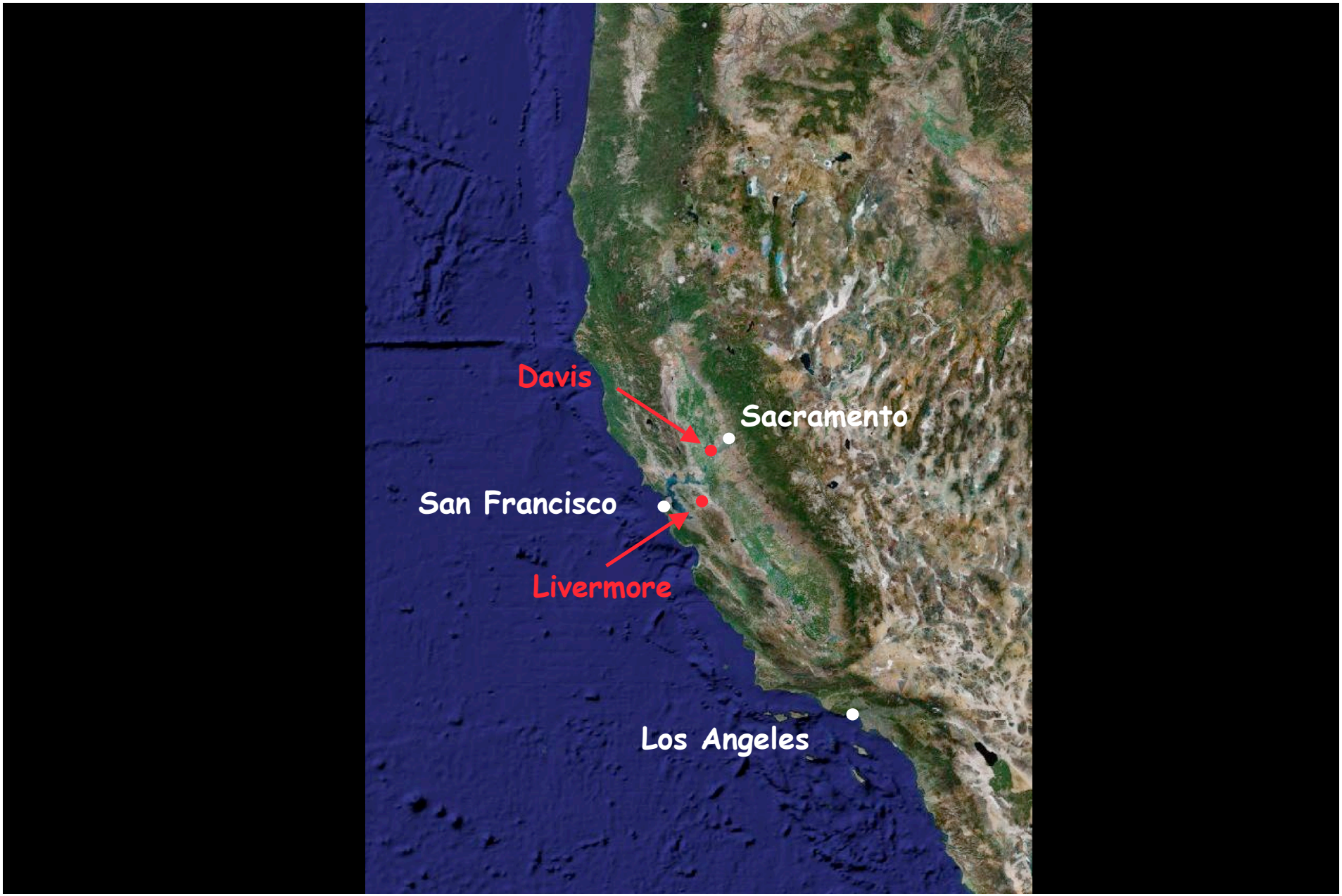
LLNL October 16, 2007

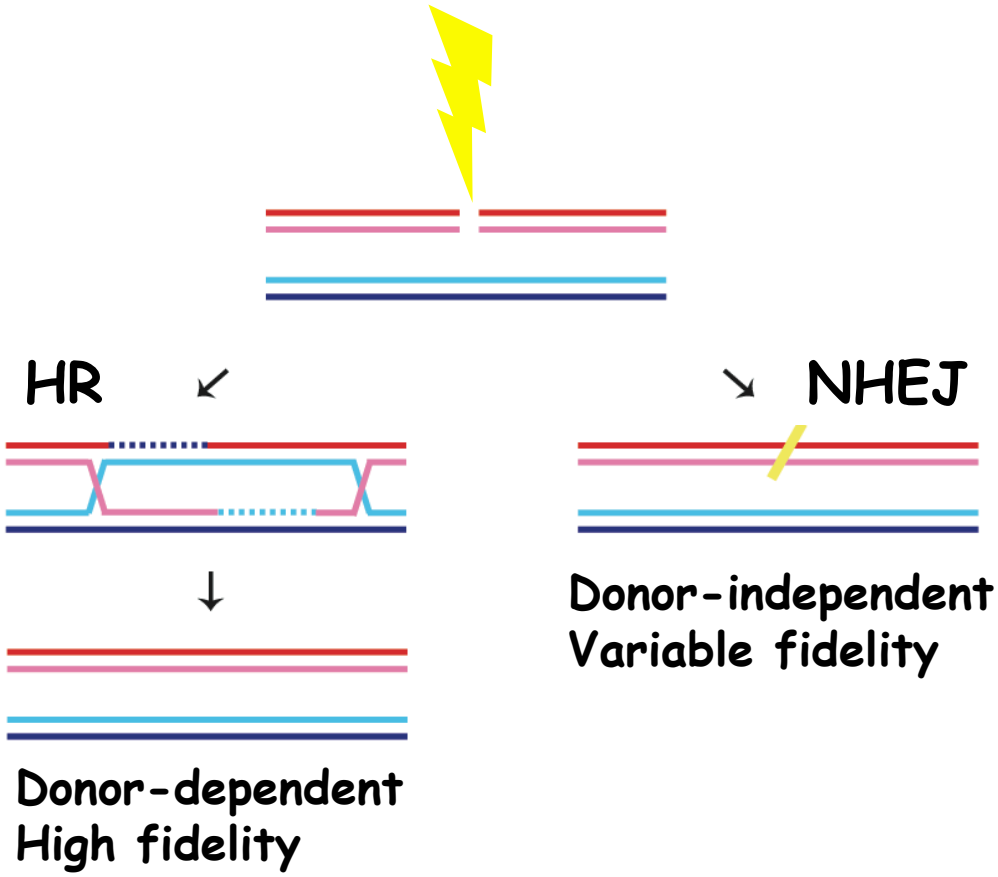
Double-strand DNA break repair by homologous recombination: Roles of the Snf2-like motor protein Rad54

Wolf-Dietrich Heyer
University of California, Davis

wdh 10/10/07


Davis
Sacramento
San Francisco
Livermore
Los Angeles

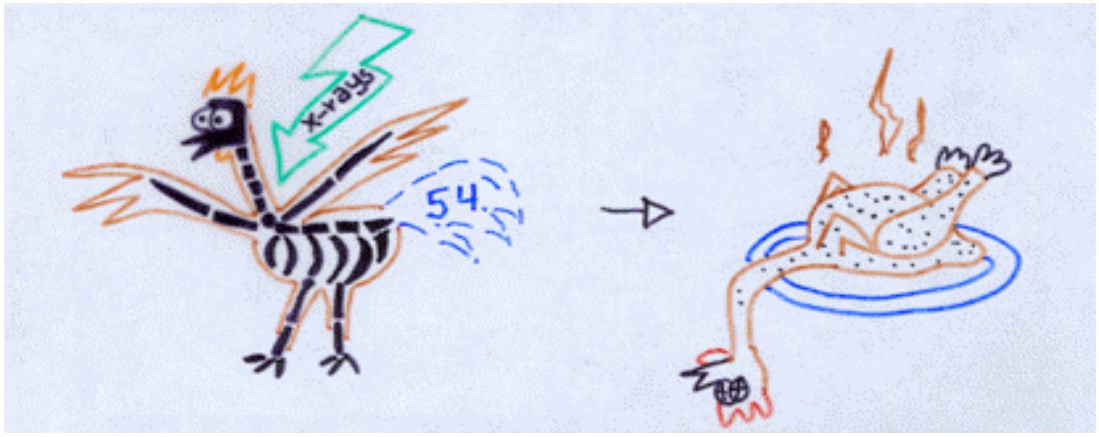
Pathways of DSB repair



***RAD54* is a member of the *RAD52* epistasis group and is critical for DSB repair and recombination *in vivo*.**

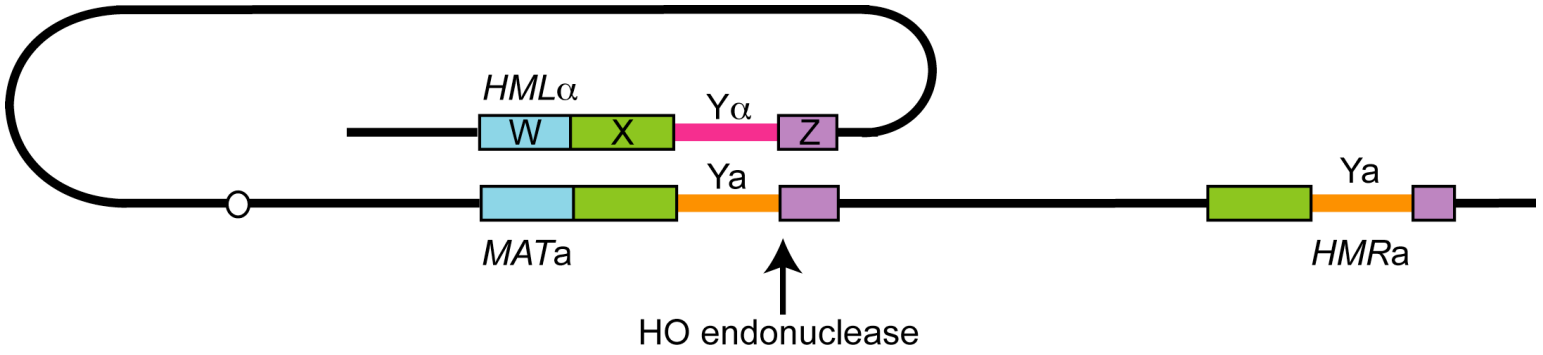
RAD54

Ionizing radiation induces DSBs and other types of DNA damage.



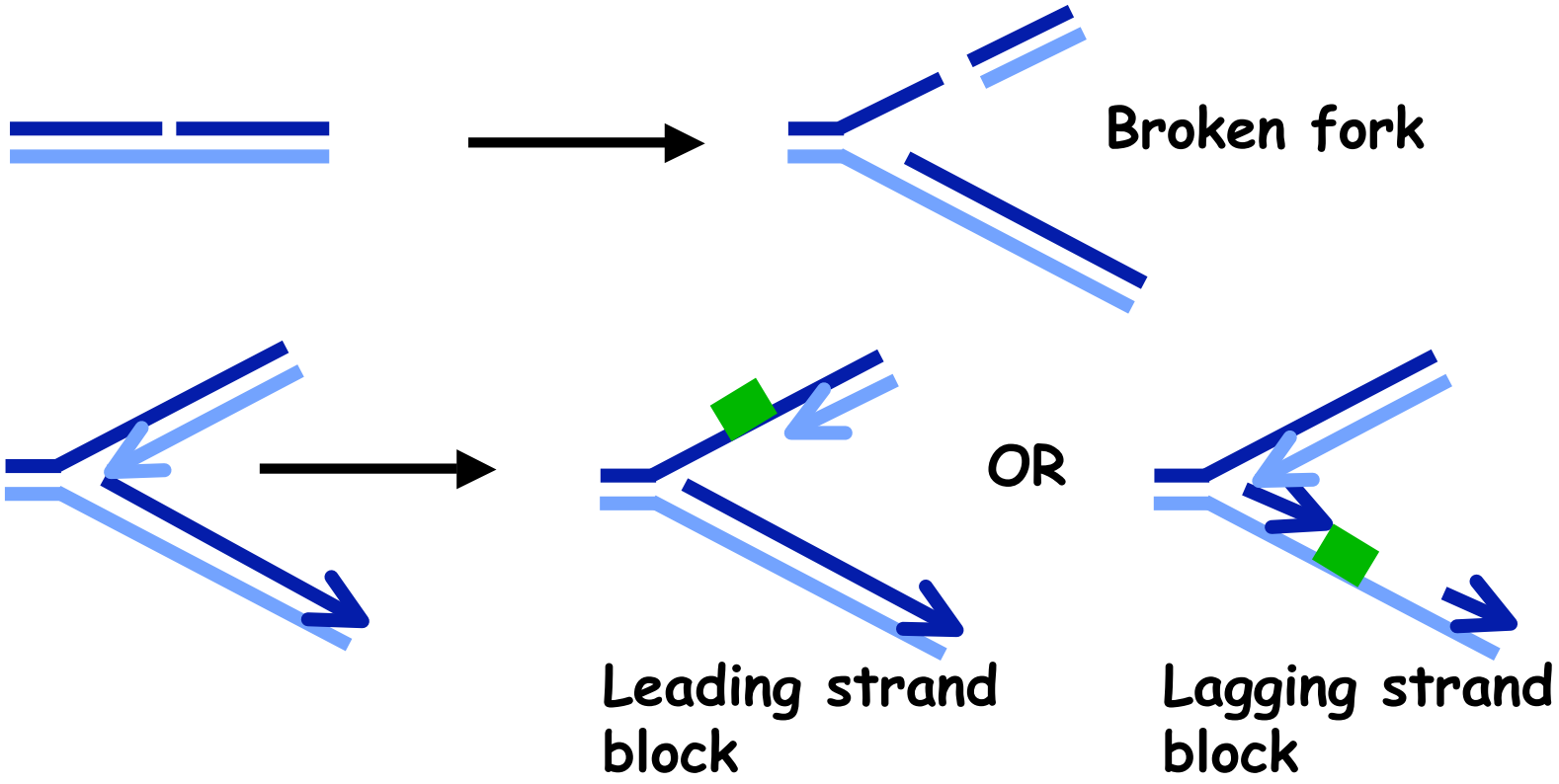
rad54 mutants are among the most ionizing radiation sensitive mutants in budding yeast
(to a similar extent as *rad51* and *rad52* mutants).

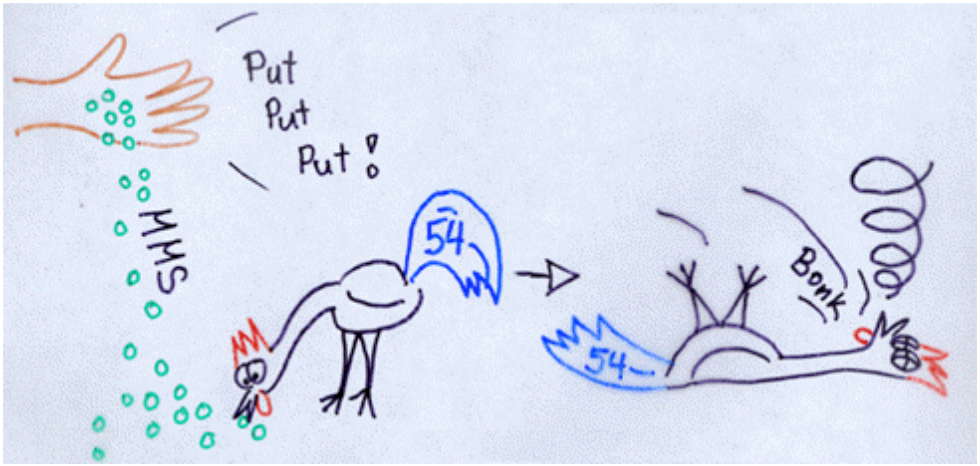
rad54 cells are defective in repairing a single DSB.



S.cerevisiae rad54 mutants are defective in mating-type switching (repair of a single DSB), similar to *rad51* and *rad52* mutants.

MMS-, CPT-, and UV-induced DNA damage leads to stalling and breaking of replication forks.



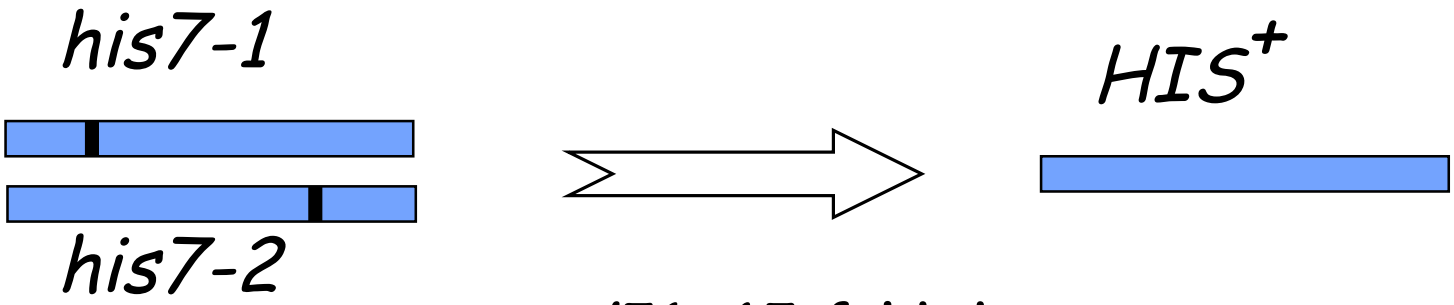


rad54 mutants are very sensitive to methylmethane sulfonate (MMS) (to a similar extent as *rad51* and *rad52* mutants).

rad54 mutants are sensitive to UV (to a similar extent as *rad51* and *rad52* mutants).

jas 2/20/01

Rad54 is important for homologous recombination.



rad51: **15 fold down**
rad54: **14 fold down**

S. cerevisiae rad54 mutants are defective in mitotic intragenic recombination (heteroalleles) to a similar extent as *rad51* (and *rad52*) mutants.

Mammalian Rad54 (Wesoly et al. 2006)

2 paralogs Rad54 and Rad54B

***rad54*$^{-/-}$ mouse ES cells IR^{s}, MMC^{s}, hyporec**

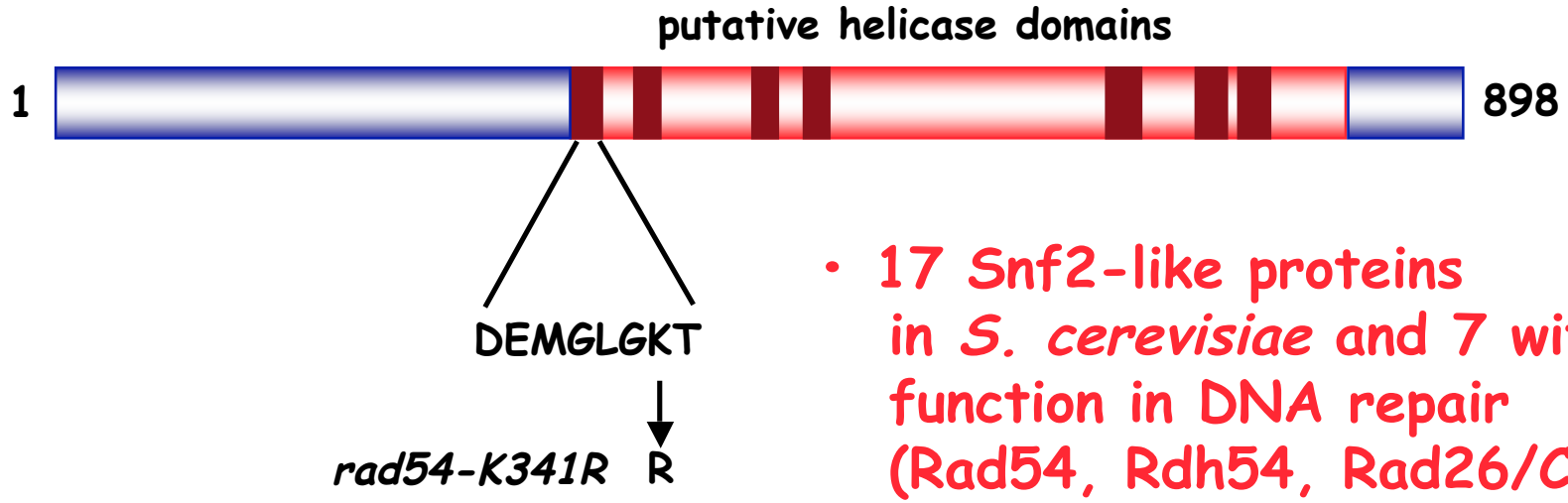
***rad54B*$^{-/-}$ mouse ES cells less IR^{s}, MMC^{s}, rec^{+}**

***rad54*$^{-/-}$ *rad54B*$^{-/-}$ enhanced IR^{s}, MMC^{s}, very hyporec**

animals synergistic MMC^{s} - tissue specificity?

Mutations in *RAD54* and *RAD54B* found in primary human cancers.

Rad54 is a Snf2-like member of the DNA helicase Superfamily 2.

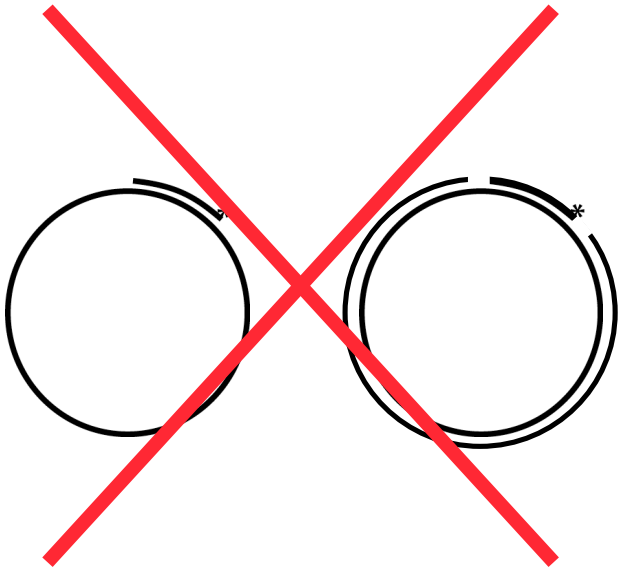


- 17 Snf2-like proteins in *S. cerevisiae* and 7 with function in DNA repair (Rad54, Rdh54, Rad26/CS-B, Rad5, Rad16, Ino80, Swr1)

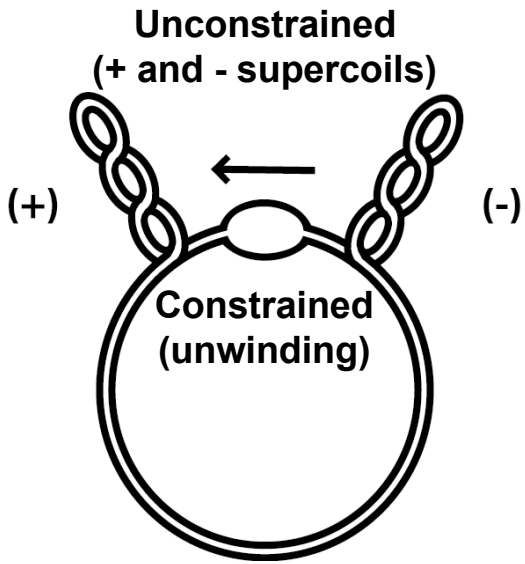
- 53 Snf2-like protein in humans

(Flaus et al. 2006 NAR)

Rad54 is not a DNA helicase but a dsDNA translocase.

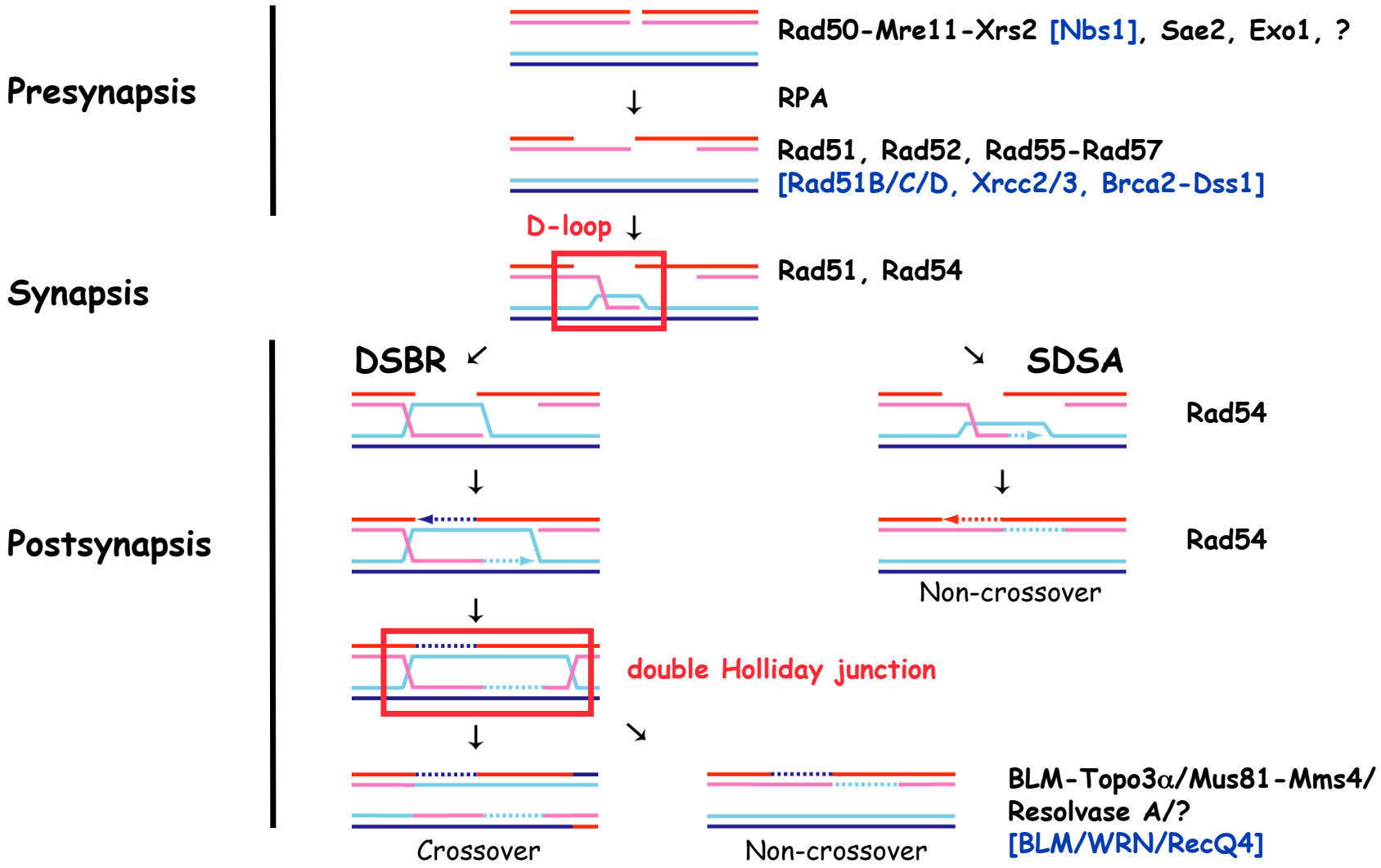


No helicase activity



Translocation on dsDNA at 300 bp/sec causing topological changes
(Amitani et al. 2006)

DSB repair by homologous recombination



wdh 4/20/07

Complex reactions with interacting components

Interactions all at the same time: Stable complex?

"Recombinosome"

Sequential transient interactions: Assembly line?

Pathway

Facts:

- Rad54 interacts with Rad51
  (Clever et al. 1997 EMBO J)
- Rad54 is a dsDNA-specific ATPase
  (Sagemakers et al. 1998 JBC)
- The Rad54 ATPase is essential for *in vivo* function
  (Clever et al. 1999 Yeast)
- Rad54 is a Snf2-like remodeling factor

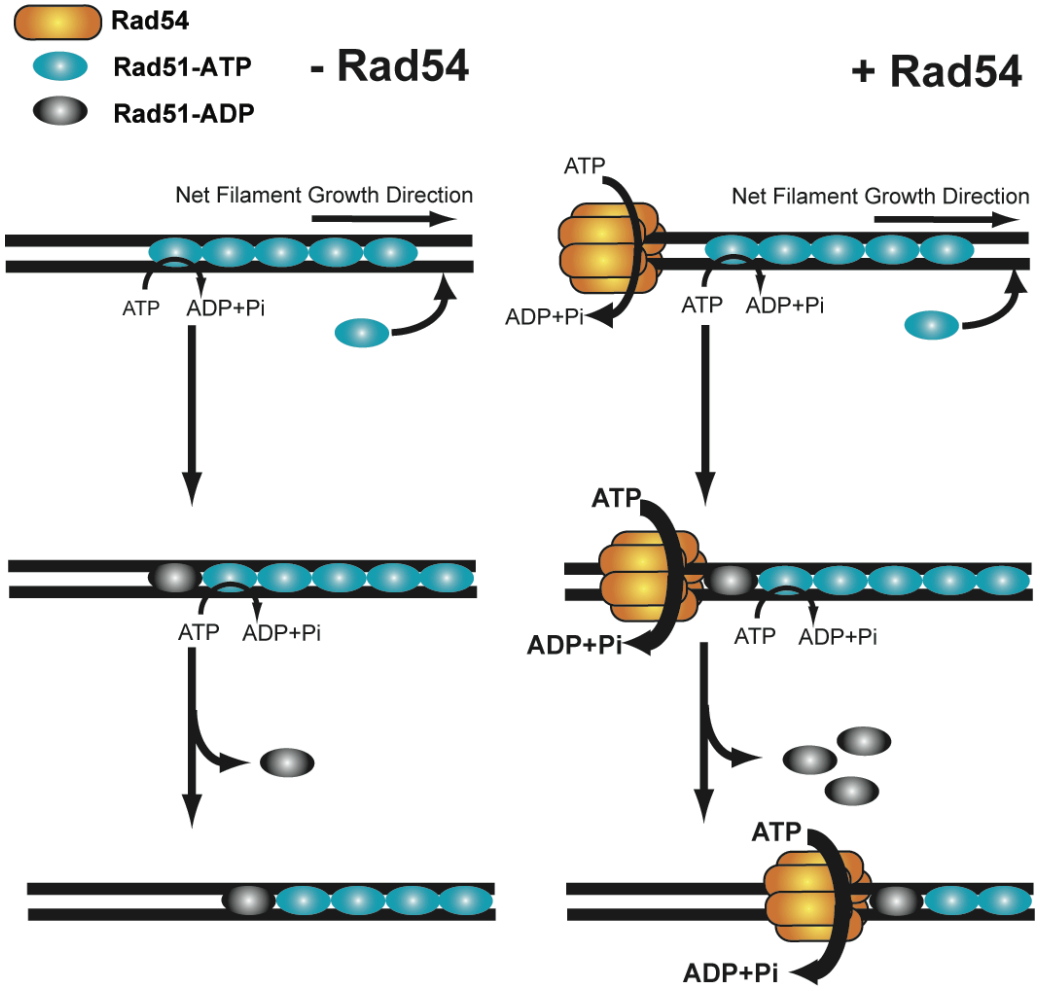
Hypothesis: Rad54 dissociates Rad51-dsDNA filaments.

Relevance of Rad51-dsDNA complexes:

- Dead-end complexes due to dsDNA binding by Rad51 (unlike RecA)

- Product complex of DNA strand invasion (Rad51 bound to heteroduplex DNA)

Conclusions from data not shown



Solinger et al. 2002
Mol. Cell

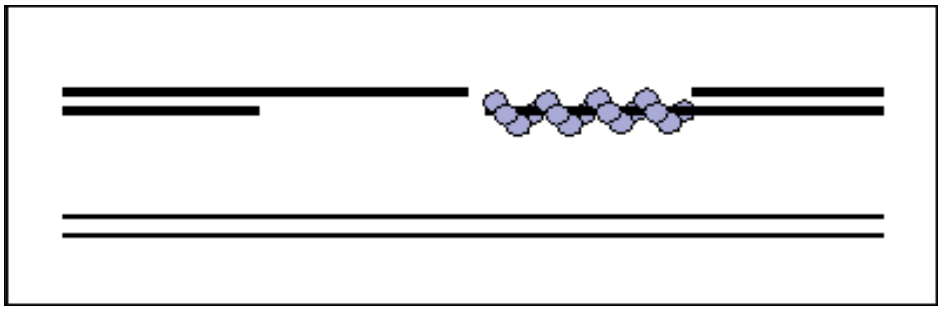
Kiianitsa et al. 2002
JBC

Kiianitsa et al. 2006
PNAS

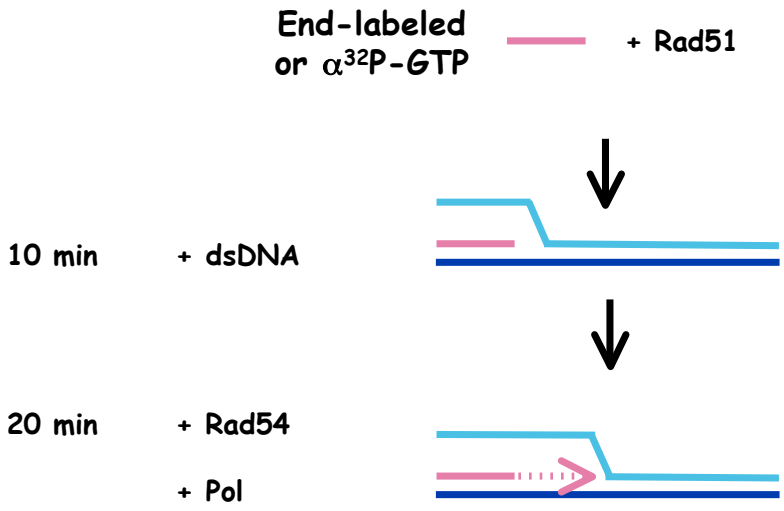
Li et al. 2007 NAR

Xuan Li

Working model:
Rad54 turns over the Rad51-dsDNA product complex to allow access to the 3'-OH end of the invading strand



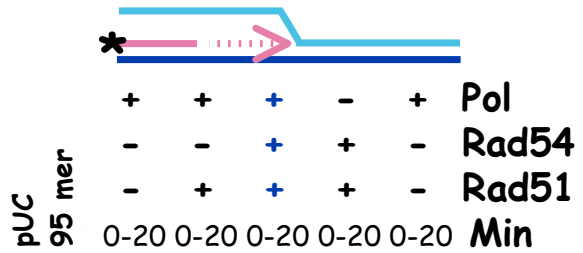
Assay system



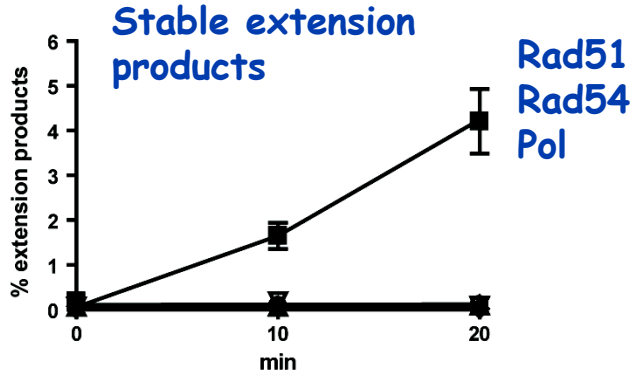
2 μM (nts) 95 mer
pUC19 1:1 molecule
0.67 μM Rad51 (1:3 nts)
72 nM Rad54
25 nM DNA Polymerase (Klenow)

Native or
denaturing gel

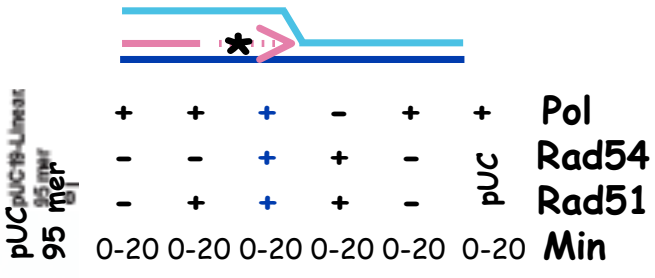
Extension product depends on Rad51, Rad54 and Pol



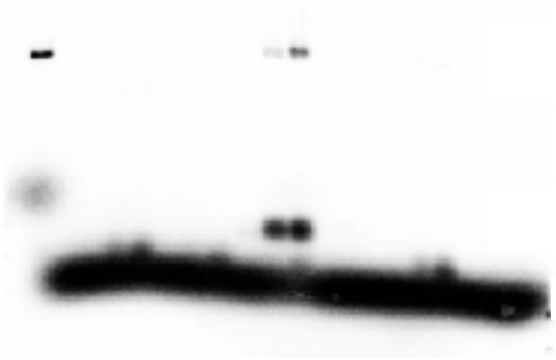
Native gels

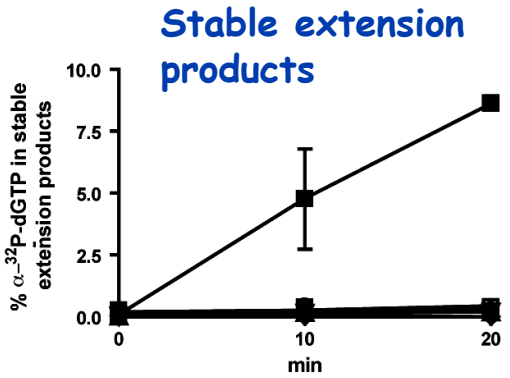


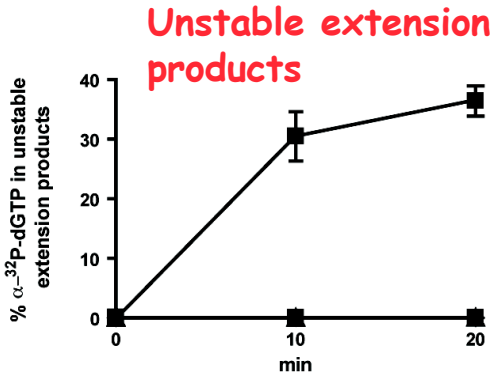
Extension product depends on Rad51, Rad54 and Pol



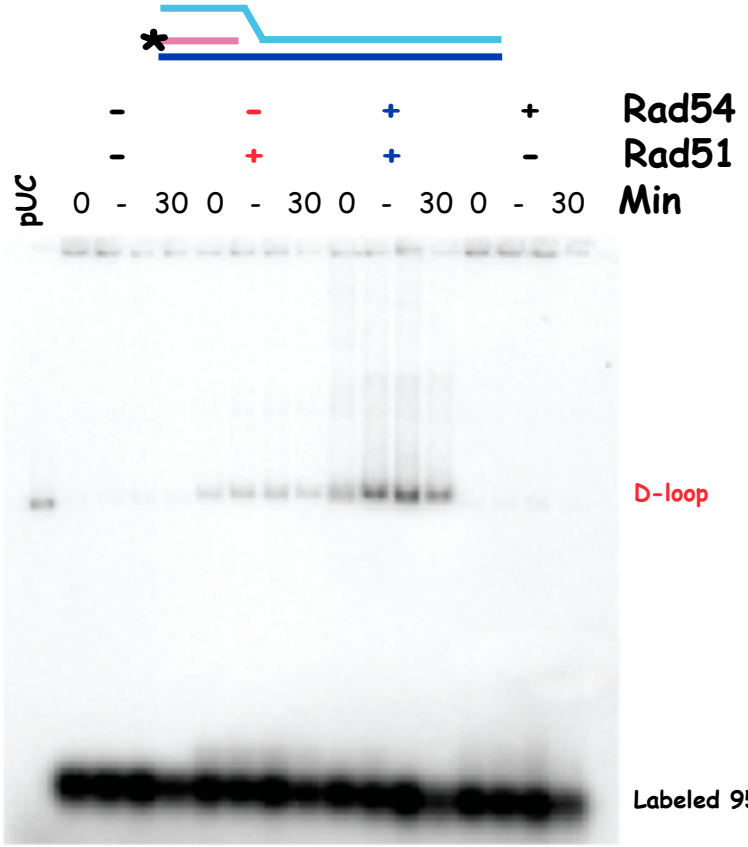
Native gels

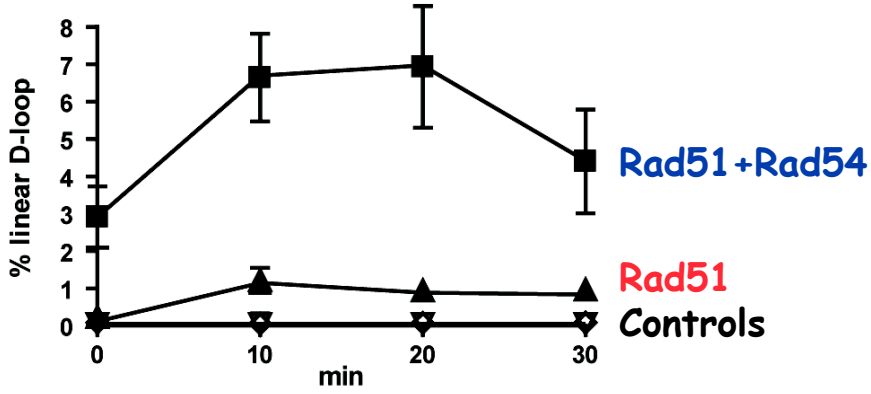






Formation of D-loops by Rad51
Analysis of psoralene-crosslinked products

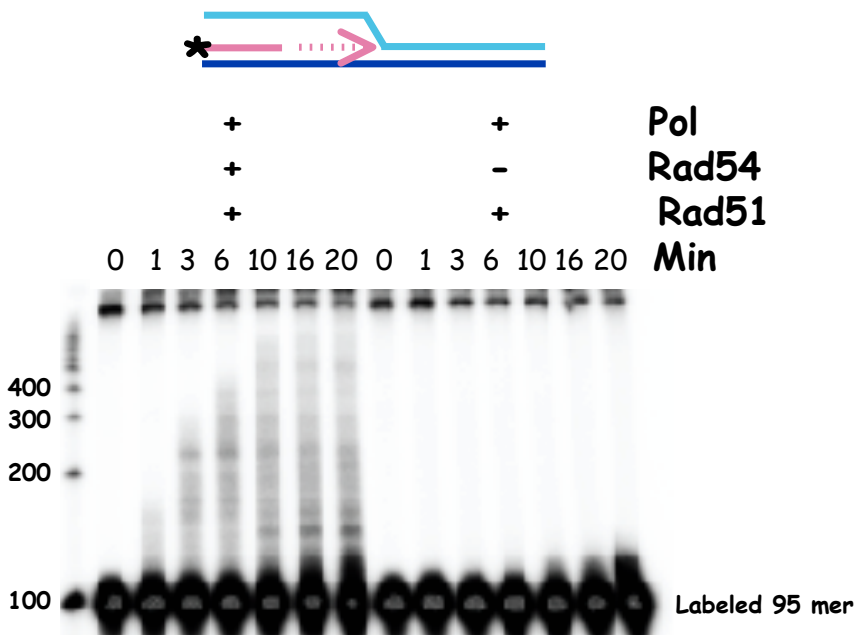


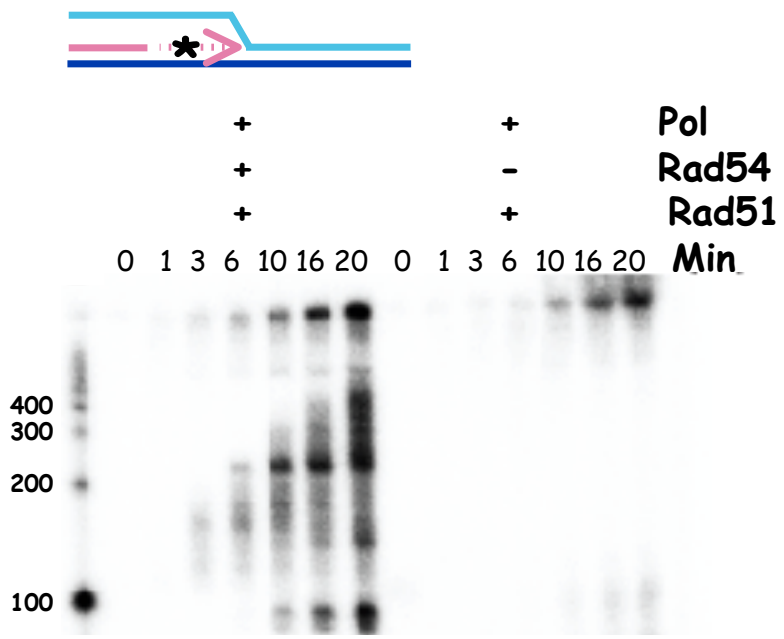


Rad54 stimulates D-loop formation 6-7 fold, but extension by Pol >100 fold.

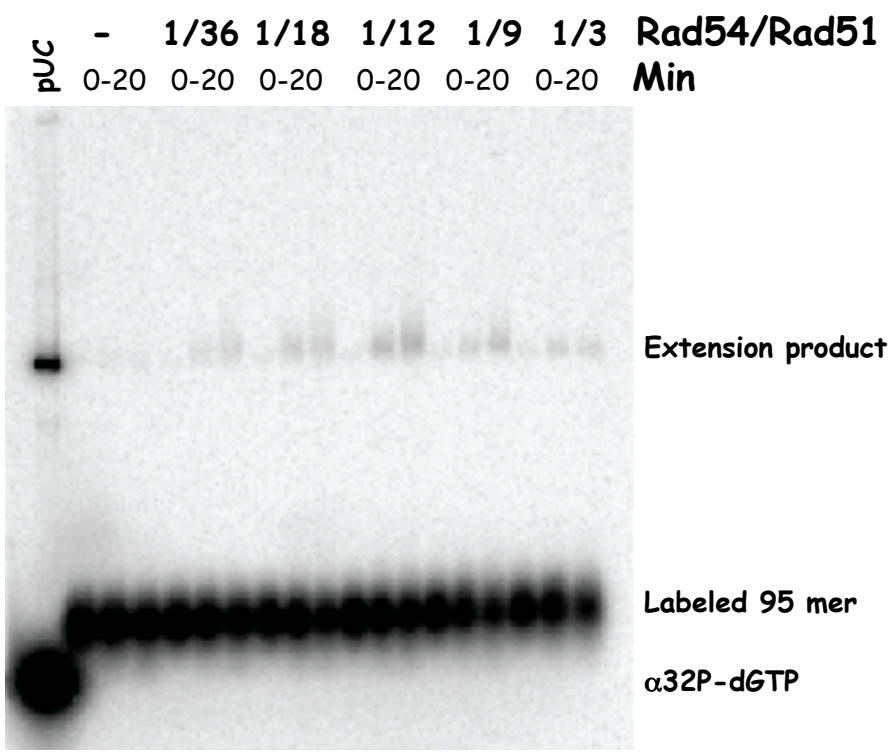
Rad54 is required for extension.

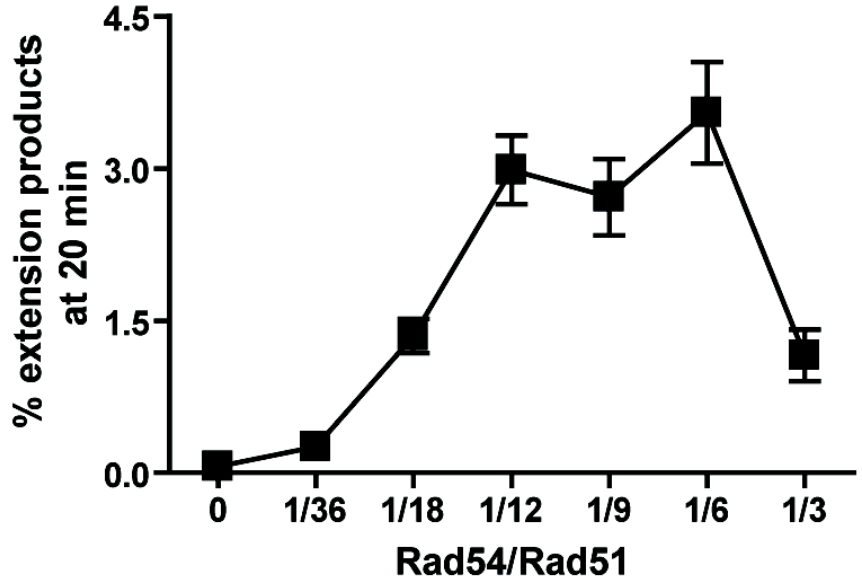
Analysis on denaturing gels





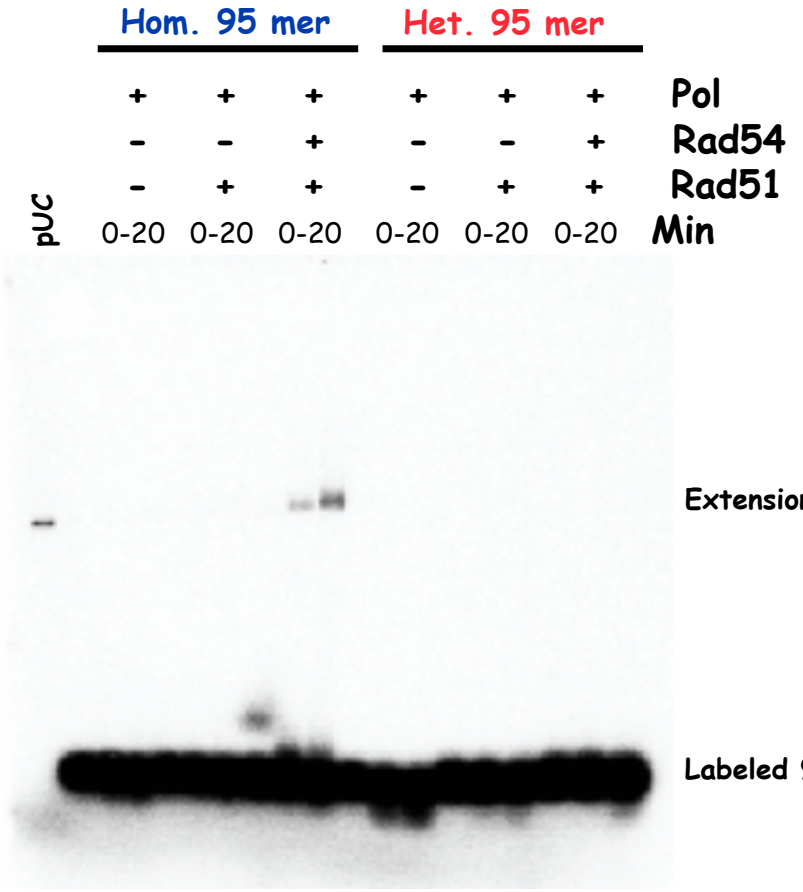
Extension is optimal at sub-stoichiometric Rad54:Rad51 ratio

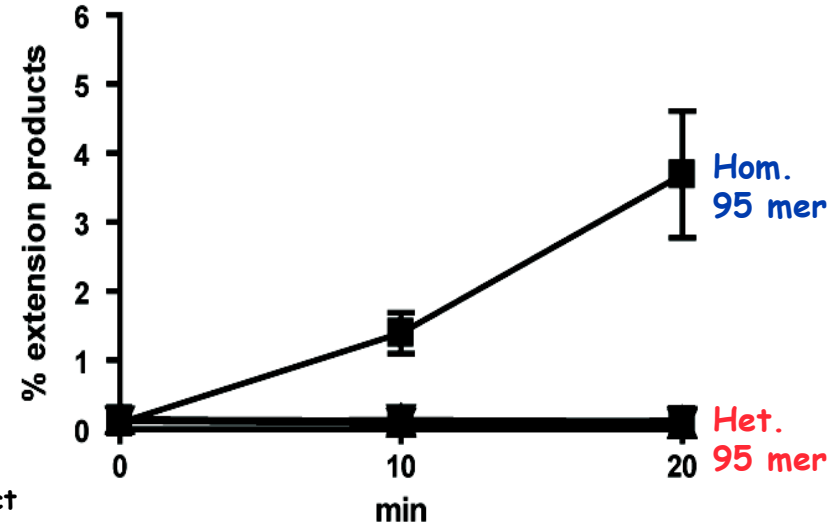




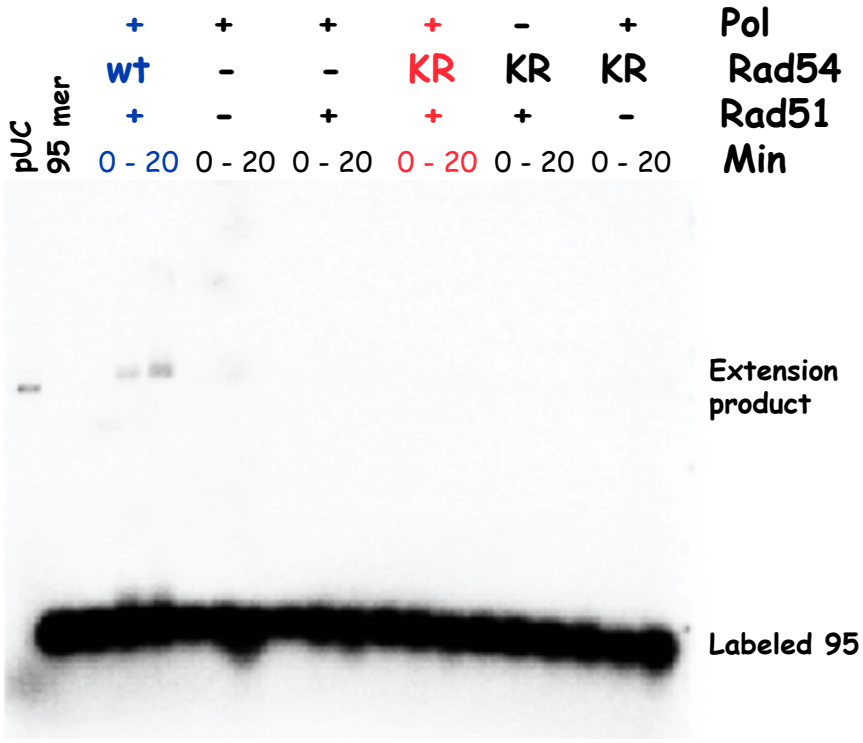
Optimal Rad51 (1/3 nts)

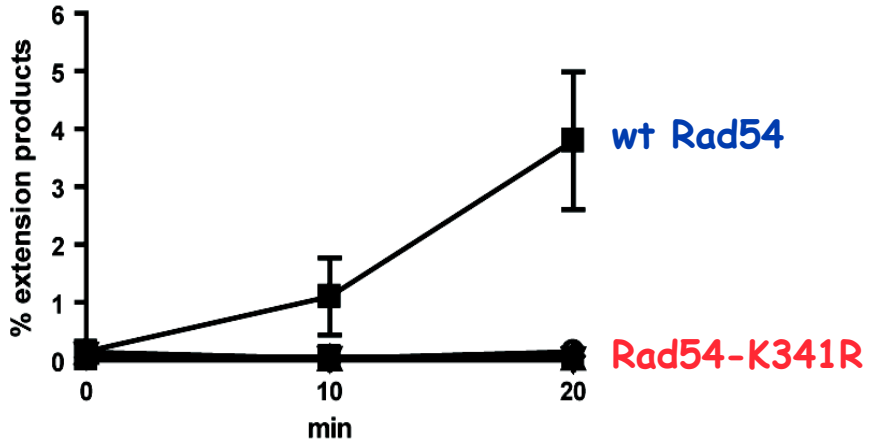
Extension is homology dependent





Extension requires Rad54 ATPase activity





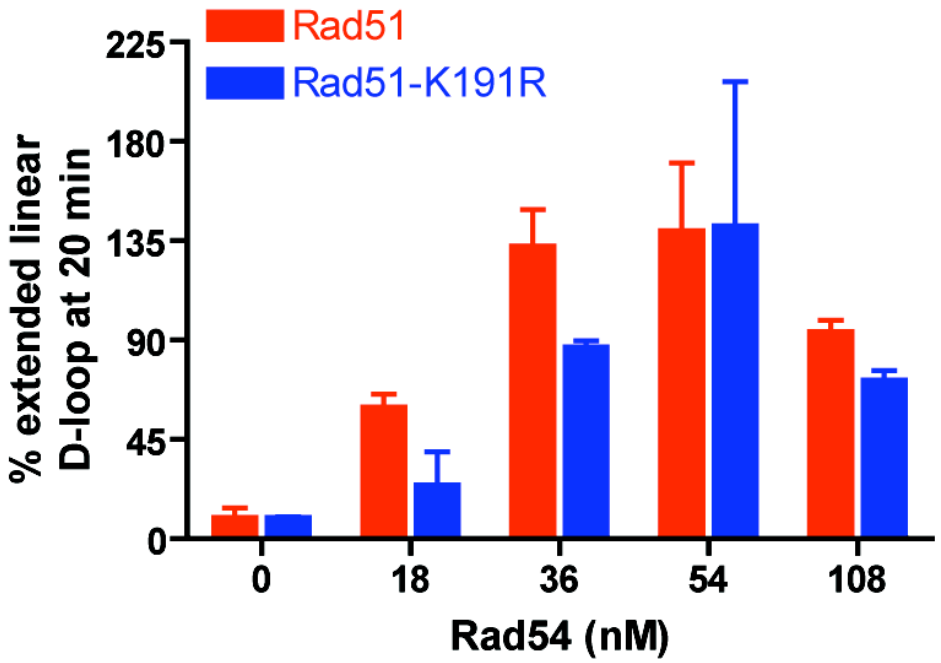
Rad51-K191R requires more Rad54 for extension

Rad51-K191R forms very stable dsDNA complexes that are less well dissociated by Rad54

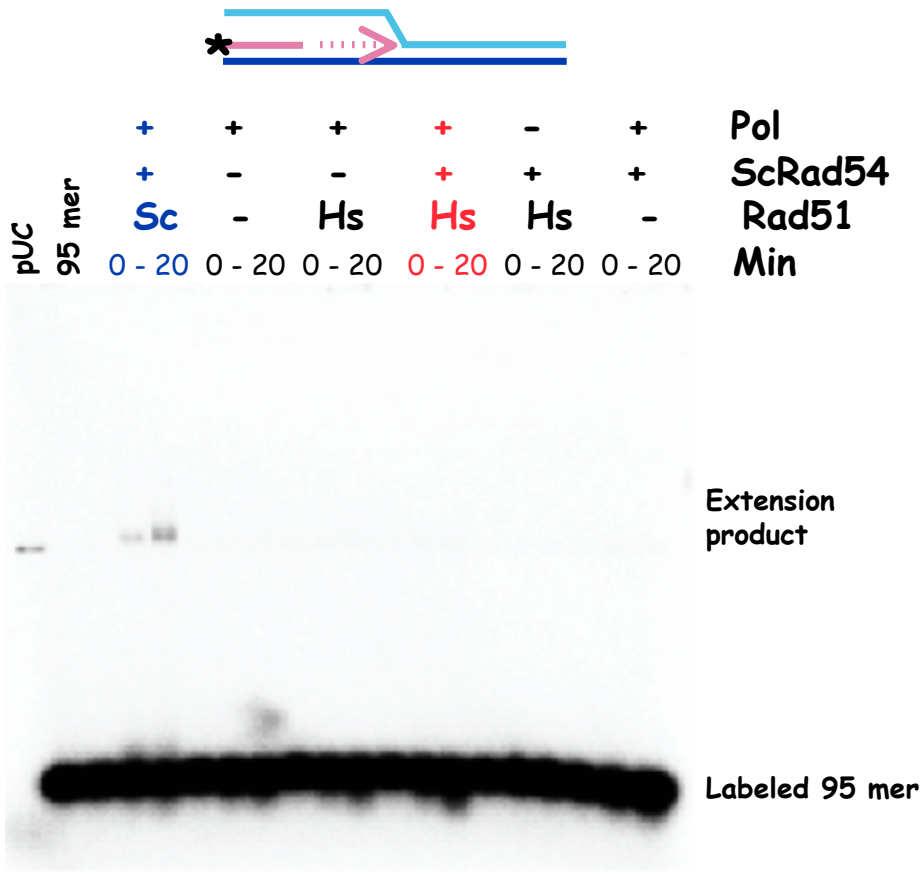
(Li et al. 2007 NAR)

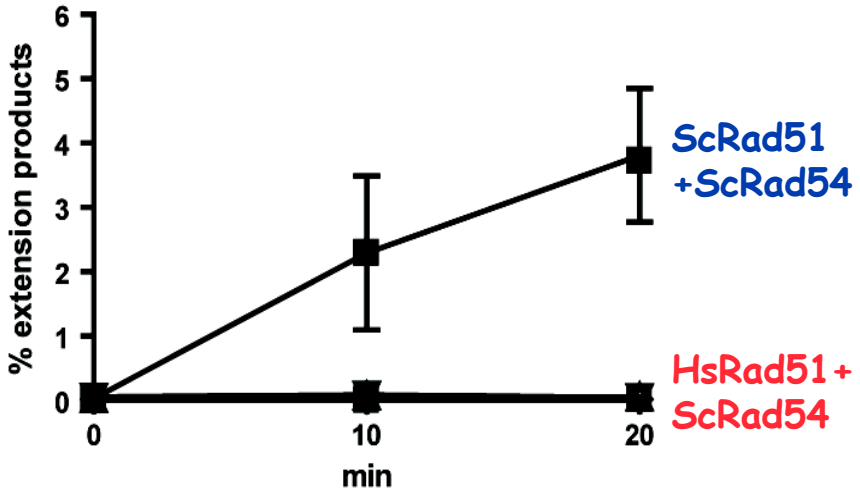
Rad54 overexpression suppresses *rad51-K191R in vivo*

(Morgan et al. 2002 MCB)

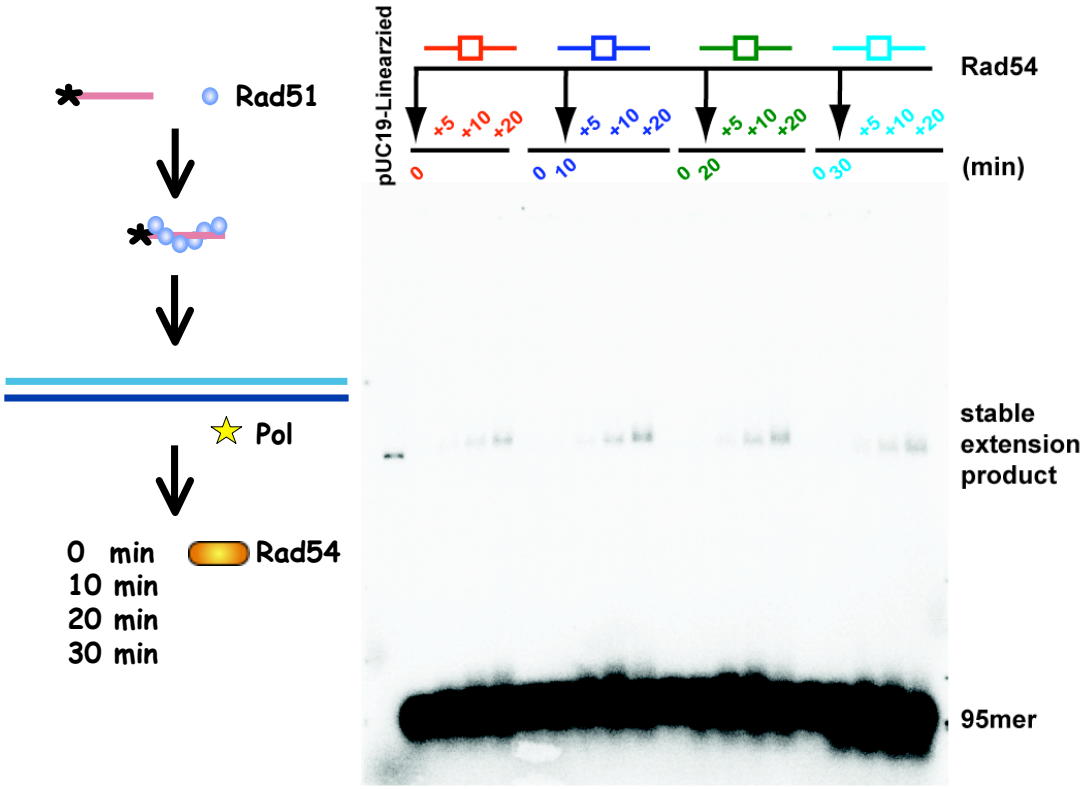


Extension requires species-specific protein interactions





Time point of Rad54 addition determines extension



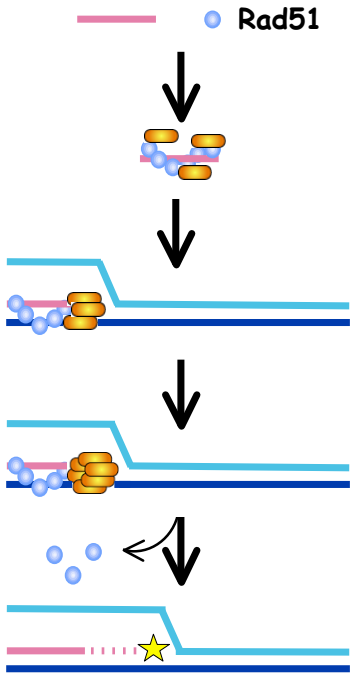
Model: Rad54 modulates access to the invading 3'-OH end

Filament formation

DNA strand invasion

Rad51 product release mediated by Rad54

DNA repair synthesis stabilizes D-loop



Rad54 stabilizes Rad51 filament

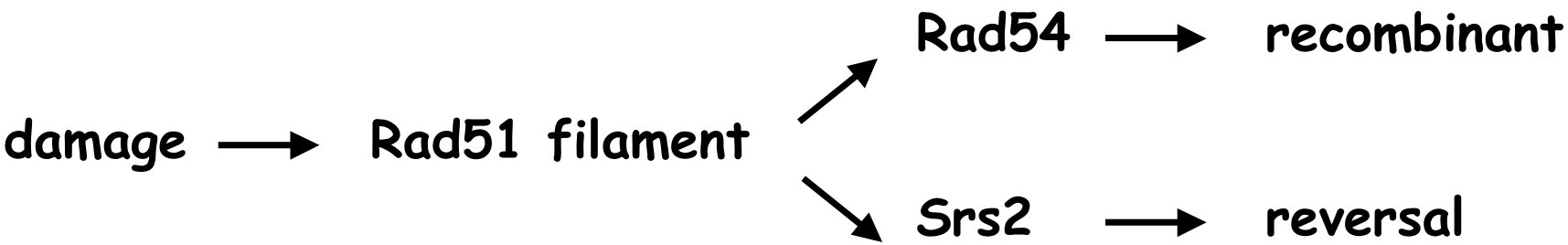
Rad54 is targeted to pairing site

Rad54

Pol

Does this make sense *in vivo* ? YES !

Genetics: Pathway analysis + Rad54 overexpression suppresses *rad51-K191R* (Symington).
Rad54 overexpression shortens conversion tracks, *rad54-KR* has longer conversion tracts (Nickoloff)
rad54 srs2 are synthetically lethal, suppressed by *rad51, rad52, rad55, rad57* (Klein, Schild)



In vivo: ChIP data (Haber, Peterson/Sung)

Cytology: Formation of Rad51 foci is independent of Rad54 in yeast, chick (DT40), and mouse (ES cells).
Rad51 foci have longer half life in *rad54.*
(Bishop, Kanaar, Shinohara, Takeda)

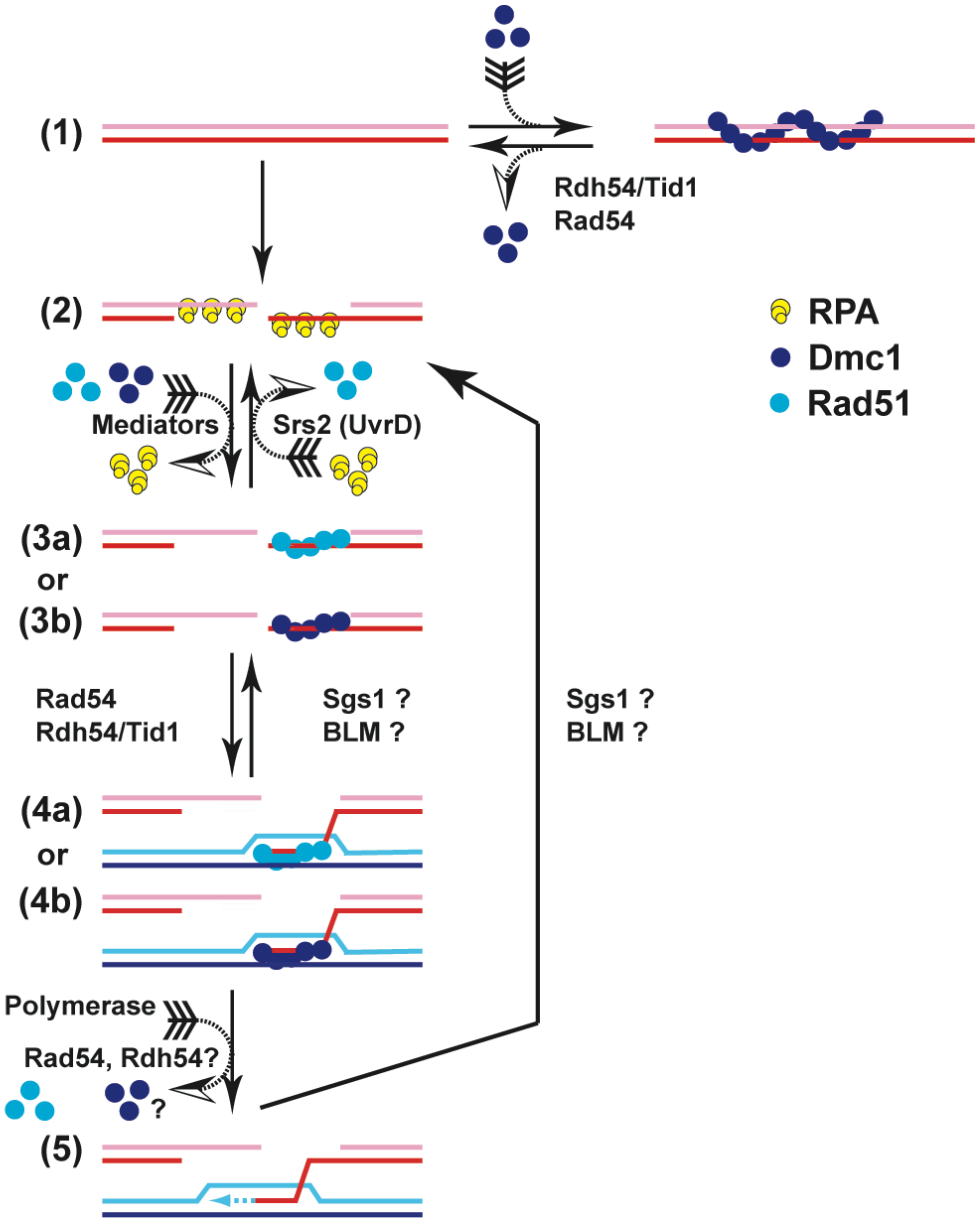
Summary

- Through its interaction with the Rad51 DNA strand exchange protein Rad54 protein enhances homologous recombination at various stages during recombination *in vitro*.

- Rad54 dissociates the Rad51-dsDNA filament, which represents the product complex of DNA strand exchange.

- Rad54 modulates the access to the 3′-end of the invading strand to make stable D-loops by DNA polymerase extension.

- Rad54 (and other Snf2-related proteins) are target-specific motor proteins that remodel protein-dsDNA complexes.

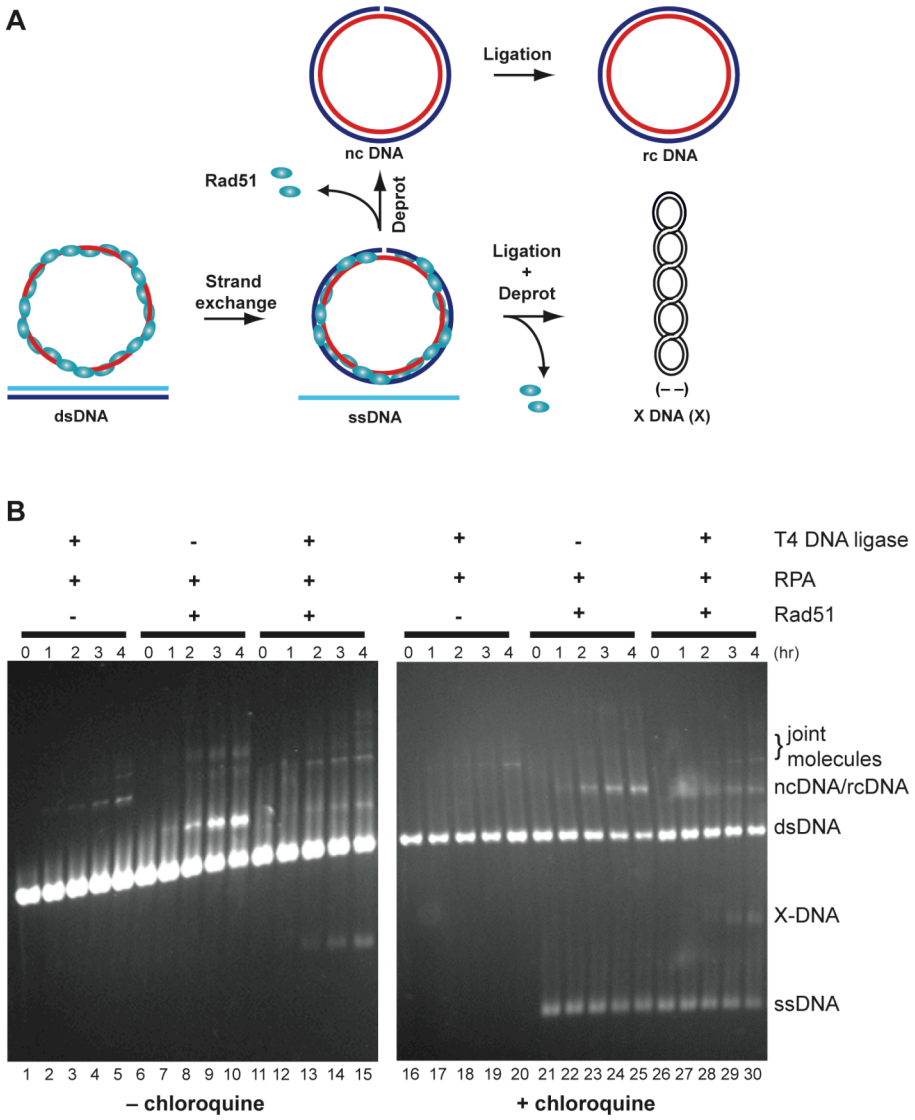


The recombination pathway:

Metastable, reversible intermediates

Motor proteins (helicases, translocases) toggle between intermediates

(Symington & Heyer 2006 Genes Dev)


A
Ligation
nc DNA
rc DNA
Rad51
Deprot
Strand
exchange
Ligation
+
Deprot
dsDNA
ssDNA
(– –)
X DNA (X)
B
T4 DNA ligase
RPA
Rad51
(hr)
joint
molecules
ncDNA/rcDNA
dsDNA
X-DNA
ssDNA
– chloroquine
+ chloroquine