# Repair and maintenance of eroded telomeres in mice

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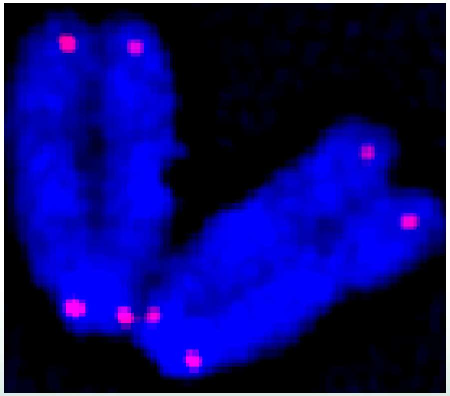

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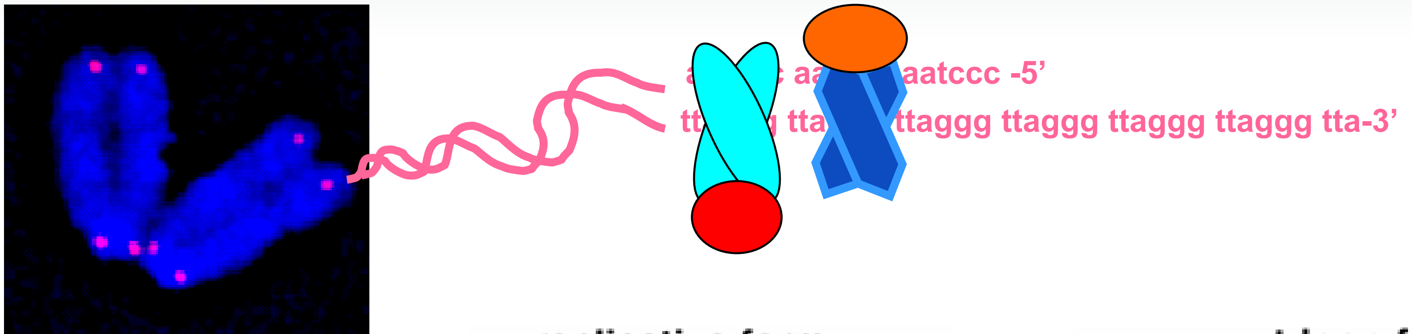
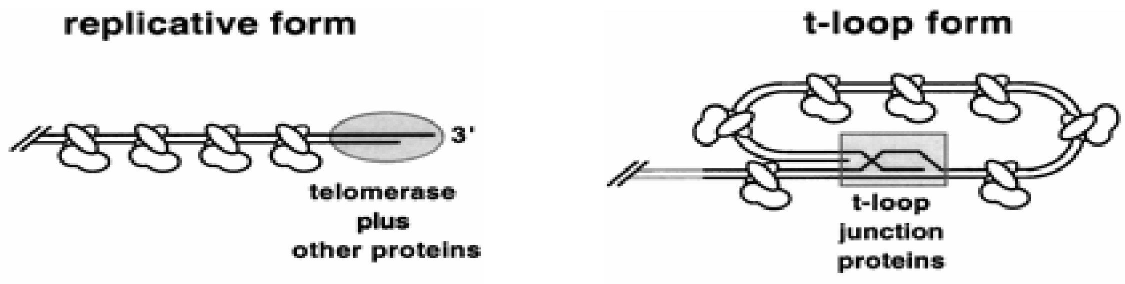
(PARP1)

# Telomere = “end-part” of chromosomes
## (end structures made up telomeric DNA + Proteins)




-Telomeric DNA contains simple, tandemly repeated sequences, *TTAGGG* in human

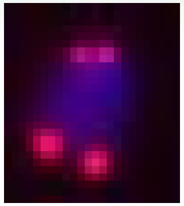
-Double strand (several Kbs) and single strand overhang (50-100 bps)

-Telomere binding proteins and associated Proteins

-Special structure (heterochromatin or T-loop…)

# Telomeres cap and protect chromosome ends

**Normal telomere**

**Cellular senescence**

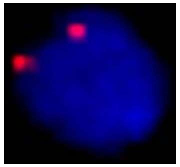
**Premature aging syndromes**

**Aging**

**DNA damage**

**Telomere capping defect**

- Loss of protection by telomere associated proteins
- Loss of telomeric DNA

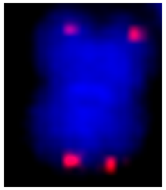


DNA damage response

Cell death

cell senescence

DNA damage repair
at chromosome ends
(chromosome end-to-end fusions)

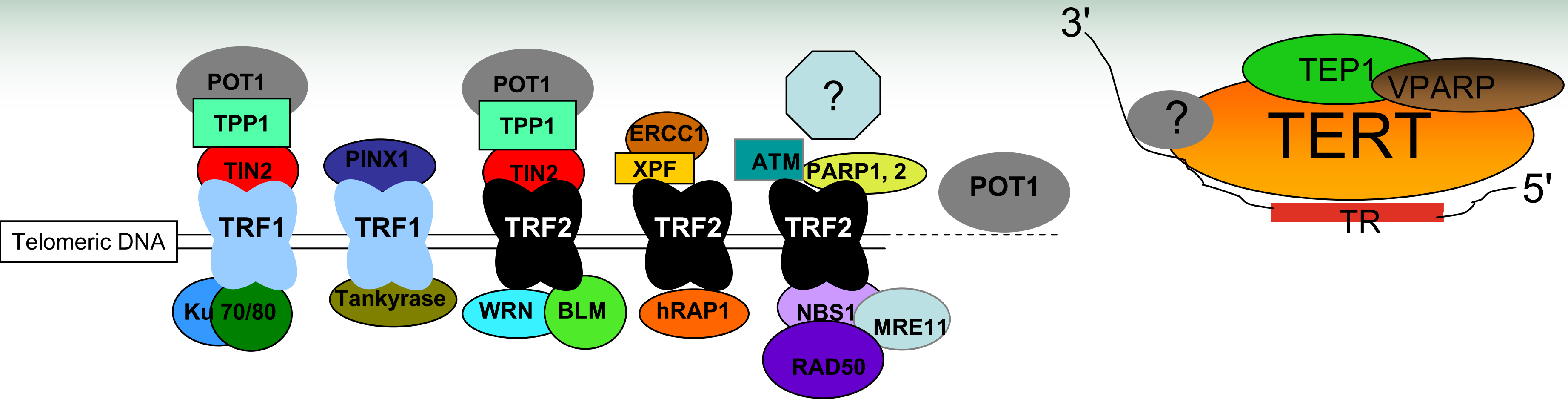


fusion/breakage cycles

genomic instability

Cancer

# Mammalian telomere maintenance




**<u>Telomere maintenance</u>**

1. Telomerase
2. Telomerase independent, alternative lengthening of telomeres (ALT)
3. Telomere protein complex: protect and regulate telomere and its structure

• ***Telosome or Shelterin***

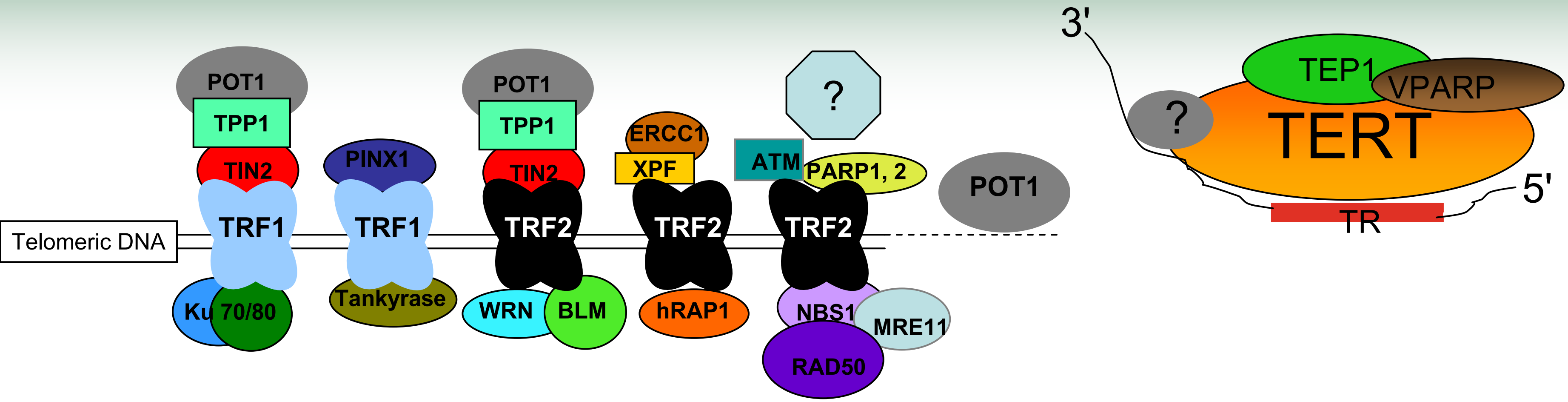
*-Telomere binding proteins (TRF1, TRF2, POT1) & their associated proteins (TIN2, TPP1, RAP1)*

•***Telomere associated proteins***

*-Tankyrase, hnRNPs*

*-DNA damage response/repair (WRN, BLM, ERCC1/XPF, RAD50/MRE11/NBS1, KU/DNA-PKC, ATM, PARP2)*

# Mammalian telomere maintenance




Research directions

1. Telomerase
2. DNA damage response/surveillance/repair proteins
3. Posttranslational modifications (PTMs) of telomerase and telomere associated proteins affect telomere capping function

Recent studies of telomere capping in mice

1. DNA damage repairs activated at eroded telomeres
2. Mechanisms of DNA damage repair/response proteins in repairing eroded telomeres

# PART I
# DNA damage repairs at critically shortened telomeres caused by telomerase deficiency in mice

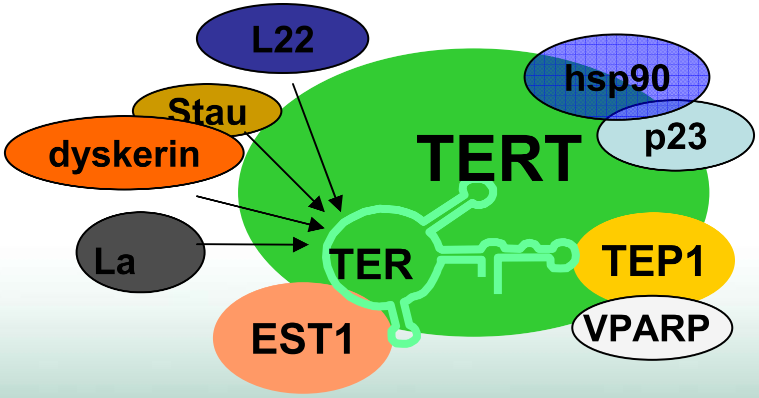
## Telomerase: Telomere terminal transferase (RNP)

•Core components: telomerase RNA (TER) & telomerase reverse transcriptase (TERT). Evolutionally conserved.

•Both essential for reconstitution of telomerase activity *in vitro* and *in vivo*

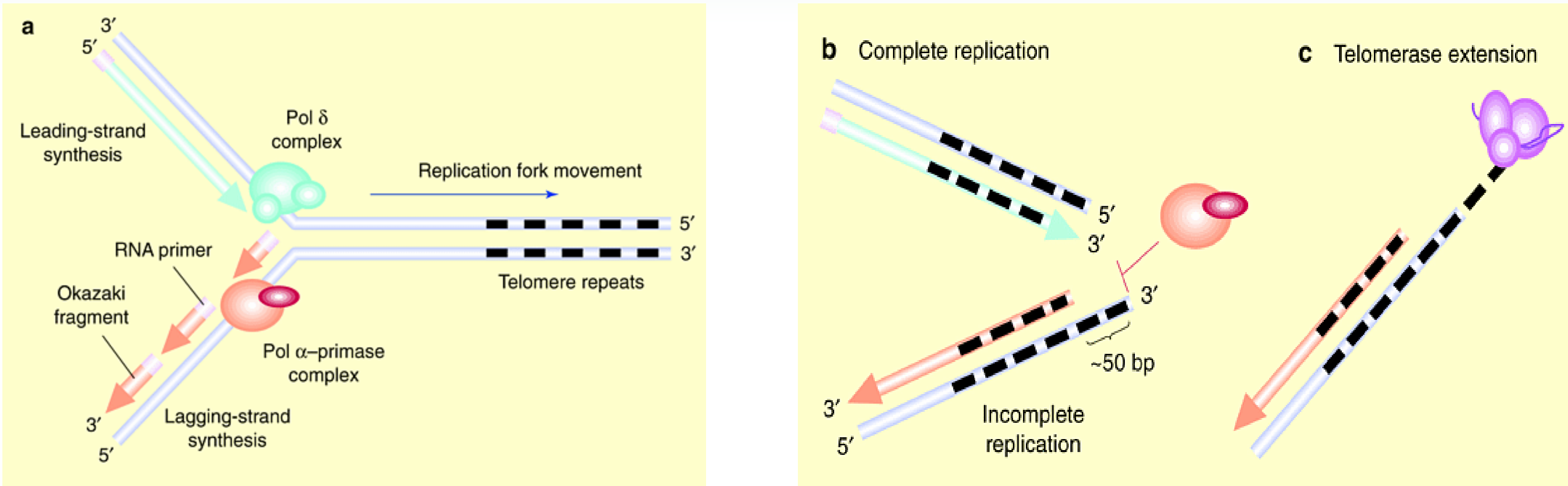
•Other telomerase associated proteins

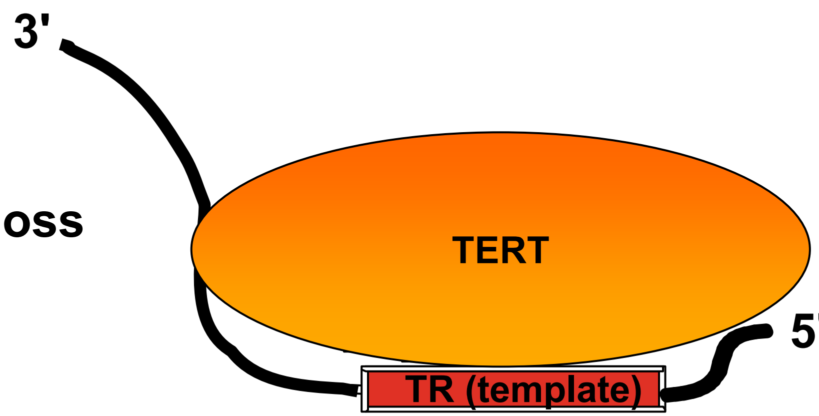
**Telomerase assembly,**
**Telomerase folding,**
**processivity & activity,**
**substrate recognition,**
**recruiting telomerase to telomere**



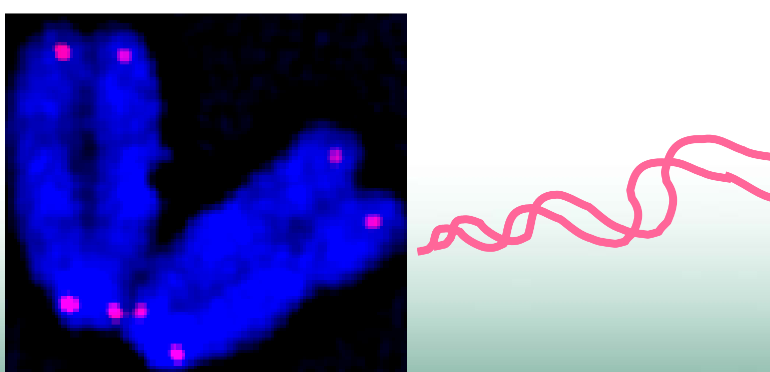
# End-replication problem-telomere loss in cell division

## Lost at a rate of 40 - 200 bp per cell division




## Telomerase – replenish telomere loss



ttaggg ttaggg ttaggg tta-3’
aatccc aatccc-5’

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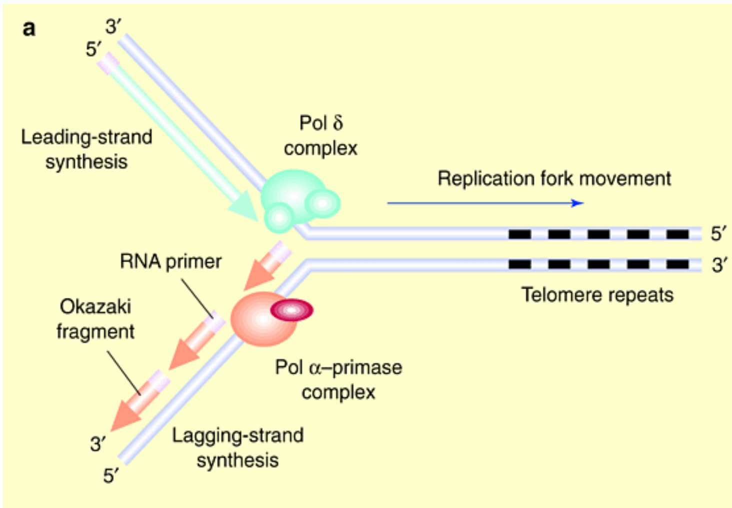


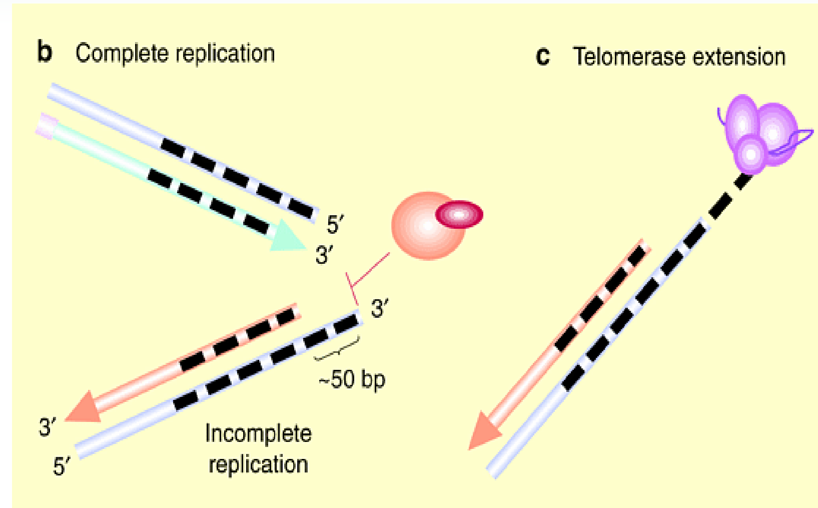


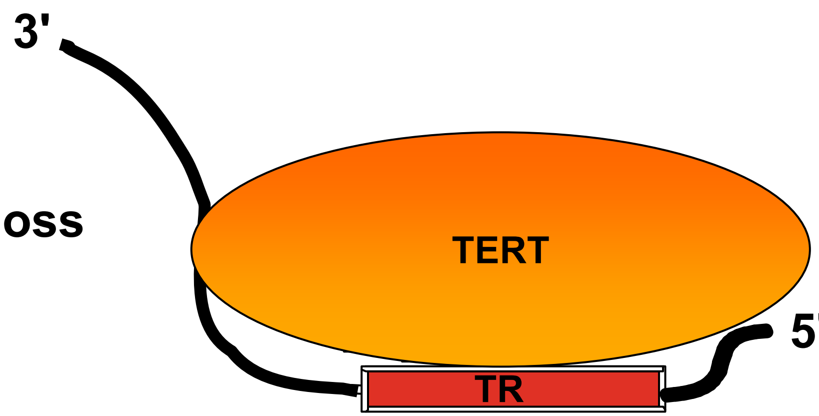


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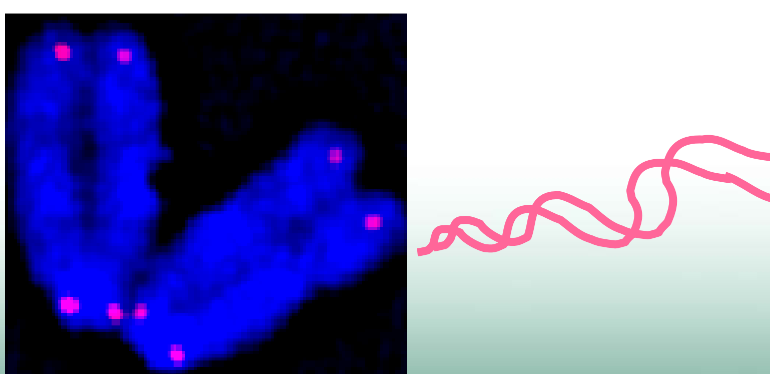

**ttaggg ttaggg ttaggg ttaggg ttaggg tta-3’**
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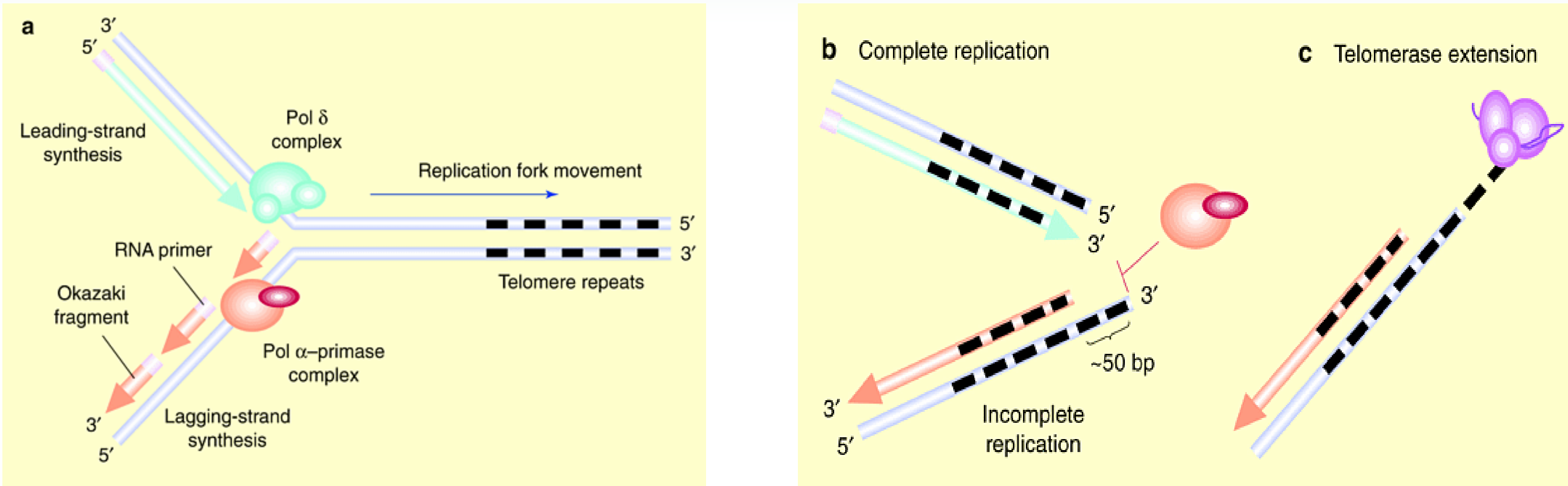
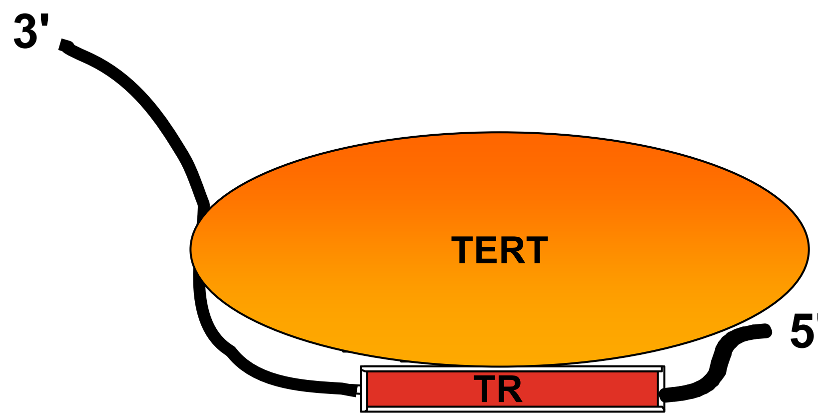
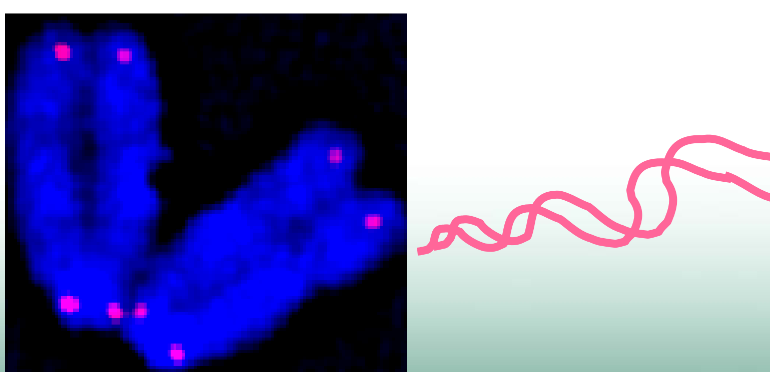



## Telomerase – replenish telomere loss



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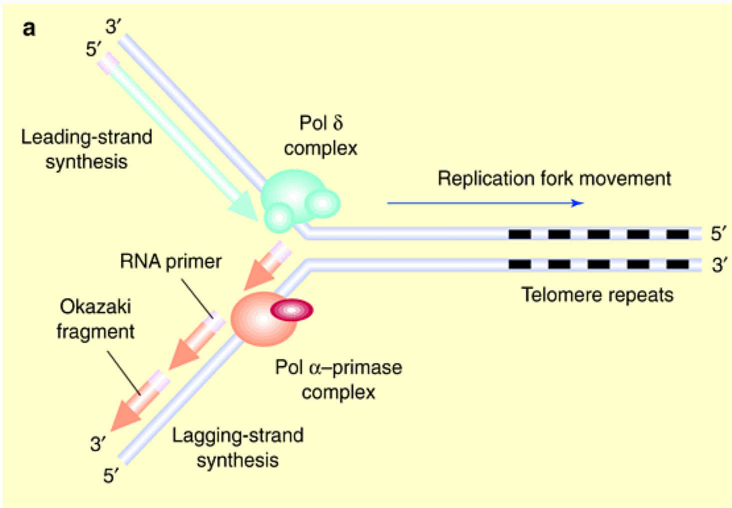
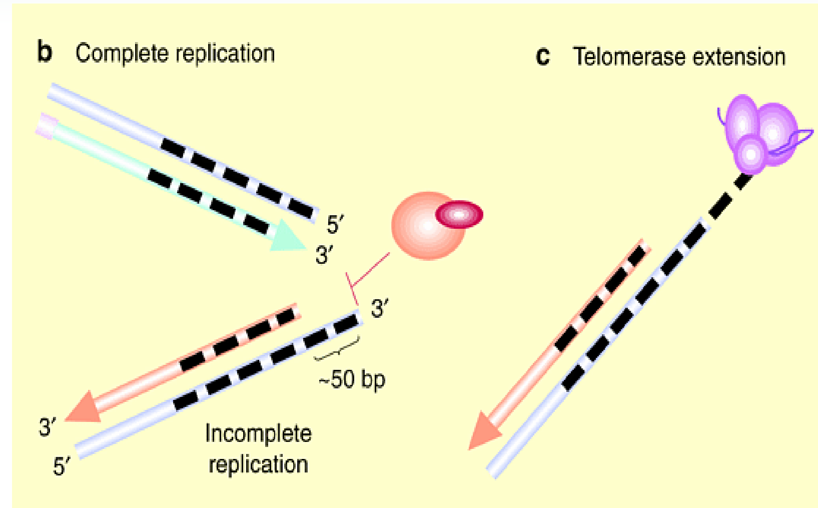
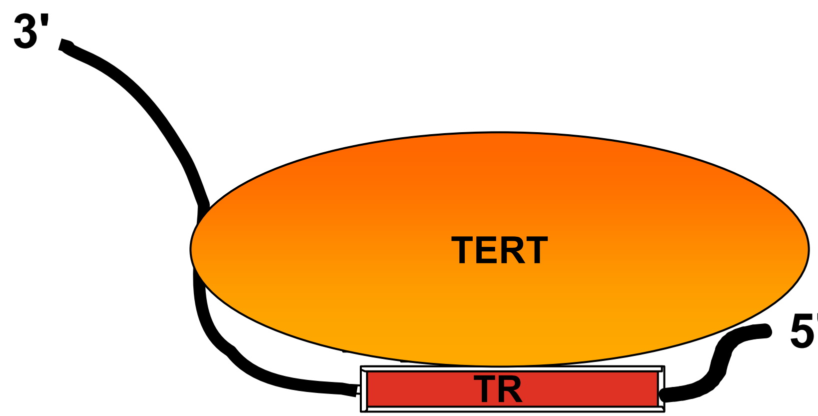
# End-replication problem-telomere loss in cell division

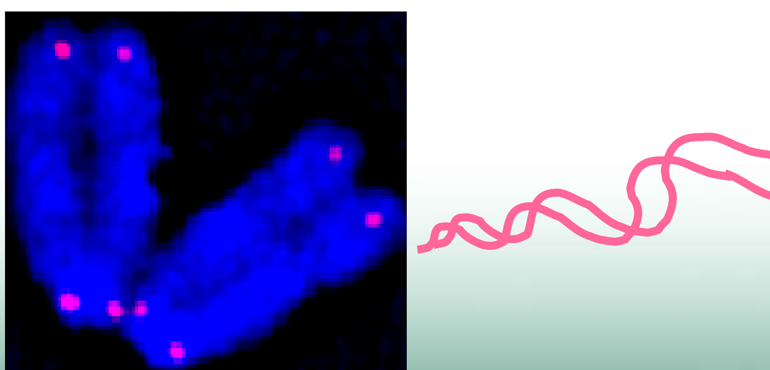
**Lost at a rate of 40 - 200 bp per cell division**



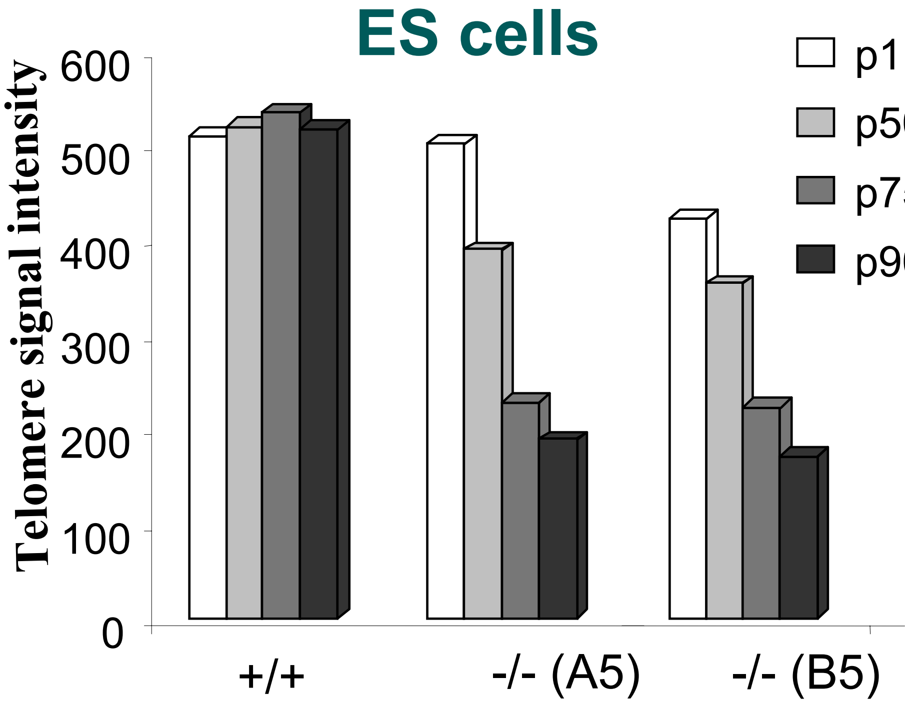



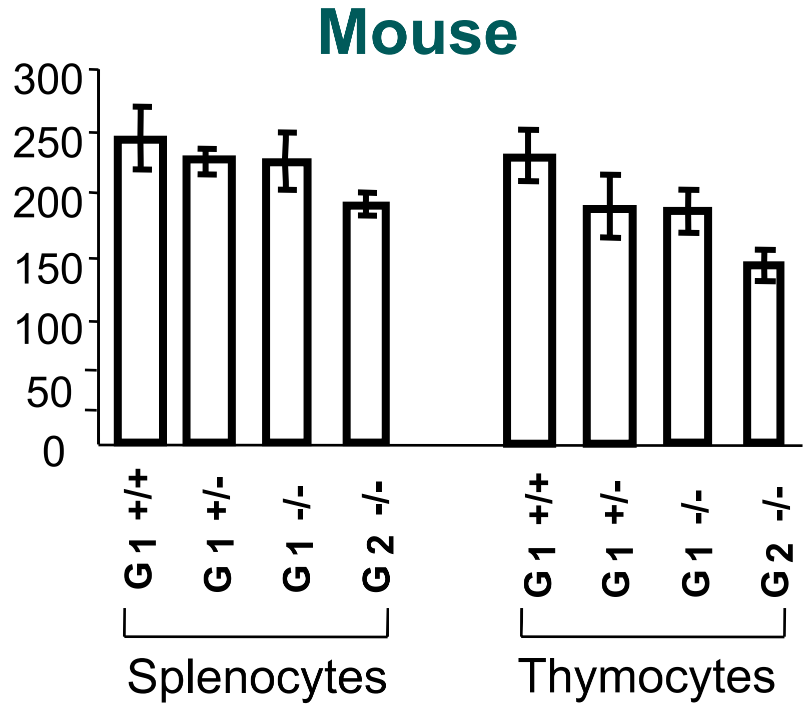

**Telomerase – replenish telomere loss**

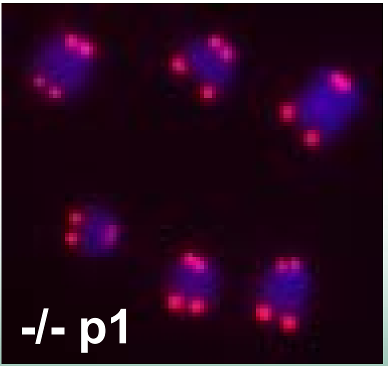


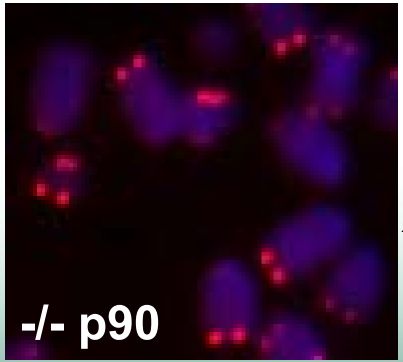
**ttaggg ttaggg ttaggg ttaggg ttaggg ttaggg ttaggg tta-3’**
**aatccc aatccc-5’**

## Progressive telomere loss in *mTert* -/- ES cells during prolonged culture and in successive generations of *mTert* -/- mice



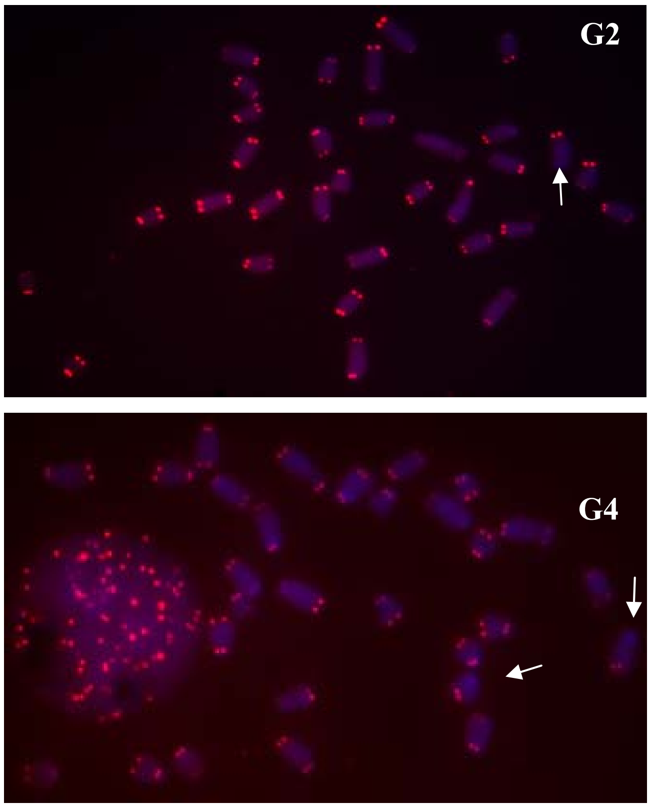




SFEs

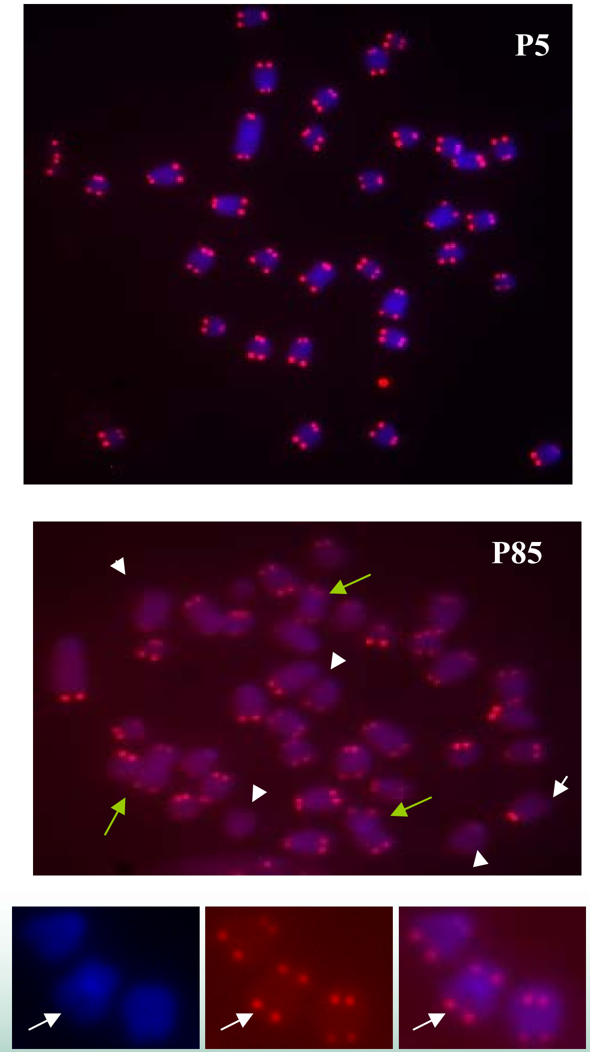
Liu Y, et al *PNAS* 99:3597, 2002
Liu Y, et al *Current Biology* 10:1459

# Chromosome end-to-end fusions (mostly end product of NHEJ) at ends with telomere loss in *mTert* -/- ES cells during prolonged culture, not in late generations of *mTert* -/- mouse

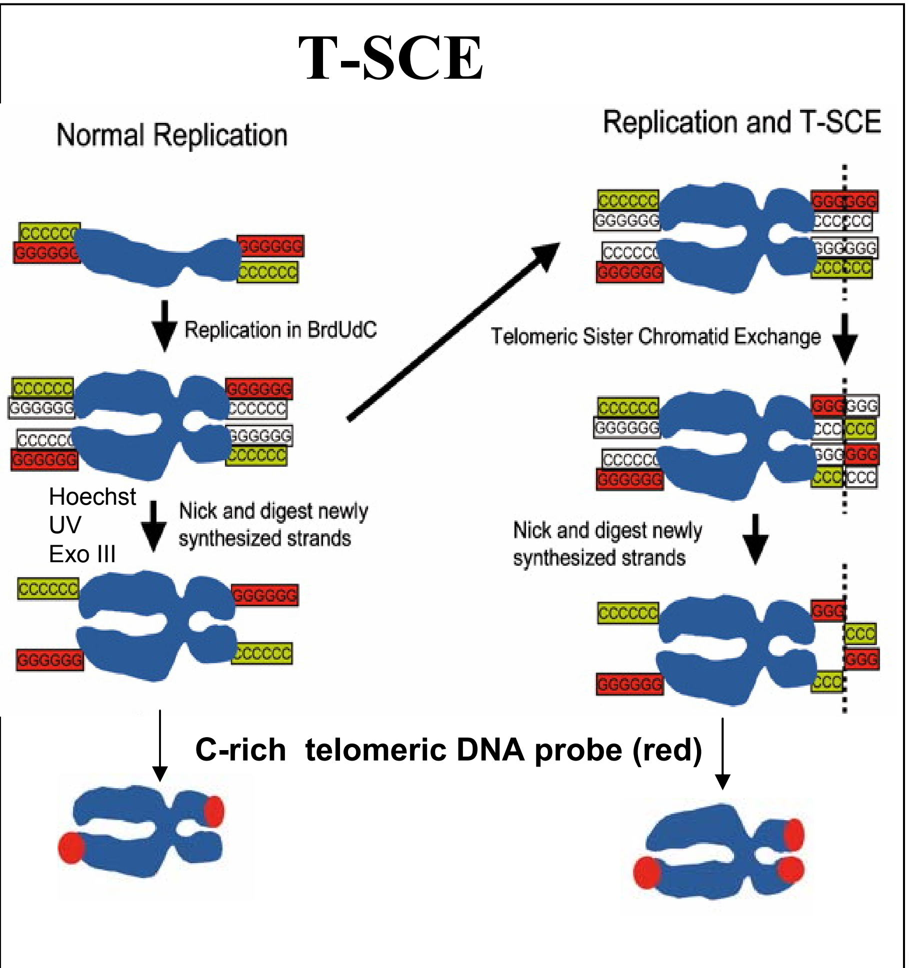
**Tert$^{-/-}$ mouse splenocytes**



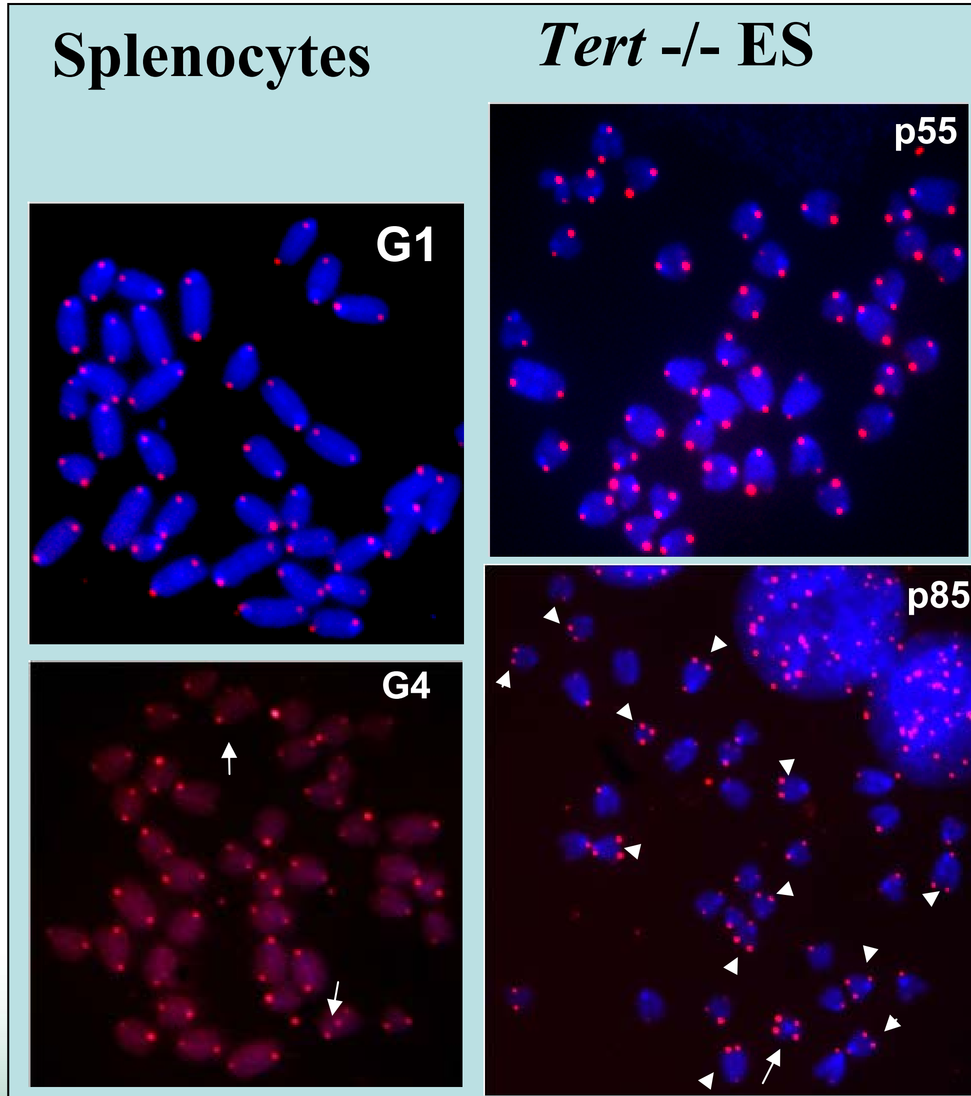

**Tert$^{-/-}$ ES cells**



# Telomere sister chromatid exchange in telomere length maintenance in *mTert* -/- ES cells and animals




Bechter OE et al, Cancer Res. 64:3444

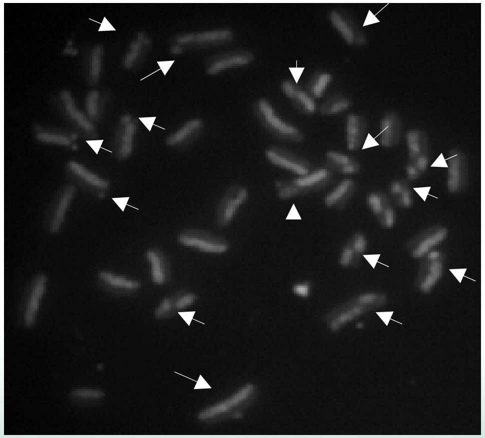


# T-SCEs are not the solo byproducts of G-SCE or end joining of extra chromosomal telomeric DNA fragments in *mTert* deficient ES cells

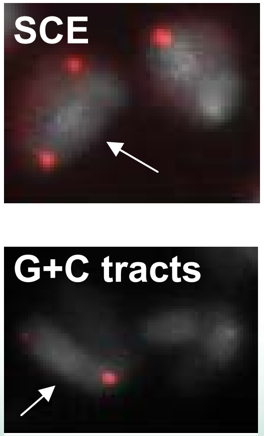
**Frequencies of T-SCEs, SCEs, and G+C telomere tracts in ES cells with telomerase deficiency**

| Cell type | % (No. of G-SCEs /chromosomes) | % (No. of interspersed G+C telomere tracts /chromosomes) |
|---|---|---|
| ES cells | | |
| Wt p5 | 42.9% (1735/4049) | 0% (0/2014) |
| Wt p85 | 35.0% (1361/3888) | 0.9% (18/1943) |
| *mTert-/-*p5 | 33.1% (1225/3701) | 0.9% (17/1855) |
| *mTert-/-*p85 | 44.0% (1756/3992) | 2.6% (52/2001) |

After removing influential factors (SCE & interspersed G+C tracts), the rate of T-SCEs was 4.8 times higher in *mTert-/-* ES cells p85 than *mTert-/-* ES cells p5 (*P<0.01*).

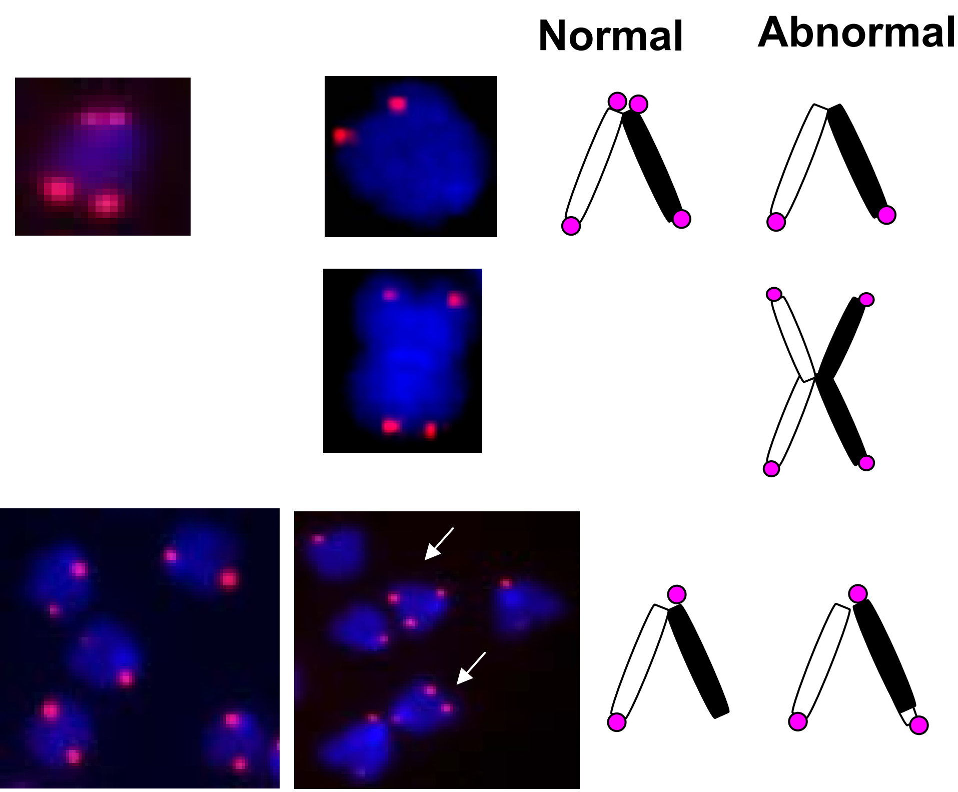


SCE




CO-FISH/SCE

# Cellular response and genomic rearrangements at critically shortened telomere differ in *mTert* -/- ES cells and animals




| | Animal | ES cells |
|---|---|---|
| Critically shortened telomeres | + (17.9%) | + (20.2%) |
| End-to-end fusion | - | + |
| T-SCE | ± | +++ |
| Cell viability | - (spleen atrophy) | + |

Liu Y, et al *PNAS* 99:3597, 2002
Wang Y, Erdmann N, Giannone JR, Wu J, Liu Y. *PNAS* 102:10256, 2005. *Cell Cycle*, 4:1320. 2005.

# Conclusion

1. Critically shortened telomeres appear to trigger NHEJ and homologous recombination between telomeres in mouse ES cells during long term culture.

2. These genomic rearrangements may help mask and maintain critically shortened telomeres, thus cell viability.

3. There may be different cellular response to critically shortened telomeres, depending on cell types or on animals/culture cells.

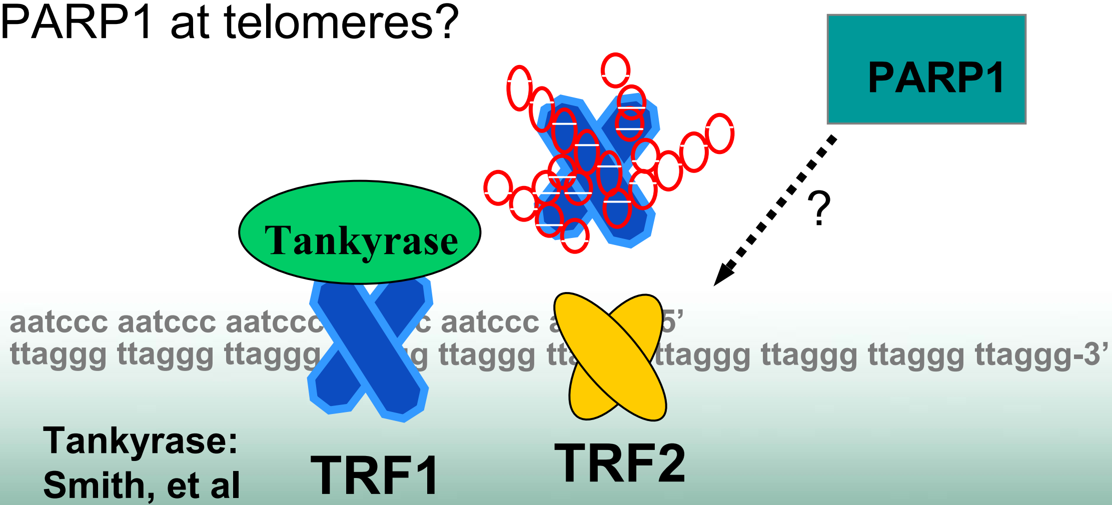
# PART II
# A base-excision DNA repair protein, PARP1, associates with eroded telomeres and involves in telomere capping *in vivo*

**PARP1**

● activated by and bound to DNA strand breaks, involved in DNA damage repair

● Poly(ADP-ribose) polymerases (family members: >18) catalyze the addition of poly(ADP-ribose) to acceptor proteins (itself and other proteins, Histones, in response to DNA damage)

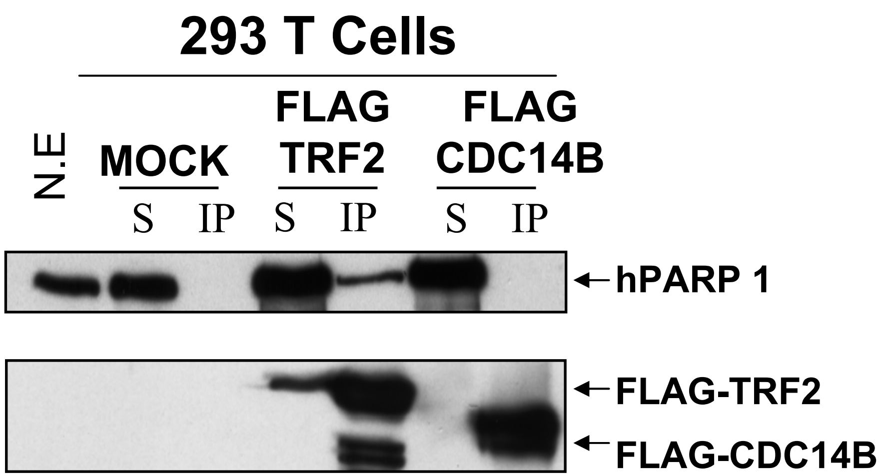
● Mass Spec. identification of PARP1 in TRF2 pulldown
● Is PARP1 associated with telomeres?
● when is PARP1 associated with telomeres?
● What is the role of PARP1 at telomeres?



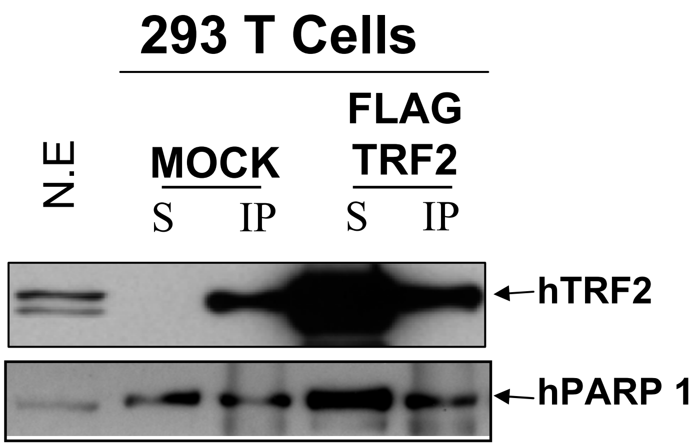

Tankyrase:
Smith, et al

# PARP1 interacts with telomere associated protein, TRF2, in human cells

## FLAG-TRF2 IP



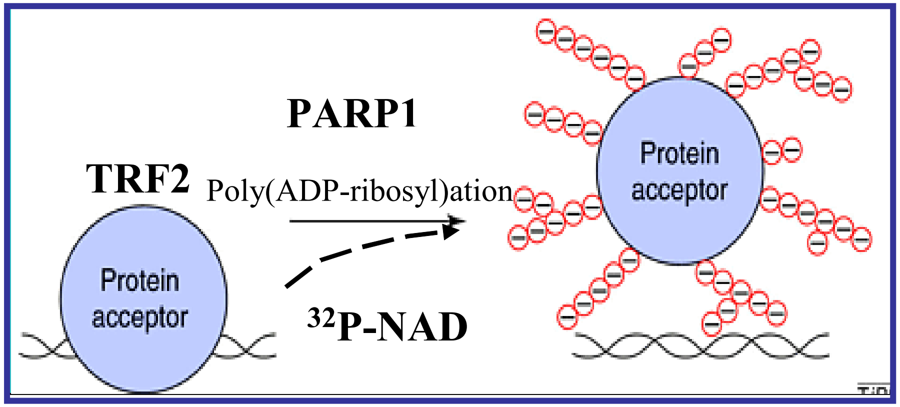

## Anti-PARP IP

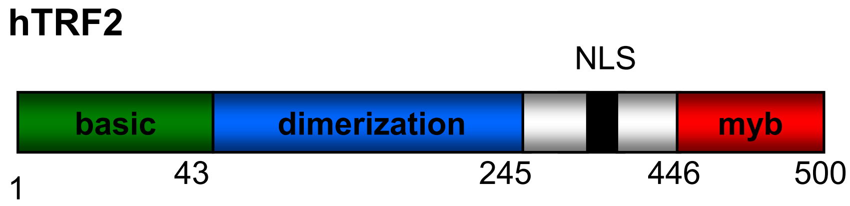


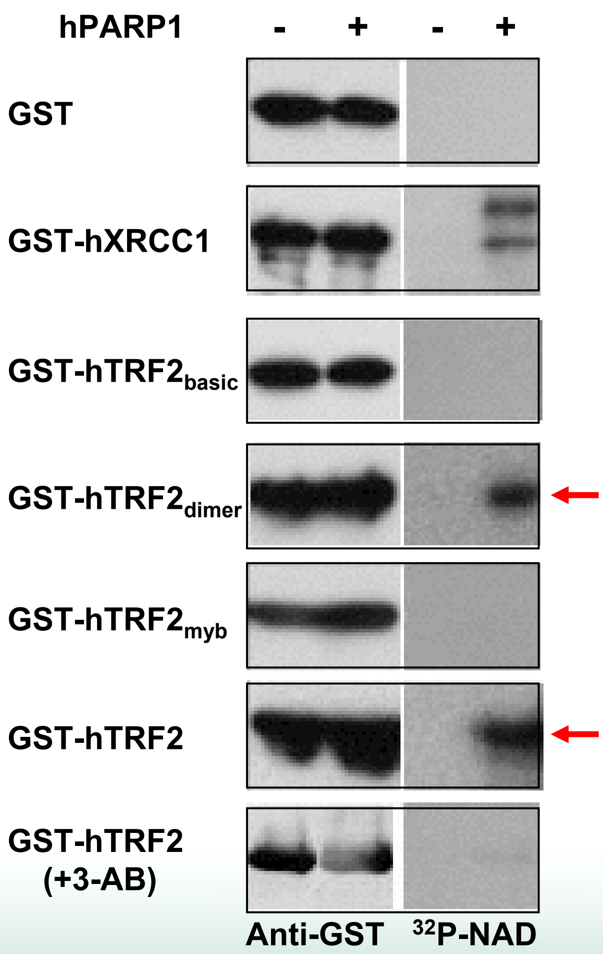

-Interacting via DNA binding and BRCT domains of PARP1 & Myb domain of TRF2

-Interaction is not mediated by DNA

# Poly(ADP-ribosyl)ation of TRF2 by PARP1

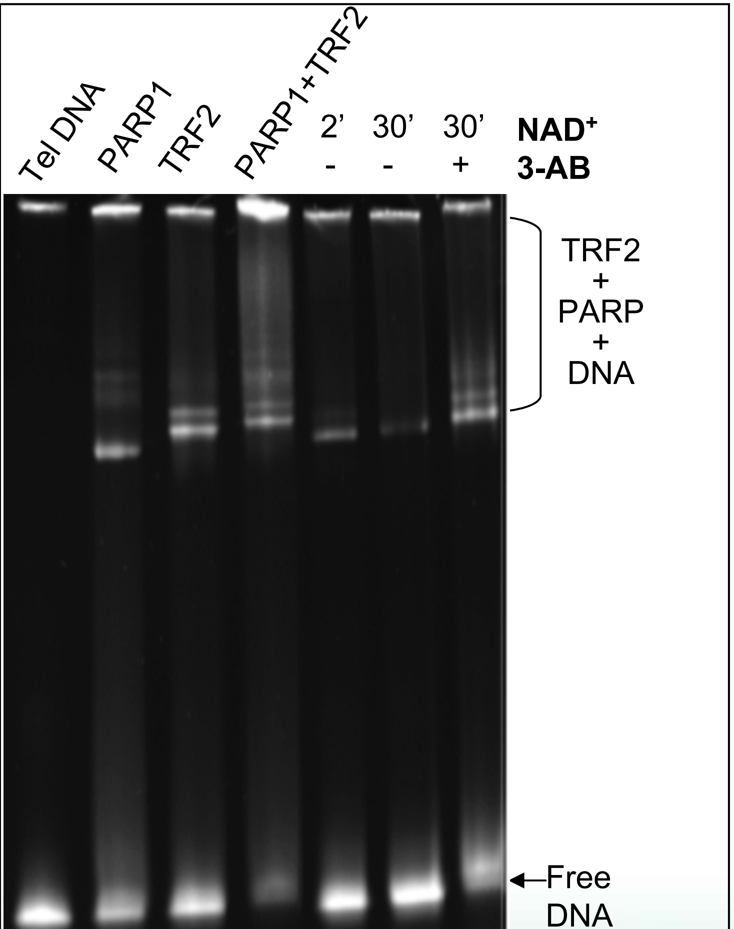


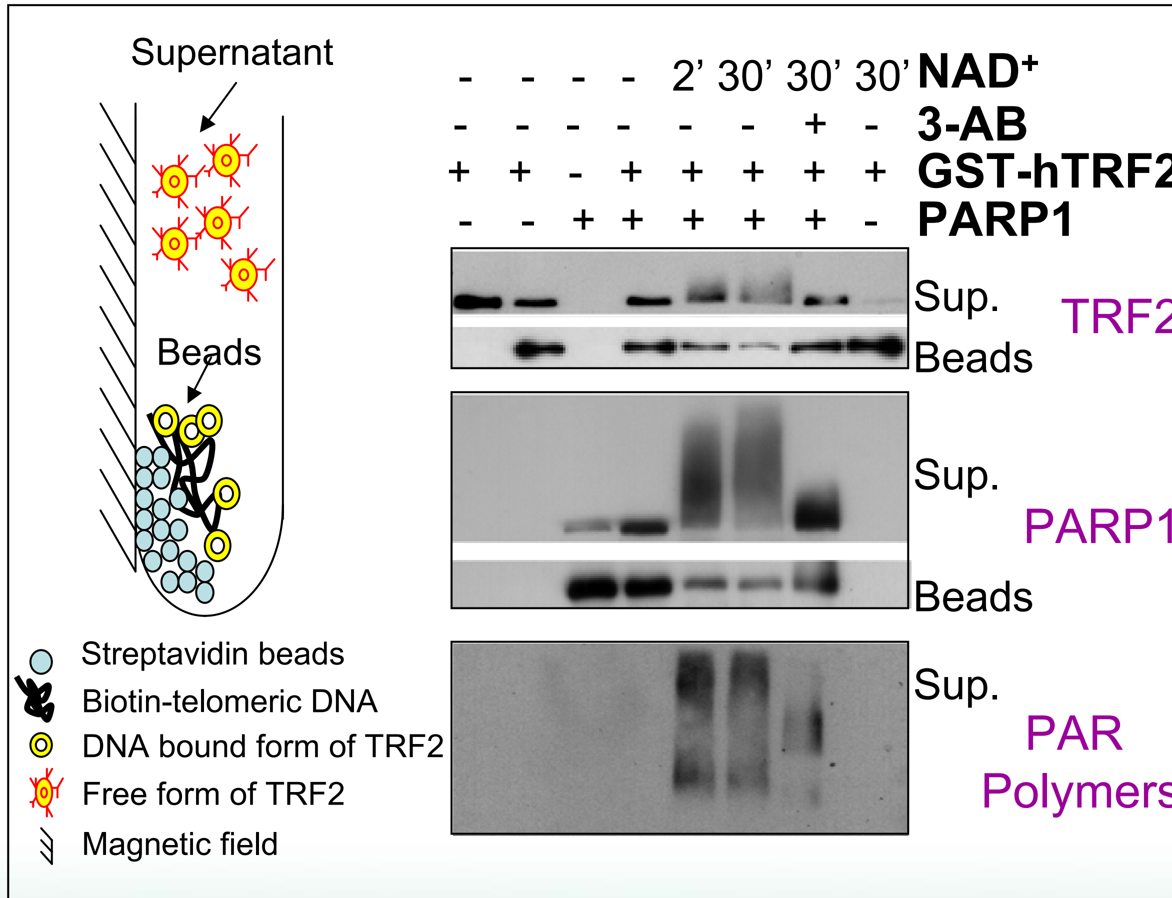



# Poly(ADP-ribosyl)ation affects the TRF2 bound to telomeric DNA

## Electrophoretic Mobility Shift Assay

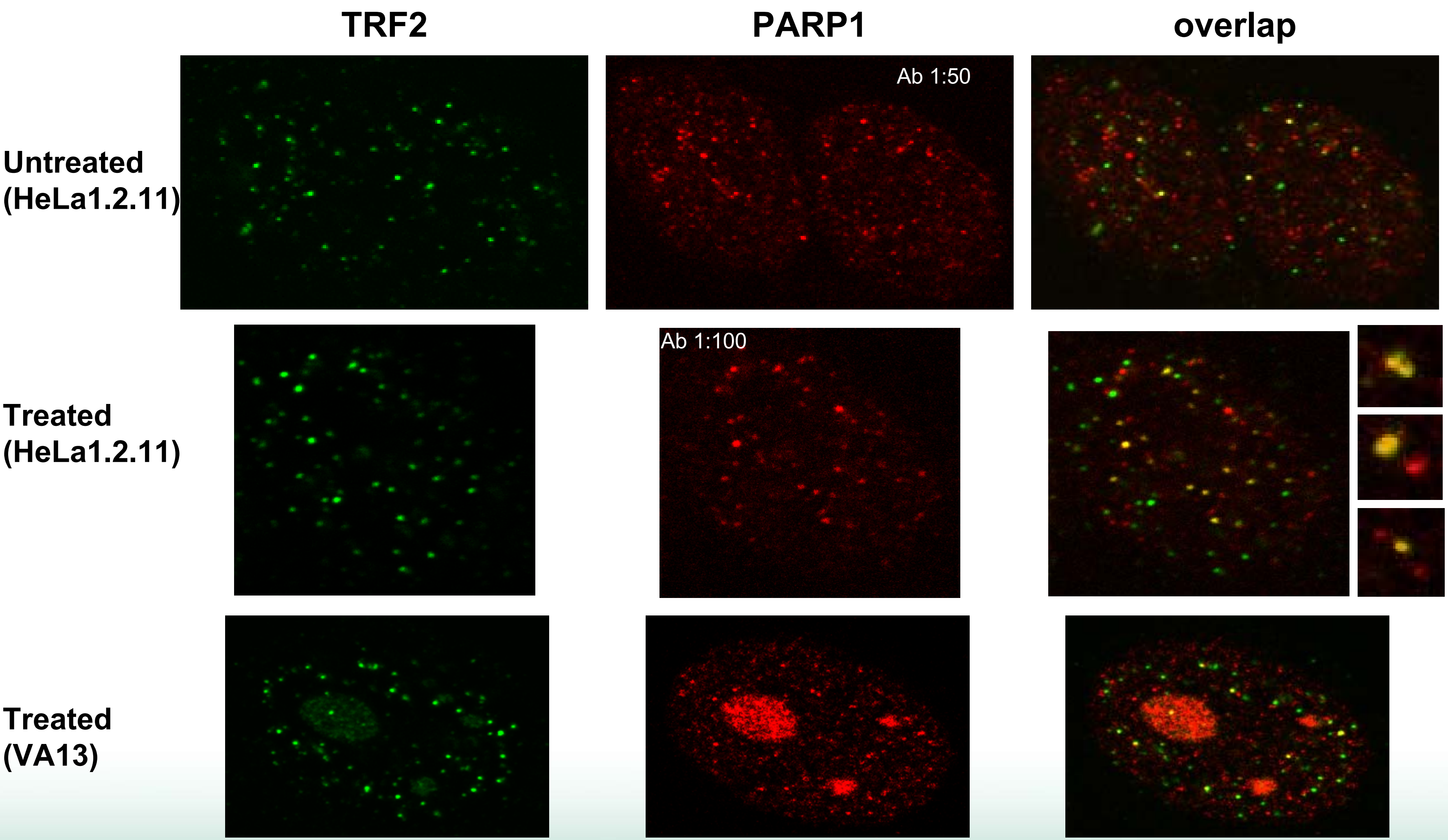



## Immobilized dsTelomeric DNA binding Assay




5’-GGCTGCTACCGGCACATCGTCCTAGCAAGG**TTAGGGTTAGGGTTAGGGTTAGGG**-3’
3’-CCGACGATGGCCGTGTAGCAGGATCGTTCC**AATCCCAATCCCAATCCC**-5’

# PARP1 rarely co-localizes with TRF2 in normal cells, but in cells exposed to DNA damaging reagents

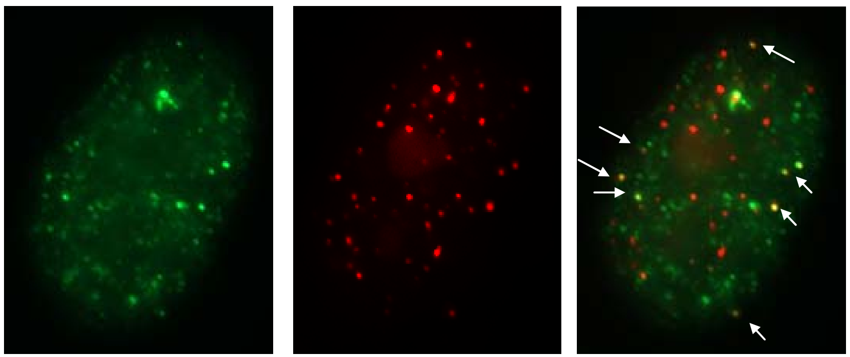



DNA damaging reagents: X-ray, MMS, $H_2O_2$

# PARP1 was detected at telomeres, with DNA damage

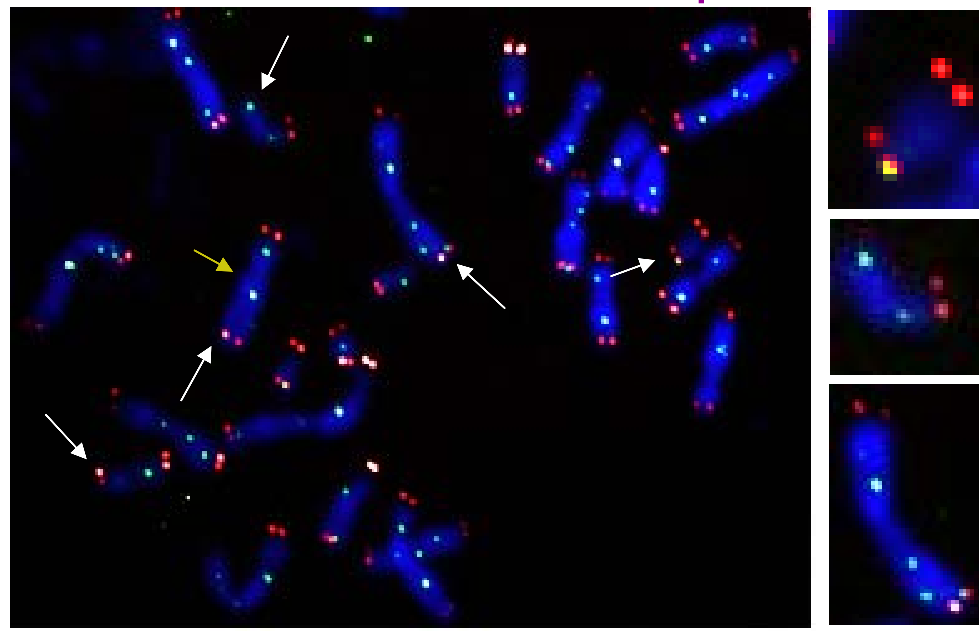
## HeLa 1.2.11 Interphase

**PARP1** **Telomeric DNA** **Merge**



10mM $H_2O_2$

## HeLa 1.2.11 Metaphase



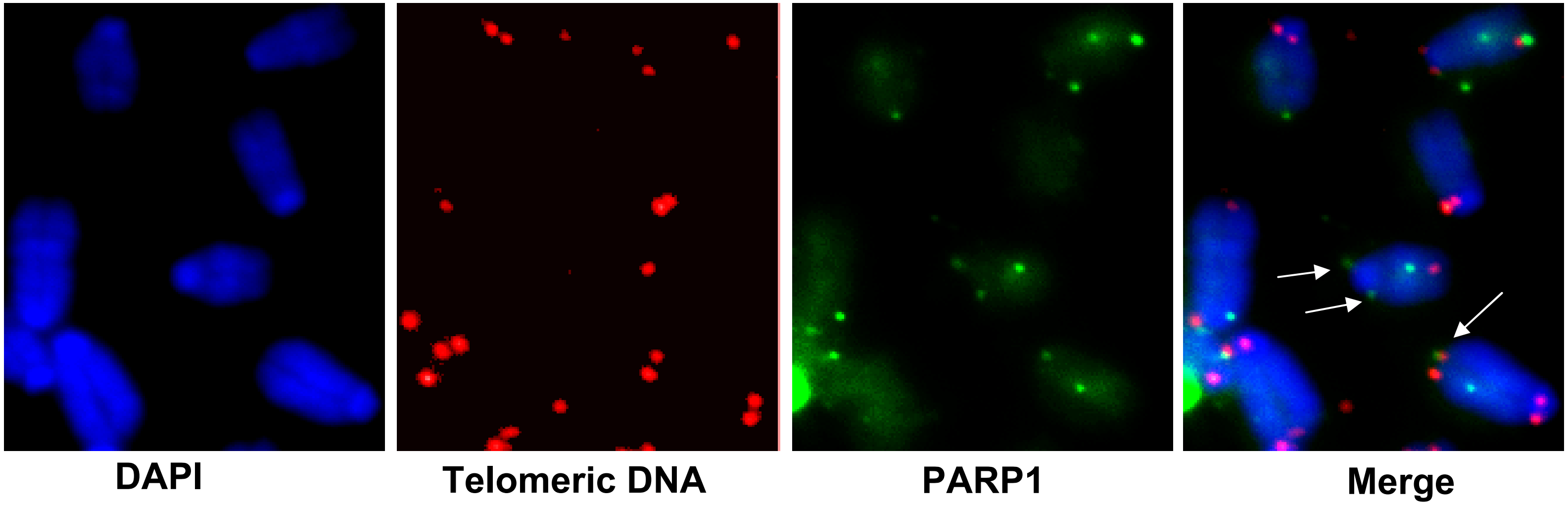
5 Gy X-ray

**PARP1 signals at telomeres in untreated or radiation-treated HeLa1.2.11 cells**

| Cell type | #Chromosome-associated PARP1 /# chromosomes | #Telomere-associated PARP1 /# chromosomes | #SFE-associated PARP1 /# SFEs |
|---|---|---|---|
| Untreated | 22/1523 (0.014) | 3/1523 (0.002) | 0/4 (0%) |
| X-ray (5 Gy) | 1552/1666 (0.932)[d] | 329/1666 (0.197)[d] | 58/196 (30%) |

# PARP1 was detected at critically shortened telomere (i.e. aging)

Tert-/-
ES cells
p90



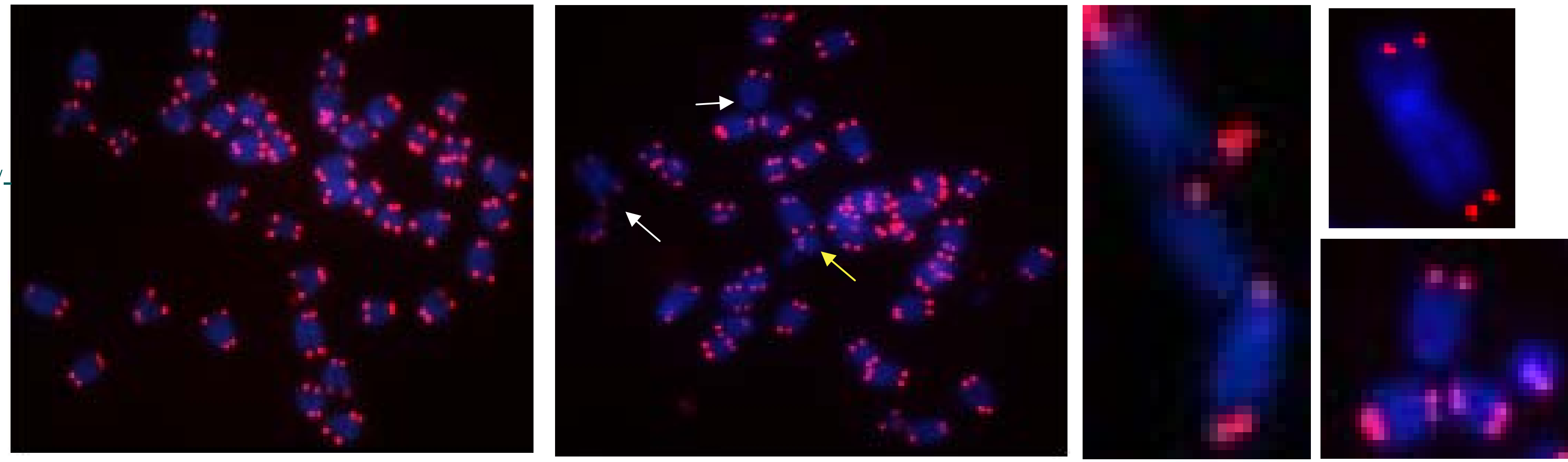

Increased PARP1 signals at eroded telomeres in *mTert* deficient mouse ES cells during culture

| Cell type | #Chromosome-associated PARP1 /# chromosomes ([a]) | #Telomere-associated PARP1 /# chromosomes ([b]) | #SFE-associated PARP1 /# SFEs (%[c]) |
|---|---|---|---|
| *Wild type* | | | |
| P30 (Normal) | 187/1010 (0.185) | 15/1010 (0.0149) | 0/0 |
| P90 (Normal) | 231/1002 (0.231) | 51/1002 (0.051) | 0/0 (0%) |
| *mTert$^{-/-}$* | | | |
| P30 (short) | 313/1043 (0.3) | 78/1043 (0.075) | 5/9[e] |
| p90 (critically short) | 946/1025 (0.923) | 315/1025 (0.307) | 131/526[e] (25%) |

# PARP1 deficiency leads to telomere capping defects and genomic instability *in vivo*

Untreated | X-ray

primary *mParp1*$^{-/-}$ MEFs (p2)



Chromosome abnormalities in untreated or radiation-treated *mParp1* deficient primary MEFs

| Cell type | End-to-end fusions /chromosomes | SFEs /chromosomes | Chromosome breakages /chromosomes |
|---|---|---|---|
| **Untreated (3% oxygen)** | | | |
| Wild type | 0/2034 (0) | 6/2034 (0.003) | 1/2034 (0) |
| *mParp*$^{-/-}$ | 2/2087 (0) | 8/2087 (0.004) | 17/2087 (0.008) |
| **X-tray (5 Gy)** | | | |
| Wild type | 7/2050 (0.003) | 192/2050 (0.094) | 86/2050 (0.042) |
| *mParp*$^{-/-}$ | 44/2071 (0.021) | 352/2071 (0.017) | 269/2071 (0.13) |

# PARP1 is a TRF2-associated poly(ADP-ribose) polymerase and protects eroded telomeres

1. PARP1 interacts with TRF2 *in vivo*

2. PARP1 poly(ADP-ribosyl)ates the dimerization domain of TRF2 and dissociate TRF2 from telomeric DNA

3. PARP1 colocalizes with TRF2 in cells exposed to DNA damaging reagents

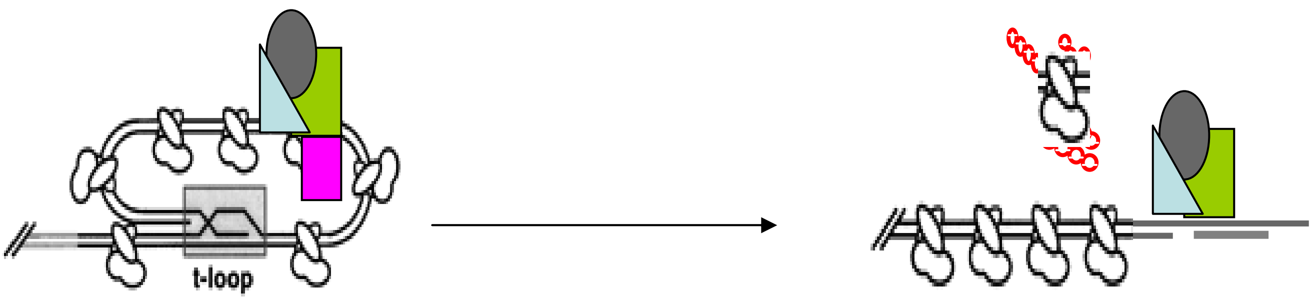
4. Damaged or critically short telomeres can recruit PARP1

5. PARP1 is dispensable for normal telomere function, but is involved in repairing damaged telomeres

**Gomez M, Schreiber V, Wu J, Dunlap J, Dantzer F, Wang Y, Liu Y**
***MBC,* Jan 25, 2006.**

# PARP1 involves in repairing eroded telomeres

*PARP1 at critically short or damage telomeres (aging)*




**TRF2'release leads to T-loop resolution**

↓

**DNA repair machinery (DNA pol. XRCC1, DNA lig, et al) repairs damaged telomere**

# PART III

# Genetic analysis of a key mismatch repair (MMR) protein, MSH2, in telomere capping *in vivo*

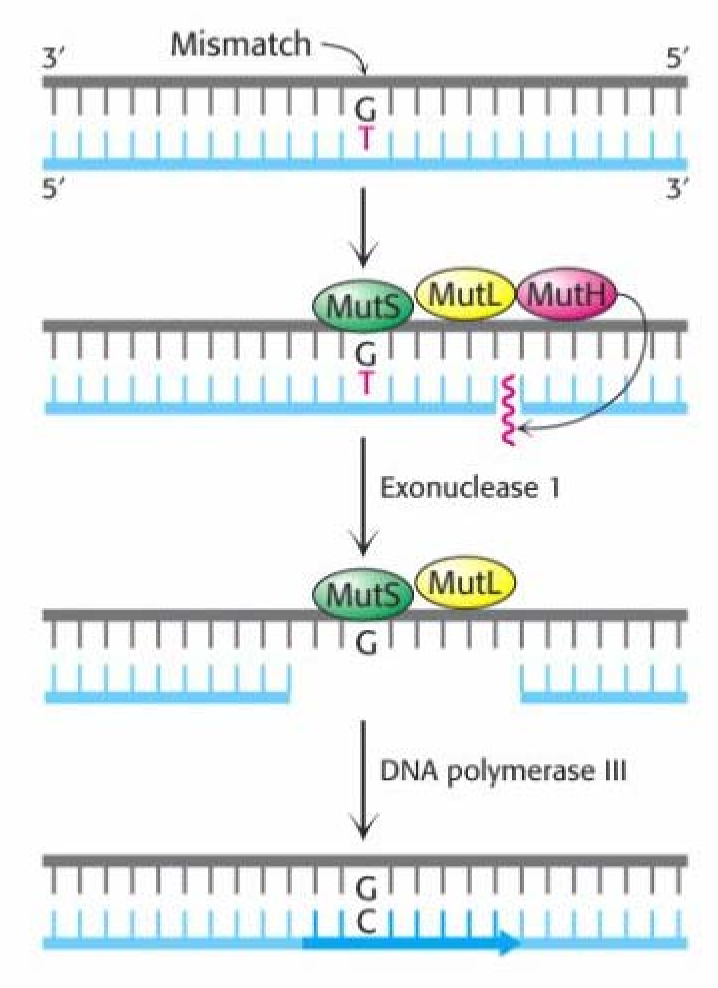
**•*MMR (Msh2, Msh3, Msh6, Mlh1, Pms1, Pms2,* and *Mlh3)***

**• Msh2, bound to either Msh6 (MutSα) or Msh3 (MutSβ), initiates the recognition of a base mispair and the subsequent recruitment of additional MMR proteins to complete the repair process**

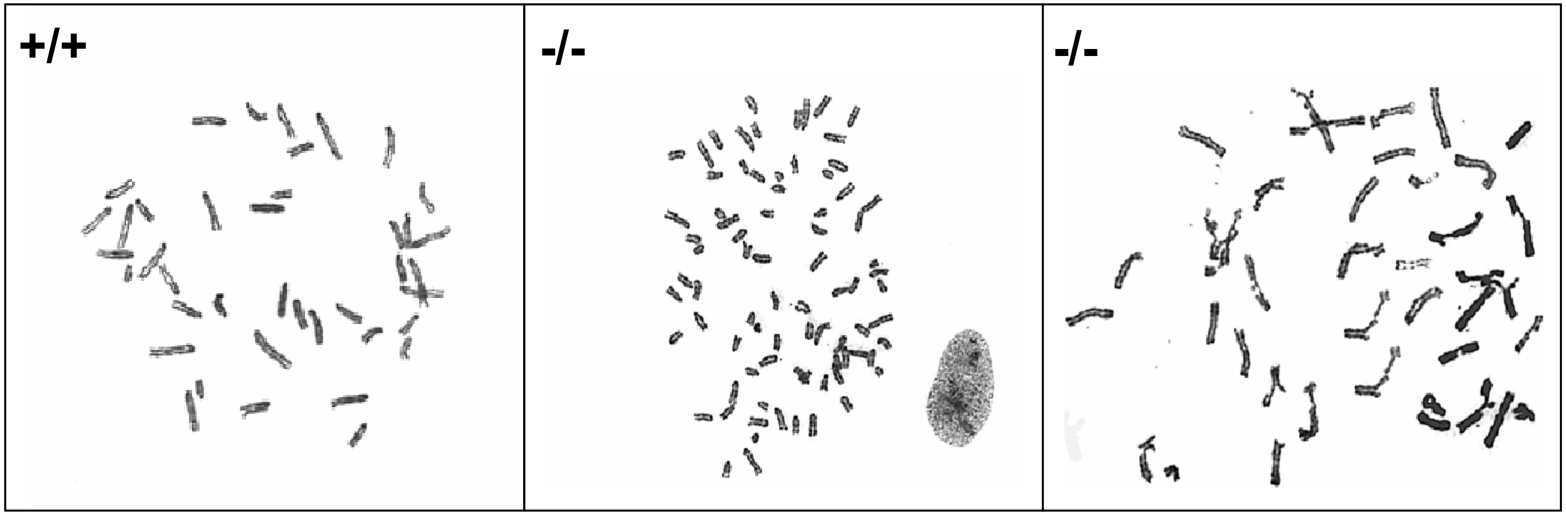
**•Human patients inherit a heterozygous mutation in one of the MMR genes, most commonly *hMSH2* or *hMLH1*, develop the human cancer syndrome Hereditary Non-Polyposis Colorectal Cancer (HNPCC)**

**•Mice completely deficient in one of the MMR genes, *Msh2, Mlh1* or *Msh6*, most commonly develop early onset thymic lymphomas**

***E.coli* MMR model**



# Increased chromosomal abnormalities, mainly aneuploidy, in *Msh2* deficient primary mouse embryonic fibroblasts



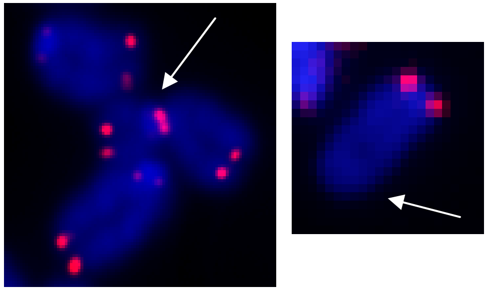

**Normal** **Aneuploid** **Chromosomal Breakage & fusion**

**Increased chromosome aneuploidy detected by Giemsa staining in primary *Msh2*$^{-/-}$ MEFs**

| Cell type | #Metaphases | #Aneuploid | # Diploid | Percentage |
|---|---|---|---|---|
| *Wild type* | 40 | 12 | 28 | 30% |
| *Msh2*$^{-/-}$ | 44 | 35 | 9 | 79.5% |

# Moderate telomere capping defect in *Msh2* deficient primary mouse embryonic fibroblasts

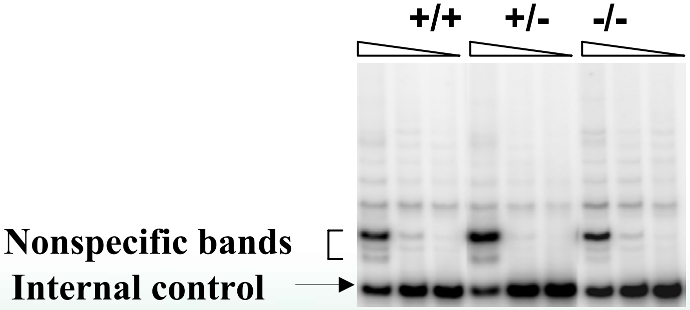
## Telomere capping defects



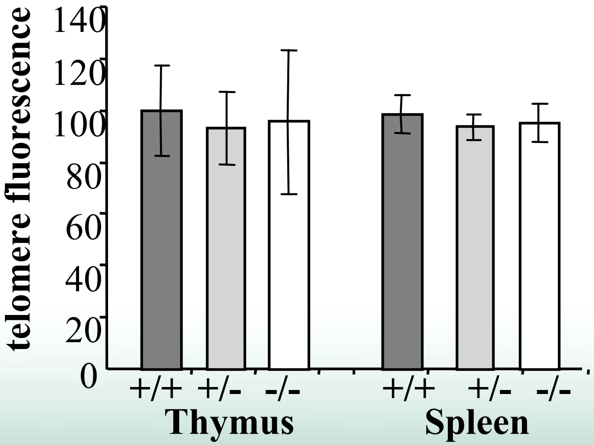
## Telomere capping detected in primary *Msh2*$^{-/-}$ MEFs

| Cell type | #Metaphases | #End-to-end fusions | # Telomere signal free ends |
|---|---|---|---|
| *Wild type* | 126 | 4 (3%) | 0 (0%) |
| *Msh2*$^{-/-}$ | 170 | 11 (6.5%) | 14 (8.2%) |

## Normal telomerase




## Normal telomere length



# Putative function of MSH2 at telomeres

## *<u>MSH2 is involved in telomere capping?</u>

***-Direct role: Association with telomere & telomere associated proteins?***

***-Indirect role: Increase in telomeric DNA mutation in MSH2 deficient cells alters binding affinities of telomere associated proteins to telomeres, led to loss of protection of telomere associated proteins?***

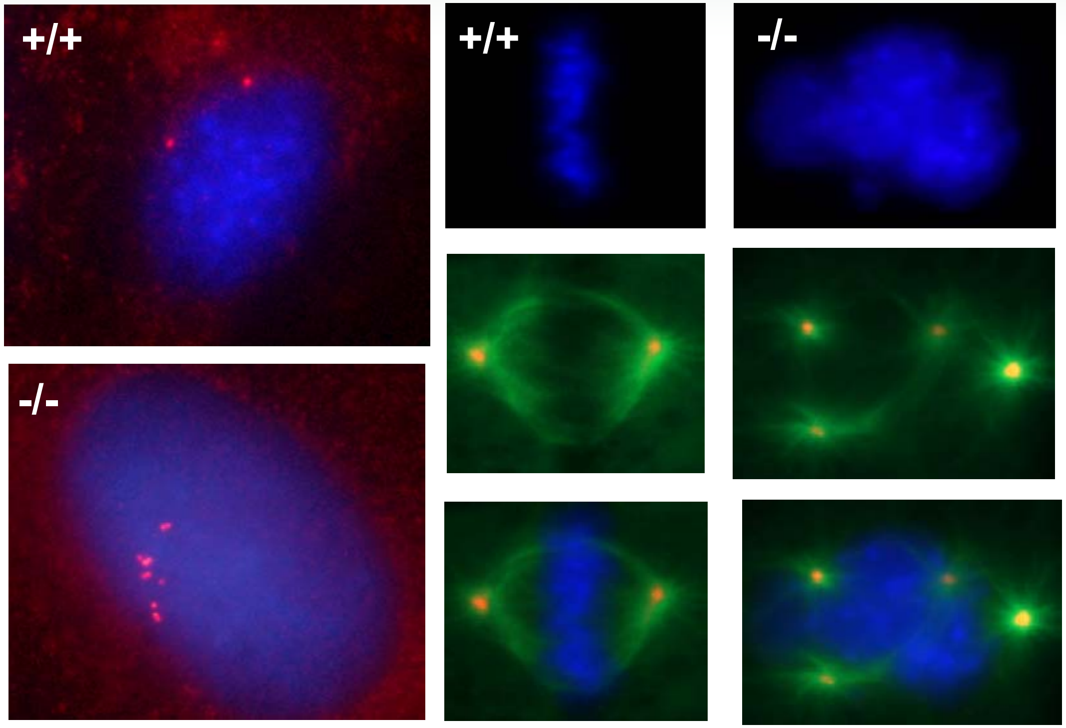
Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2.
Opresko PL, Fan J, Danzy S, Wilson DM 3rd, Bohr VA
Telomere instability detected in sporadic colon cancers, some showing mutations in a mismatch repair gene.
Pickett HA, Baird DM, Hoff-Olsen P, Meling GI, Rognum TO, Shaw J, West KP, Royle NJ

## *<u>Anti-recombination activity of MSH2 affect telomere maintenance in mammals?</u>

***Yeast: Yes***

***-MSH2 deficiency promotes recombination (or ALT) of critically shortened telomere in telomerase deficient cells in mice?***

# Centrosome amplification in *Msh2* deficient primary mouse embryonic fibroblasts




## Centrosome amplification in primary *Msh2*$^{-/-}$ MEFs

| Cell type | Number of centrosomes | | | | | Summary | |
|---|---|---|---|---|---|---|---|
| | n=1 | n=2 | n=3 or 4 | n=5-6 | n≥7 | n=1 or 2 | n≥3 |
| *Wild type* | 21.9% | 66% | 9.9% | 1.8% | 0.4% | 87.9% | 12.1% |
| *Msh2*$^{-/-}$ | 11.3% | 54.8% | 23.7% | 6.6% | 3.5% | 66.1% | 33.9% |

# MSH2-conclusion

1. A novel role of *Msh2* gene in maintaining chromosome stability, through regulating centrosome fidelity and telomere function in mice.

2. These findings suggest that defects in MMR can contribute to oncogenesis through multiple pathways including centrosome amplification, telomere capping defects and chromosomal abnormalities

3. The use of isogenic non-tumor cells for these experiments demonstrates the importance of MMR in early instabilities that ultimately lead to tumorigenesis