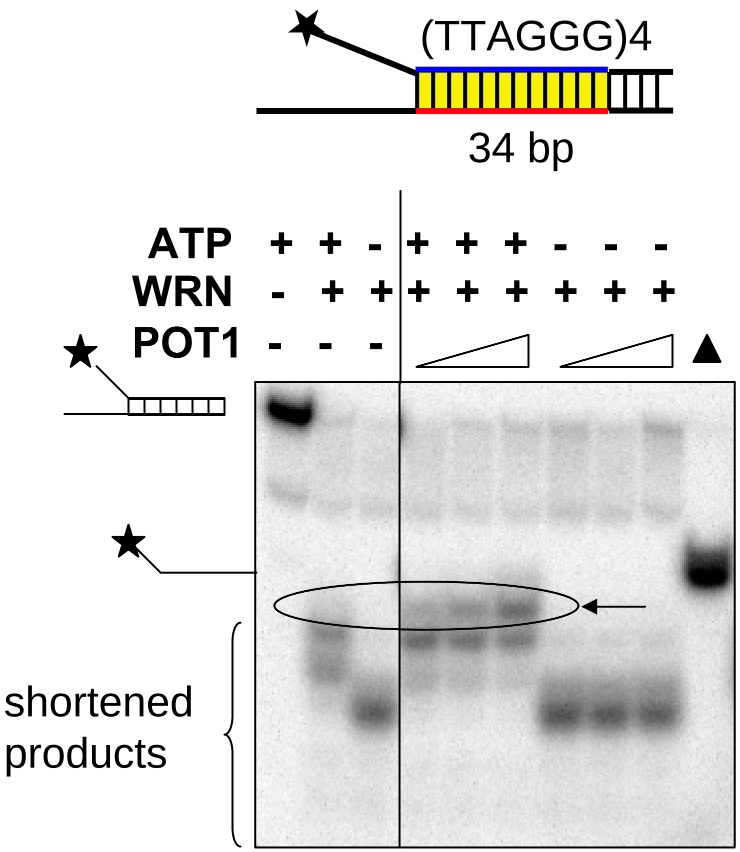
WRN Helicase and Exonuclease Cooperate to Dissociate Fork-like Substrates



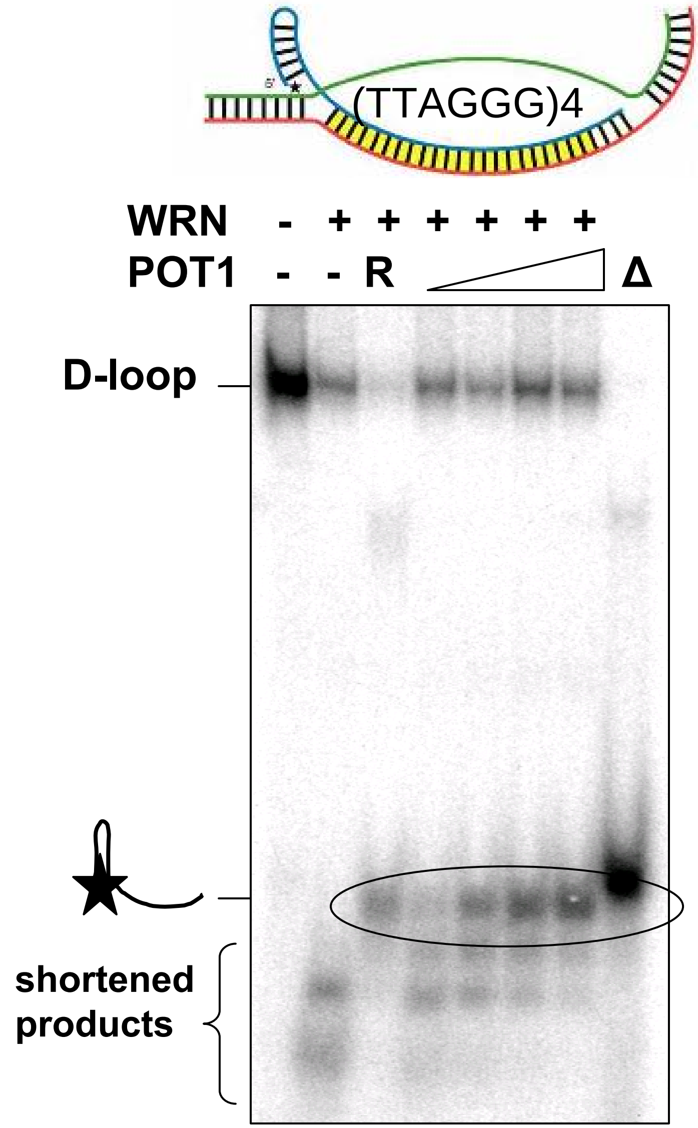
- **WRN exonuclease is inefficient on short ssDNA**
- **WRN is inactive on blunt ended duplex DNA**
- **Junctions in the substrate active the exonuclease at blunt ends**
- **Also cooperate to release invading strand of a D-loop**



POT1 Limits WRN Exonuclease by Stimulating WRN Helicase



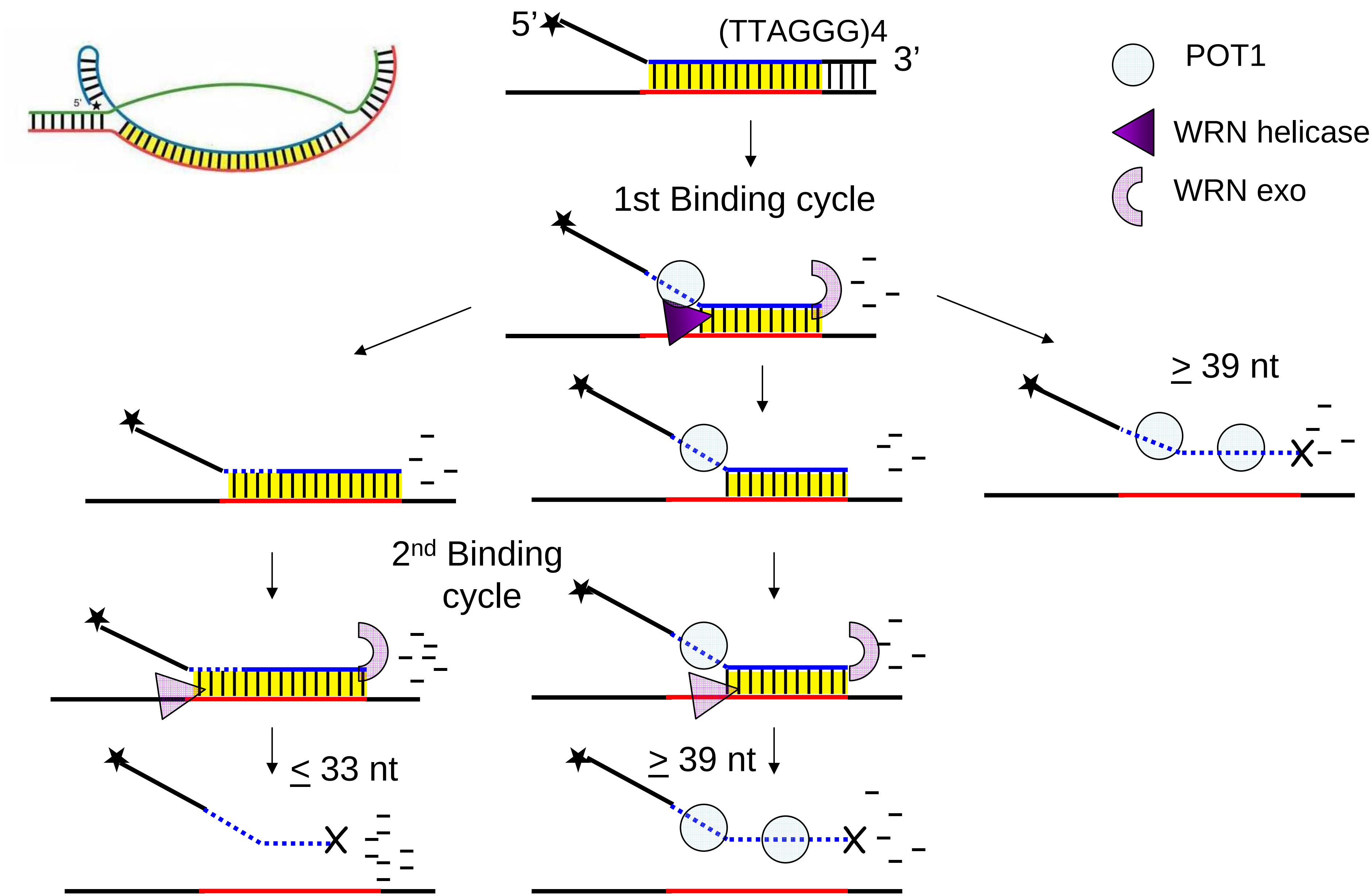
Native Gel



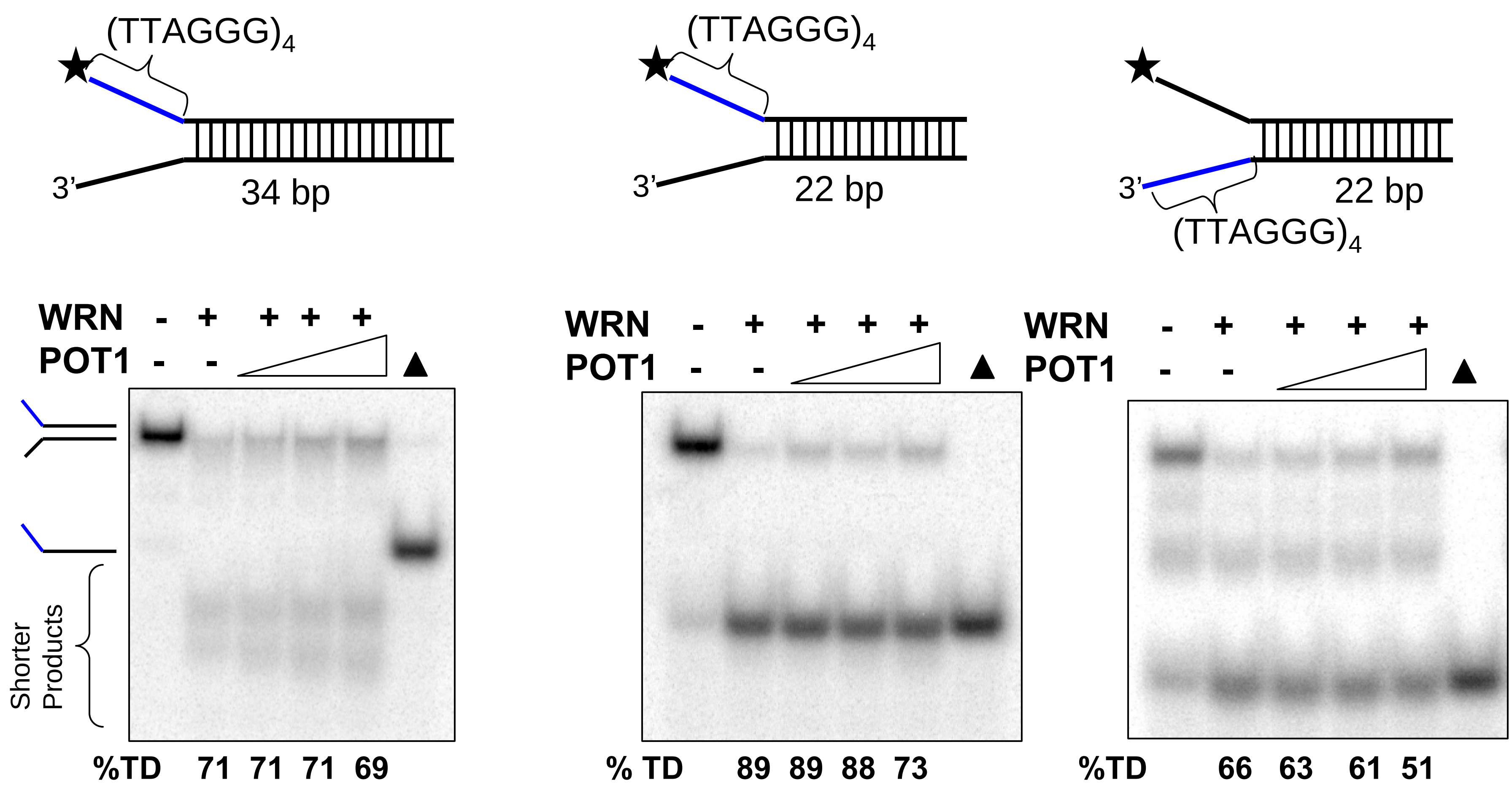
- **POT1 does not alter the WRN exonuclease in the absence of ATP/helicase activity**

(Opresko, JBC 2005)

Possible Mechanisms of WRN Helicase Stimulation by POT1



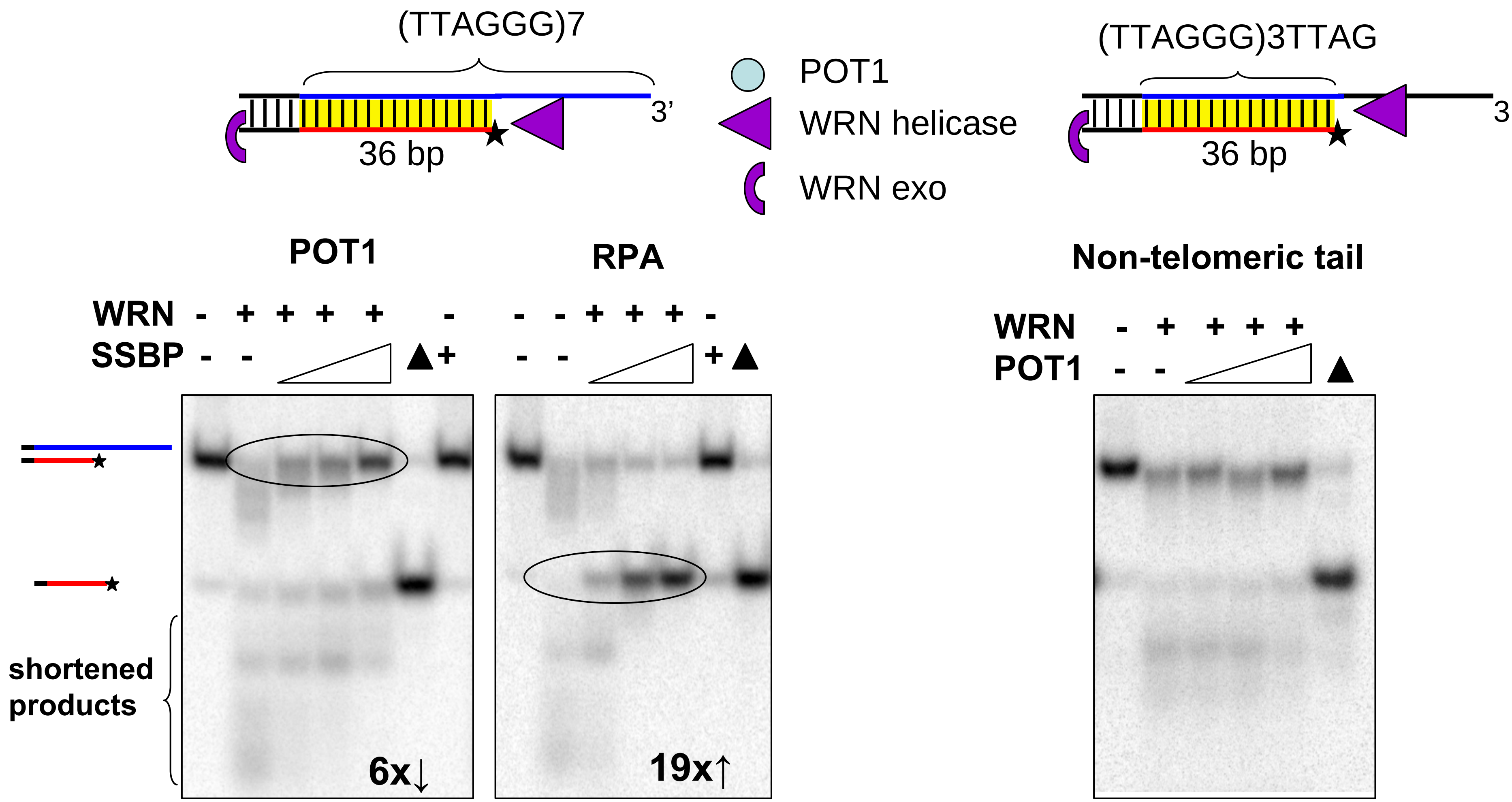
Can POT1 Pre-loading Stimulate WRN Helicase ?



POT1 pre-loading - is not sufficient to stimulate WRN helicase

- does not prevent WRN activity

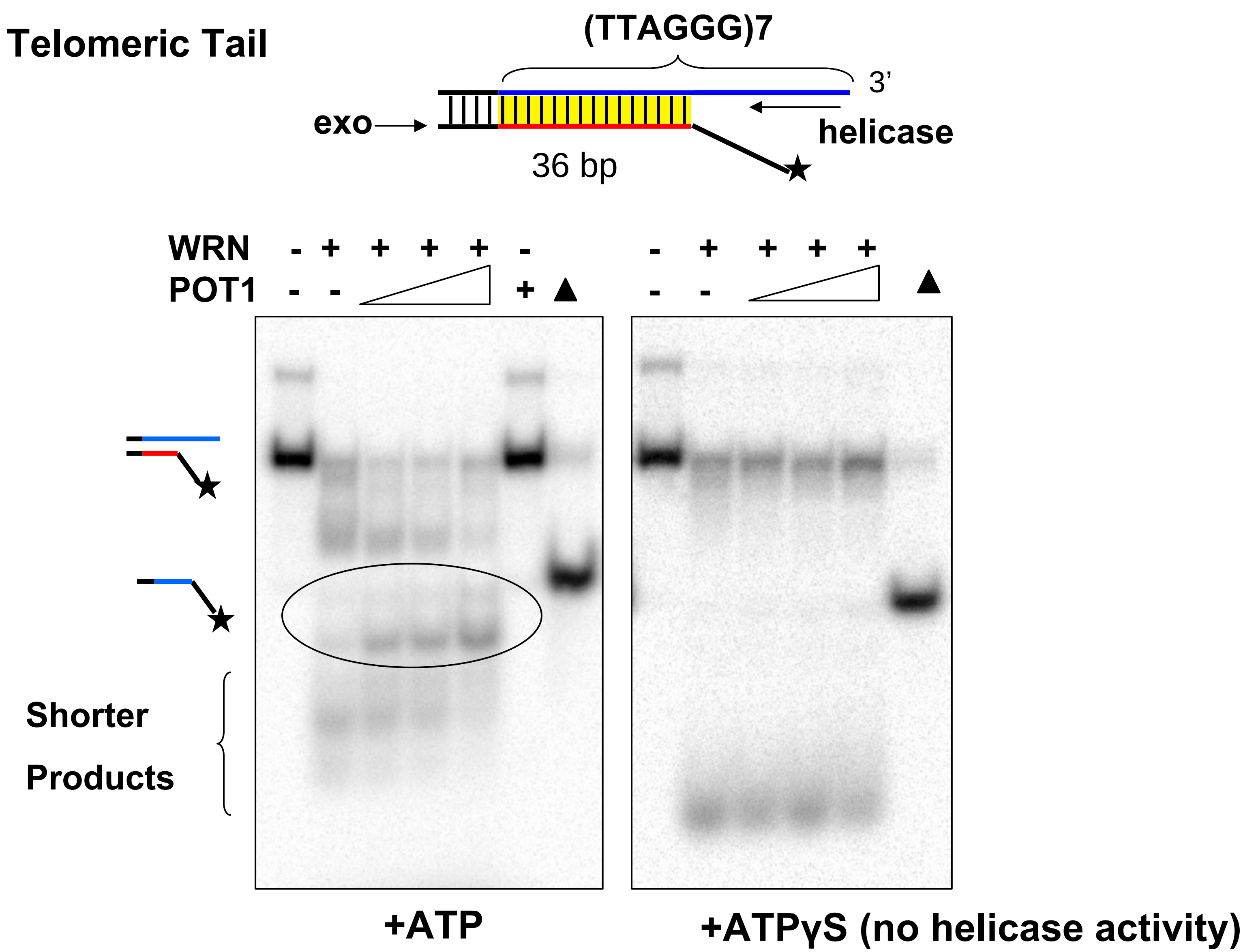
POT1 and RPA Differ in Regulation of WRN on Open Telomeric Ends



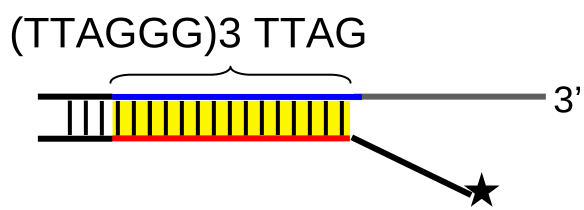
POT1 inhibits WRN, RPA stimulates

POT1 inhibition requires a telomeric tail

Addition of a 5' ssDNA Tail (Fork) Restores POT1 Stimulation of WRN

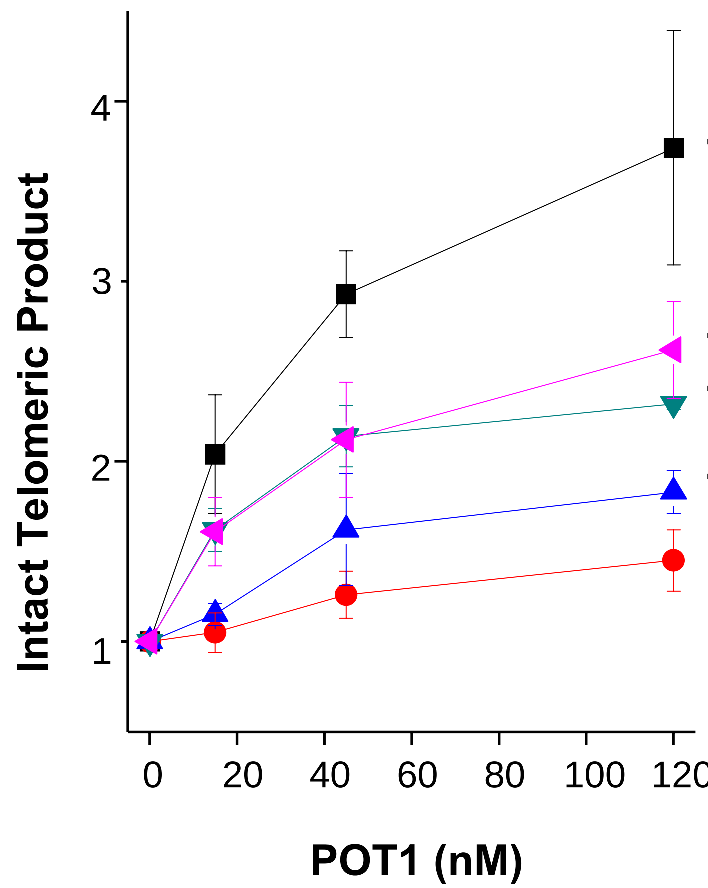


POT1 Pre-loading Promotes WRN Helicase Unwinding of Telomeric Forks



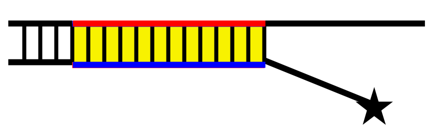
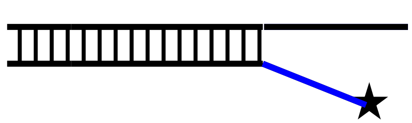
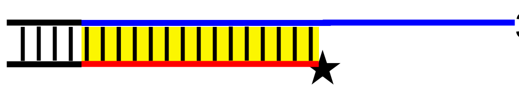
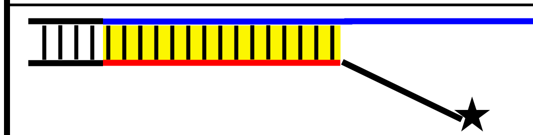
	3’ tail sequence	Fold Increase
Mix	CTGTTTGCATCGATCTGC	**1.5**
Tel	GGTTAGGGTTAGGGTTAGGG	**3.7**
Tel-A	GGTTACGGTTAGGGTTAGGG	**1.8**
Tel-B	GGTTAGGGTTAGGCTTAGGG	**2.3**
Tel-G	GGTTAGGGTTAGGGTTAG	**2.6**

= POT1 binding site



POT1 loading near the junction (Tel-B) is more important for WRN stimulation

Summary of POT1 Modulation of WRN Activity

Substrate =(TTAGGG)n	Effect on WRN Helicase
	Stimulation
	No effect
	Inhibition of activity
	Stimulation

Exonuclease not altered directly

POT1 binding mode may regulate WRN activity

POT1 May Protect Telomeric Ends from Fraying by DNA Helicases

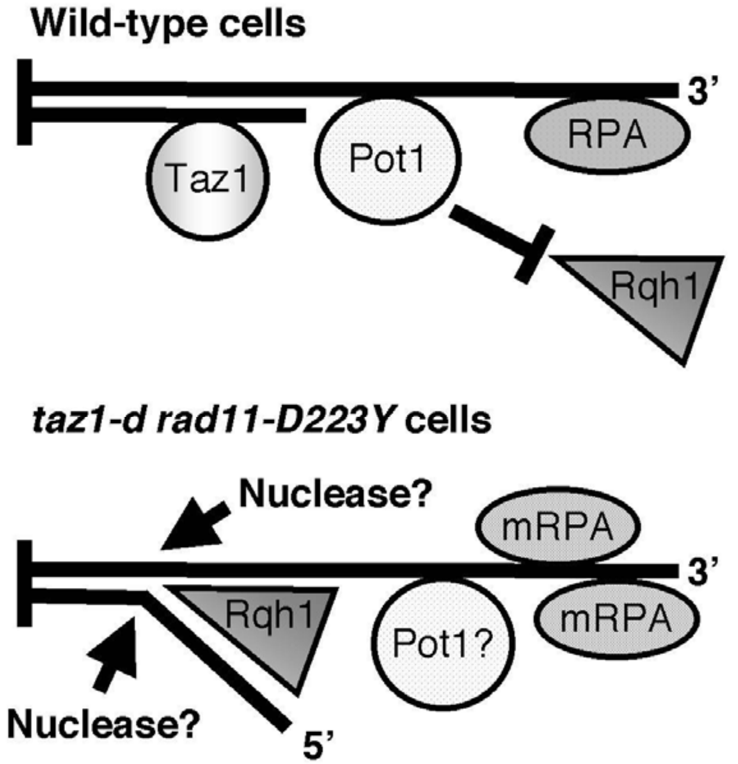


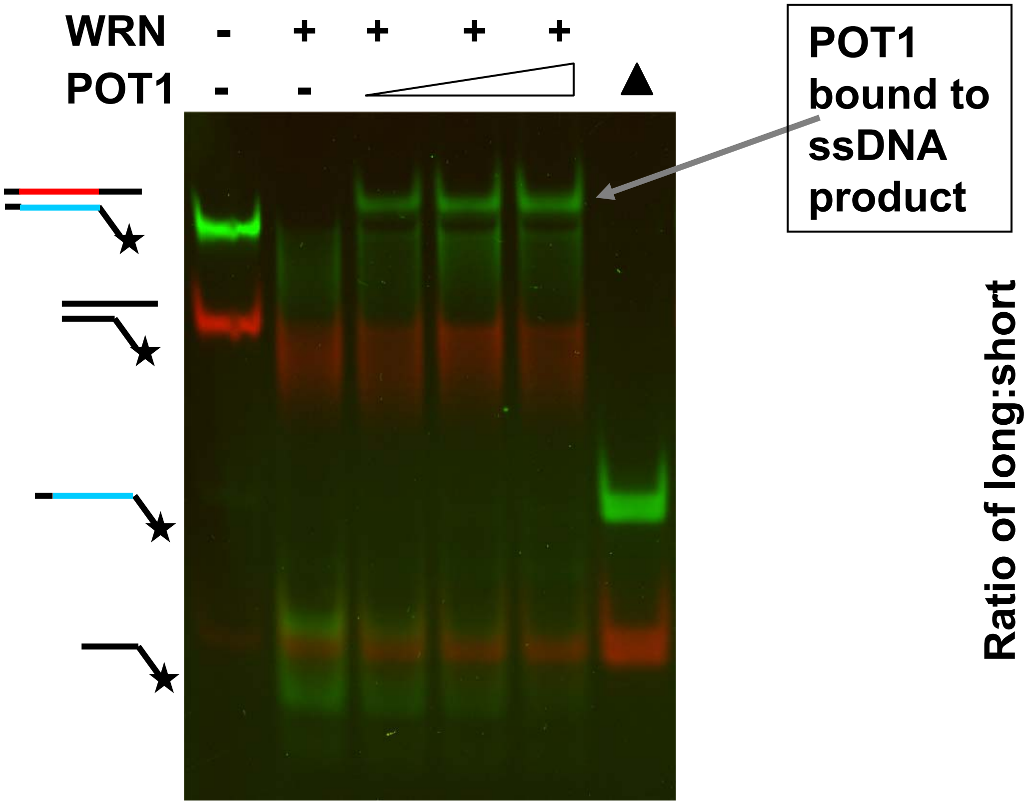
Figure 6

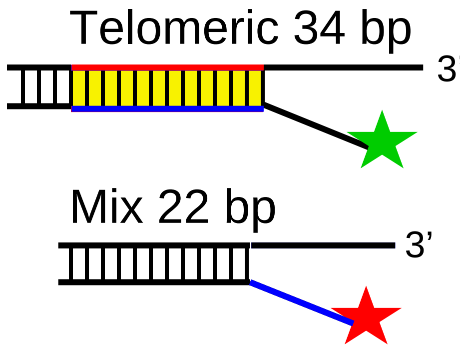
- Yeast lacking Taz1 (TRF2) and expressing mutant RPA (mRPA) exhibit rapid telomere loss
- Telomere loss is suppressed if Rqh1 (RecQ) is knocked out OR POT1 is overexpressed
- Coating of the telomeric tail with POT1 vs. RPA has profound consequences for WRN helicase activity

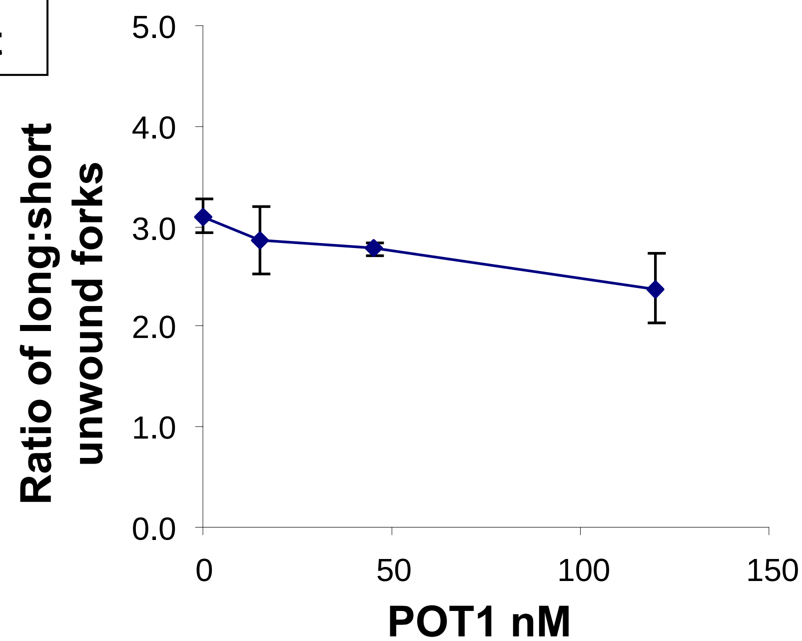
Kibe et al MBC, 2007, p. 2378.

Fission Yeast Taz1 and RPA Are Synergistically Required to Prevent Rapid Telomere Loss

POT1 Does Not Retain WRN on Telomeric Forks During Unwinding







POT1 does not alter the ratio of unwound long telomeric forks to short mixed sequence forks

MCM helicase N-terminus increases the ratio of unwound long:short duplexes - acts as a processivity clamp (Barry et al 2007, NAR)

Summary and Conclusions

1. POT1 stimulates WRN and BLM helicases, but not E. coli RecQ
   - species specific

2. POT1 pre-loading on telomeric tails:
   A. is not sufficient to stimulate WRN; does not recruit WRN

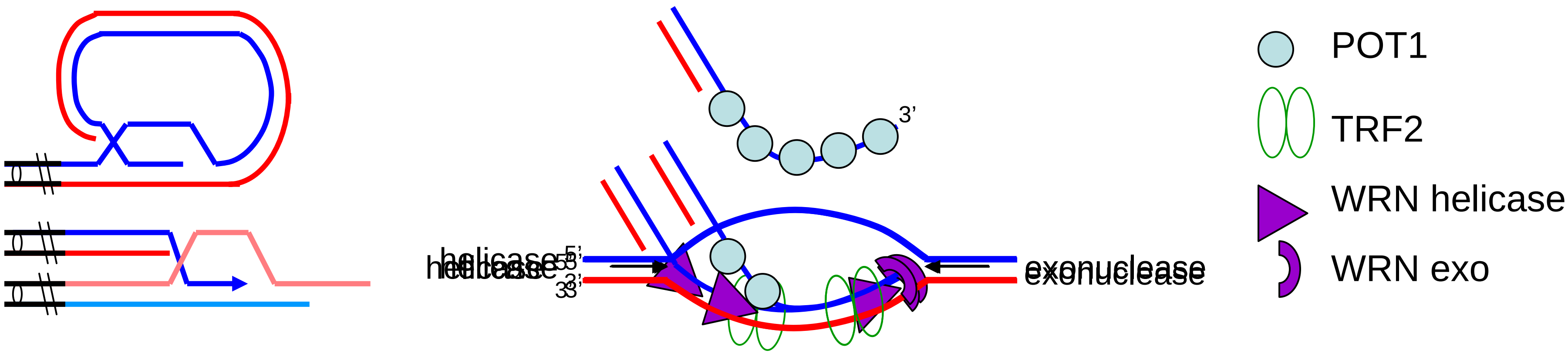
   B. inhibits WRN activity on 3' tailed telomeric duplexes
      - POT1 protects telomeres in the OPEN form

   C. stimulates WRN unwinding of telomeric forks
      - POT1 interaction with the ssDNA/dsDNA junction regulates WRN

3. POT1 does not retain WRN on telomeric forks during unwinding
   - stimulation is by preventing strand re-annealing rather than WRN dissociation

4. WRN show increased processivity on plasmid D-loops compared to oligomeric D-loops
   - POT1 stimulates WRN helicase on telomeres in the CLOSED form

Roles for WRN Protein at Telomeric Ends



+ WRN ⊣ Replication stall in telomere

↓ - WRN

Fork collapse and DS break → Initiation of HR mediated repair

telomerase ⊣ ↓ - WRN

Sister telomere loss

Initiation of HR mediated repair ↑ +WRN → Restore replication fork

Dissociate without strand cross over

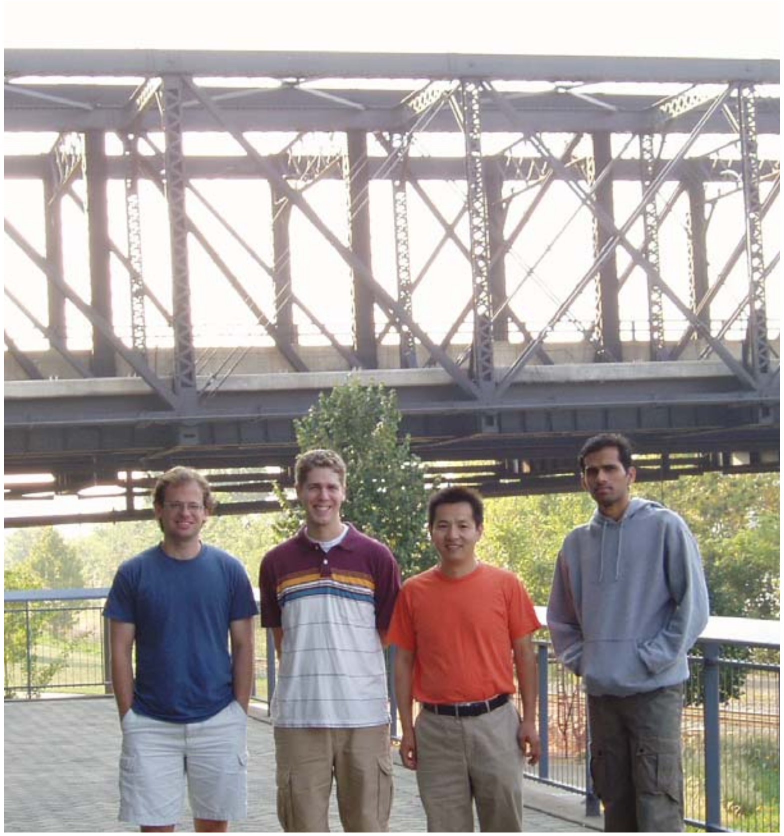
Initiation of HR mediated repair ↓ - WRN → T-SCE shortened telomere

Resolve with strand cross over

Acknowledgements

Opresko lab

- Jerry Nora
- Greg Sowd
- Fujun Liu
- Rama Damerla



- Vilhelm Bohr, NIA
- Ming Lei, U. of Michigan
- Peter Baumann, Stowers Inst.
- James Keck, U. of Wisconsin
- Walter Chazin, Vanderbilt

Funding

- Ellison Medical Foundation
- NIEHS