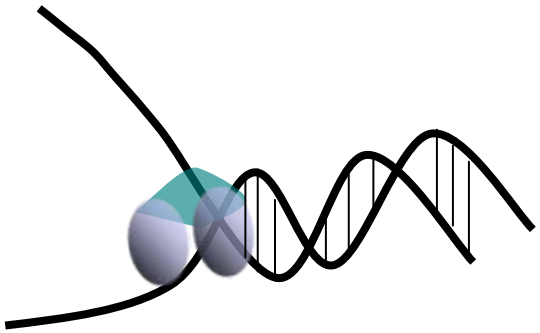
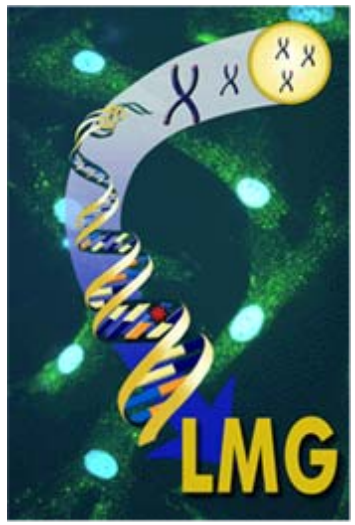
So Much to Do, So Little Time… Unraveling the Molecular Mechanisms of DNA Helicases Associated with Human Disease and Aging





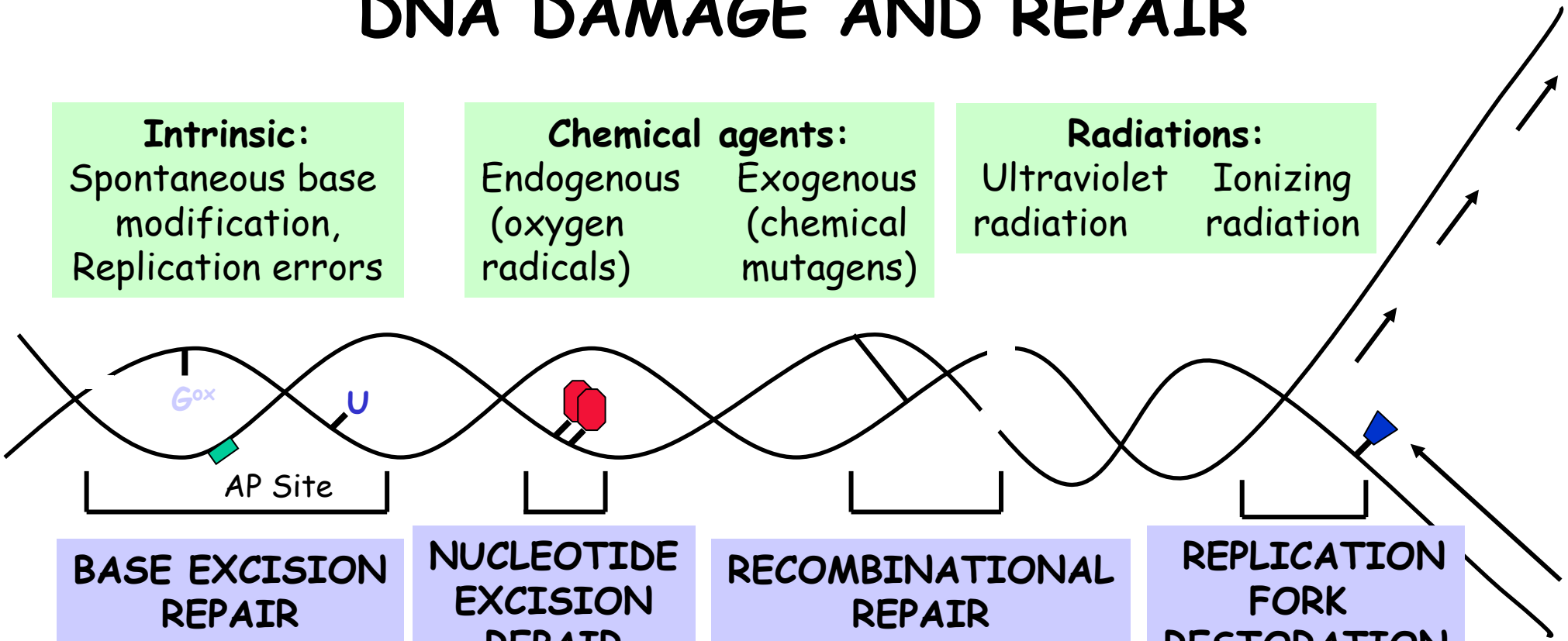
Robert M. Brosh Jr.
Section on DNA Helicases
Laboratory of Molecular Gerontology
National Institute on Aging, NIH

DNA DAMAGE AND REPAIR

Intrinsic:
Spontaneous base modification, Replication errors

Chemical agents:
Endogenous (oxygen radicals)
Exogenous (chemical mutagens)

Radiations:
Ultraviolet radiation
Ionizing radiation



BASE EXCISION REPAIR

NUCLEOTIDE EXCISION REPAIR

RECOMBINATIONAL REPAIR

REPLICATION FORK RESTORATION

Cell survival

Genomic instability
Mutation
Chromosomal aberrations

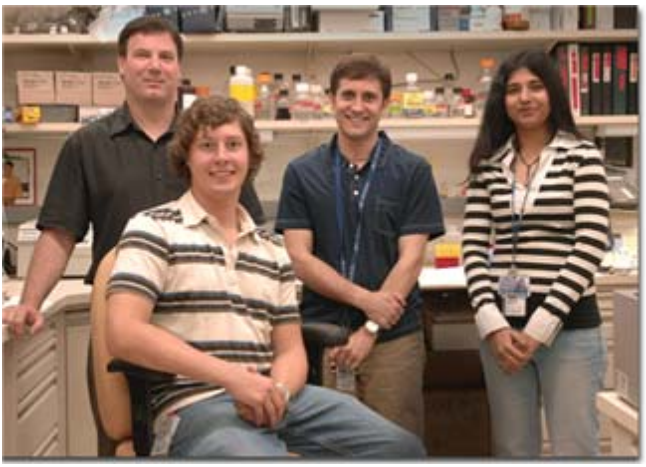
Cell death

Aging

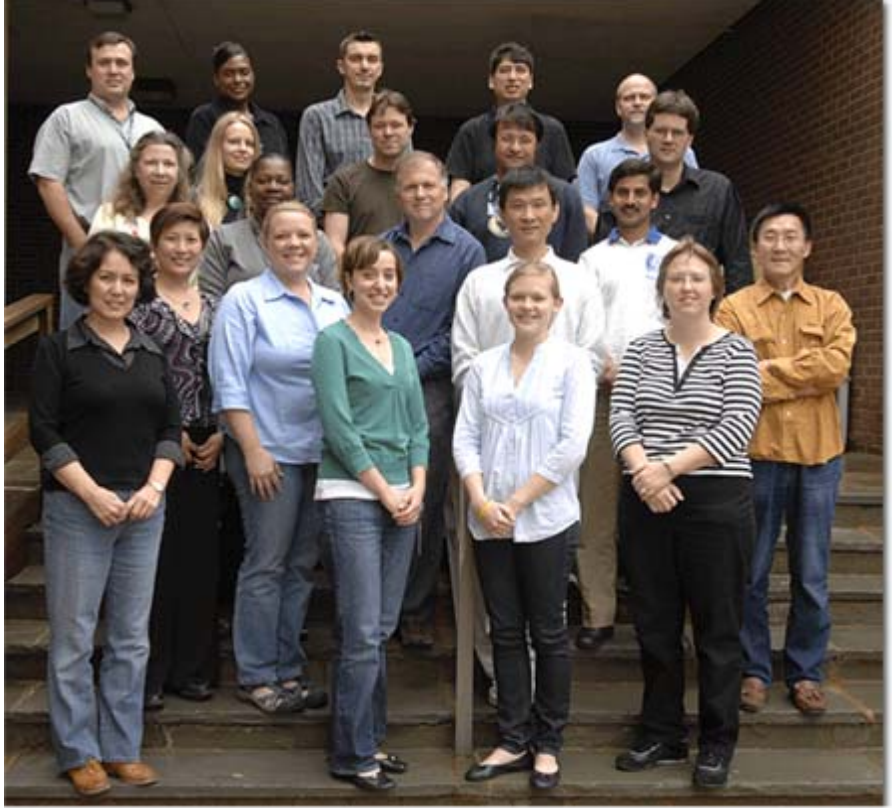
Cancer

Disease

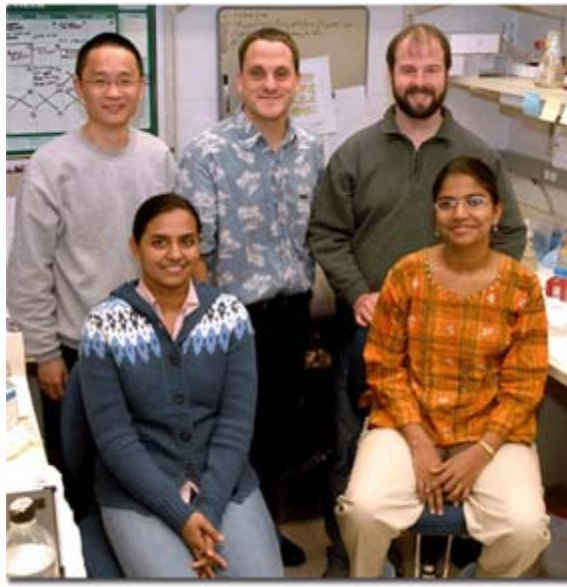
LABORATORY OF MOLECULAR GERONTOLOGY



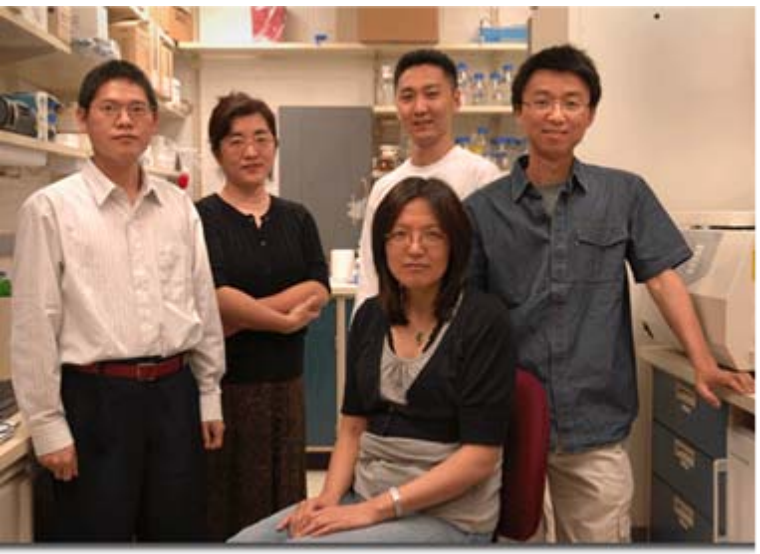
Section on Base Excision Repair



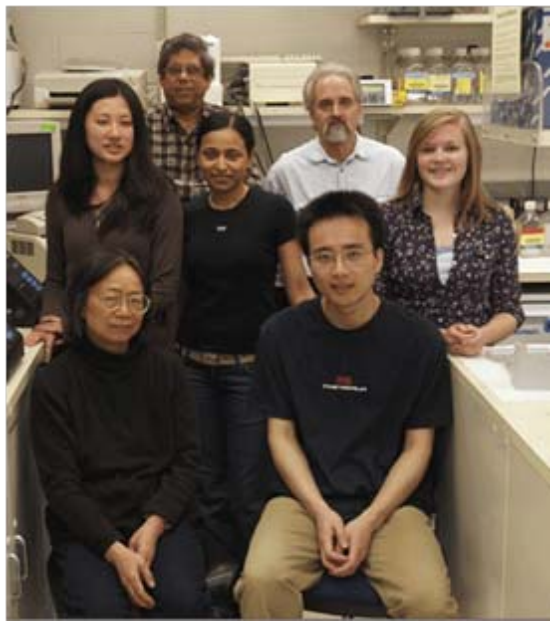
Section on DNA Repair



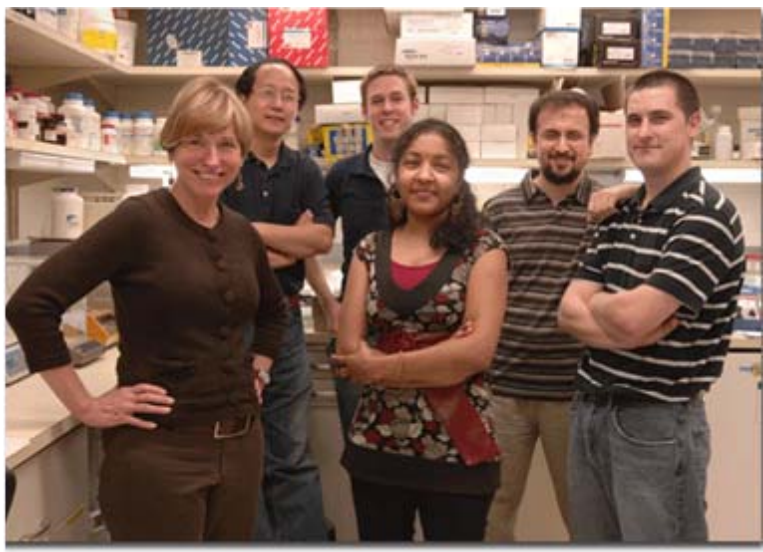
Section on DNA Helicases



Unit on Telomeres



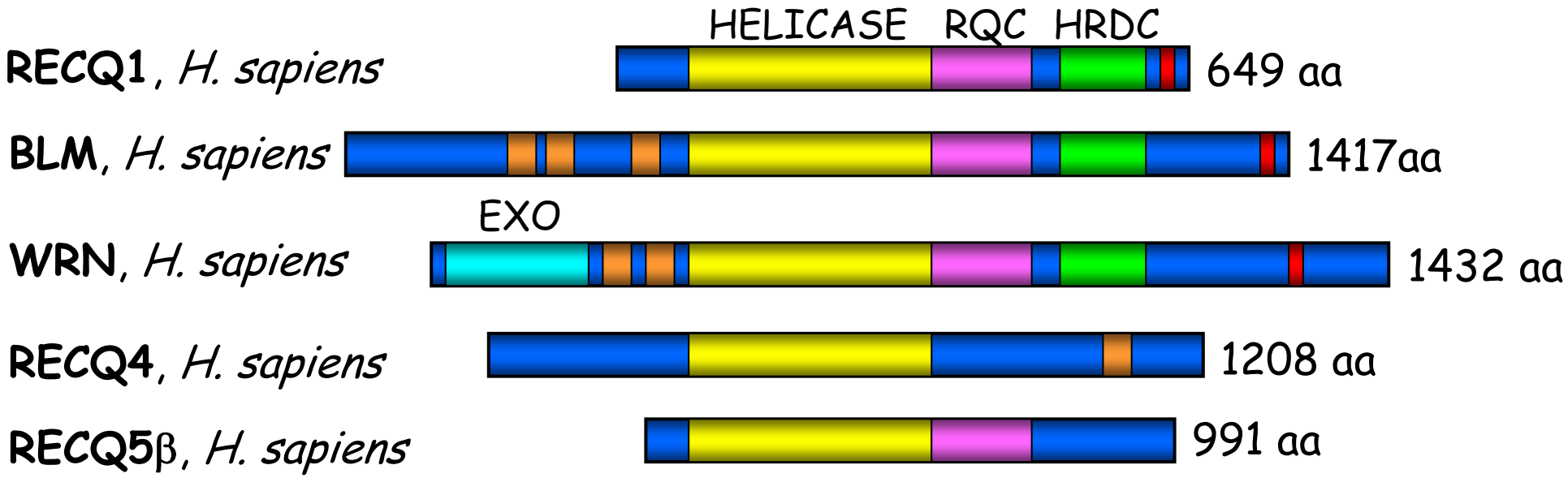
Section on Gene Targeting



Section on Antibody Diversity

NIH Biomedical Research Center

Question: Why are there unique clinical and cellular phenotypes in the RecQ helicase disorders?

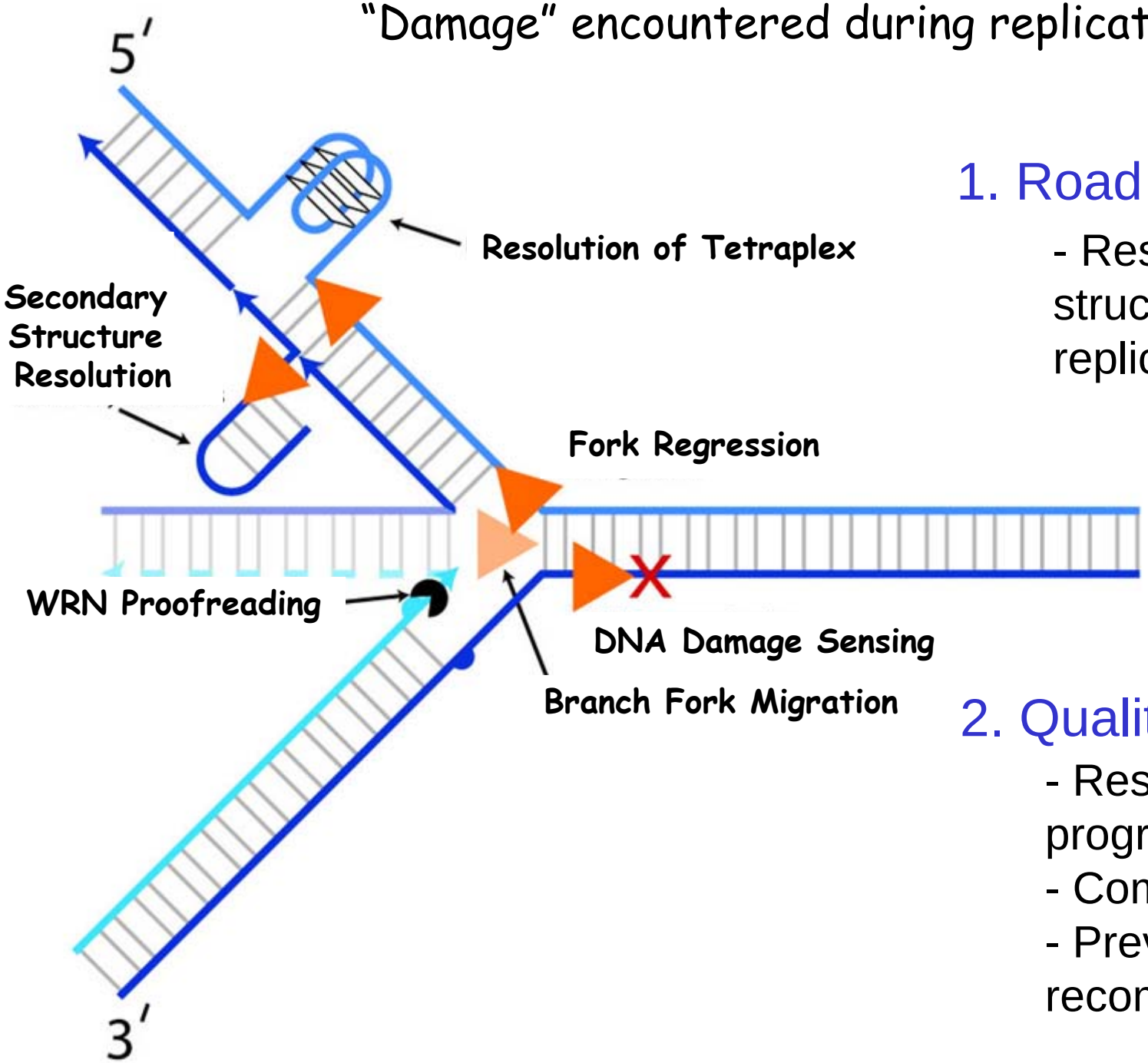


Objective: To delineate and characterize unique roles of RecQ helicases in genomic stability maintenance

Today:
1. RECQ1 cellular phenotypes
2. Model system to study WRN genetics
3. Novel functions of FANCJ helicase

Roles for RecQ Helicases in DNA Metabolism

"Damage" encountered during replication of the genome



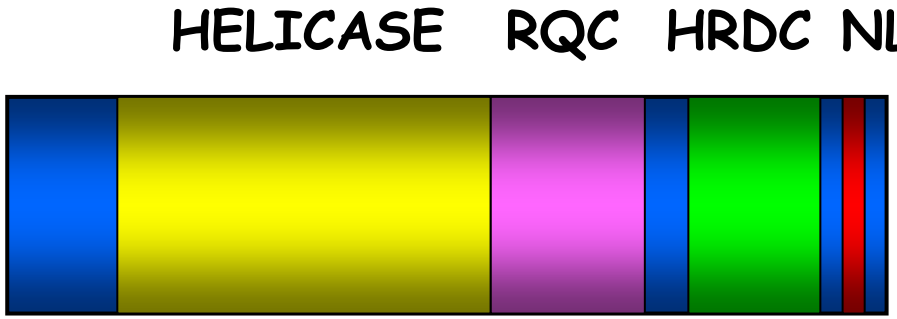
1. Road Block Remover

- Resolve alternate DNA structures for proper replication progression

2. Quality Control

- Restore replication fork progression
- Complete proper repair
- Prevent inappropriate recombination

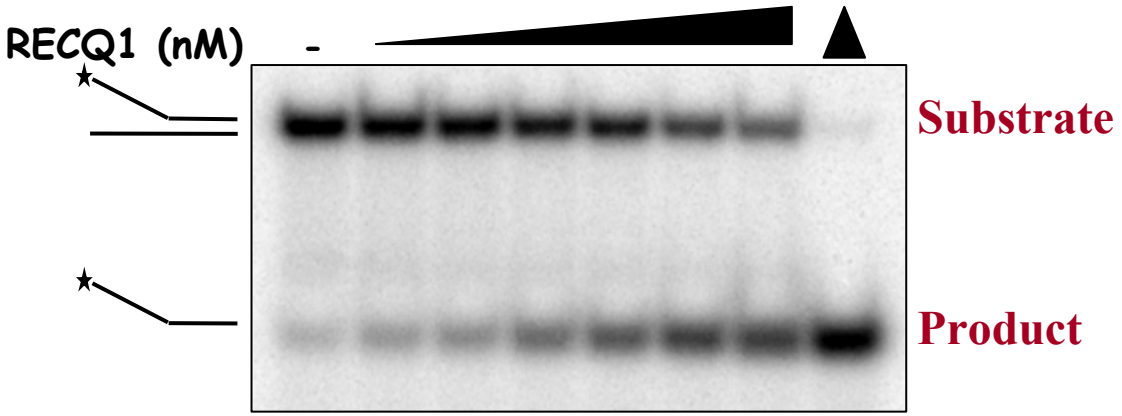
RECQ1, *H. sapiens*



- First RecQ helicase discovered in the early 1970's by Blackshear and Okumura Labs
- Smallest RecQ helicase
- Most abundant human RecQ helicase
- Not genetically linked to a human disease
- No organismal phenotypes for RECQ1 KO mouse detected by Blackshear Lab, NIEHS

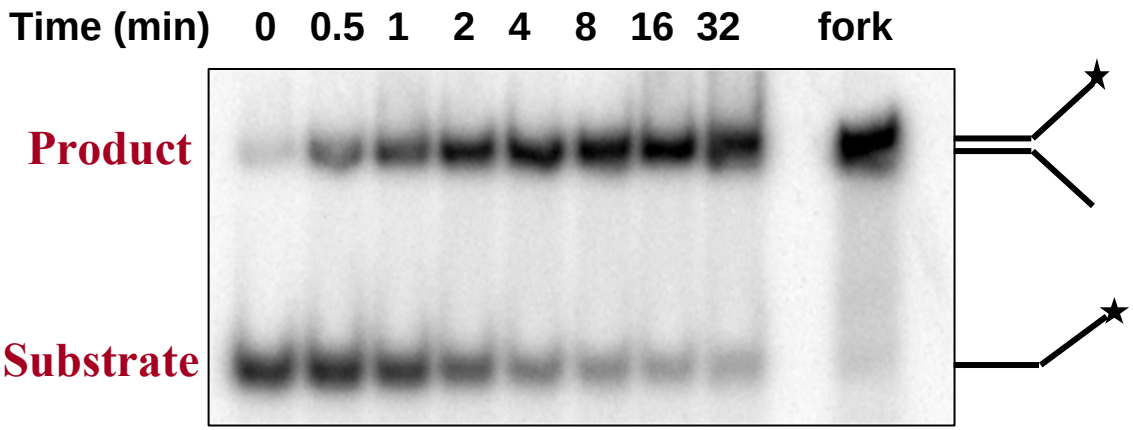
Biochemical Activities of Recombinant Human RECQ1

Helicase Activity



- Unwinds synthetic replication forks and Holliday Junctions
- Melts D-loop recombination intermediate

Strand Annealing Activity



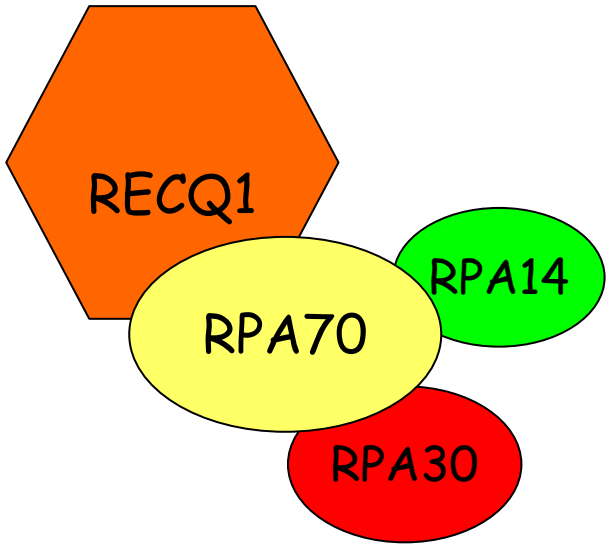
- Modulated by ATP binding due to conformational change in RECQ1

Sharma et al., J. Biol.Chem., 2005

What DNA metabolic pathways does RECQ1 participate in?

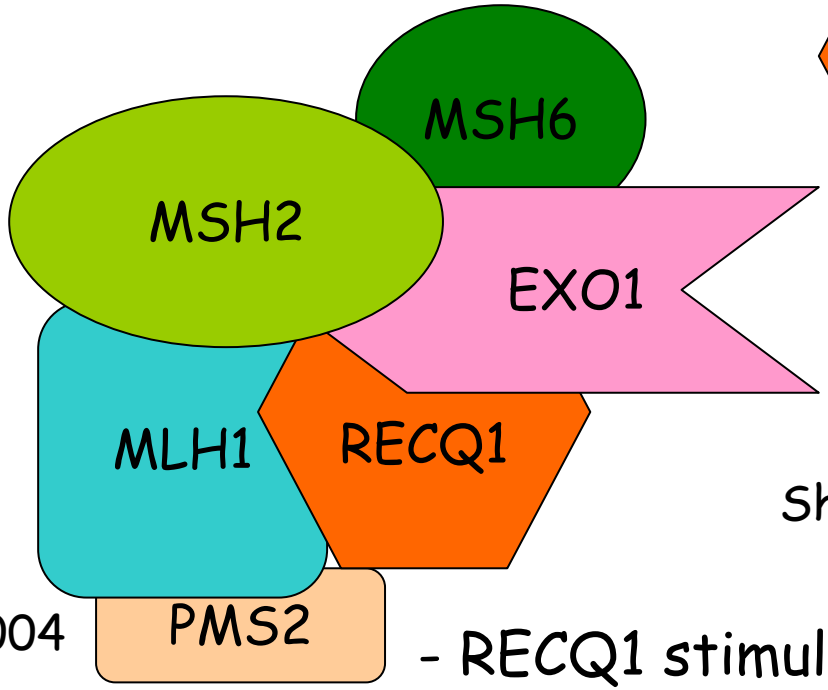
- Identify RECQ1 interacting partners and characterize their functional interactions

RECQ1 helicase interacts with DNA repair factors that regulate genetic recombination



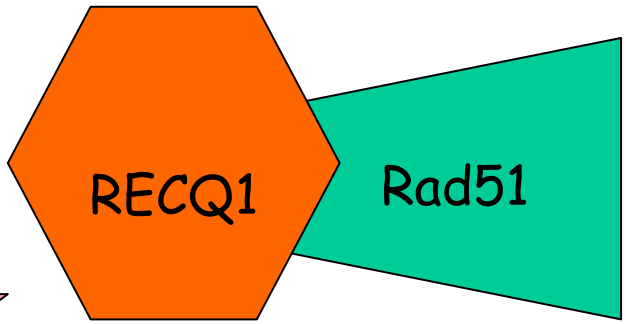
RPA stimulates RECQ1 helicase

Cui et al., Nucl. Acid Res. 2004



- RECQ1 stimulates EXO-1 incision
- MSH2/6 stimulates RECQ1 helicase

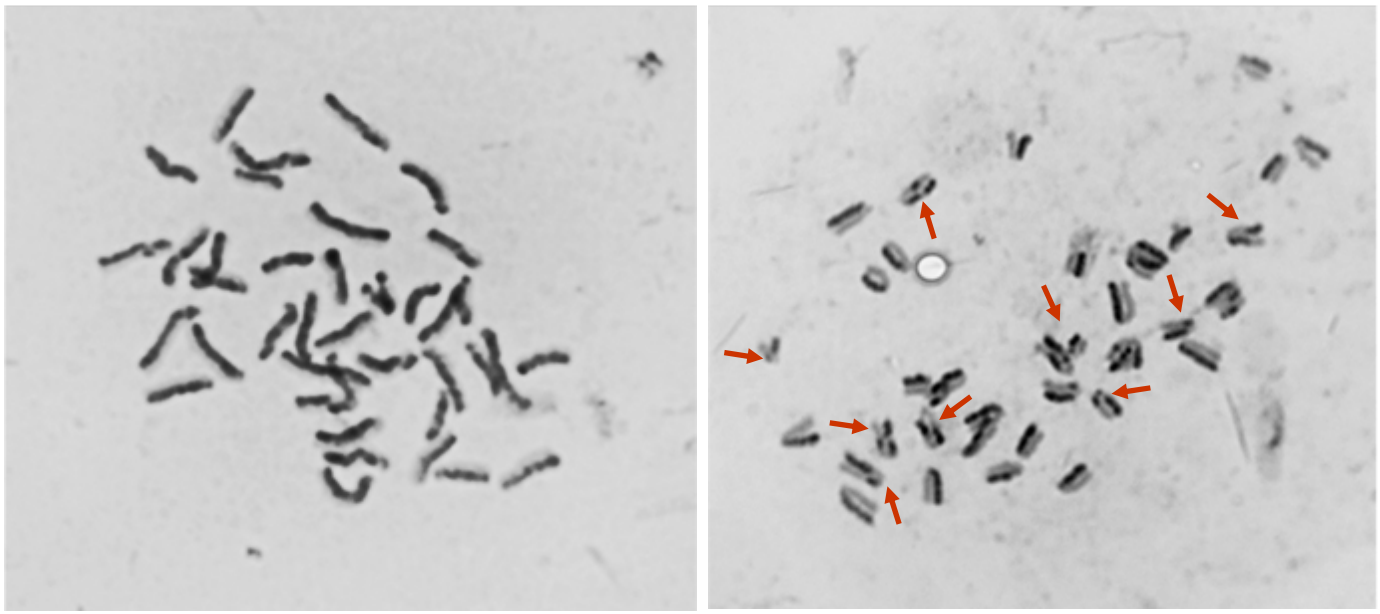
Doherty et al., J.Biol.Chem. 2005



RECQ1-Rad51 found in MutSα / MLH1-PMS2 complex

Sharma et al., PLOS One 2007

RECQ1 Suppresses Sister Chromatid Exchanges



Cell line	metaphases scored	No. of SCE/metaphase
Wild type MEF	20	2.71 ± 0.932
RECQ1 knockout MEF	25	12.32 ± 1.416

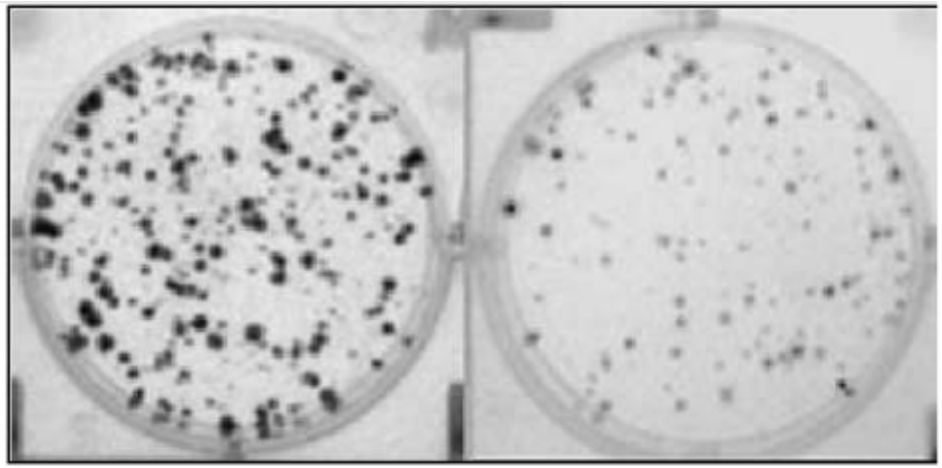
Sudha Sharma

Sharma et al., MCB, 2007

Spontaneously elevated γH2AX and Rad51 foci in RECQ1 knockout MEFs

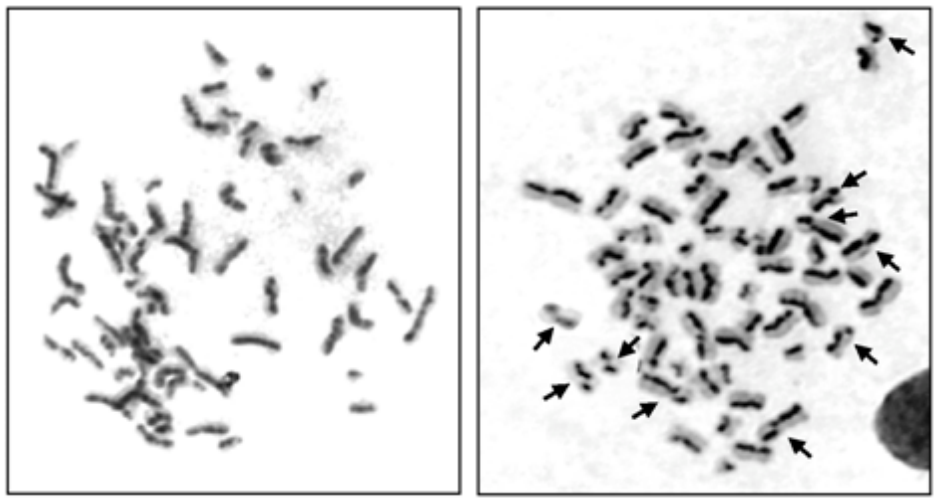
Reduced cell growth and elevated sister chromatid exchange in RECQ1-depleted cells

Colony forming assay



Control shRNA RECQ1 shRNA

BrdU labeled metaphase chromosome spreads

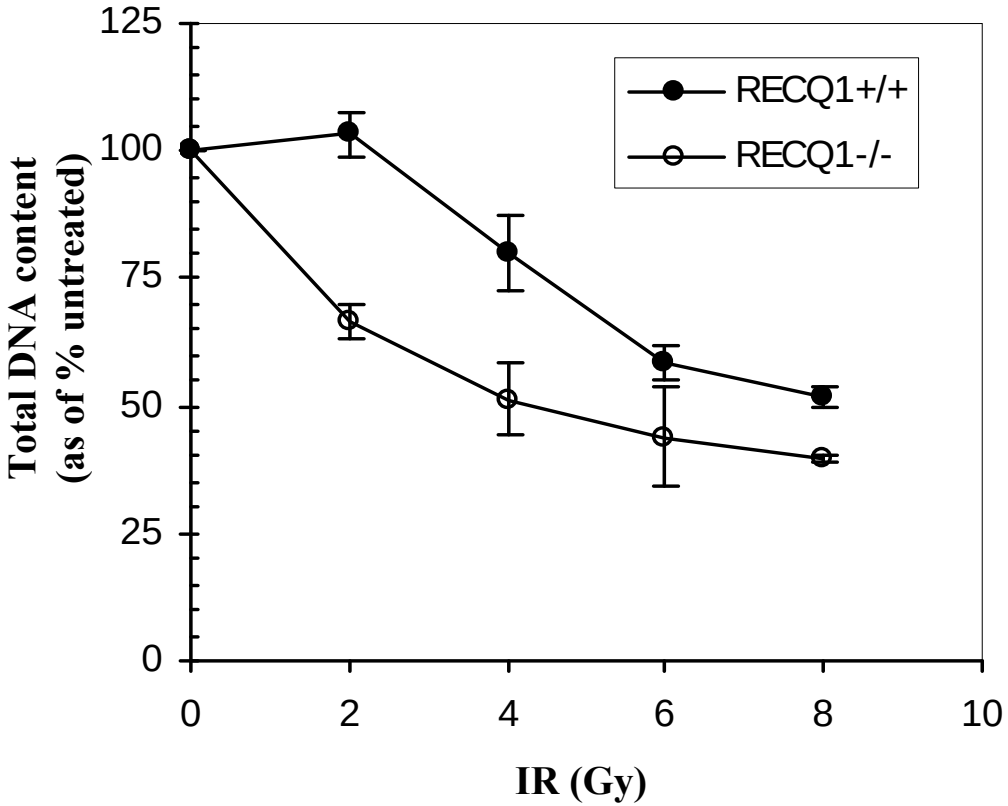


Control siRNA RECQ1 siRNA

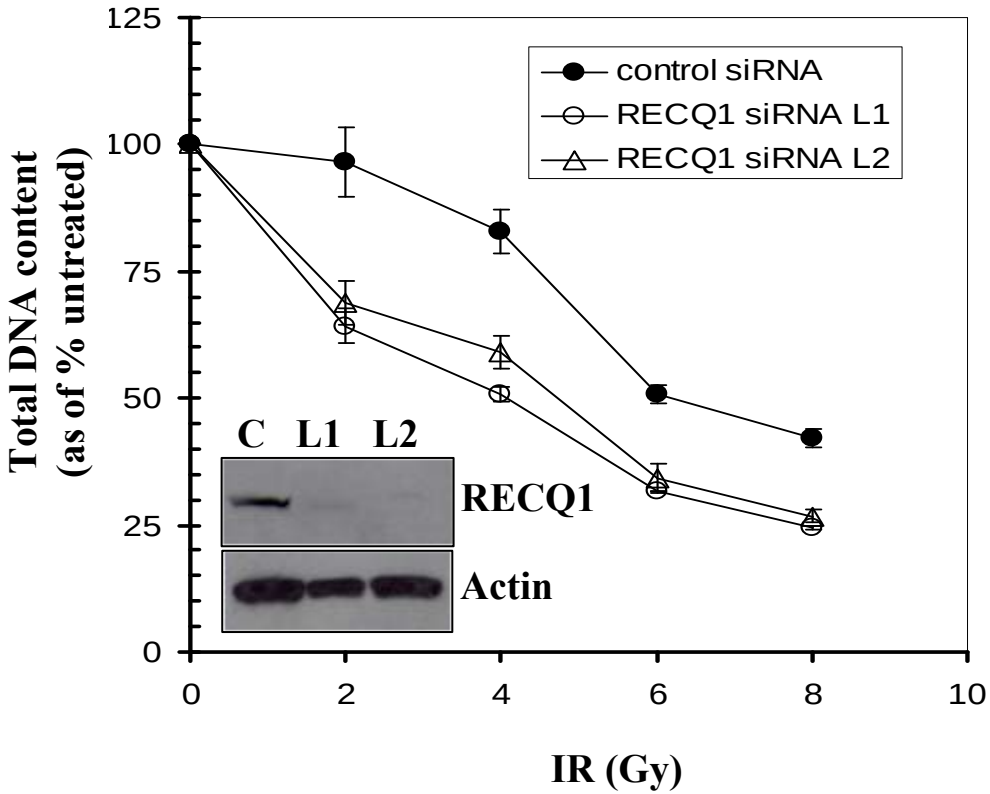
Sharma et al.,
PLOS One, 2007

Cellular Deficiency of RECQ1 Leads to Increased IR Sensitivity

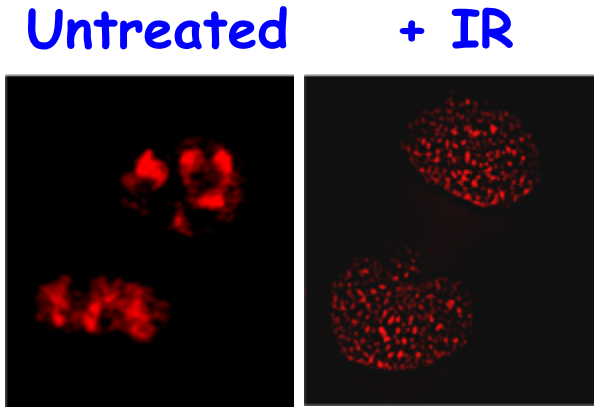
primary MEFs of RECQ1 knockout mice are sensitive to IR



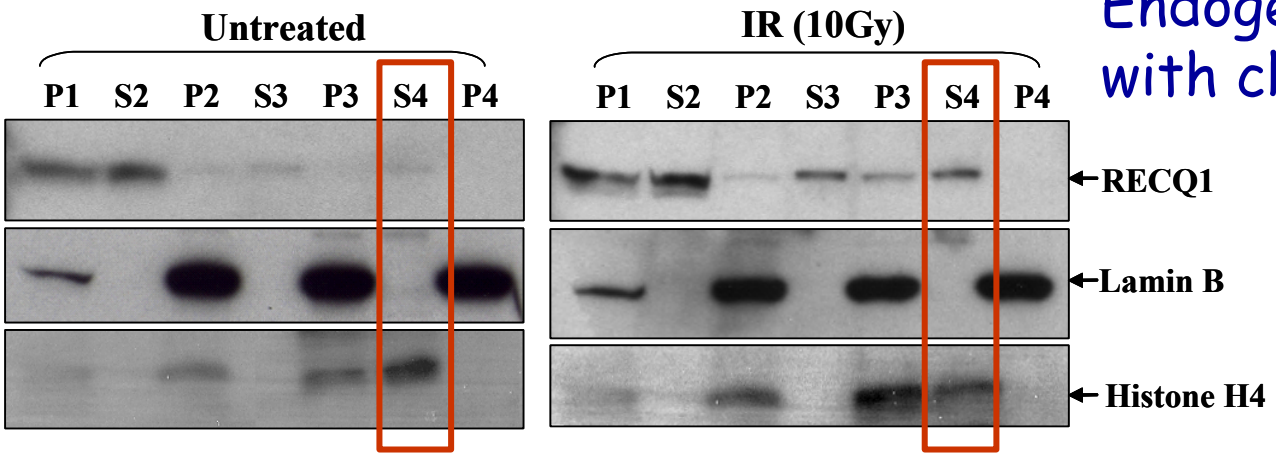
siRNA knockdown of RECQ1 leads to increased IR sensitivity in HeLa cells



Does RECQ1 respond to DNA damage?

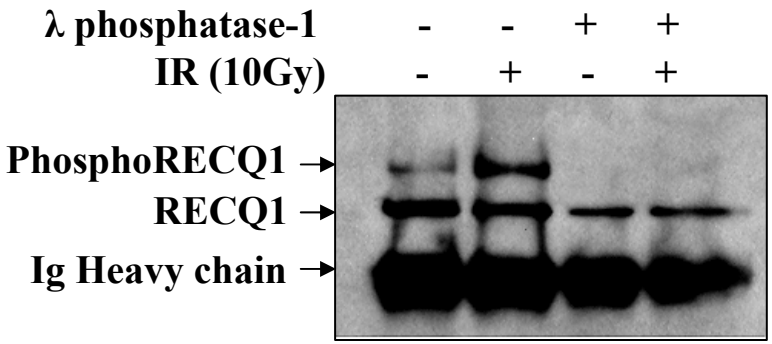


RECQ1 is a nucleolar protein and relocates to form chromatin bound foci upon DNA damage

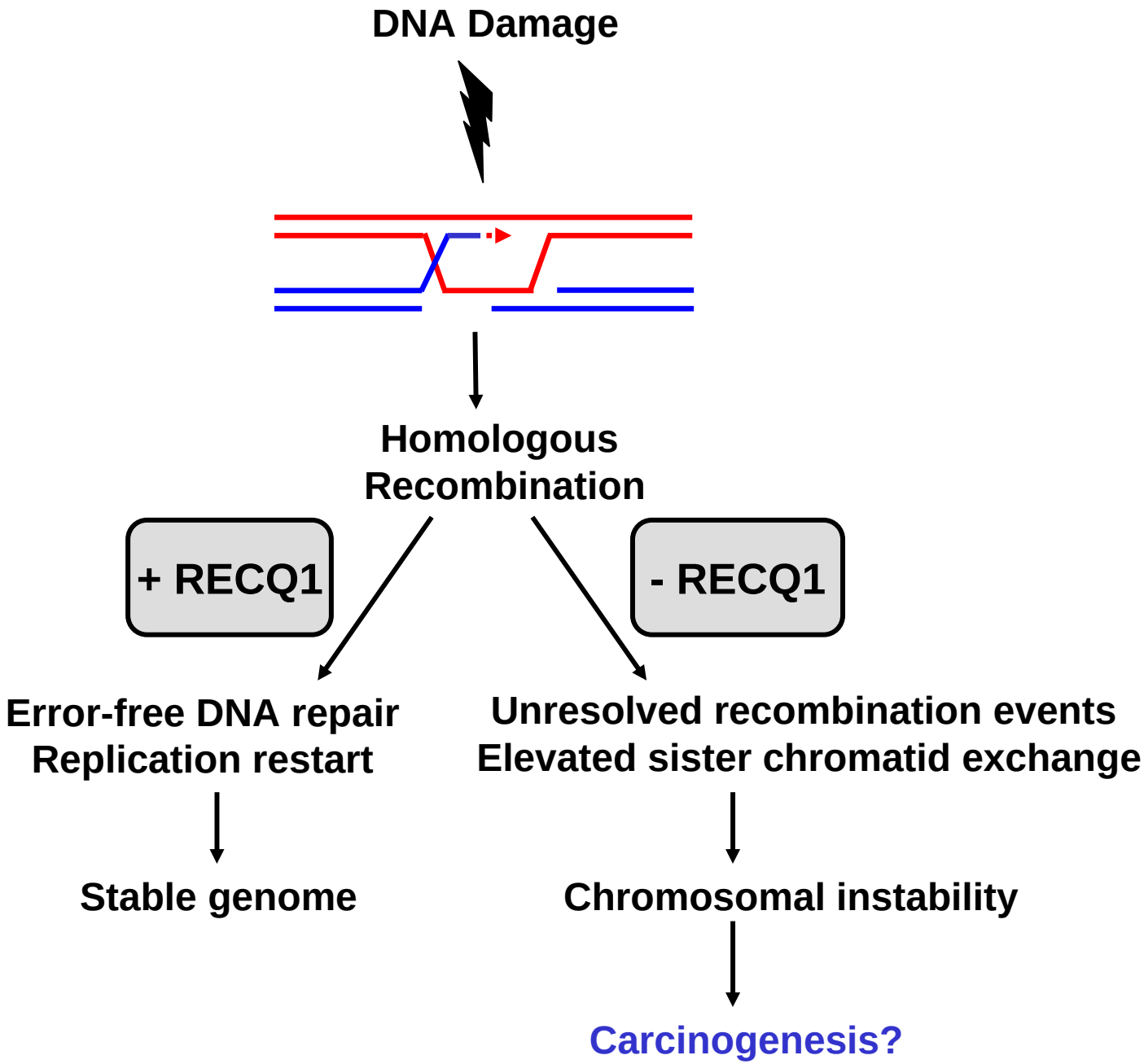


Endogenous RECQ1 associates with chromatin in response to IR

Who phosphorylates RECQ1, and is this important?



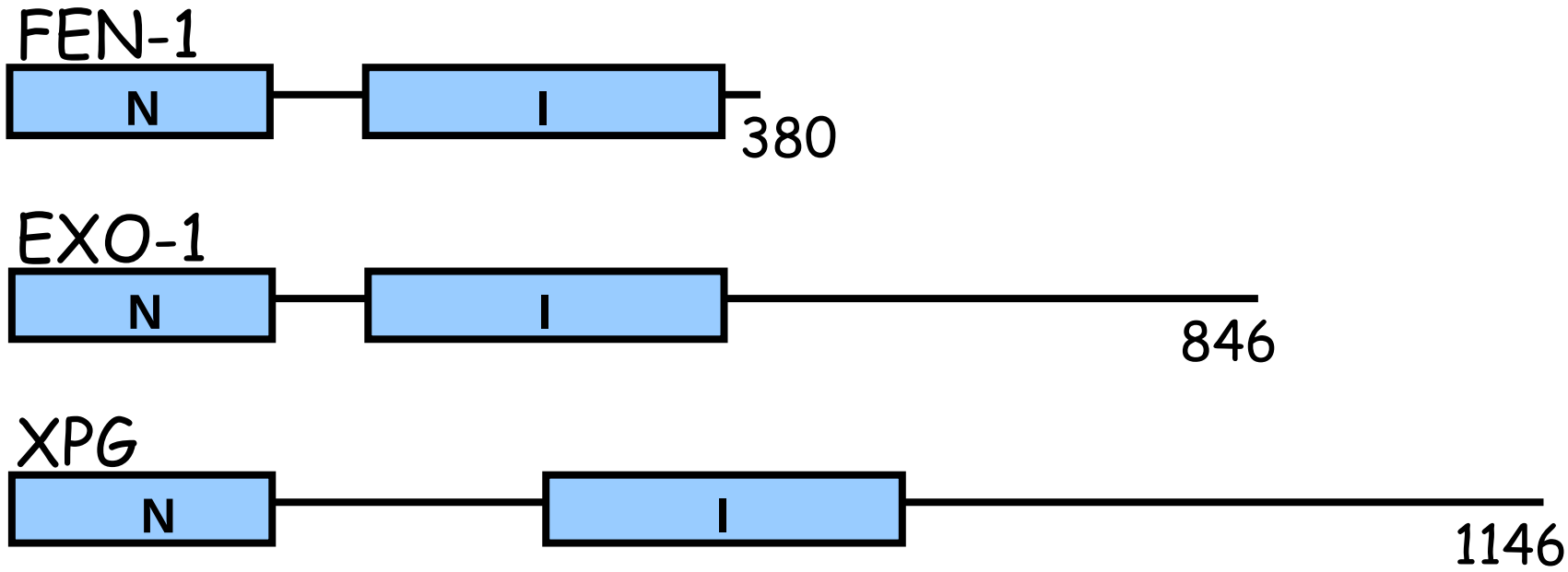
RECQ1 is phosphorylated in response to IR



RECQ1 preserves genomic integrity through its role in homologous recombinational repair

We are interested in the importance of protein interactions between RecQ helicases and structure specific nucleases.

Human Rad2 Structure-Specific Nucleases



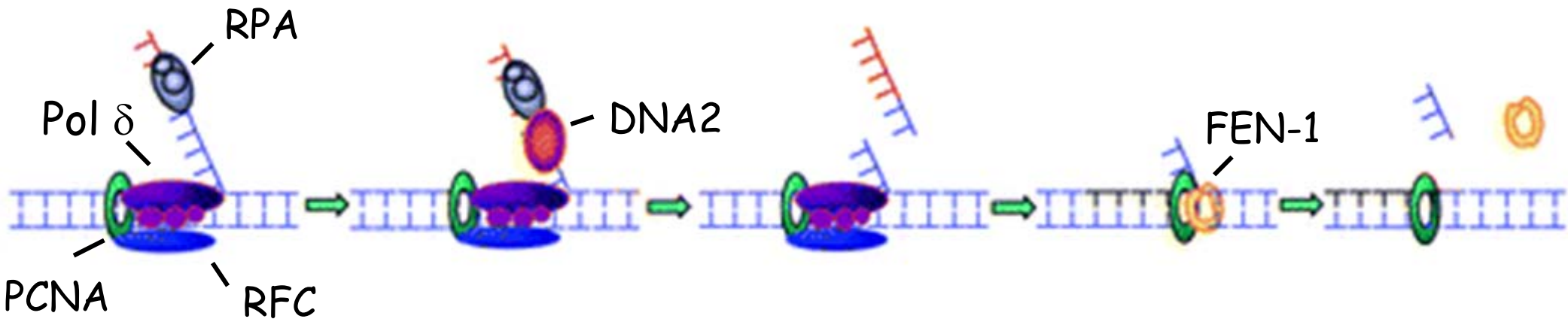
WRN and BLM helicases interact with human FEN-1 and stimulate FEN-1 nucleolytic activities.

WRN and RECQ1 helicases interact with human EXO-1 and stimulate EXO-1 nucleolytic activities.

How are these interactions important in vivo? Perhaps under conditions of replicational stress.

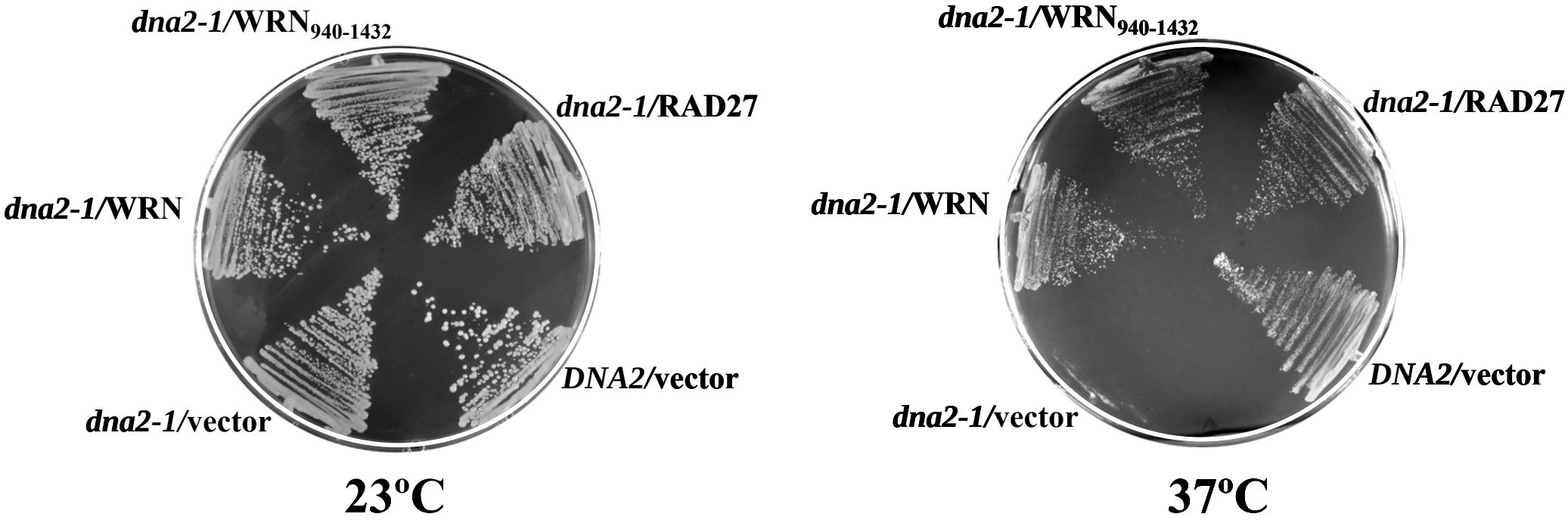
Hypothesis: WRN stimulates FEN-1 cleavage *in vivo* to rescue the DNA replication and repair phenotypes of the *dna2* replication mutant

Okazaki fragment processing model



- FEN-1 over-expression rescues *dna2* mutant phenotypes
- Functionally conserved roles of human and yeast FEN-1 in DNA replication and repair

WRN Rescues Replication Defects of *dna2*

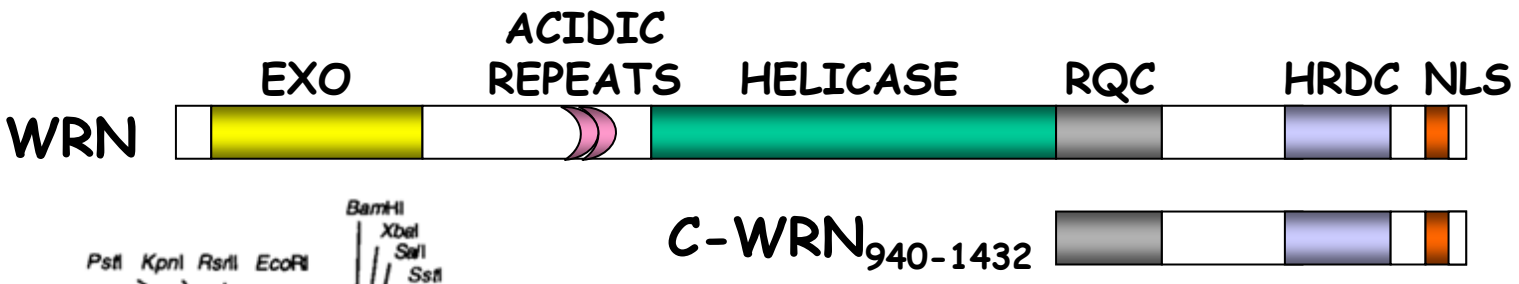


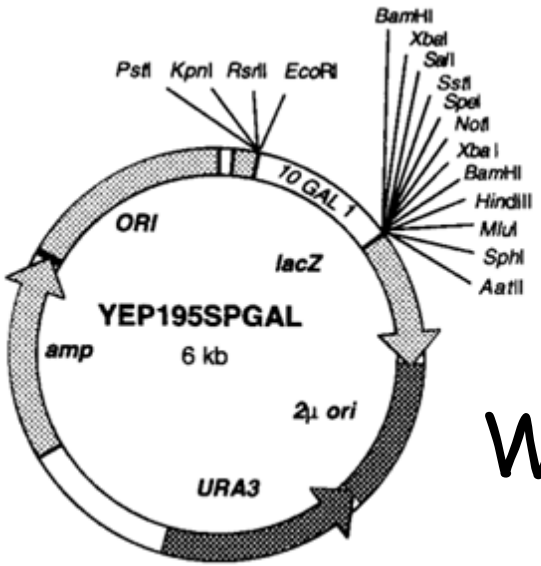
- Complementation regulated by level of WRN expression
- FEN-1 interaction domain of WRN sufficient for rescue
- WRN rescues cell cycle progression defect
- WRN rescues sensitivity to replication inhibitor HU or DNA damaging agent MMS

Sharma et al., 2004 Human Mol. Genet.

Does the *WRN: EXO1* interaction play a role in the replication stress response?

We chose to use yeast as a model system to answer this question since it was previously shown that yeast and human EXO1 are functional homologs.



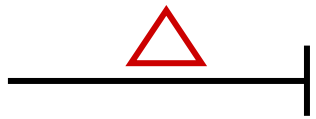


WRN expression construct

Monika Aggarwal

Yeast *rad50* mutant strain

Xrs2 Complex
(Rad50-Mre11-Xrs2)



Pleiotropic effects

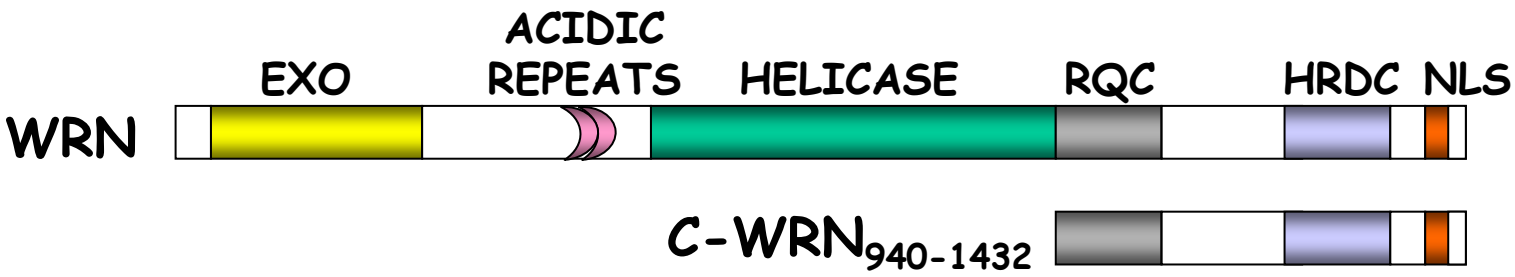
- ➢DNA repair deficiency
- ➢Hyper-recombination
- ➢Telomere shortening
- ➢Intra-S phase Checkpoint defect
- ➢DNA damage sensitivity

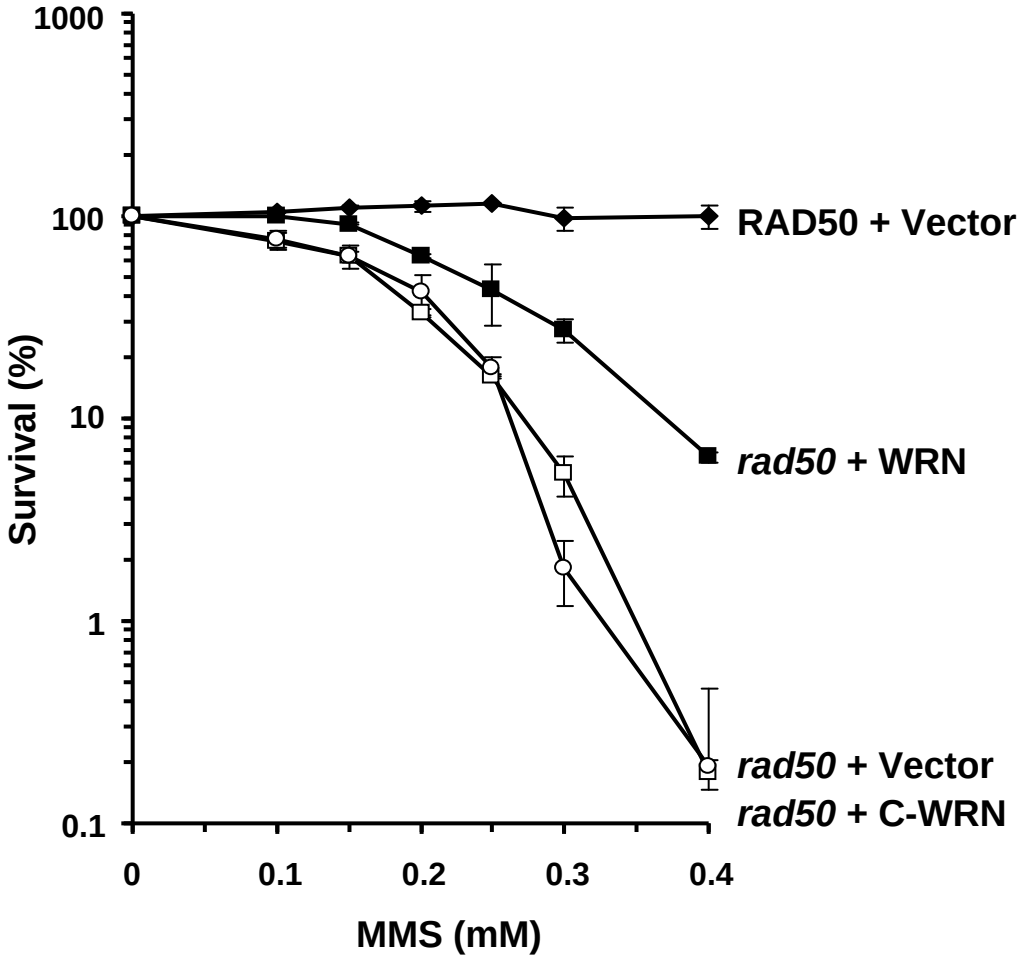
- ✓ Nonhomologous end-joining
- ✓ Homologous recombination

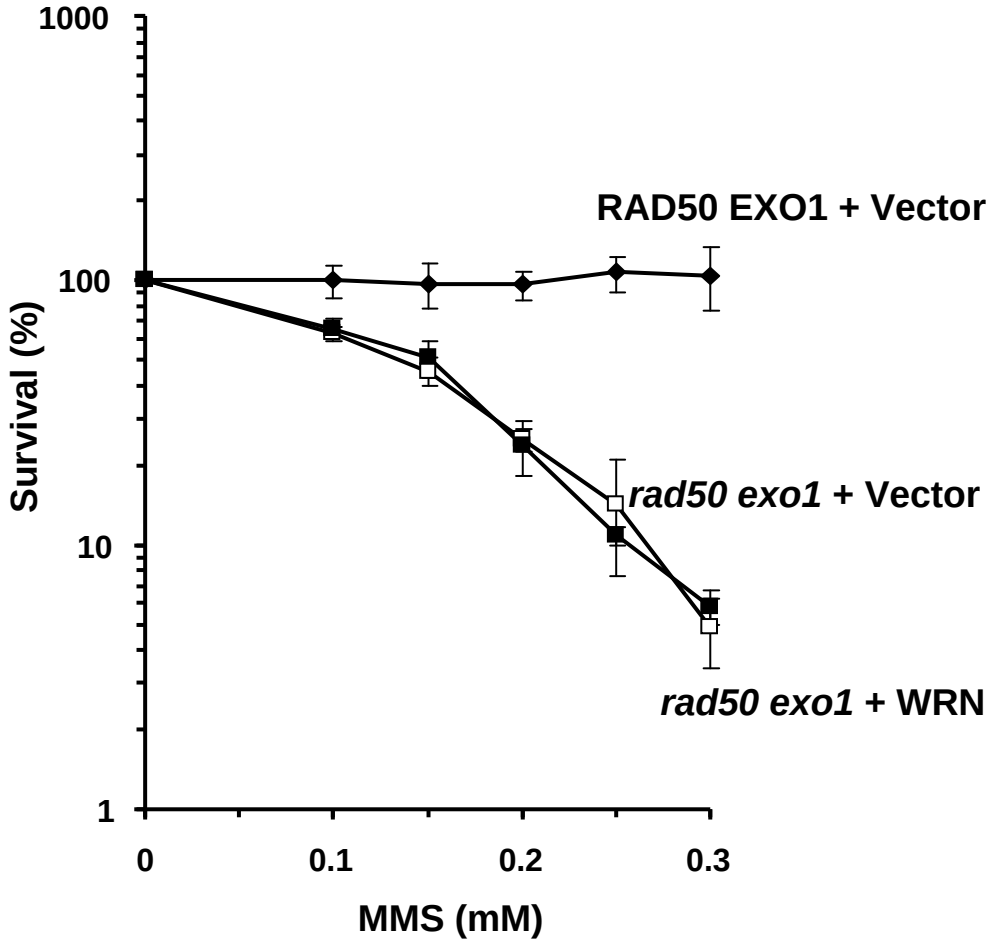
Overexpression of *Exo1* (5'-3' exonuclease) rescues MMS and IR sensitive phenotypes of Rad50-Mre11-Xrs2 complex mutants, and this is dependent on EXO-1 nuclease activity.

Tsubouchi, H and Ogawa, H. *Mol. Biol. Cell* 2000; 11:2221-33.
Moreau, S. et al., *Genetics* 2001; 159:1423-33.
Lewis, KE et al., Genetics. 2002;160(1):49- 62.
Lewis KE et al., Genetics 2004; 166(4):1701-13.

Test WRN for *rad50* rescue



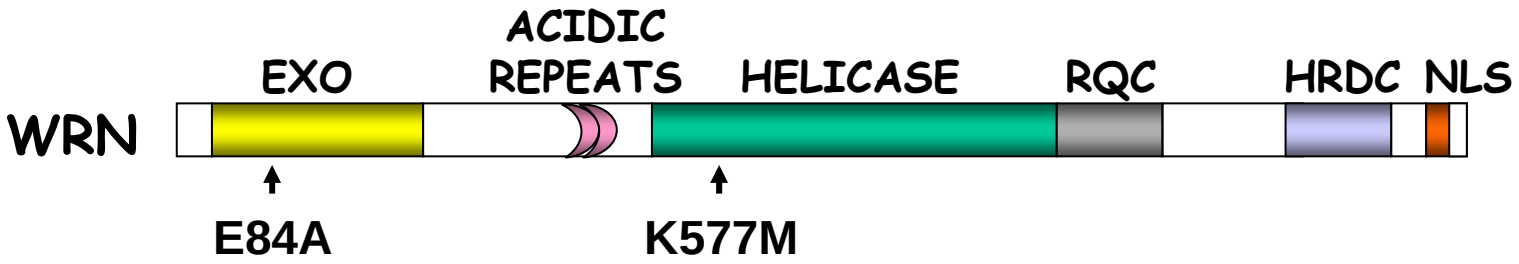


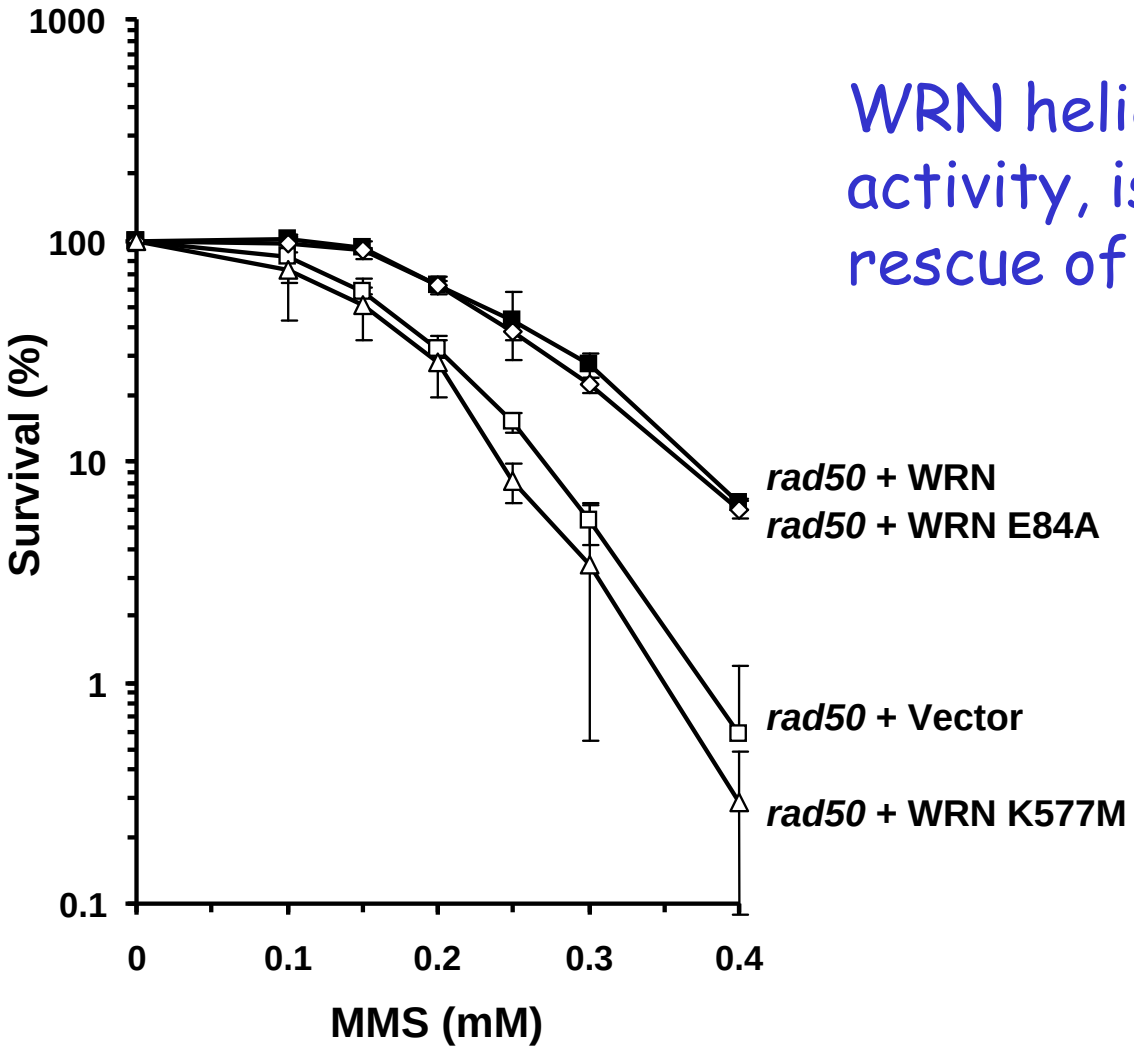


Expression of full-length WRN rescues *rad50* MMS sensitivity

WRN rescue is EXO1-dependent

Test WRN catalytic domain mutants for *rad50* rescue

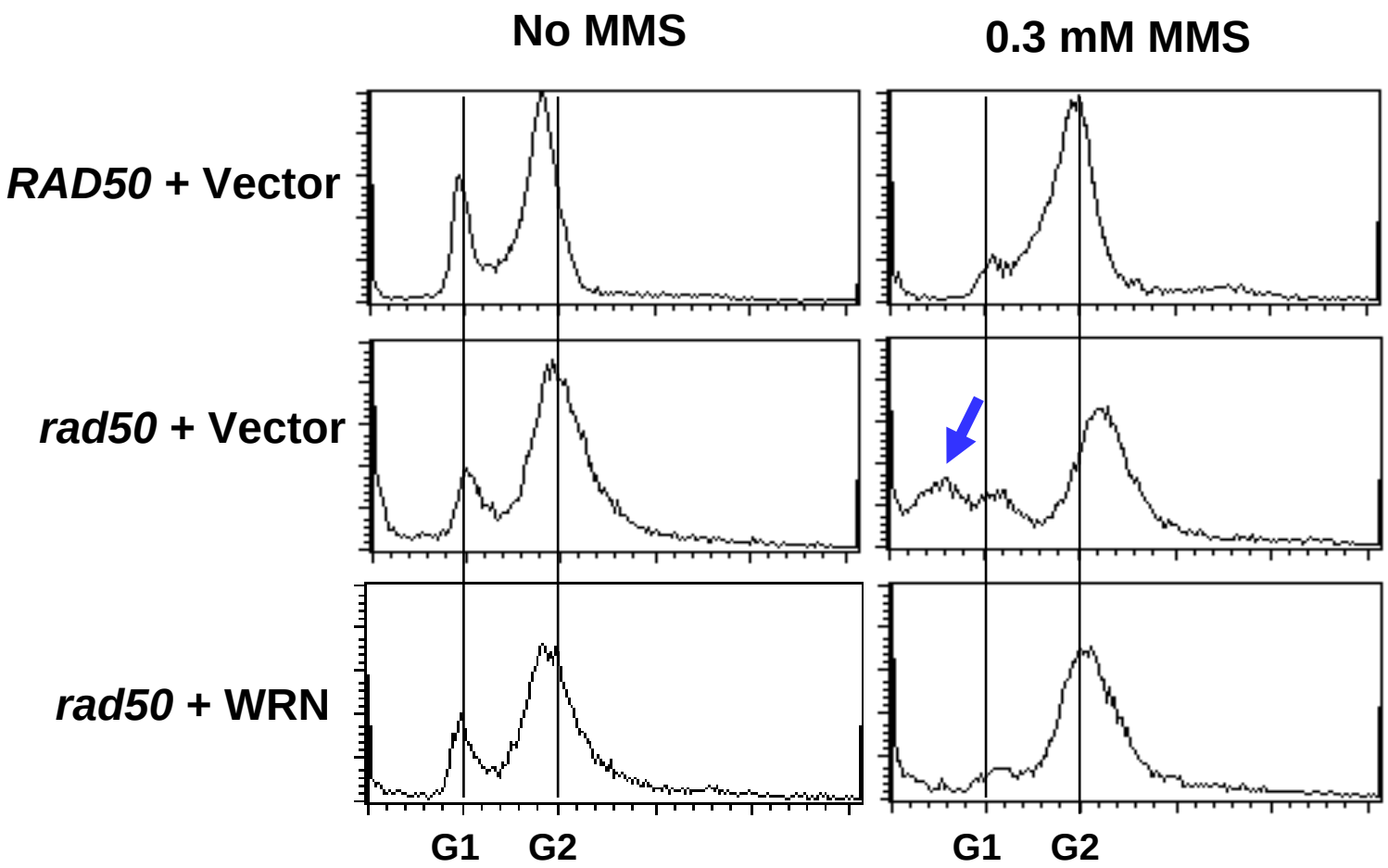




WRN helicase, but not exonuclease activity, is required for genetic rescue of *rad50* MMS sensitivity.

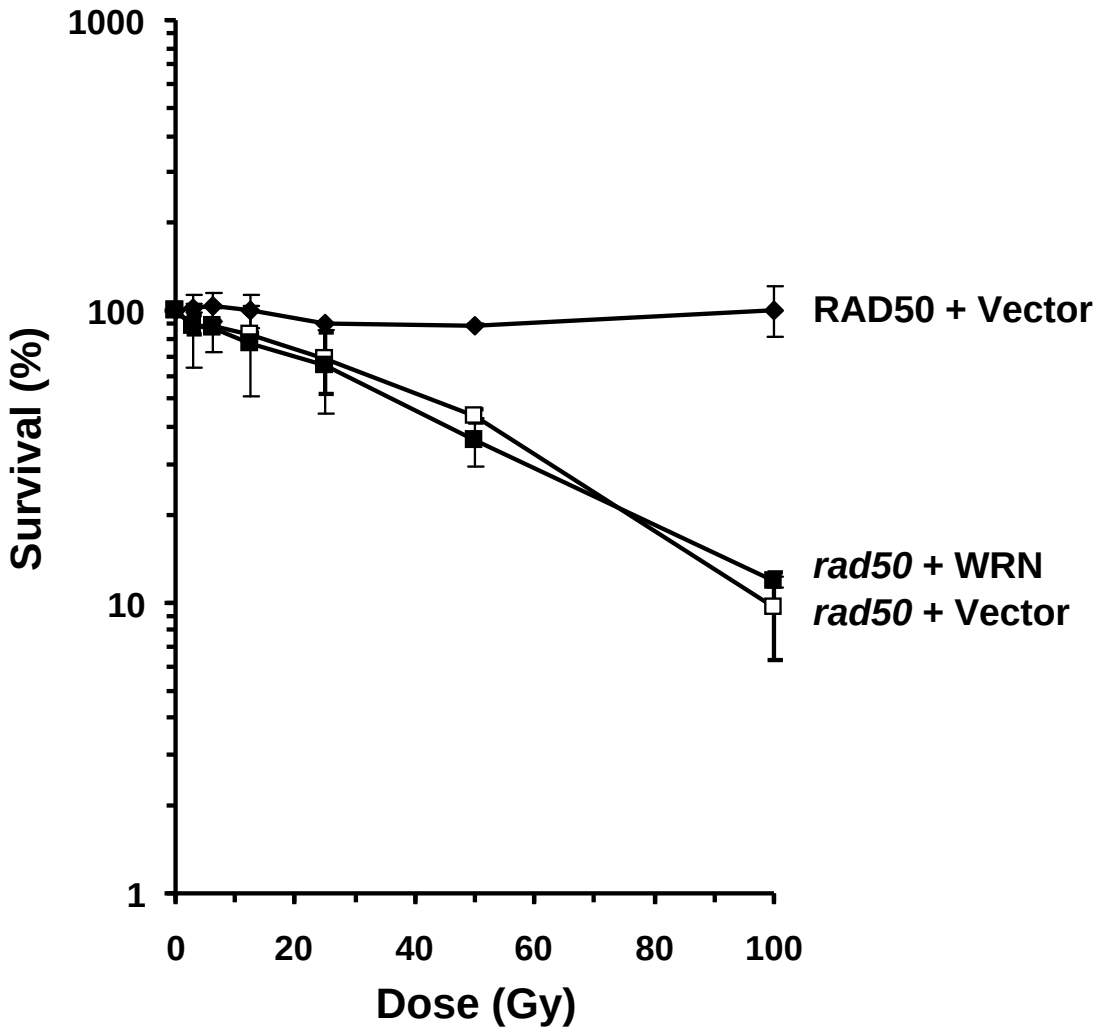
Can WRN prevent mitotic catastrophe in *rad50* mutant?

FACS Analysis



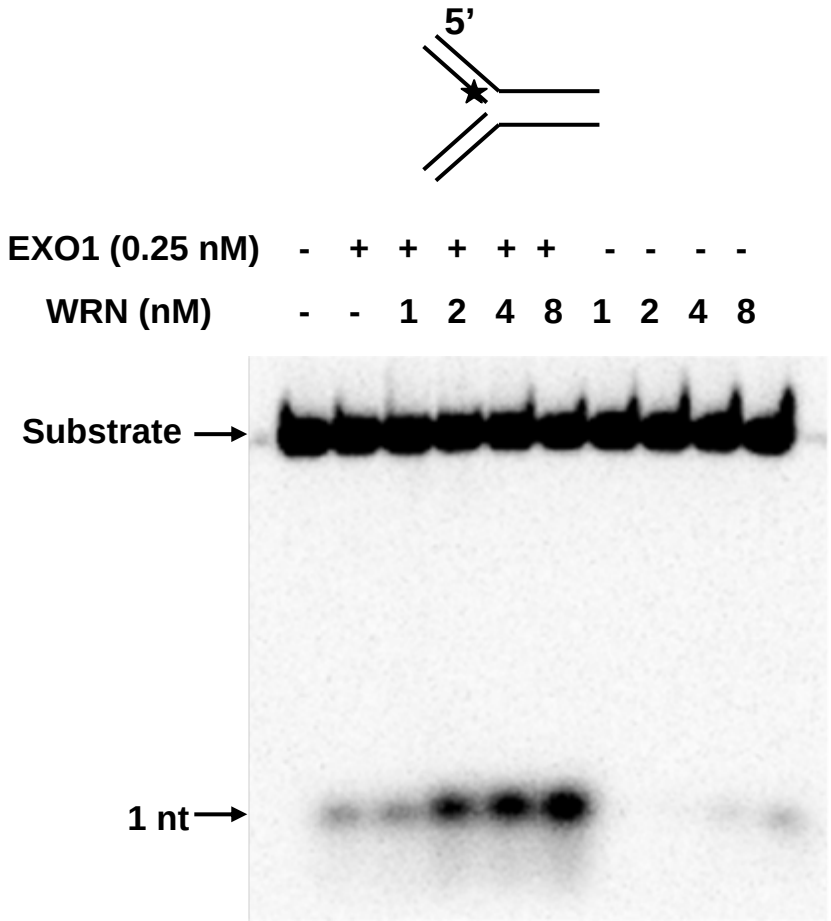
WRN expression prevents accumulation of sub-G1

Can WRN expression rescue IR sensitivity of *rad50*?



WRN expression does not rescue IR sensitivity of *rad50*, suggesting that WRN rescue is specific to agents like MMS that stall replication forks.

Can WRN stimulate EXO1 to process a stalled replication fork to counteract fork reversal?



Mol Cell. 2005 Jan 7;17(1):153-9.

Exo1 processes stalled replication forks and counteracts fork reversal in checkpoint-defective cells.

Cotta-Ramusino C, Fachinetti D, Lucca C, Doksani Y, Lopes M, Sogo J, Foiani, M.

WRN stimulates the exonuclease activity of EXO1 on replication fork lagging strand, suggesting mechanism to prevent fork regression.

Summary

- WRN rescues *rad50* MMS sensitivity in EXO-1 dependent manner

- WRN does not rescue *rad50* IR sensitivity, suggesting WRN:EXO1 interaction is important for response to agents that induce replicational stress, not direct strand breaks

- WRN rescue of *rad50* MMS sensitivity requires helicase, but not exonuclease activity

- WRN prevents MMS-induced mitotic catastrophe in *rad50* mutant

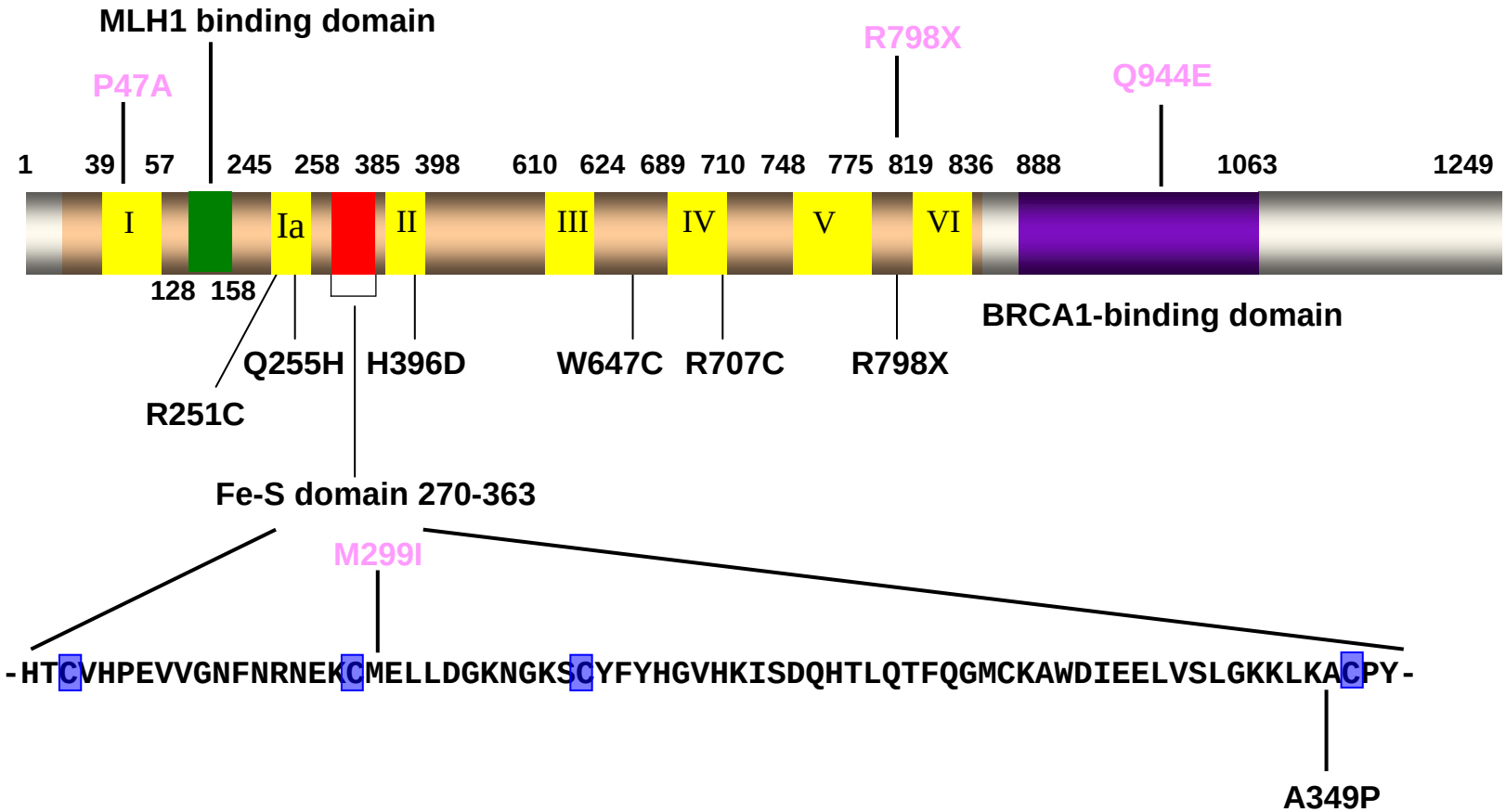
- WRN stimulates EXO1 to process replication fork structures in a manner that would counteract fork reversal

Understanding the Consequences of Helicase Dysfunction for Age-related Disease, Cancer, and Genomic Instability

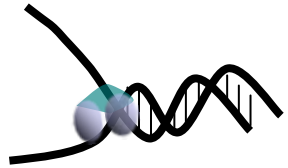
SF2 Helicase	Disease / Abnormality
WRN	Werner syndrome
BLM	Bloom syndrome
RECQ4	Rothmund-Thomson syndrome
RECQ1	?
RECQ5	?
FANCJ	Fanconi anemia, Breast cancer

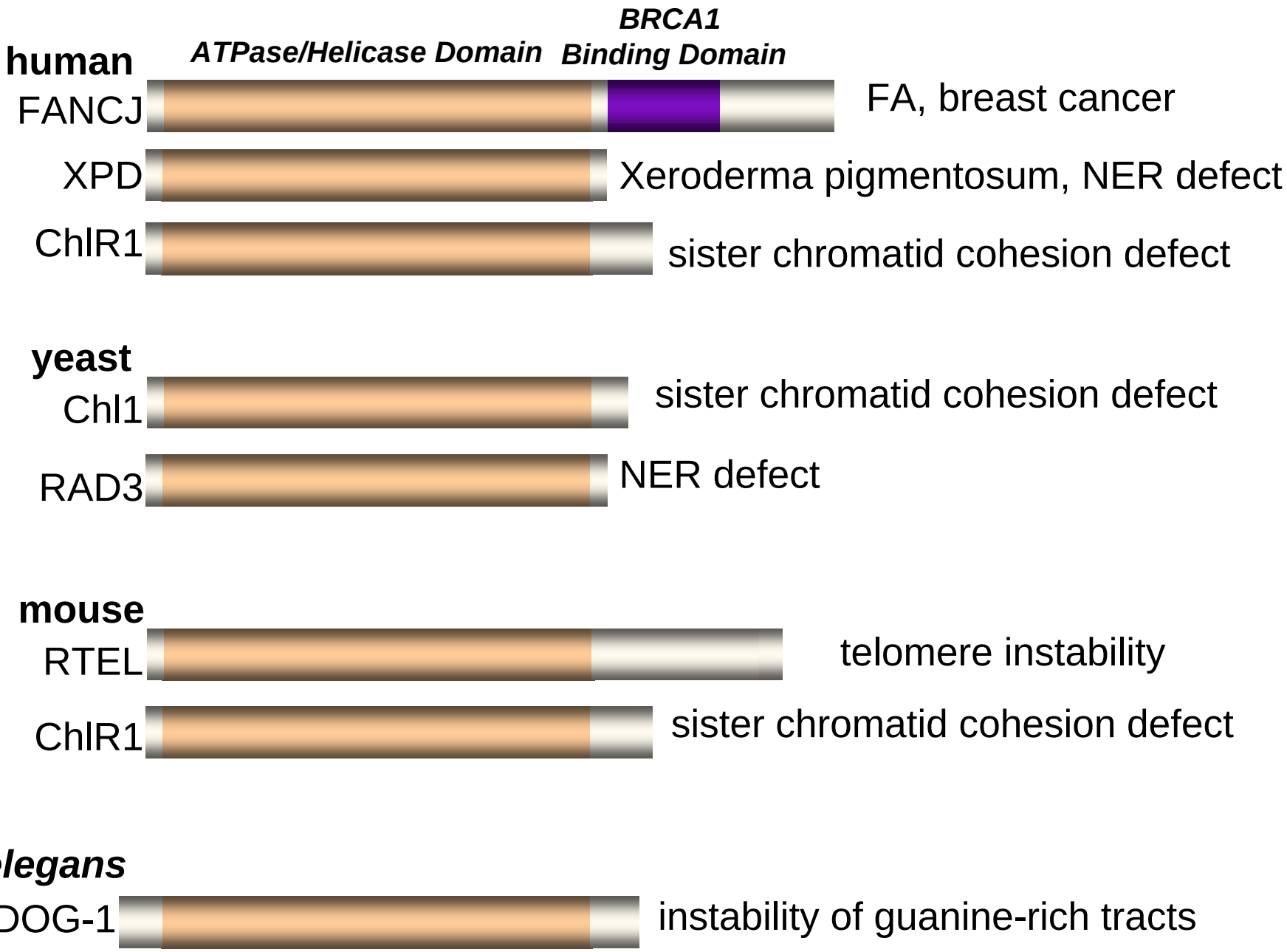
Common pathways promote chromosomal rearrangements in different genome instability syndromes

Distribution of *FANCJ* mutations and breast cancer associated sequence changes in FANCJ protein

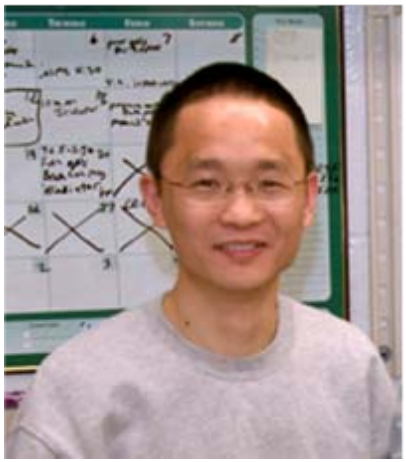


FANCJ Helicase Family

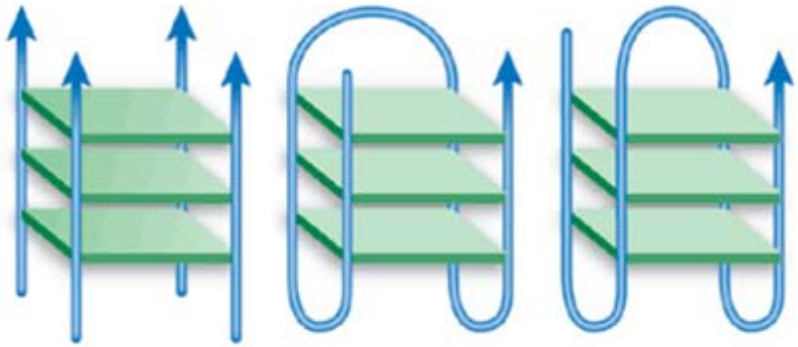


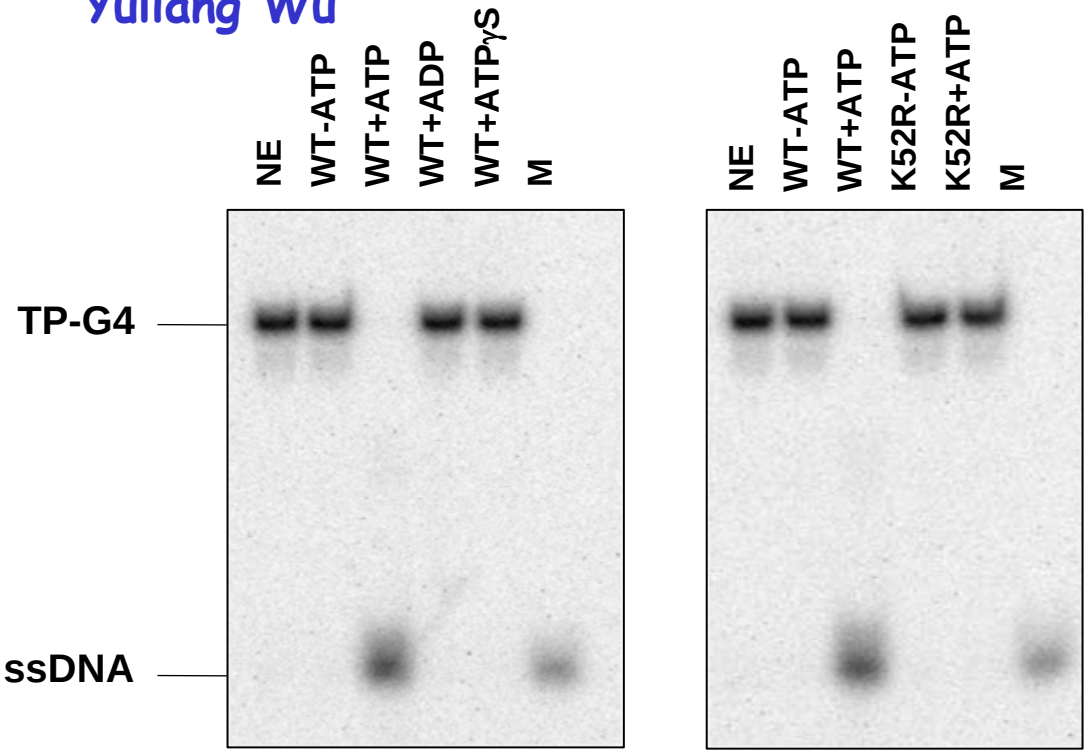


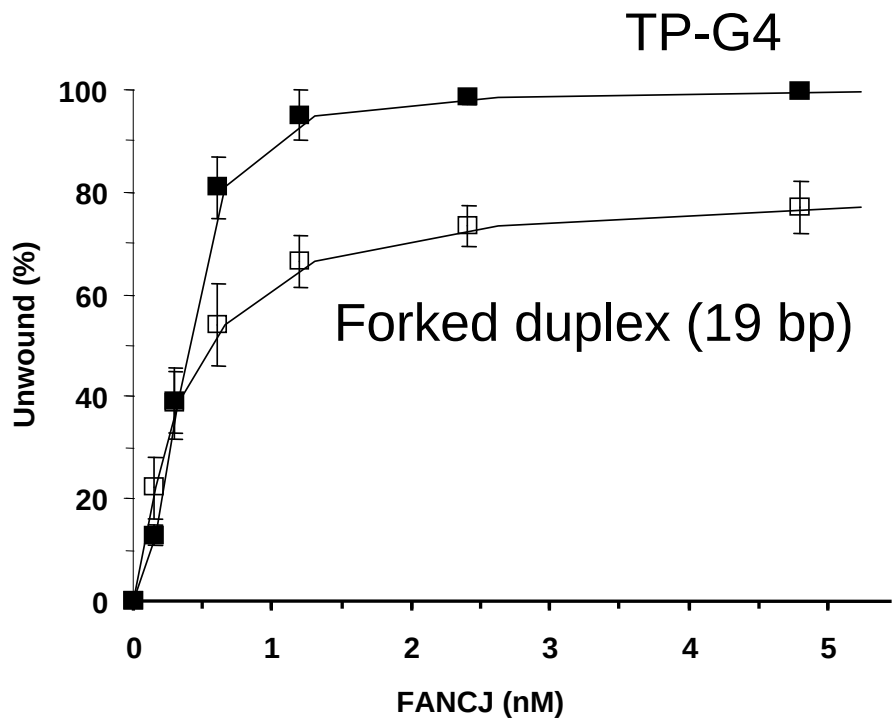
Does FANCJ Unwind G4 DNA?



Yuliang Wu



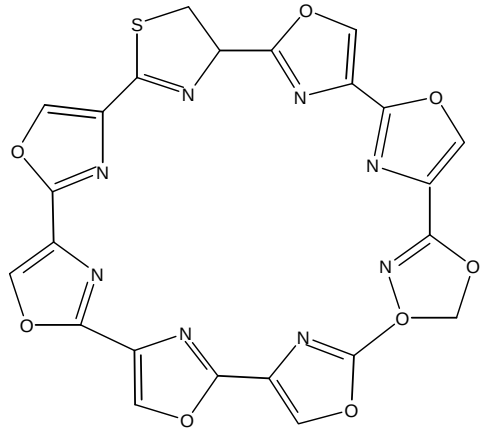




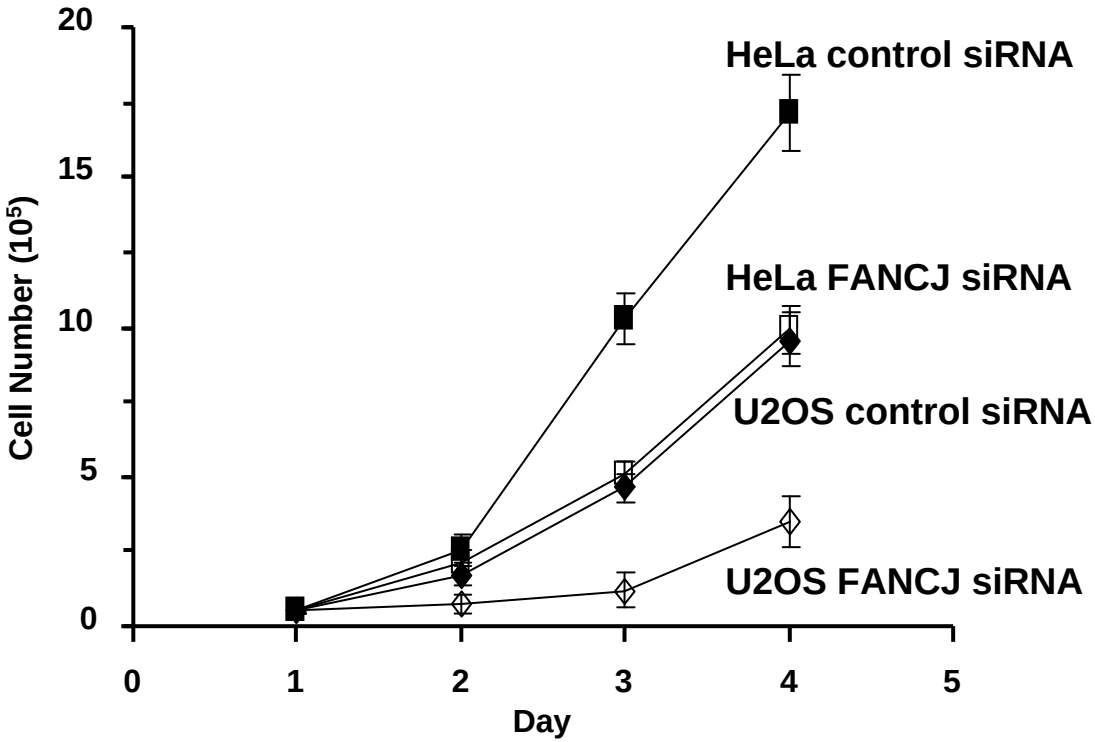
G4 unwinding by FANCJ is dependent on ATP hydrolysis and protein concentration.

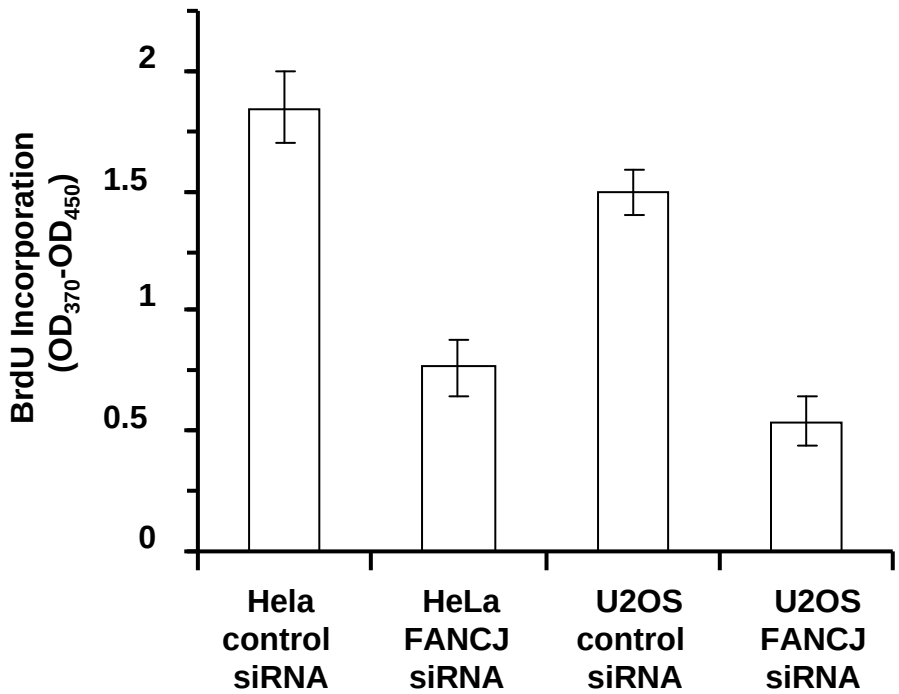
Wu et al., MCB 2008

Effect of telomestatin (5 μM) exposure on FANCJ-depleted cells



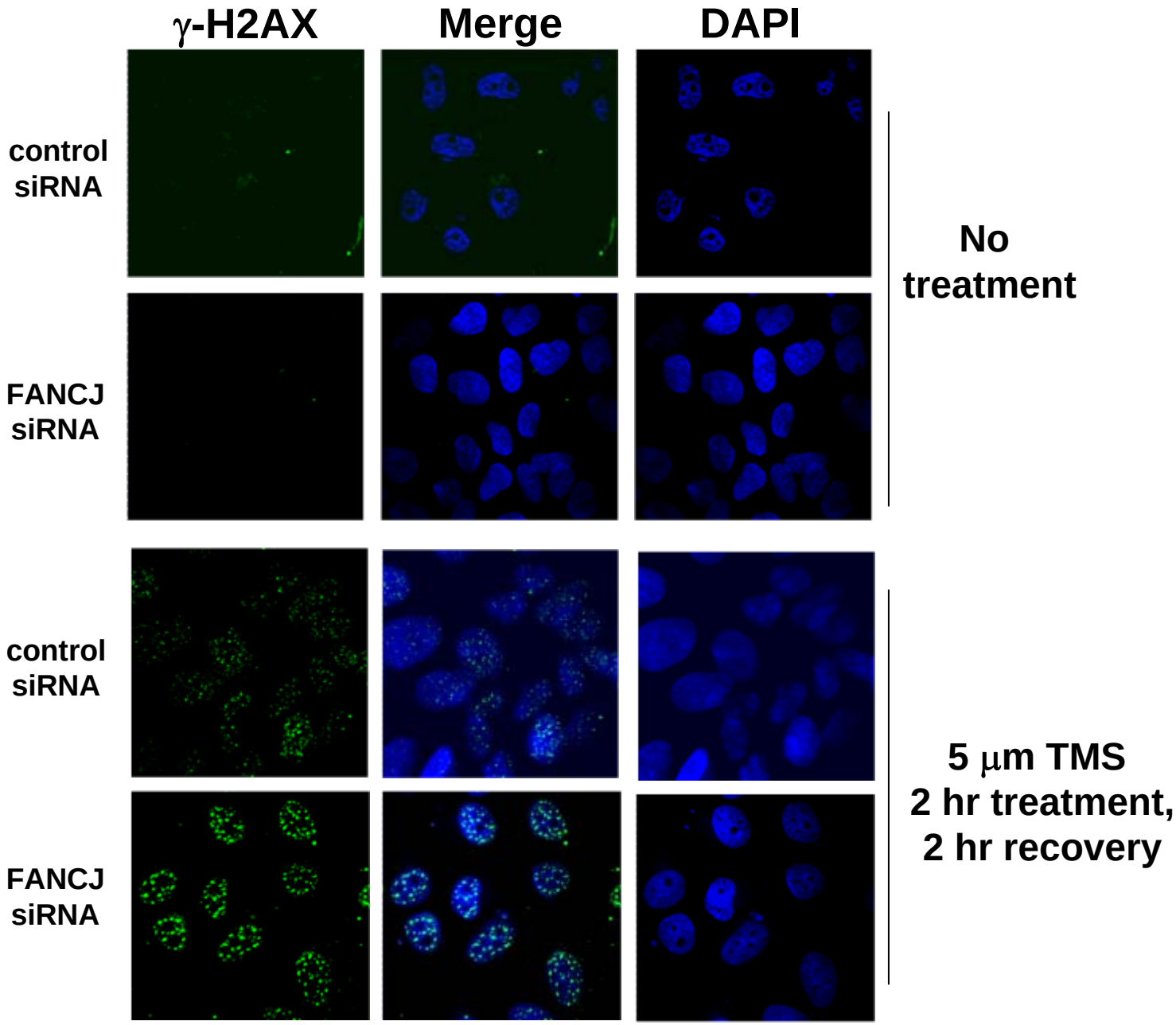
Telomestatin



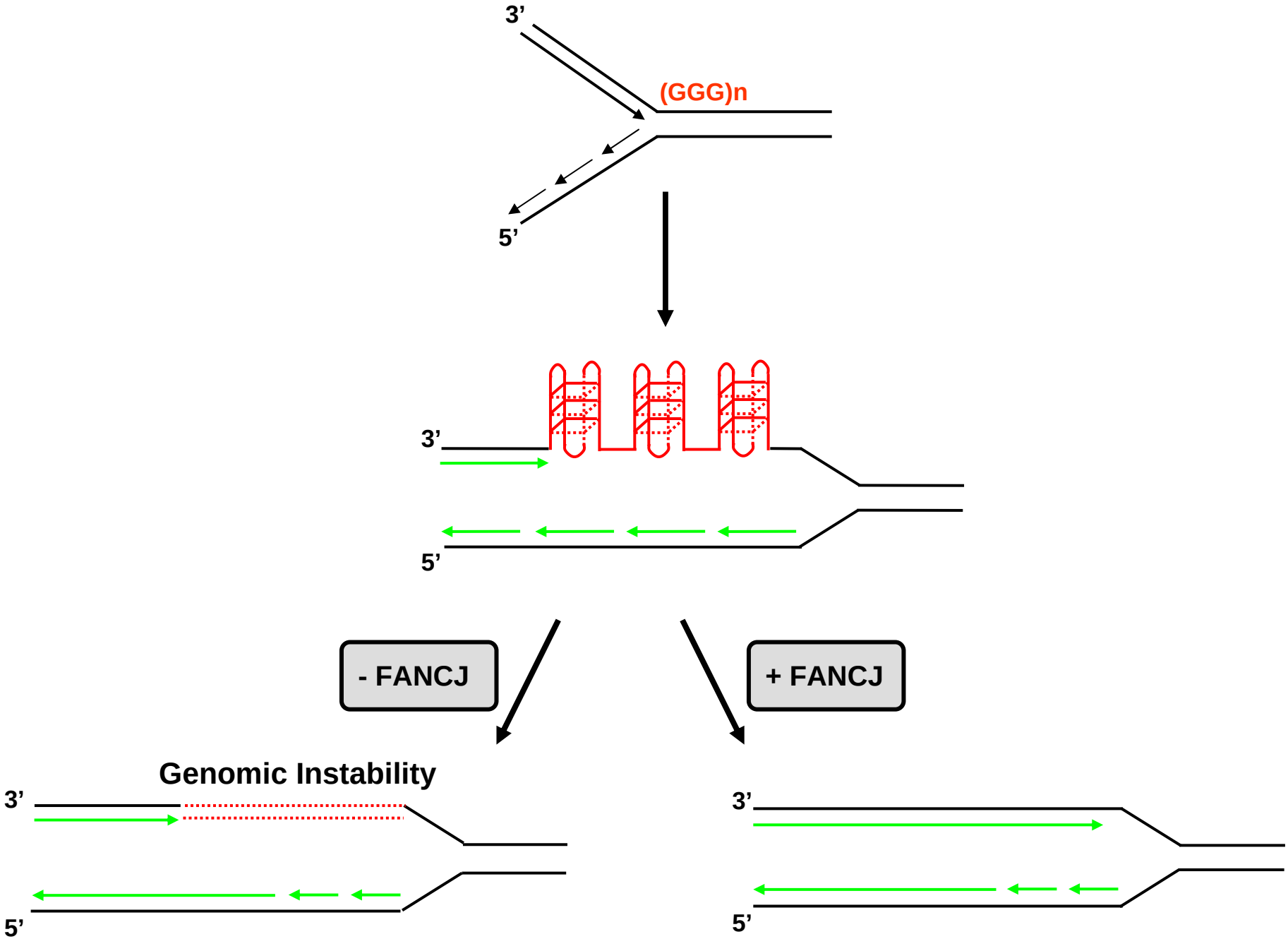


FANCJ depletion sensitizes cells to telomestatin

Telomestatin induces elevated γ-H2AX foci in FANCJ-depleted cells



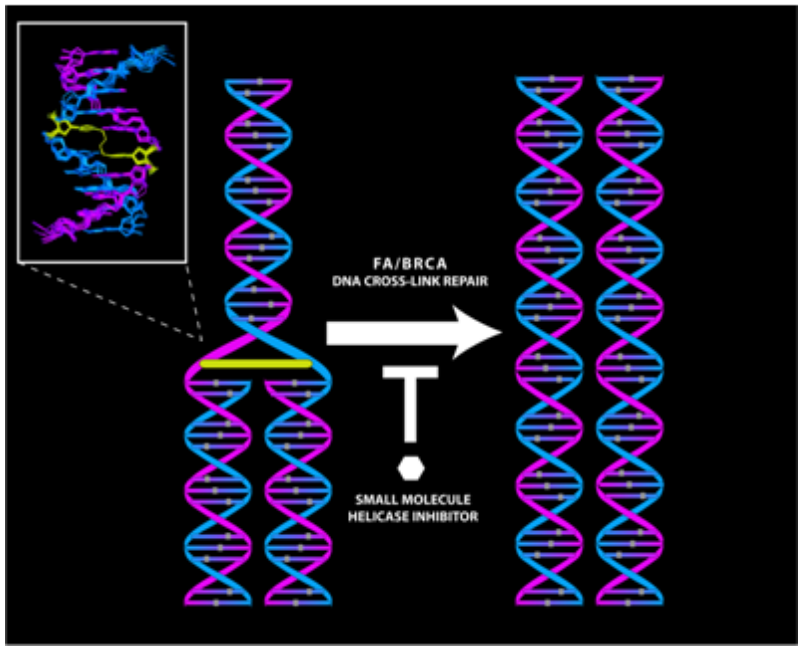
Proposed Role of FANCJ to Resolve G4 Quadruplex DNA Structures During Replication



Role of FANCJ to Preserve Chromosomal Integrity

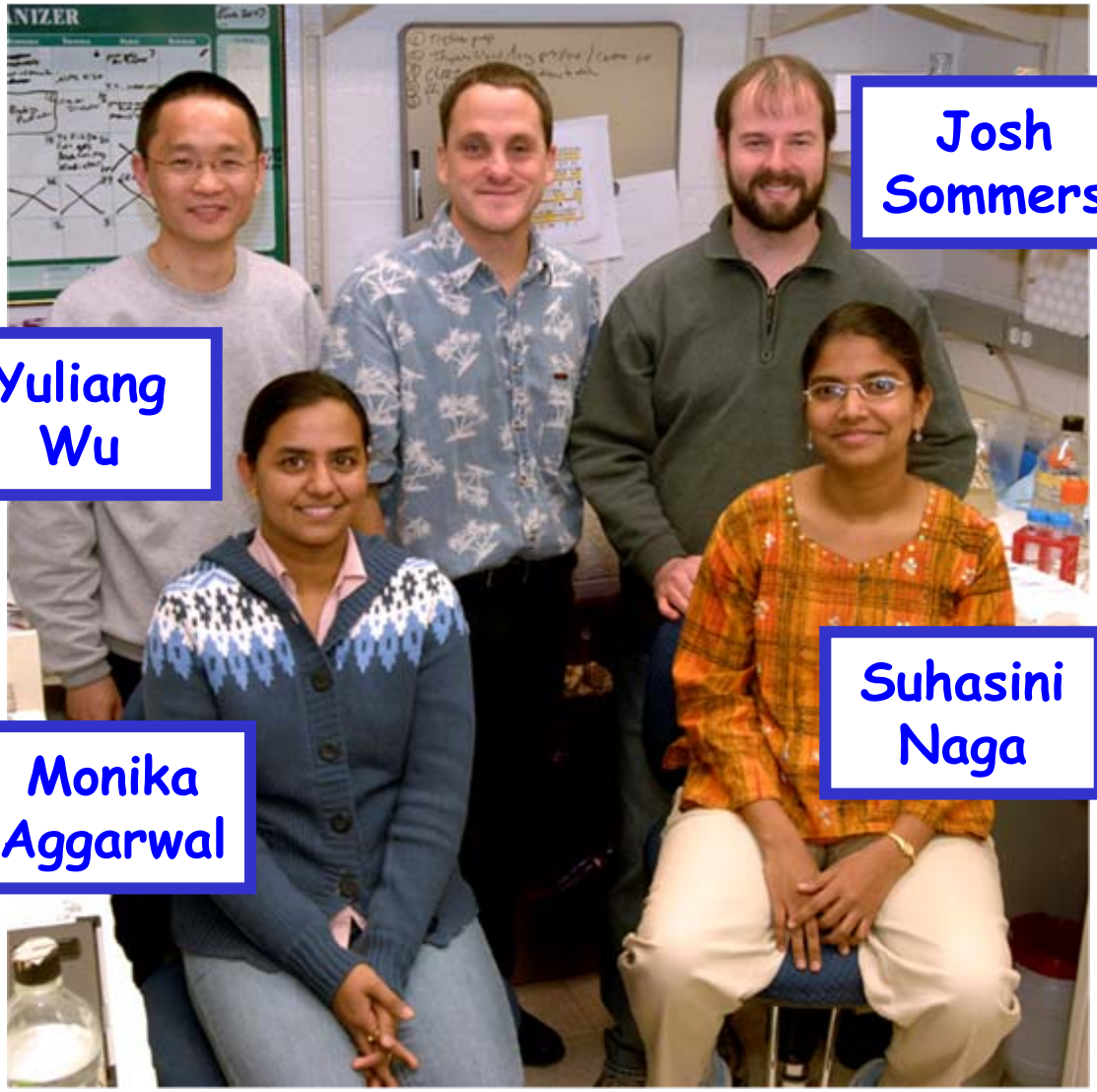
- Promote S phase progression by resolving DNA roadblocks such as G4 tetraplexes that destabilize or impede the replication fork
- Preserve genomic stability to prevent cancer susceptibility

FANCJ & Human RecQ Helicases— Potential Cancer Therapy Targets?

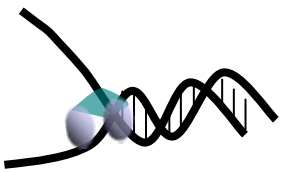


Exploit the roles of DNA helicases in DNA repair to enhance cytotoxic effects of DNA damaging agents

Gupta and Brosh, *Curr. Med. Chem.* 2007



Section on DNA Helicases
Laboratory of Molecular Gerontology
NIA-NIH



LMG, NIA-NIH
Vilhelm Bohr Michael Seidman
David Wilson Yie Liu
Pat Gearhart

Perry Blackshear, NIEHS
Debbie Stumpo, NIEHS
Ian Hickson, Univ. Oxford
Sharon Cantor, U Mass Med Ctr
Alex Mazin, Drexel Univ.