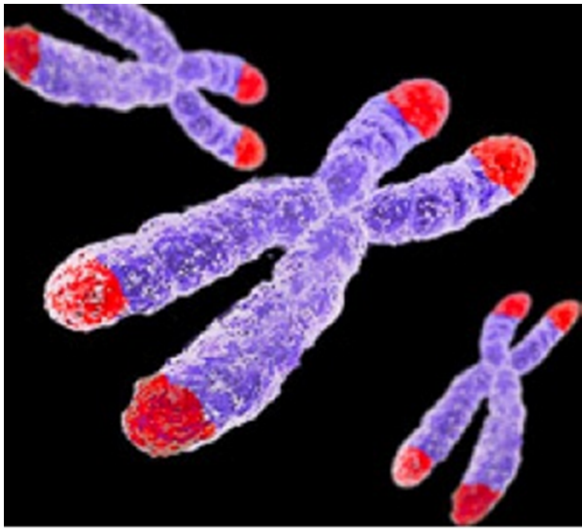
Involvement of the Werner syndrome protein (WRN) in telomeric recombination

DNA Repair Videoconference
January 17, 2017

David Orren, Ph.D.
dkorre2@uky.edu
Department of Toxicology and Cancer Biology
University of Kentucky College of Medicine

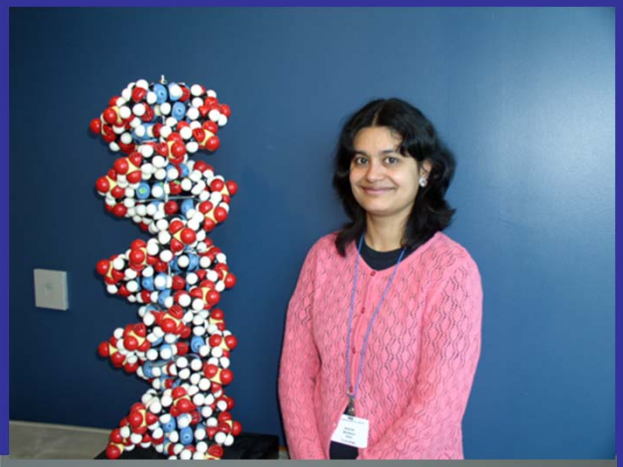


ACKNOWLEDGEMENTS

Lab Members

Amrita Machwe

Deanna Edwards



Outside Collaborators on WS Project

Vilhelm Bohr (Natl. Institute on Aging)

Joanna Groden (Ohio State)

Jianyuan Luo (UMAB)

Steve Matson (UNC)

Jack Griffith (UNC)

Titia de Lange (Rockefeller Univ.)

Robert Lloyd (Univ. of Nottingham)

Edward Bolt (Univ. of Nottingham)

Marc Wold (Univ. of Iowa)

Alexander Mazin (Drexel Univ.)

FUNDING

National Cancer Institute (R01CA113371)

National Institute on Aging (R01AG027258)

Outline

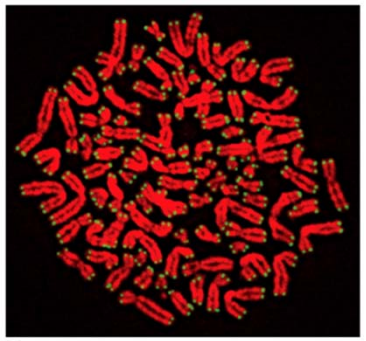
- **Telomeres: relevance to genome stability, cancer and aging**
  - **Telomere Structure and Function**
  - **Telomere Maintenance Problems**
  - **Telomerase (and its limitations)**
    - **Requirement for other telomere maintenance pathways?**

- **Werner syndrome and its telomeric abnormalities**
  - **Model to study relationships between telomere dysfunction, genome instability, aging and cancer**
  - **Phenotypes in cell and animal models**

- **Our findings support a role for WRN in telomeric recombination**
  1. **Structure and telomeric sequence specificity of WRN**
  2. **WRN and the key telomeric factor TRF2 promote telomeric strand exchange**

- **Models for telomere maintenance by WRN**

Telomere Sequence and Structure



(A)

Figure 10.12 The Biology of Cancer (© Garland Science 2014)

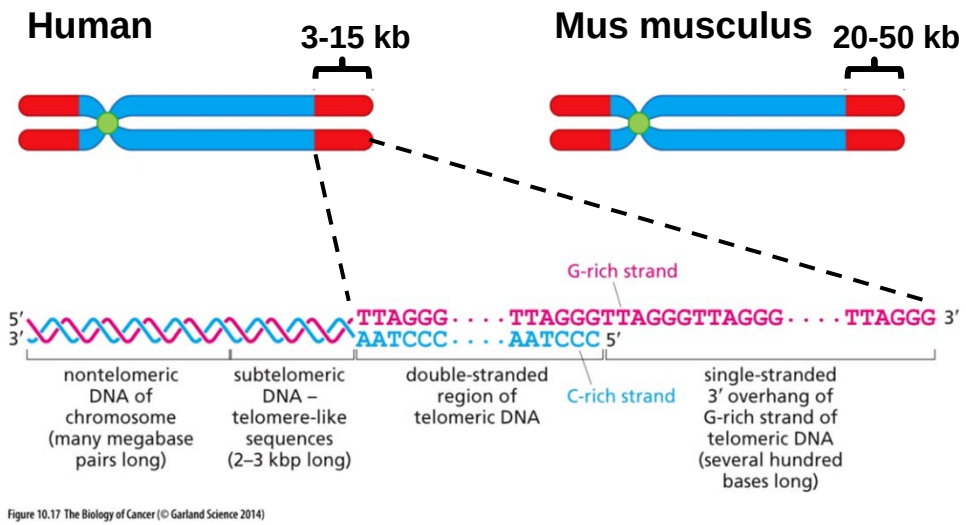


Figure 10.17 The Biology of Cancer (© Garland Science 2014)

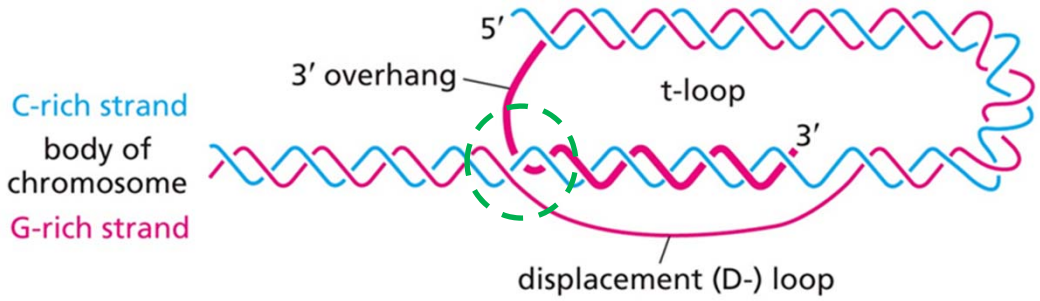
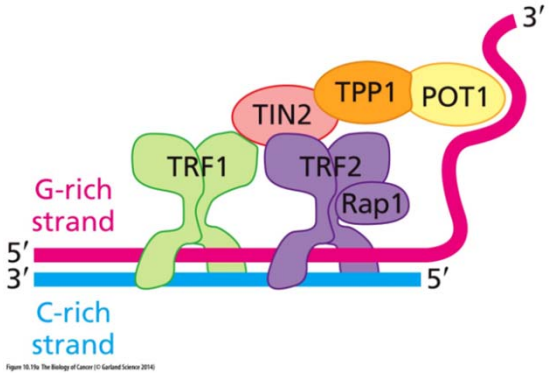


Figure 10.18c The Biology of Cancer (© Garland Science 2014)

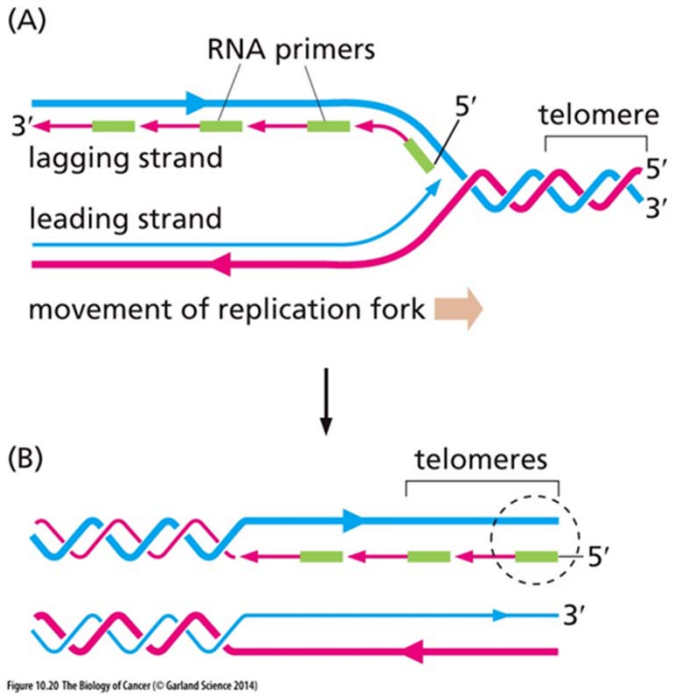
- **Chromosomes end in repeating sequences**
  - **TTAGGG/AATCCC in higher eukaryotes**
  - **ending in 3’ overhang of G-rich strand**
- **Telomere lengths vary:**
  - **between species, chromosomes, and over time**
- **Telomere-specific proteins**
  - **collectively termed “shelterin”**
  - **various functions in telomere maintenance**
  - **TRF2, TRF1 bind duplex telomeric repeats**
    - **TRF2 is essential for telomere protection**
- **Telomeres may form looped structures (T-loops) to theoretically prevent the ends from being recognized as double-strand breaks**
  - **3’-overhang inserted into telomeric duplex**



Trouble with Telomeres

- **Subject to shortening**
  1. **End (lagging strand) replication problem**
     - **Sequence loss during each replication cycle**
  2. **Stochastic (random) deletions on individual telomeres**
     - **Telomeres are fragile sites**
       - **Difficult to replicate**

- **Critically short telomeres lose "protected" status**
  - **One (or very few) unprotected telomeres triggers checkpoint response, leading to apoptosis or senescence**
  - **Loss of ATM- and p53-dependent checkpoint allows cell to enter "crisis" where telomere and chromosome instability becomes rampant**
    - **Key stage in cancer development and progression?**



Apoptosis

Cellular Senescence

Checkpoint Activation

ATM (ATR) p53

Unprotected Telomere

Telomerase (and its limitations)

- **Telomerase: reverse transcriptase containing RNA (template) component that adds back G-rich telomeric repeats to the 3' telomeric ends**
  - **Counteracts telomere loss due to end replication problem and to stochastic deletions**
- **Only normally expressed in:**
  - **germ cells**
  - **embryonic stem cells**
  - **Some but not all adult stem cell types**
- **Most somatic cells do not express telomerase**
  - **Vulnerable to gradual and stochastic telomere shortening**
- **However, 85-90% of tumor cells have significant telomerase activity**
  - **Importance of telomere length maintenance for tumor development**
  - **Anti-cancer drug target**

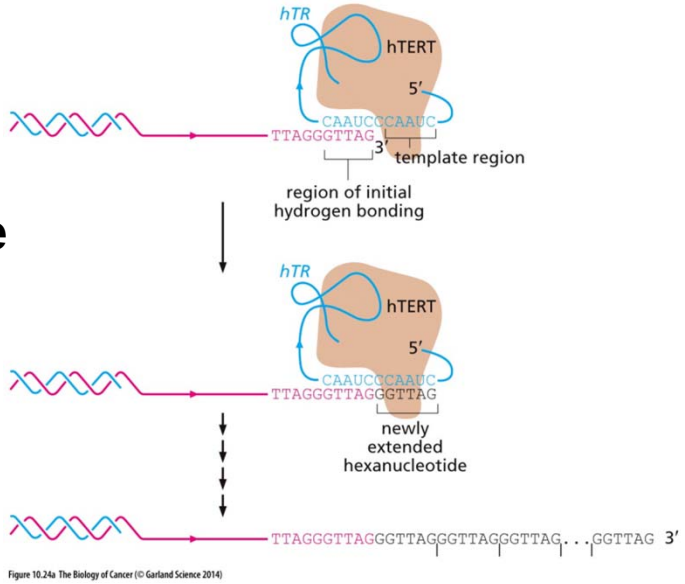
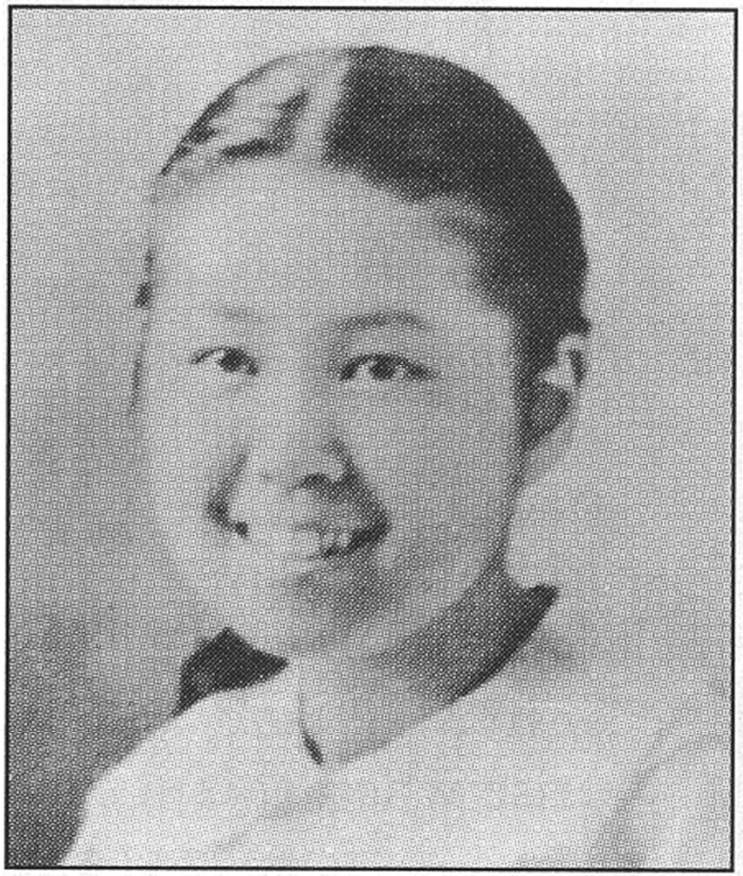


Figure 10.24a The Biology of Cancer (© Garland Science 2014)

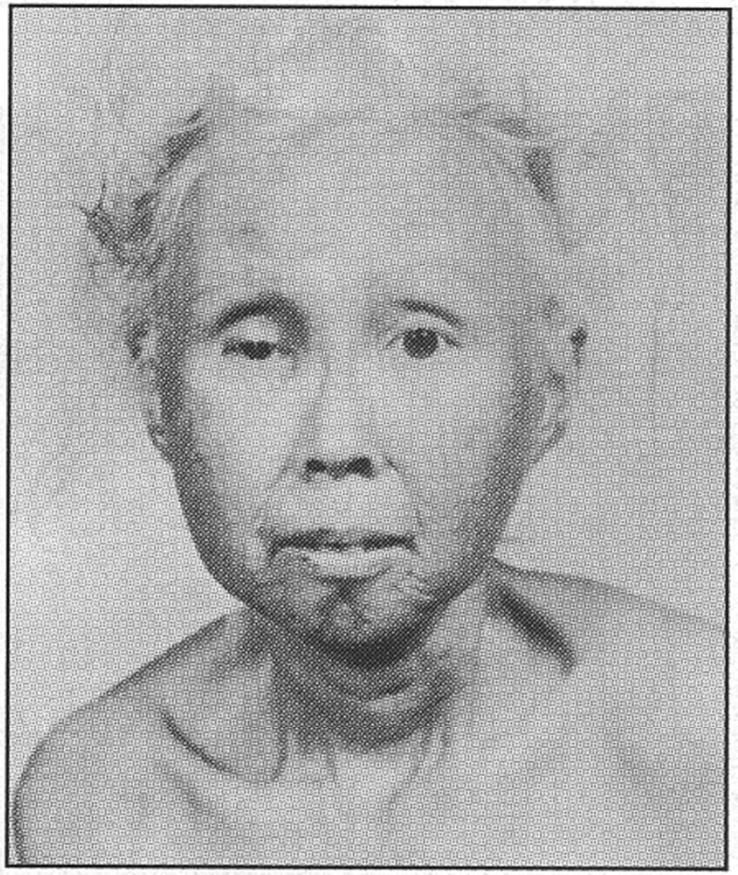
Inherent telomeric instability seems to indicate a requirement for somatic cells to have other mechanisms that minimize or rescue (stochastic) telomere loss.

If so, how do these mechanisms operate?

Werner syndrome (WS)



Age 15

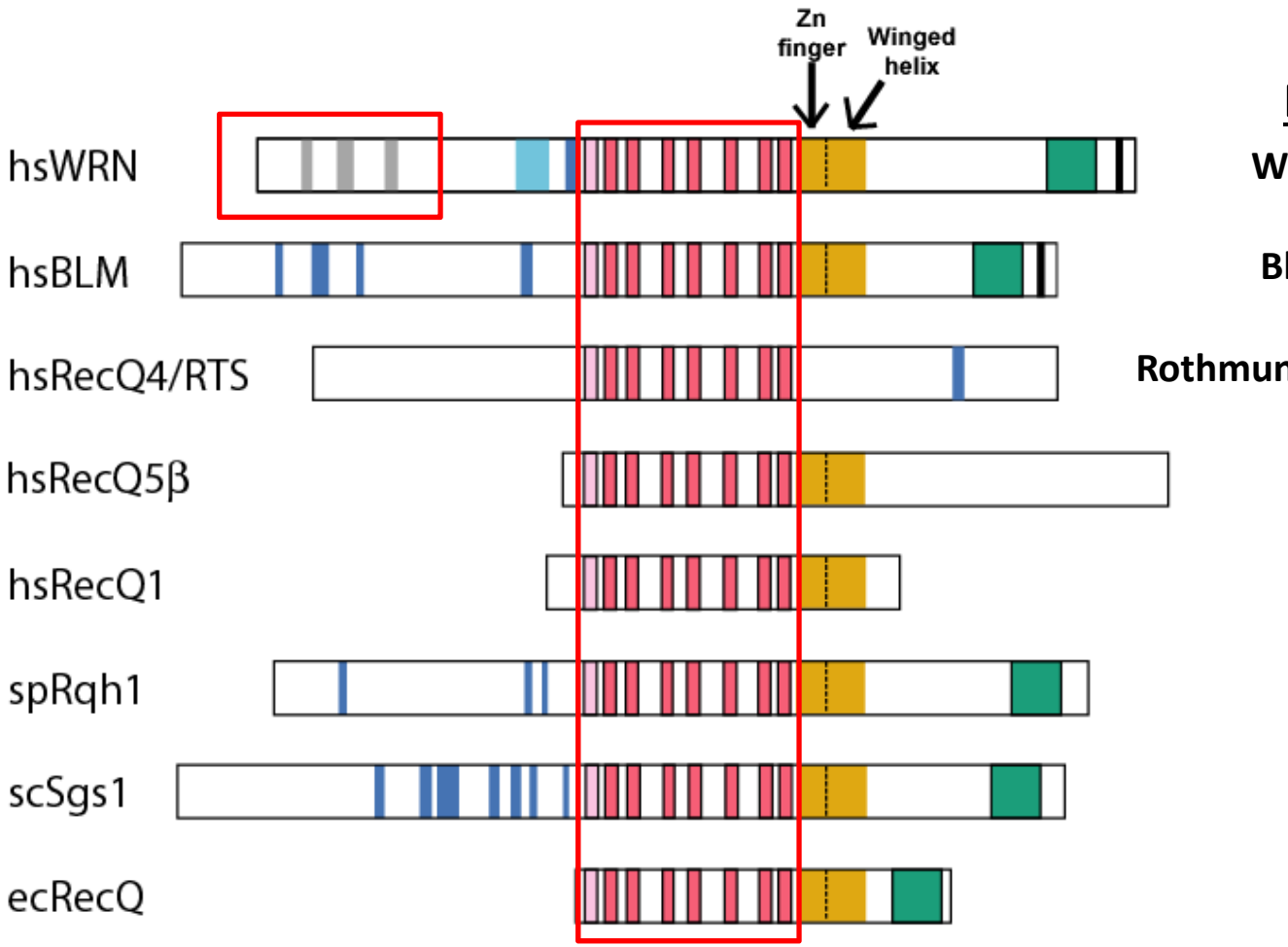


Age 48

Werner syndrome vs. normal aging

Major Clinical Features	WS	Aging
Short stature	Yes	-
Soft tissue calcification	Yes	-
Laryngeal atrophy	Yes	-
Graying and loss of hair	Early	Yes
Skin ulceration	Early↑	Yes
Malignancies	↑	Yes
Hyaluronic aciduria	Early↑	Yes
Atherosclerosis	Early↑	Yes
Hypogonadism	↑	Yes
Osteoporosis	Early↑	Yes
Cataracts	Early↑	Yes
Diabetes mellitus type II	↑	Yes
Hypertension	↑	Yes
Central nervous system degeneration	No	Yes
Inheritance	autosomal recessive	polygenic

RecQ helicase family members



Defective in

Werner Syndrome

Bloom Syndrome

Rothmund-Thomson Syndrome

WS cellular phenotypes

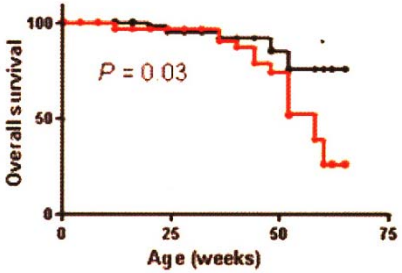
- **Hypersensitivity to certain DNA damaging agents**
  - **topoisomerase inhibitors (camptothecin)**
  - **Interstrand crosslinking agents (platinum compounds, mitomycin C)**
  - **4-nitroquinoline-oxide**
  - **hydroxyurea**
- **Genomic instability**
  - **increased chromosomal and telomeric abnormalities**
- **Slow growth**
  - **replication abnormalities and extended S phase**
  - **premature replicative senescence (primary fibroblasts)**

Telomere maintenance defects in WS

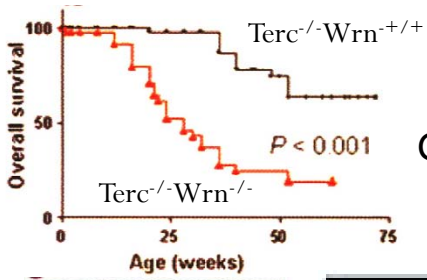
- **Dramatic premature cellular senescence in primary WS fibroblasts compared to normal fibroblasts (Martin et al., 1970; Machwe et al., 2000)**
- **Introduction of telomerase into WS primary fibroblasts prevents senescence and results in immortality, indicating that this premature senescence is associated with telomere dysfunction (Wyllie et al., 2000)**
- **WRN-deficient mice had no phenotype, but telomerase-deficient mice upon which WRN deficiency was superimposed showed stochastic telomere loss and premature aging characteristics reminiscent of WS (Chang et al., 2004; Du et al., 2004)**
- **Expression of a dominant-negative, helicase-deficient WRN protein in transformed human cells caused stochastic telomere loss (Bai and Murnane, 2003; Crabbe et al., 2004)**
  - **Telomeric loss related to lagging strand replication (Crabbe et al., 2004)**

Loss of WRN function affects telomeres *in vivo*

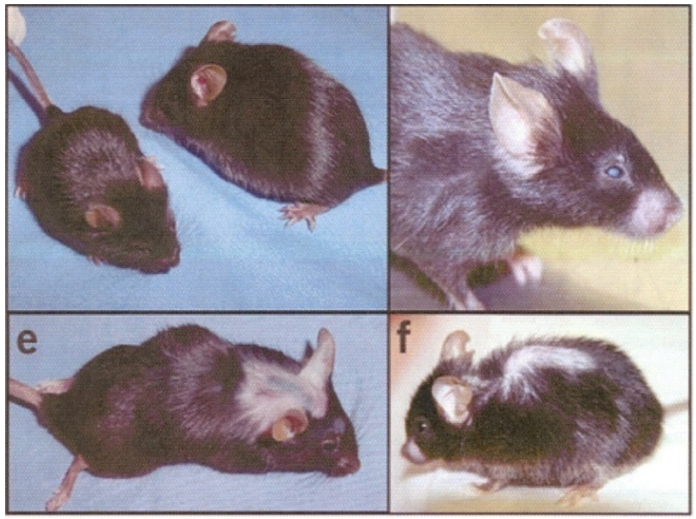
Chang et al. (2004). Nat. Genet. 36: 877-82

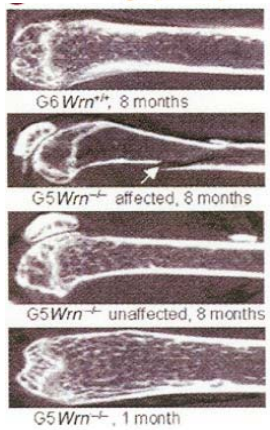


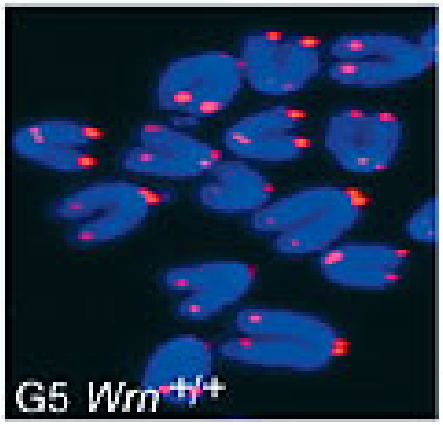
G1-3

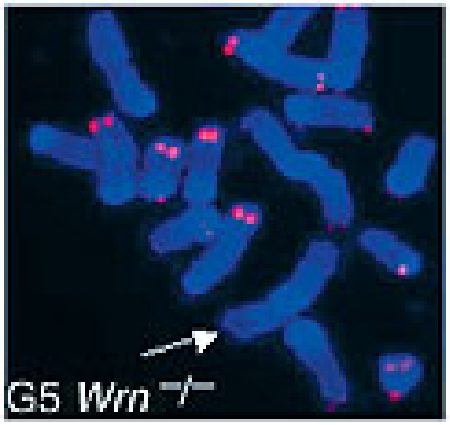


G4-6



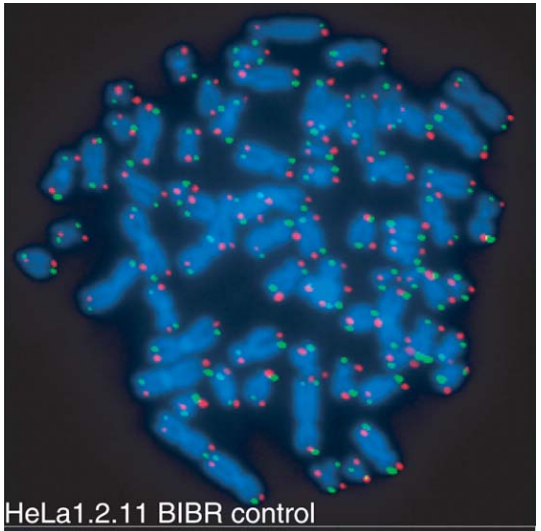


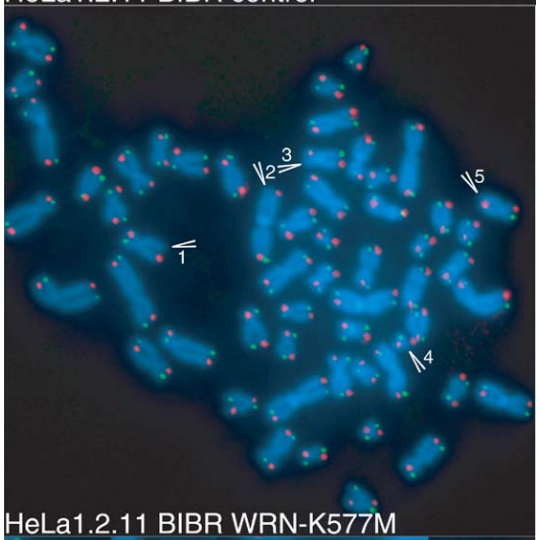




- **WRN acts to suppress stochastic telomeric deletions**
- **Suggests that telomere dysfunction elicits organismal aging phenotypes**

Crabbe et al. (2004). Science 306: 1951-3



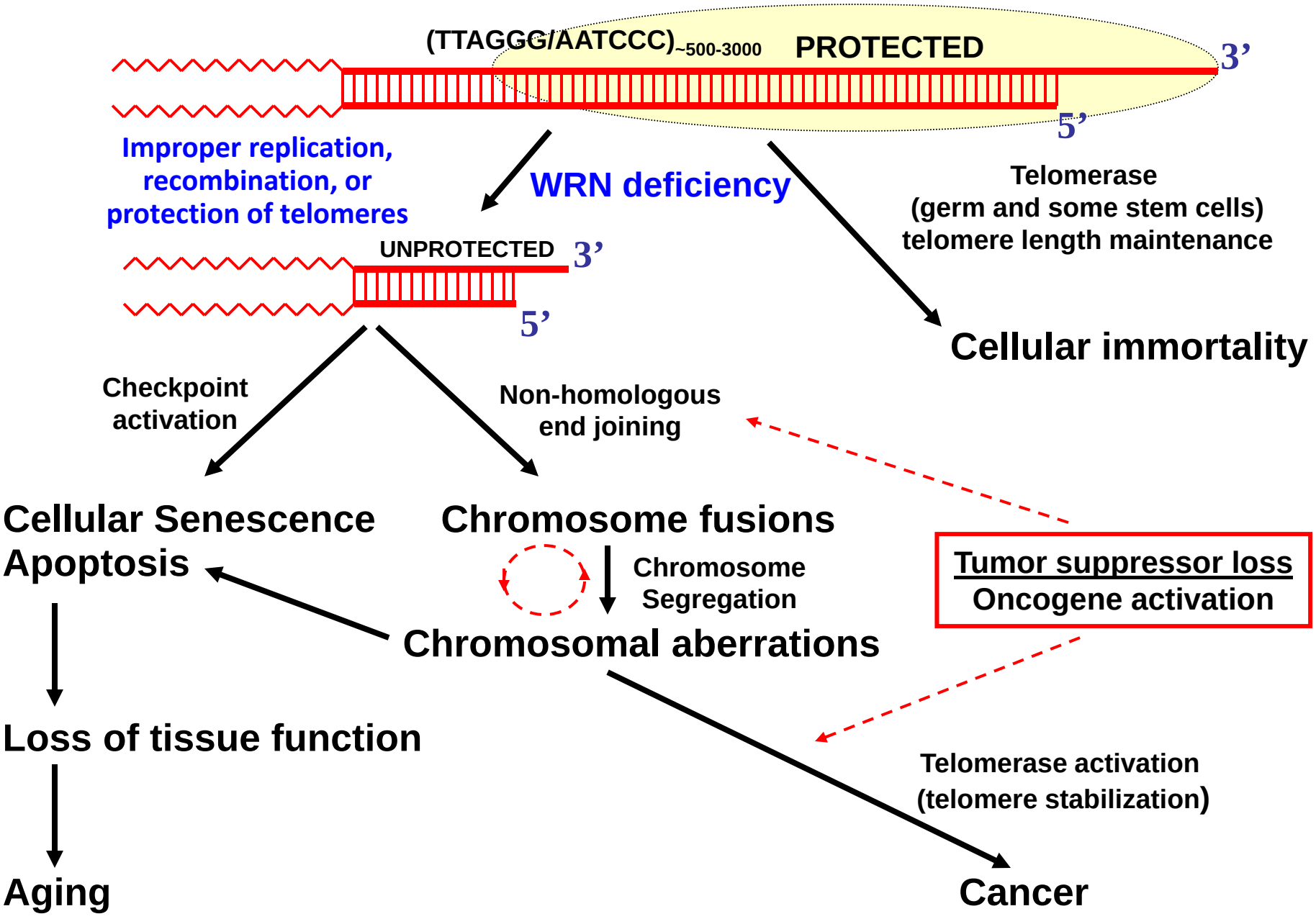


How does WRN Deficiency Result in This Sudden Telomere Loss Phenotype?

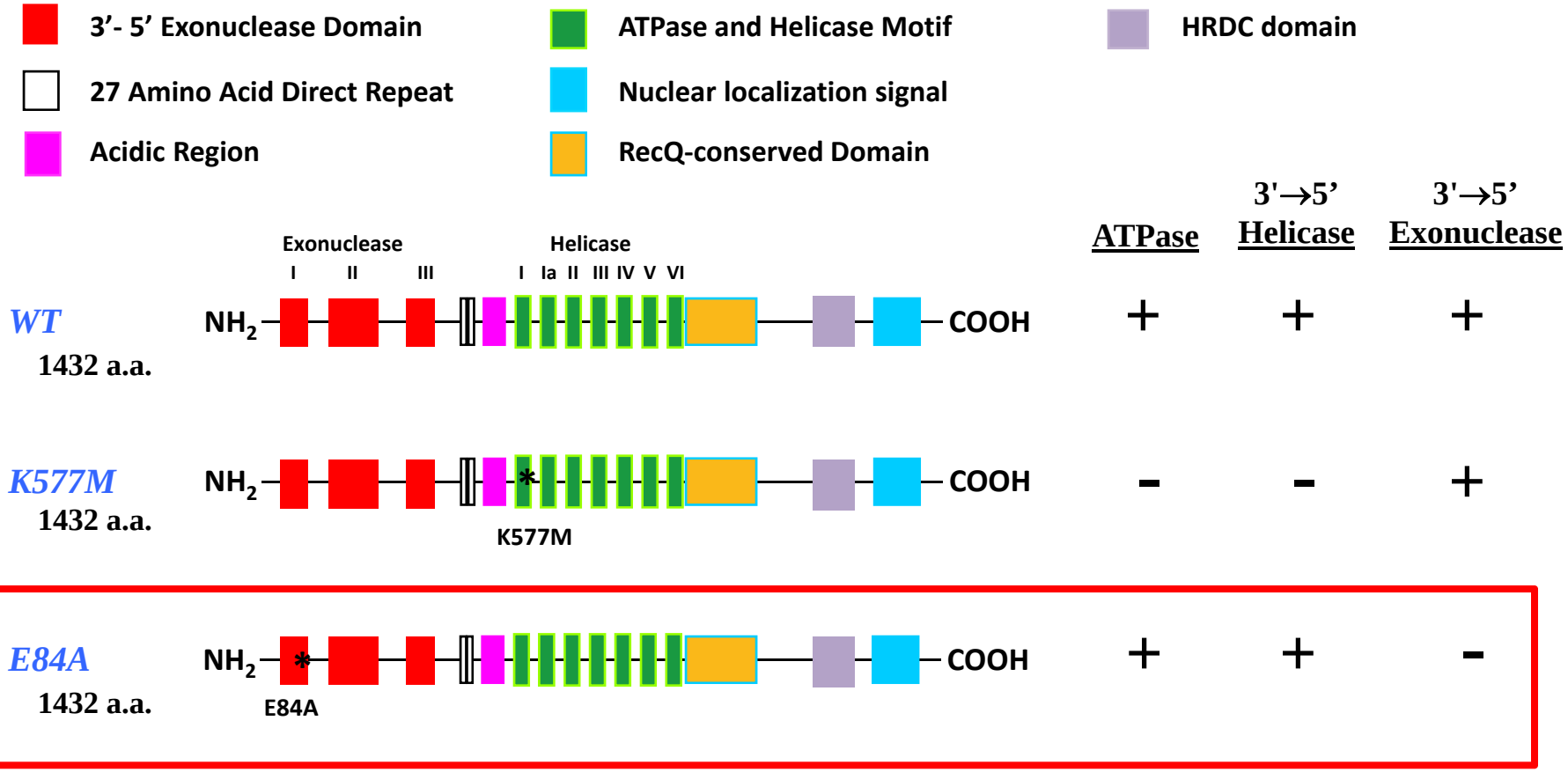
1. **Loss of WRN function increases generation of telomeric deletions**

2. **Loss of WRN function prevents rescue of sporadic telomeric deletions, resulting in their persistence**

Telomere Protection Hypothesis



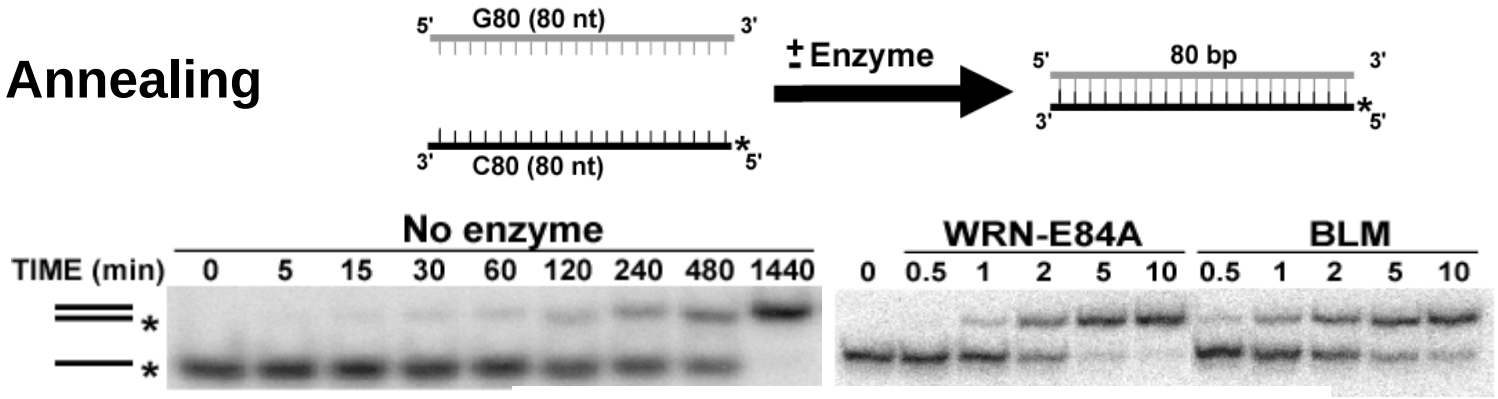
WRN Helicase/Exonuclease Mutants



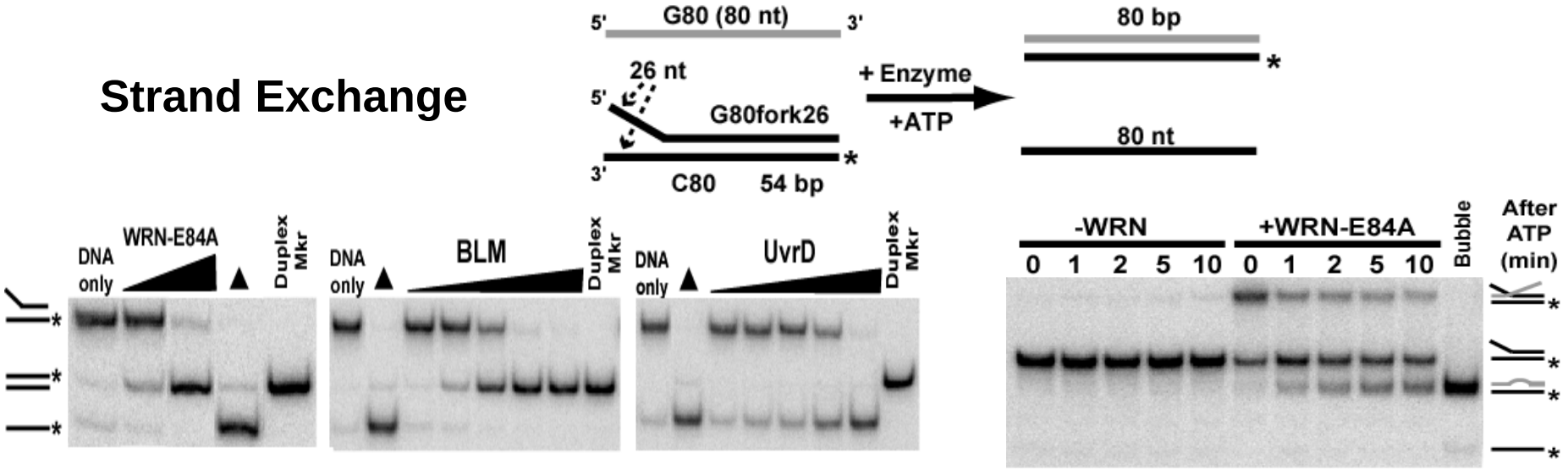
Additional DNA-dependent activities of WRN

[Machwe et al., JBC 280: 23397-23407, 2005]

- **WRN, BLM and several other RecQ helicases facilitate the annealing of complementary regions of single-stranded DNA**



***WRN- and BLM-mediated strand annealing occurs in multiple structural contexts**

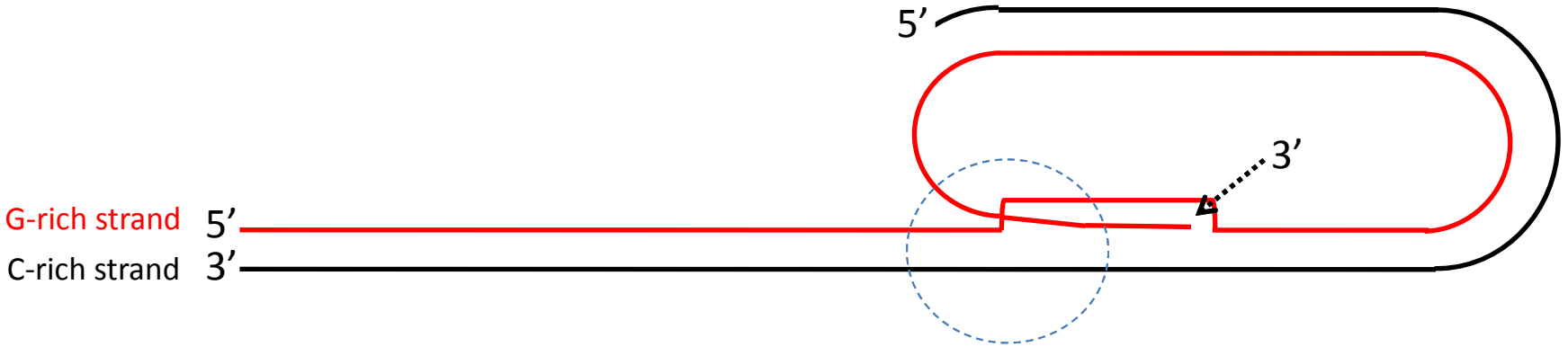


What is WRN's precise molecular function in telomere maintenance?

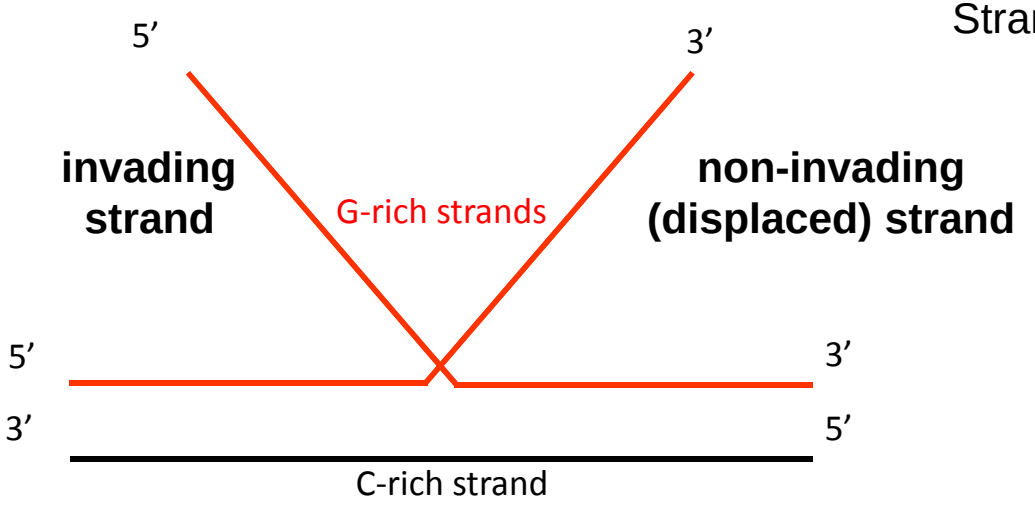
One Working Hypothesis:

Based on the observation that WRN promotes strand exchange, we initially postulated that WRN acts on telomeric recombination-related structures, such as T-loops.

Model Strand Invasion DNA Substrate

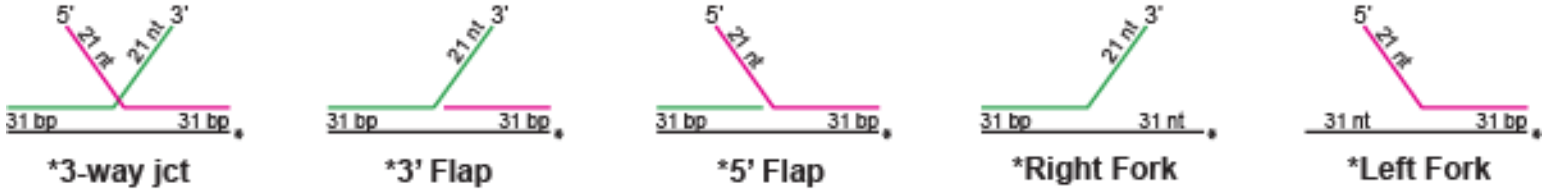


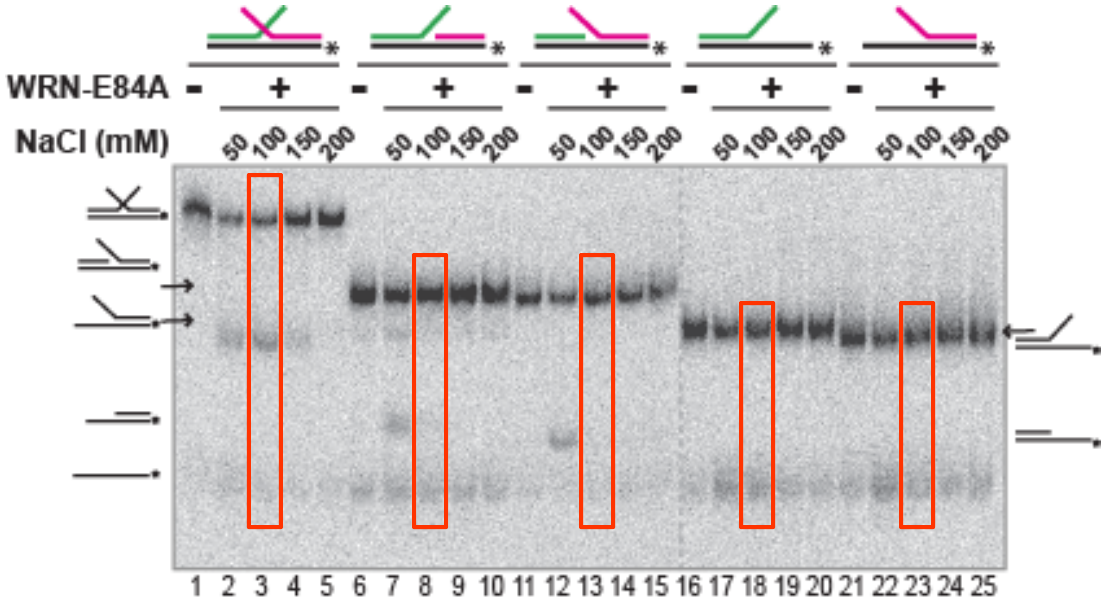
Strand invasion of G-rich 3’ overhang into duplex telomeric region

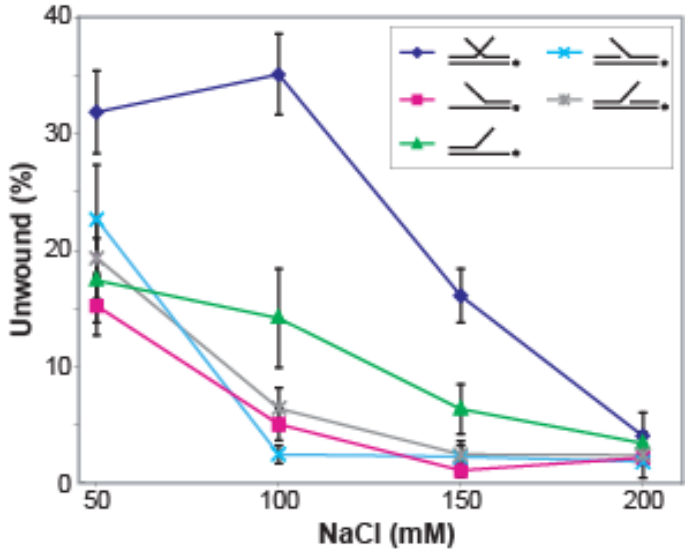


3-way junction

WRN Preferentially Unwinds Strand Invasion Intermediates (containing random sequences)



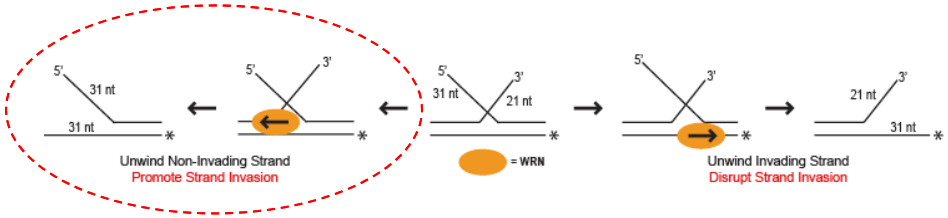


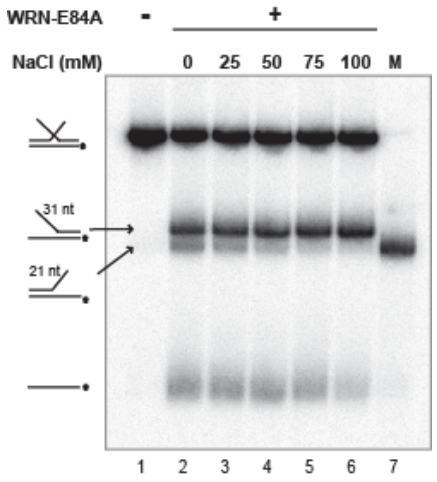


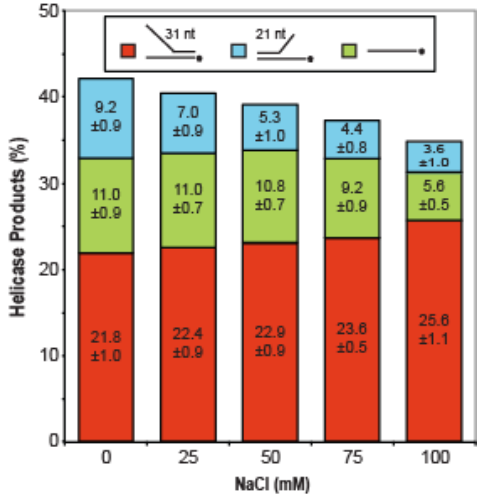
DNA binding expts also showed similar preference for strand invasion intermediates
Strand invasion structure may be a physiological target of WRN

WRN Unwinding Directionality on Strand Invasion Intermediates

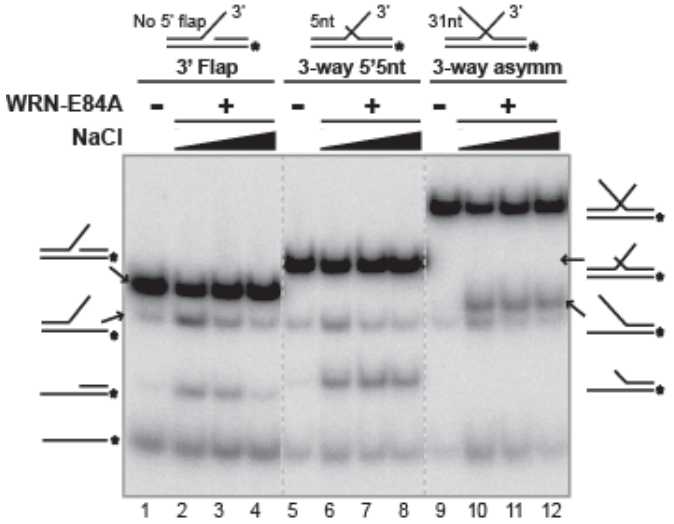
WRN preferentially unwinds the 3’ flap strand, a preference that becomes nearly absolute at physiological salt concentration

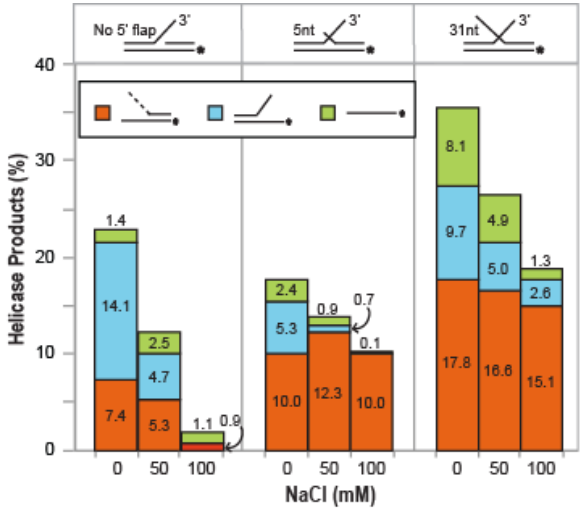






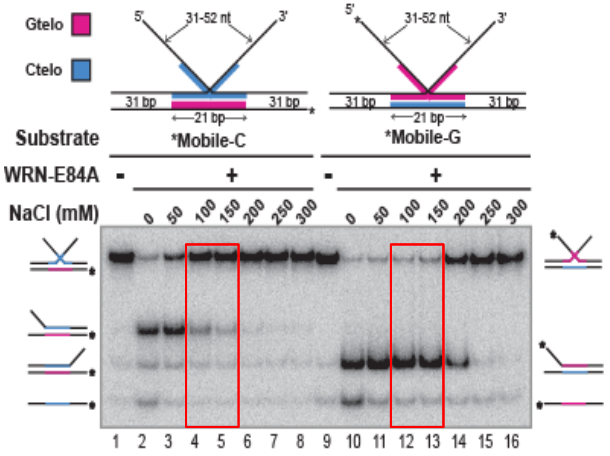
Importance of single-stranded 5’ flaps

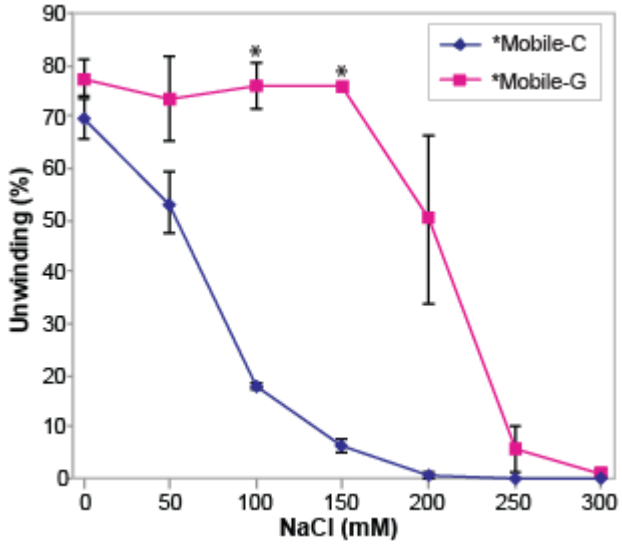




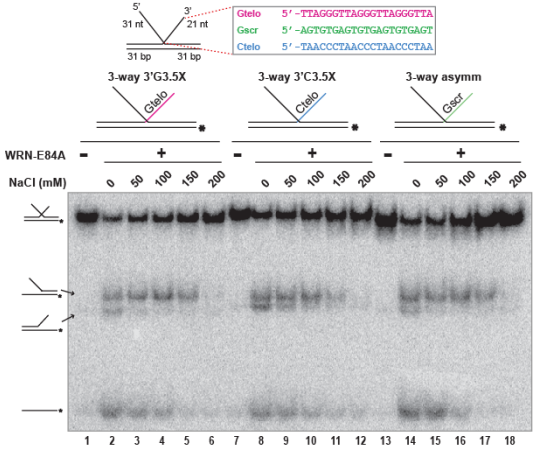
WRN preferentially unwinds strand invasion intermediates containing G-rich telomeric sequence on the 5’ flap

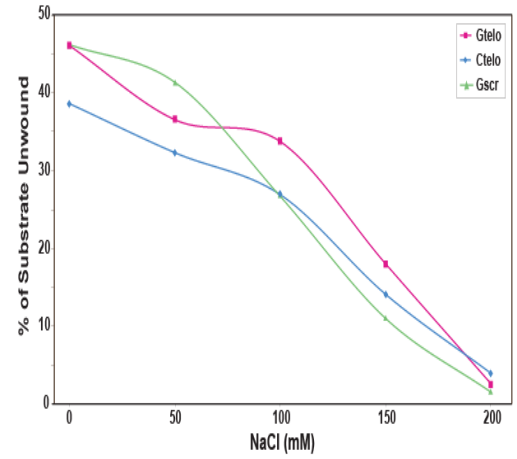
Mobile 3-way Junctions



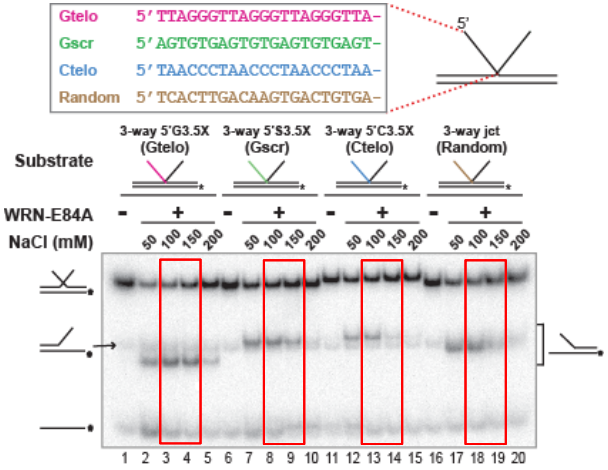


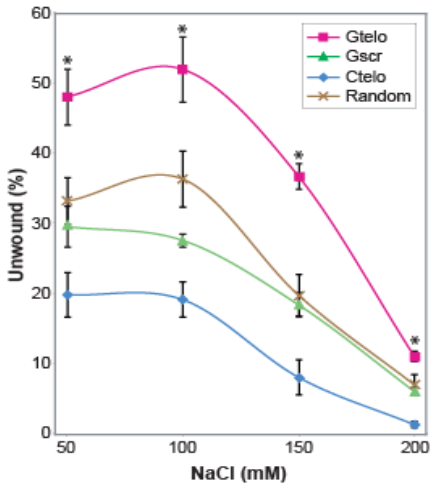
3’ Flap Variations





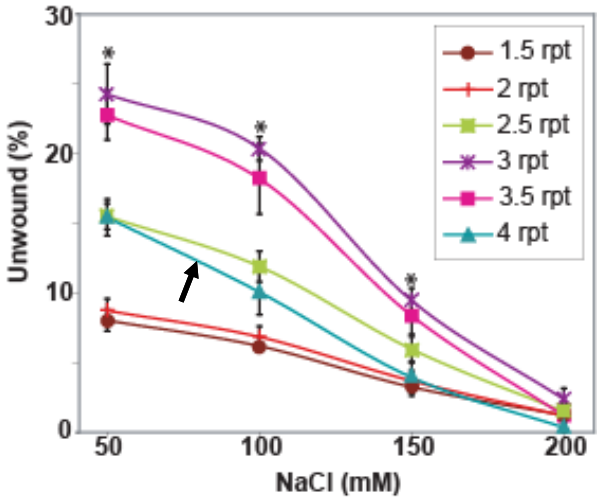
5’ Flap Variations

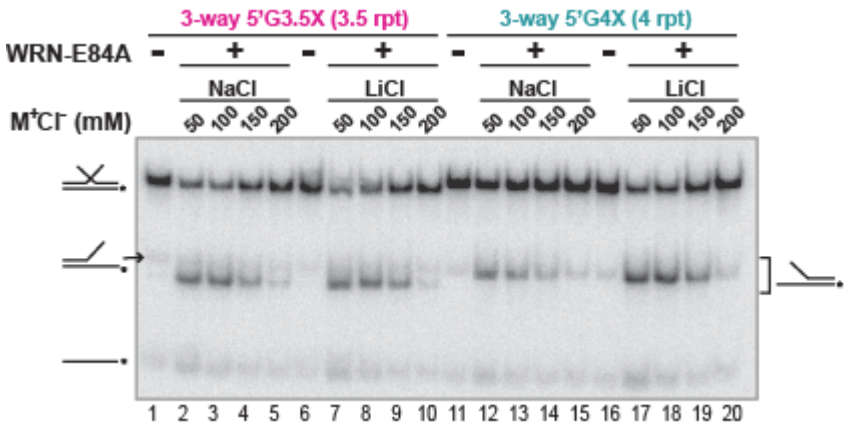


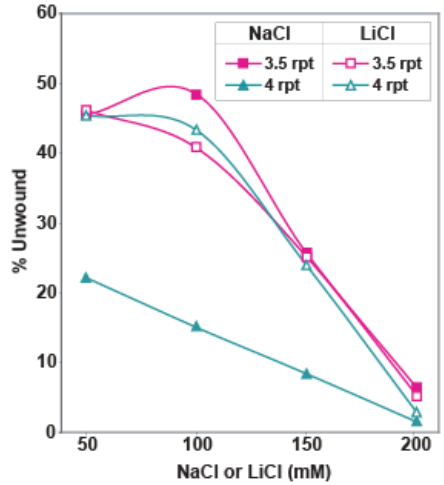


DNA binding studies support these context-specific sequence preferences

WRN preferentially unwinds strand invasion intermediates containing ≥3 G-rich telomeric repeats on the 5’ flap



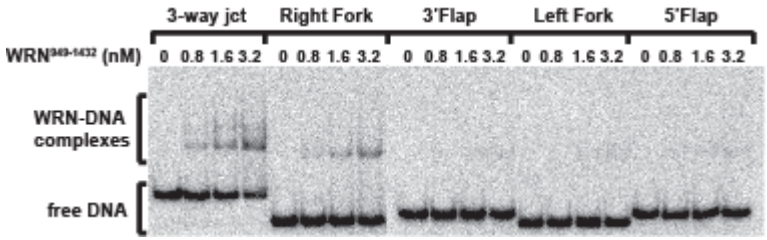


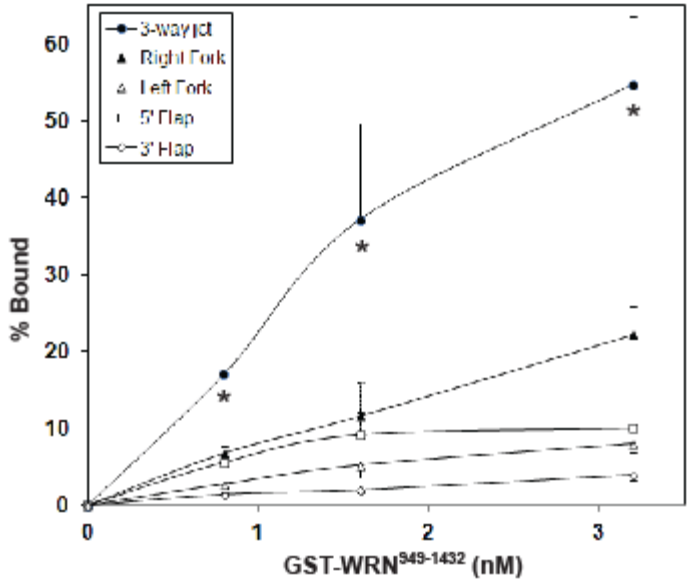


DMS assays confirmed that, under these NaCl and DNA concentration conditions, ssDNAs with 3 G-rich telomeric repeats do not form G-quadruplexes, while those with 4 repeats do. Thus, this sequence preference is due to unfolded G-rich telomeric sequence and not G-quadruplexes.

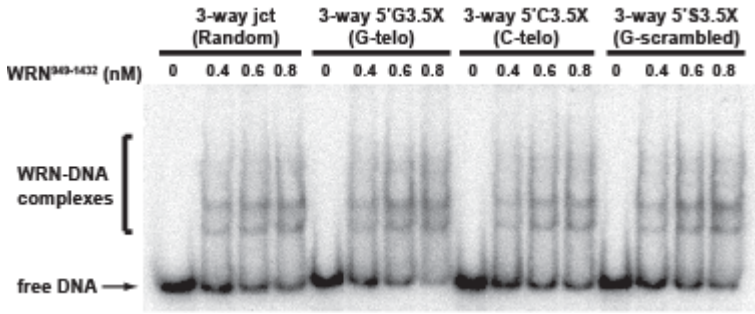
Structure and Telomeric Sequence Specificity are Conferred by WRN's C-terminal Region

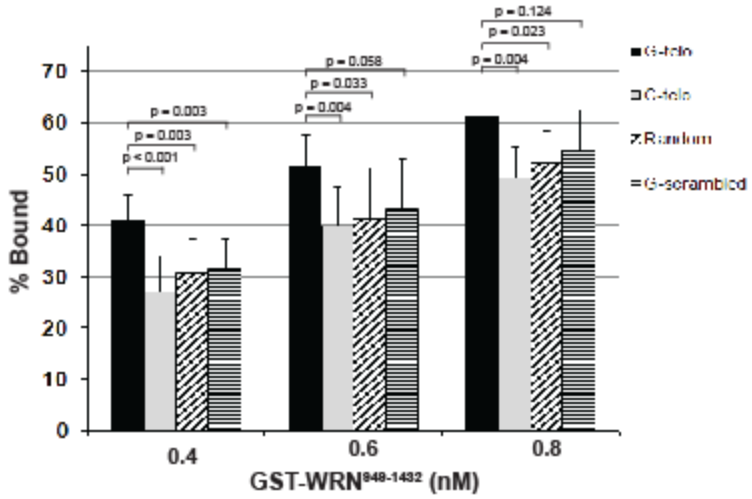
Structure Specificity





Sequence Specificity

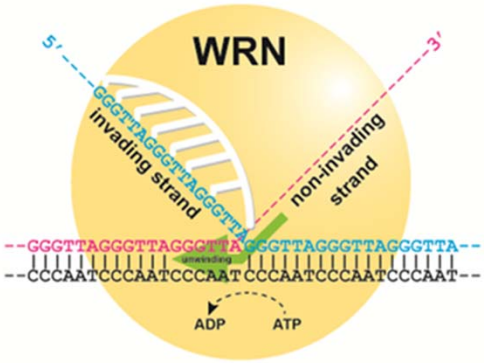




Summary of 3-way Junction Studies with WRN

Edwards et al. 2015. *Nat. Commun.* **6**:8331 and unpublished

- **WRN preferentially binds to and unwinds strand invasion intermediates with a directionality of that promotes strand invasion. The 5' single-stranded flap is a key structural element for unwinding, even though WRN action displaces the 3' flap strand of these strand invasion intermediates.**
- **WRN acts optimally on strand invasion intermediates when the invading (5' flap) strand contains G-rich telomeric sequences. First indication of sequence preference related to WRN unwinding activity, and it is consistent with WRN function at telomeres.**
- **These preferences are most pronounced at nuclear monovalent cation levels.**
- **C-terminal region of WRN mediates structure and telomeric sequence specificity, with the RQC-Winged helix domain conferring structure specificity.**
- **Strand invasion structures are likely physiological targets for WRN action, with telomeric recombination intermediates being even more enhanced targets. WRN also recognizes multiple DNA elements within these structures.**



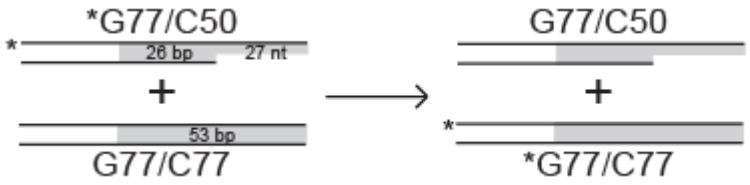
WRN Helicase Promotes
G-Rich Telomeric Strand Invasion

Since telomeres are bound with various telomere-specific proteins, do telomeric factors impact WRN activity?

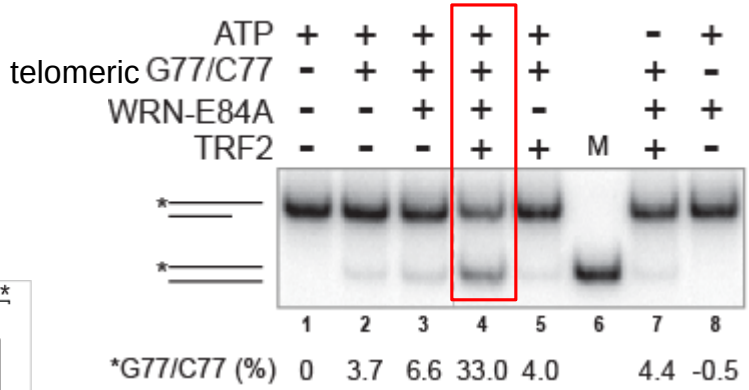
Cooperation between WRN and TRF2, the key protein for protecting telomeres

- **Previous studies identified a positive functional interaction between WRN and TRF2**
  [Opresko et al., 2002; Machwe et al., 2004; Opresko et al, 2004]
  - **Purified WRN and TRF2 bind to each other in solution**
  - **WRN and TRF2 coimmunoprecipitate from cells**
  - **TRF2 influences WRN helicase and exonuclease activities and dramatically stimulates WRN exonuclease activity specifically on telomeric substrates**
  - **WRN colocalizes with telomeres in S phase**

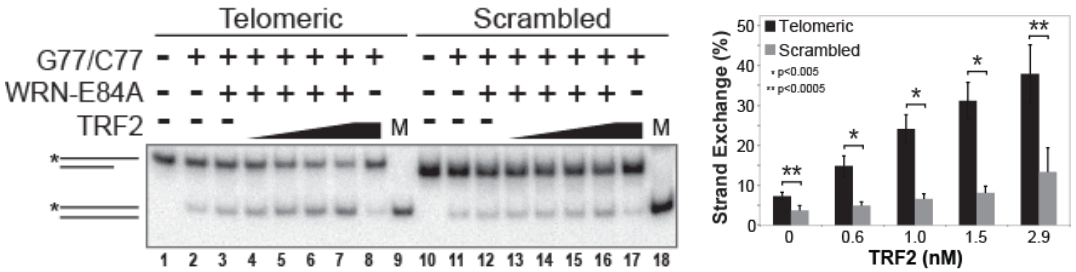
TRF2 stimulates WRN-mediated telomeric strand exchange



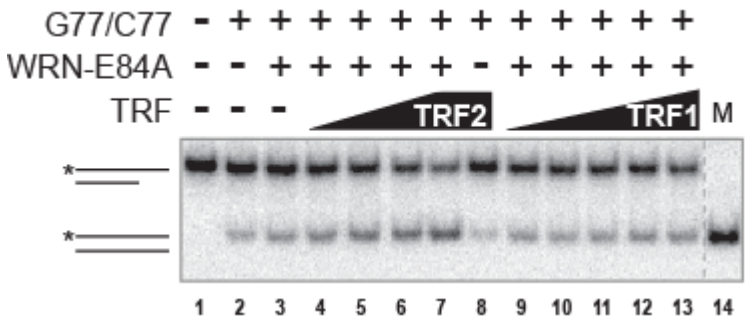
Requirements for Strand Exchange



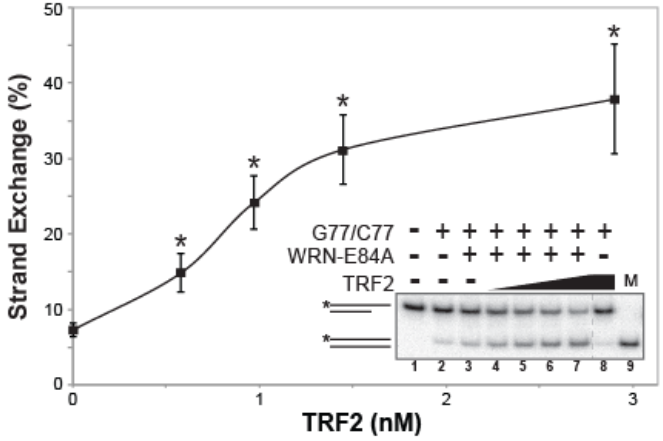
Specificity for Telomeric Substrates



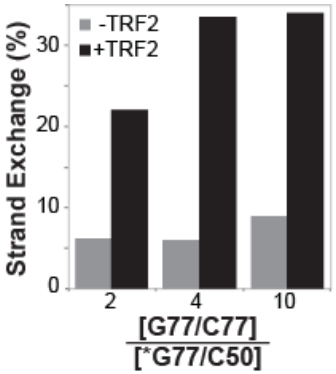
Specificity for TRF2 vs. TRF1



Dependence on [TRF2]

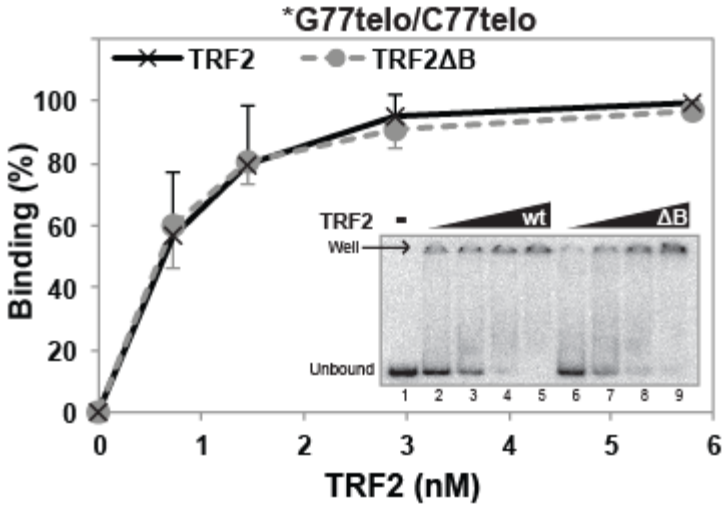


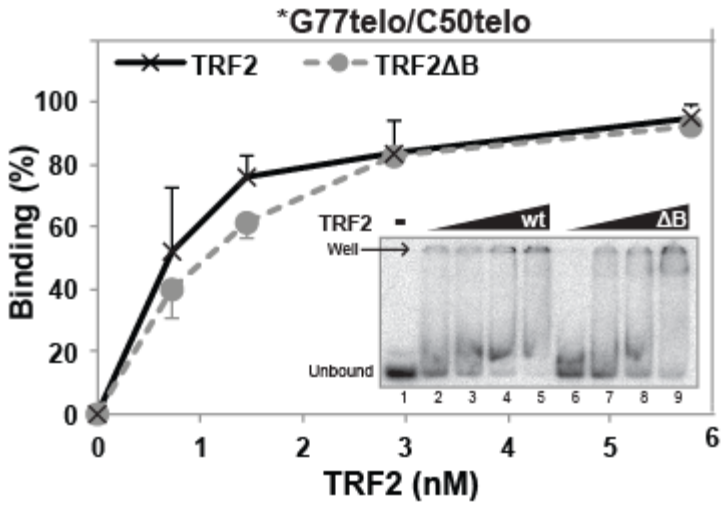
Effect of [DNA]

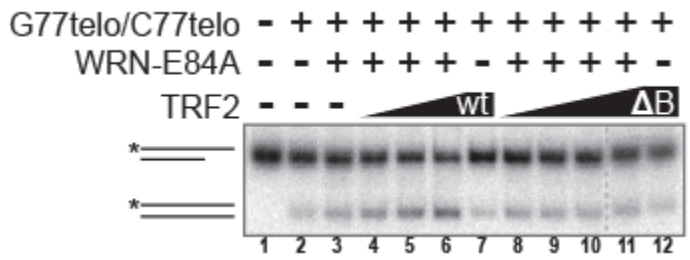


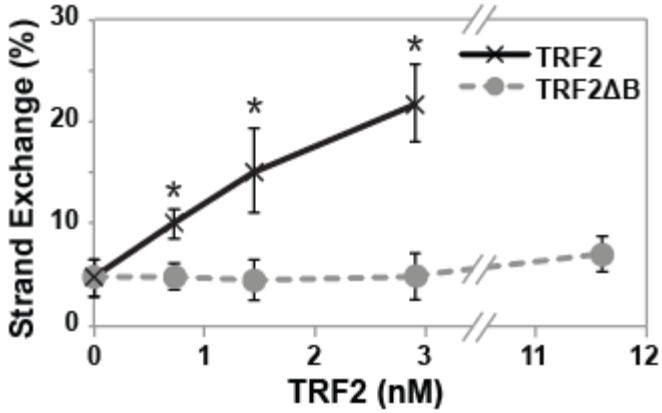
WRN-TRF2 direct interaction is needed for telomeric strand exchange

N-terminal basic region of TRF2 mediates its direct interaction with WRN







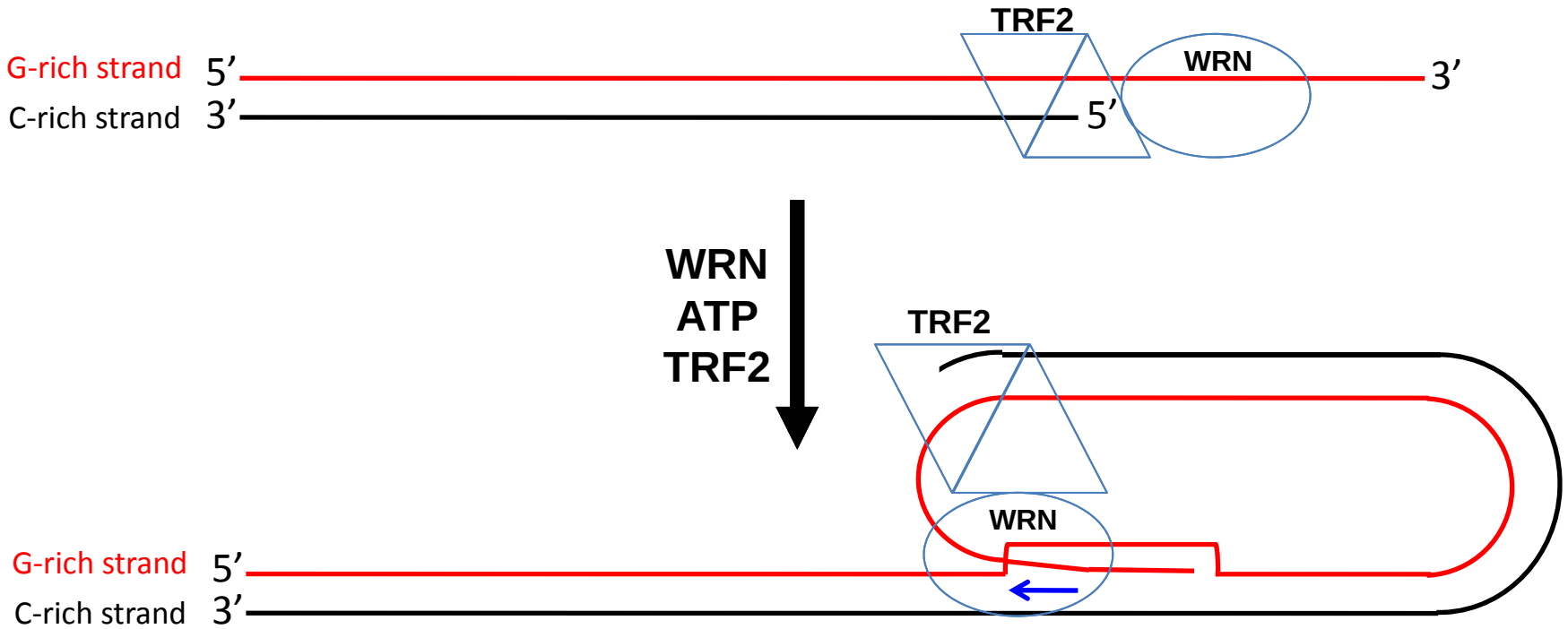


Summary of Telomeric Strand Exchange Study

Edwards et al. 2014. *Nucleic Acids Res.* **42**:7748-7761

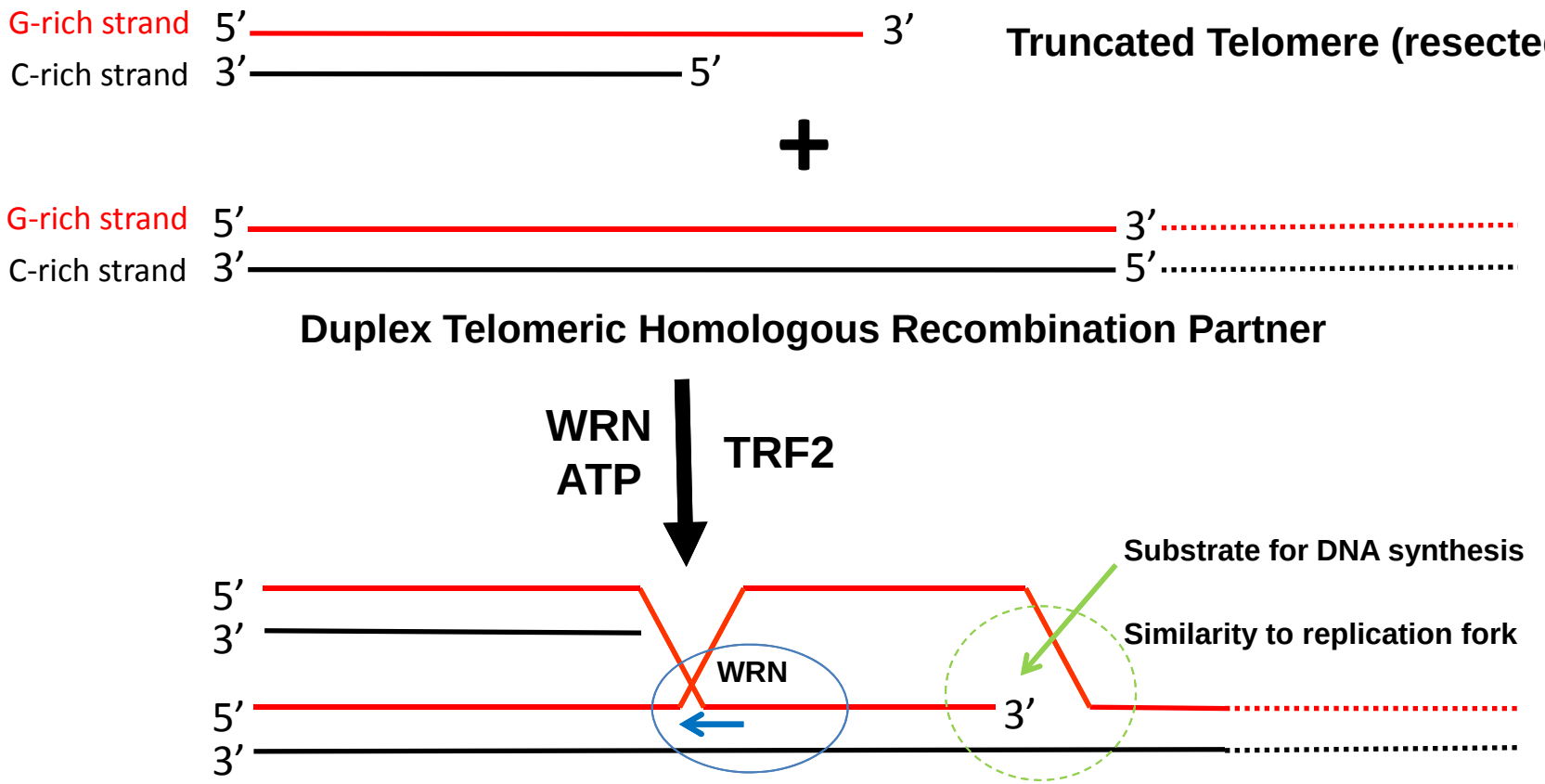
- **TRF2 stimulates WRN-mediated strand exchange specifically between telomeric DNA substrates**
  - **Dependent upon ATP hydrolysis by WRN**
  - **Dependent upon TRF2 concentration**
    - **Requires TRF2's basic domain that mediates its interaction with WRN**
  - **TRF1 has no effect, although it binds double-stranded telomeric substrates with similar affinity as TRF2**
- **WRN can displace TRF2 from telomeric DNA**
- **These findings also point to WRN function in a telomeric recombination process, probably in coordination with TRF2**
- **TRF2 recruitment of WRN to telomeres further contributes to WRN specificity for G-rich telomeric sequences by reducing competition from non-telomeric sequences**

Model 1: T-loop formation



Intramolecular strand invasion and exchange of G-rich 3’ overhang into duplex telomeric region. This mode of action is favored by WRN directionality and promoted through its interation with TRF2. Further unwinding of the duplex region promotes further strand exchange and T-loop stabilization.

Model 2: Telomeric Recombinational Repair



- **Intermolecular invasion/exchange of G-rich strand into homologous duplex**
  1. **Telomerase-independent process for telomere lengthening**
     - **G-rich strand extension followed by fill-in synthesis of C-rich strand (established mechanism)**
  2. **Re-establishment of replication fork within telomeric region**
     - **Telomere-specific break-induced replication (BIR) mechanism**

Future Directions

- **Determine whether telomeric deletions caused by WRN deficiency are exclusively linked to telomere replication**
- **Test telomeric recombination models for WRN**
- **Precisely define WRN specificity for sequences within the G-rich strand**
  - **Determine protein and exact nucleotide sequence requirements**
- **Further investigate coordination between WRN and TRF2 as well as other shelterin components or subcomplexes**

Thanks for your attention!