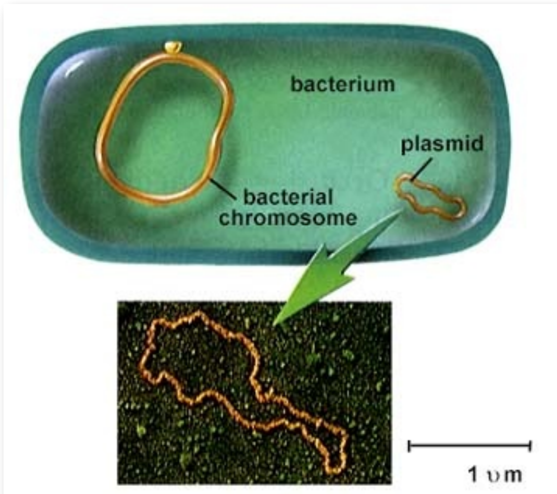
Telomeres and Telomerase in Cancer and Aging

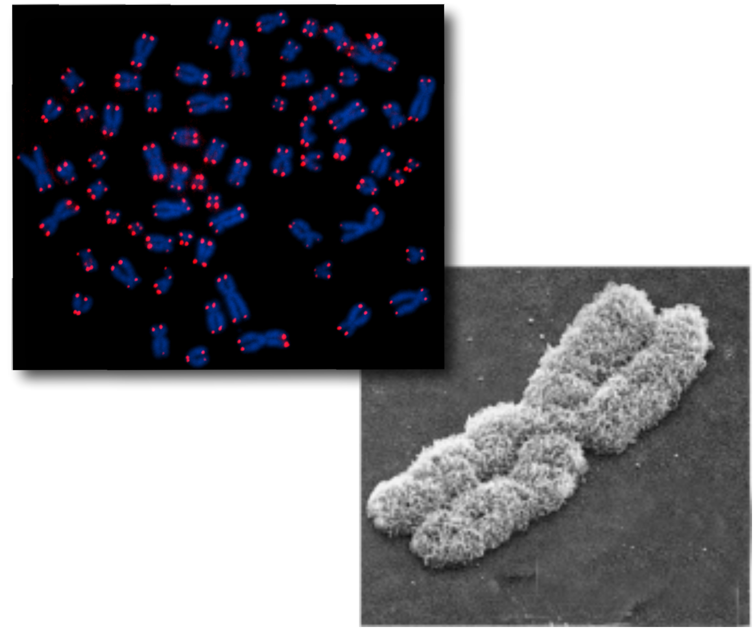
Agnel Sfeir, PhD
GSK – October 2025

Outline

- End-replication problem
- End-protection problem
- Telomeres and Telomerase
- Cellular aging (Senescence) and Telomeres
- Telomere Length Changes in Human Aging
- Telomeropathies – Dyskeretosis Congenita
- Telomere/Telomerase dynamics in Cancer
- Telomerase Inhibition in the Clinic
- Telomere Dynamics in Embryonic Stem cells

Our Genome is Linear

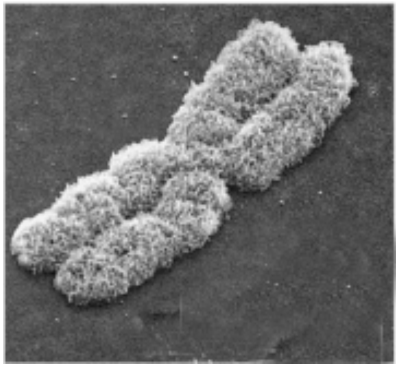




bacterial
chromosome
<u>Circle</u> of 2,000,000 bp
(1/cell)

eukaryotic
chromosome
<u>Linear</u> of 50,000,000 bp
(~40 in each cell)

Two Problems Associated with Linear Chromosomes



vs.

The end-replication problem

The end-protection problem

James Watson
(1928 -)

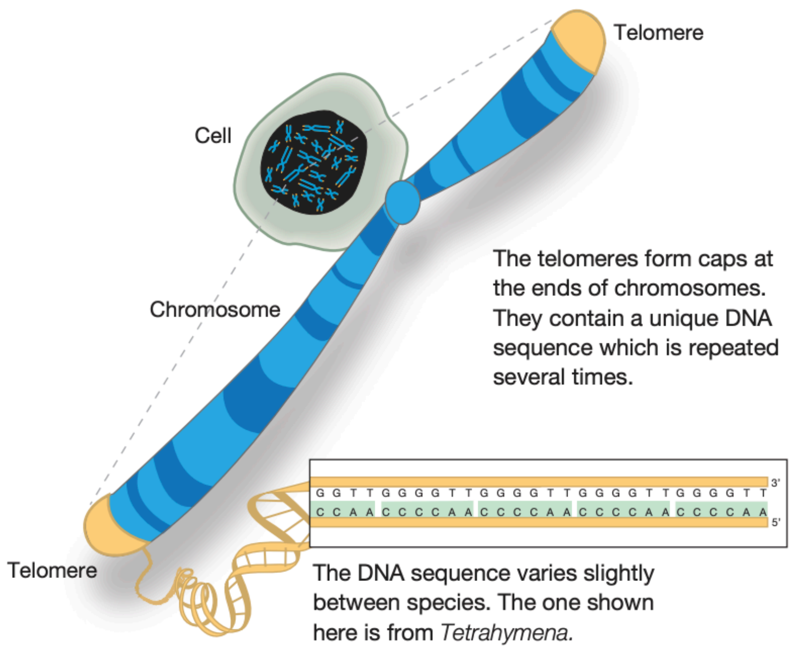
Alexei Olovnikov
(1902-1992)

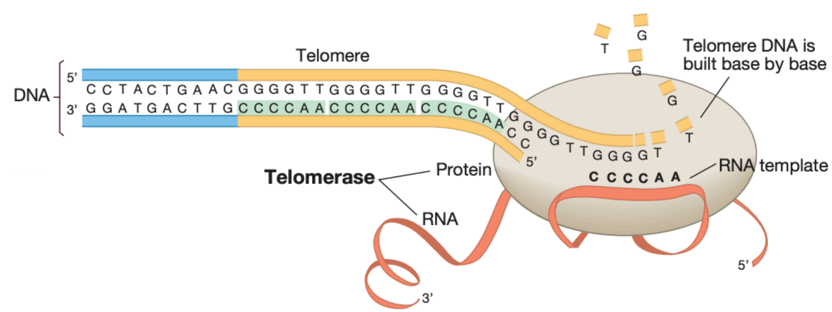
Hermann Muller (1890-1967)

Barbara McClintock
(1902-1992)

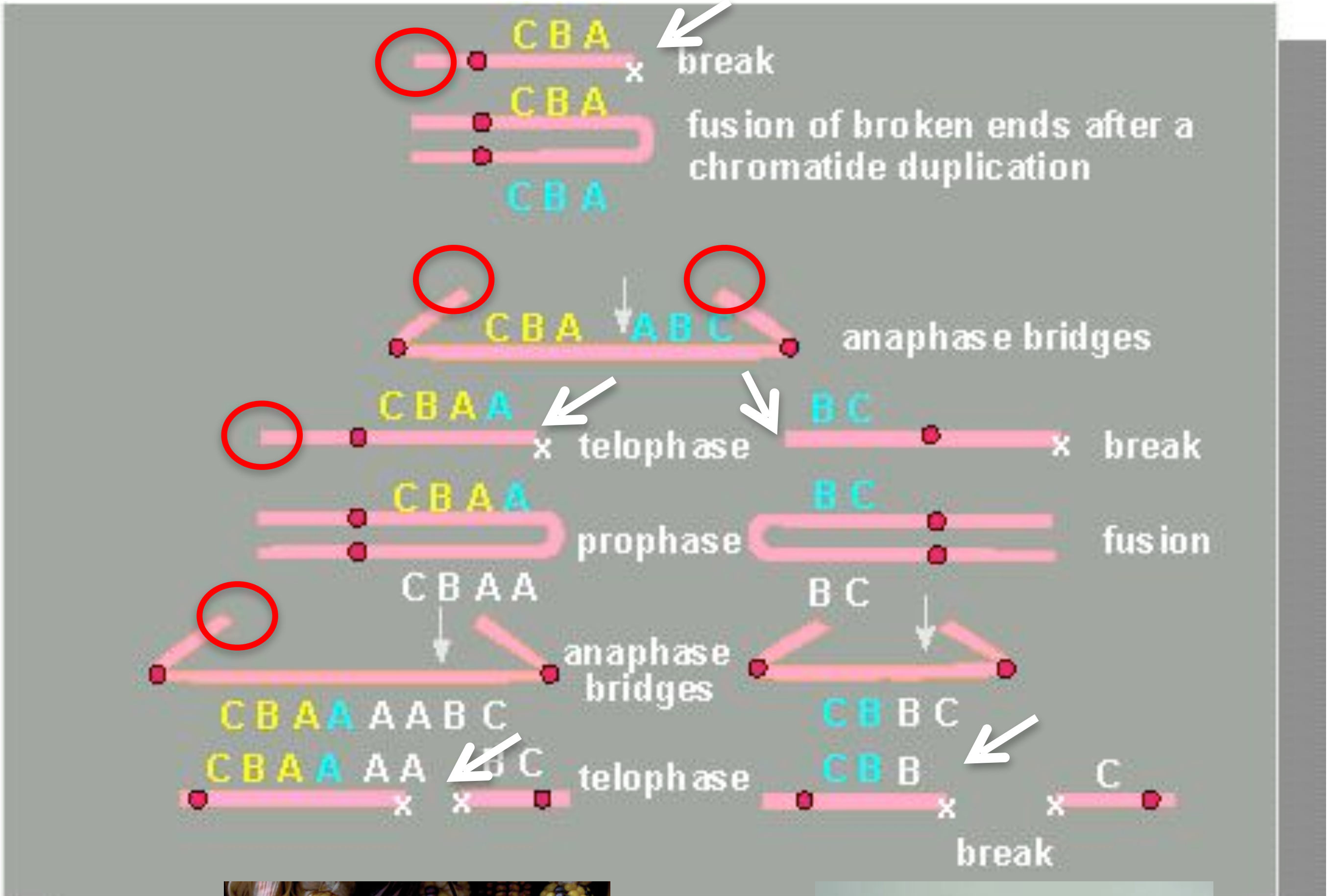
Both Problems are Solved by Telomeres/Telomerase

The Nobel Prize in Physiology or Medicine 2009
Elizabeth H. Blackburn, Carol W. Greider, Jack W. Szostak





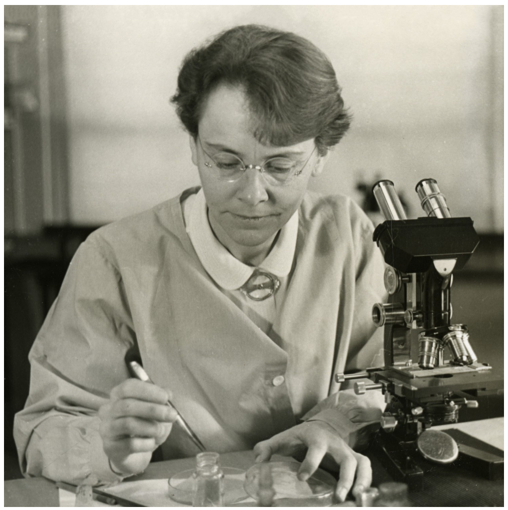
The End Protection Problem

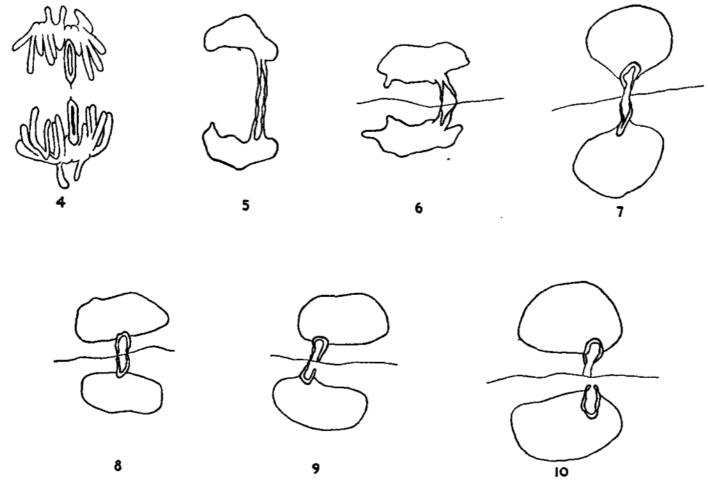


Barbara McClintock

Hermann Muller

Evidence for the “break in mitosis” model



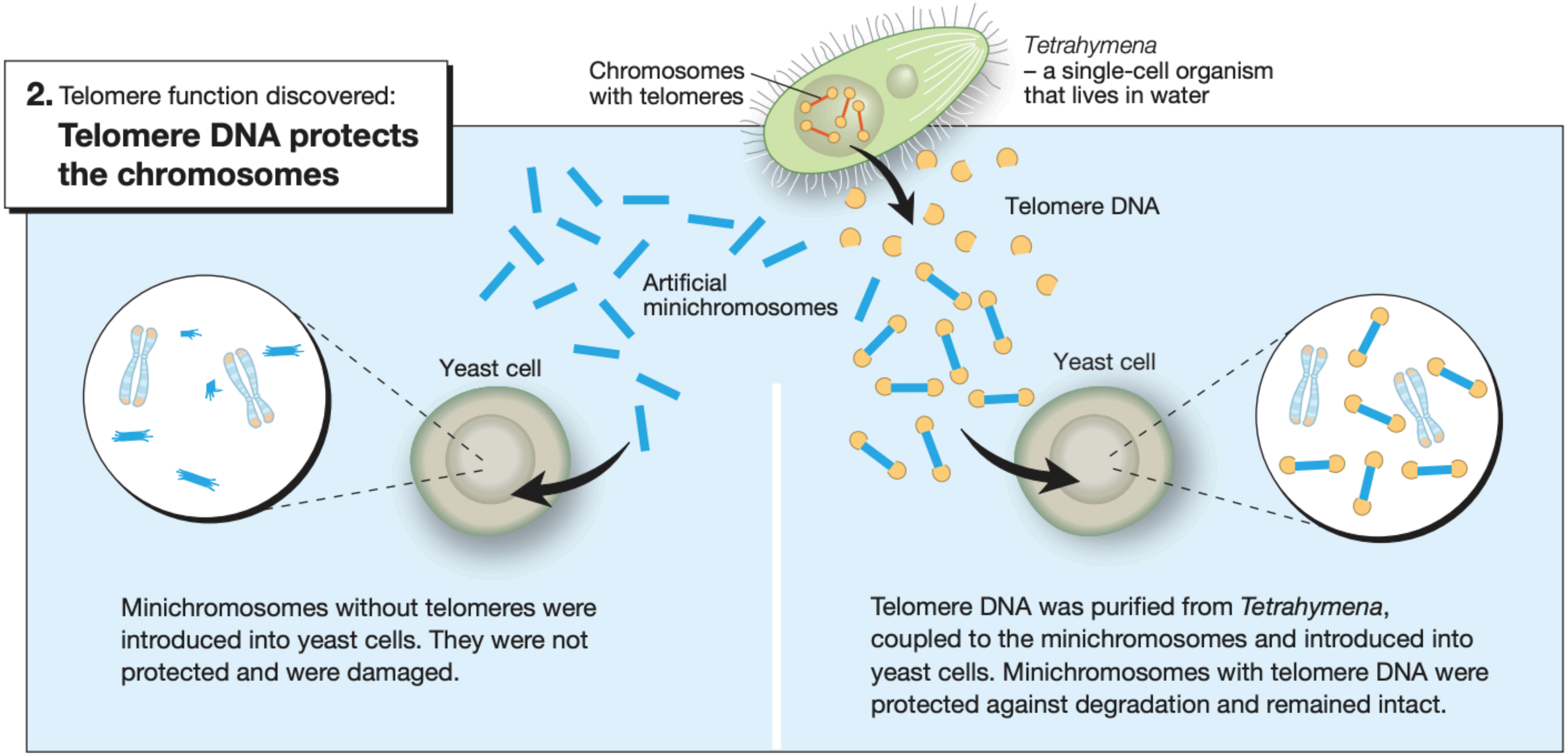


“It would be premature to draw rigid conclusions from the results so far obtained.”

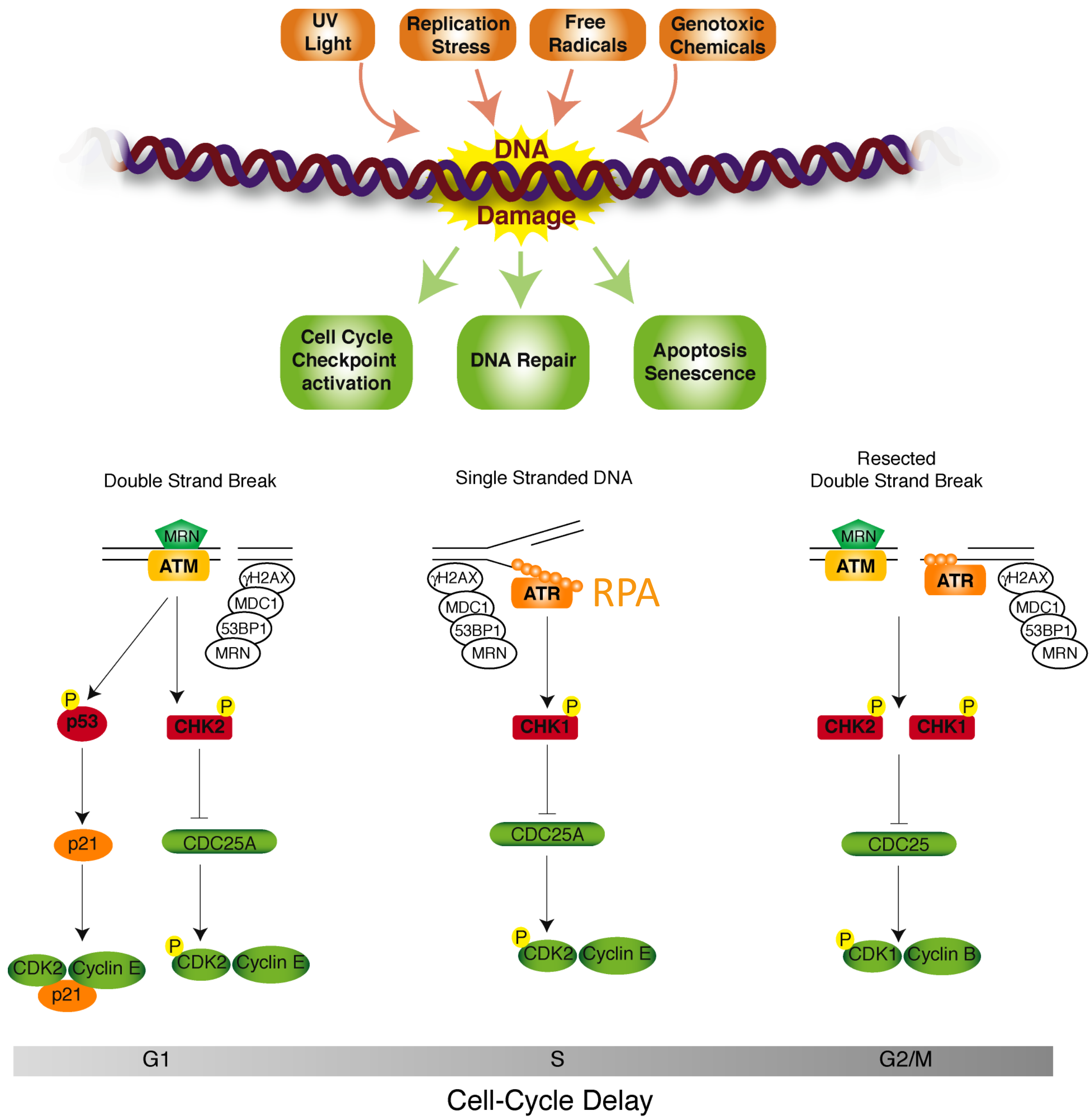
-Barbara McClintock

McClintock, B. *Genetics*. 1938a.
McClintock, B. *Missouri Agric. Exp. Station Res. Bull.* 1938b.
McClintock, B. *Genetics* 1941.

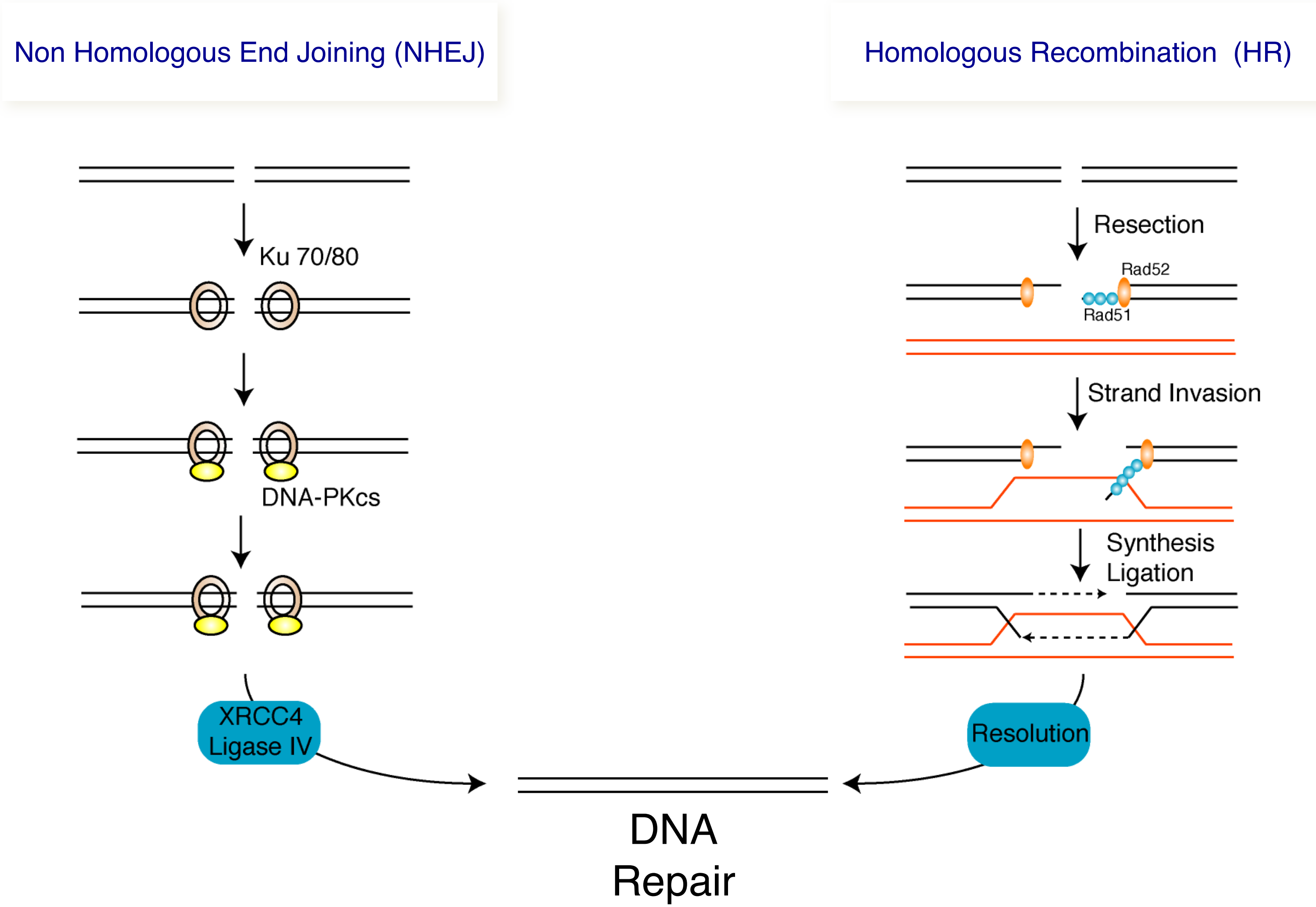
The breakthrough experiment – worth a Nobel prize



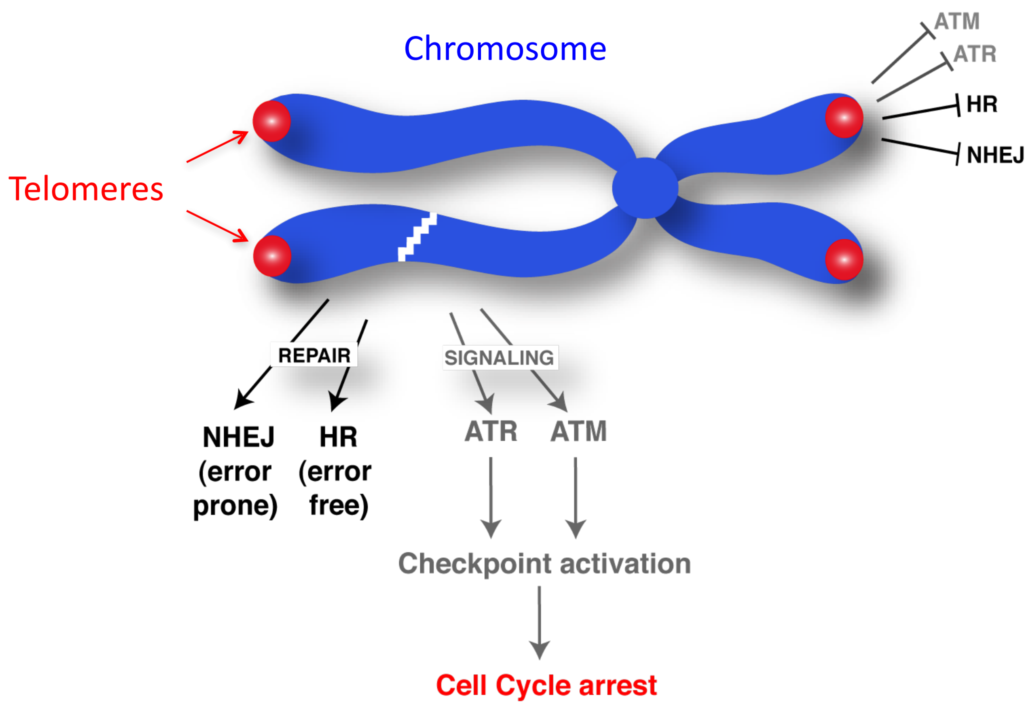
End-Protection Problem – Rooted in the DNA damage Response



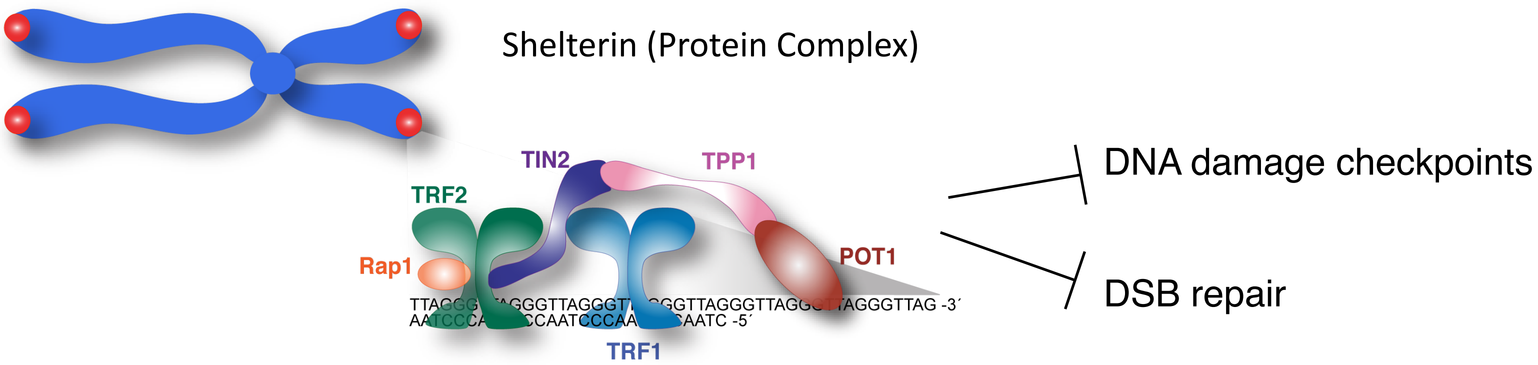
DNA Break Repair



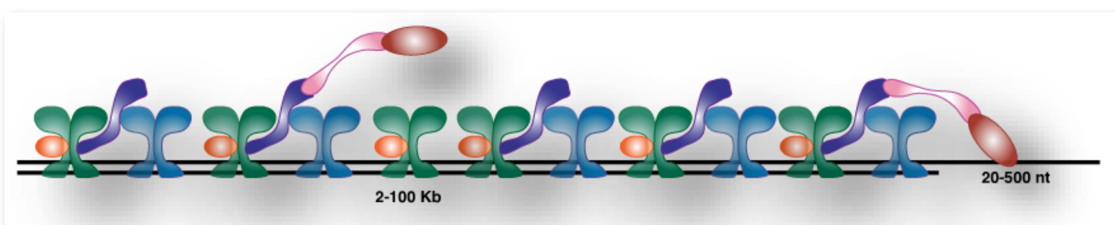
End-Protection Problem



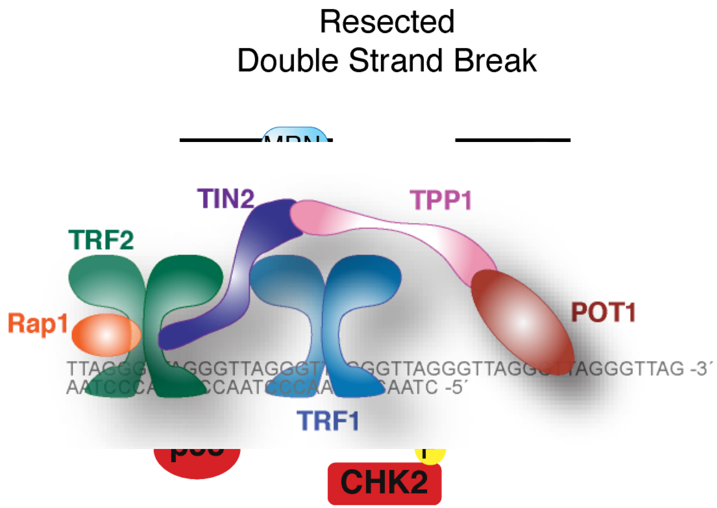
Shelterin Solves the End Protection Problem in Somatic Cells

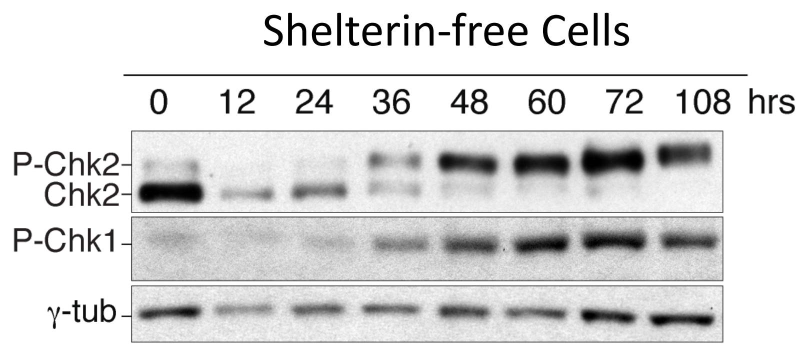


Shelterin:
- consists of 6 proteins; 3 bind directly to the DNA
- binds to the TTAGGG repeats with high specificity
- is highly abundant (100-1000 copies per cell)

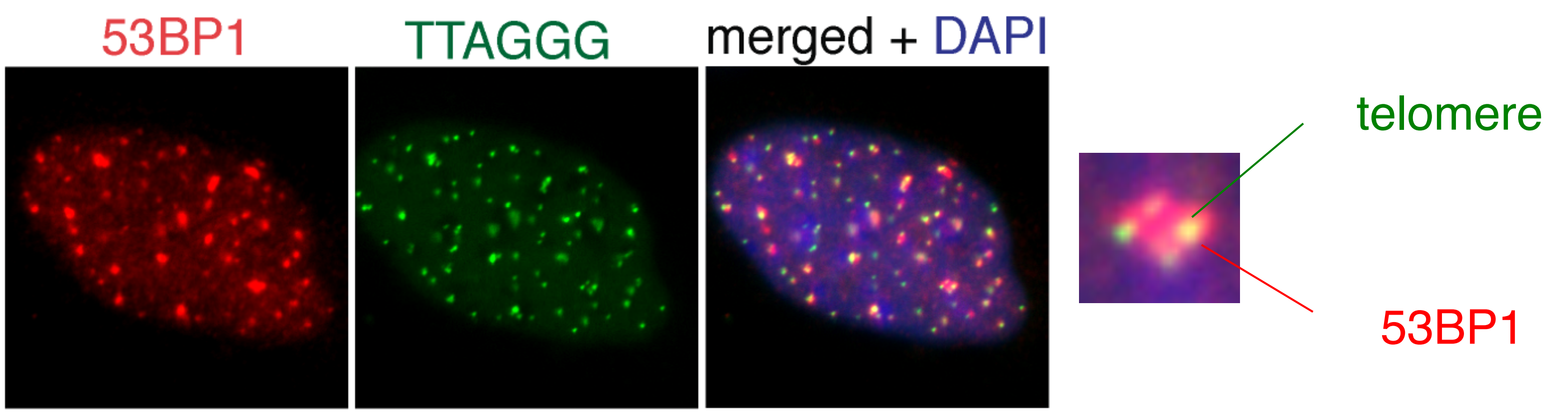


Telomeres Lacking Shelterin Activate the ATM and ATR kinases



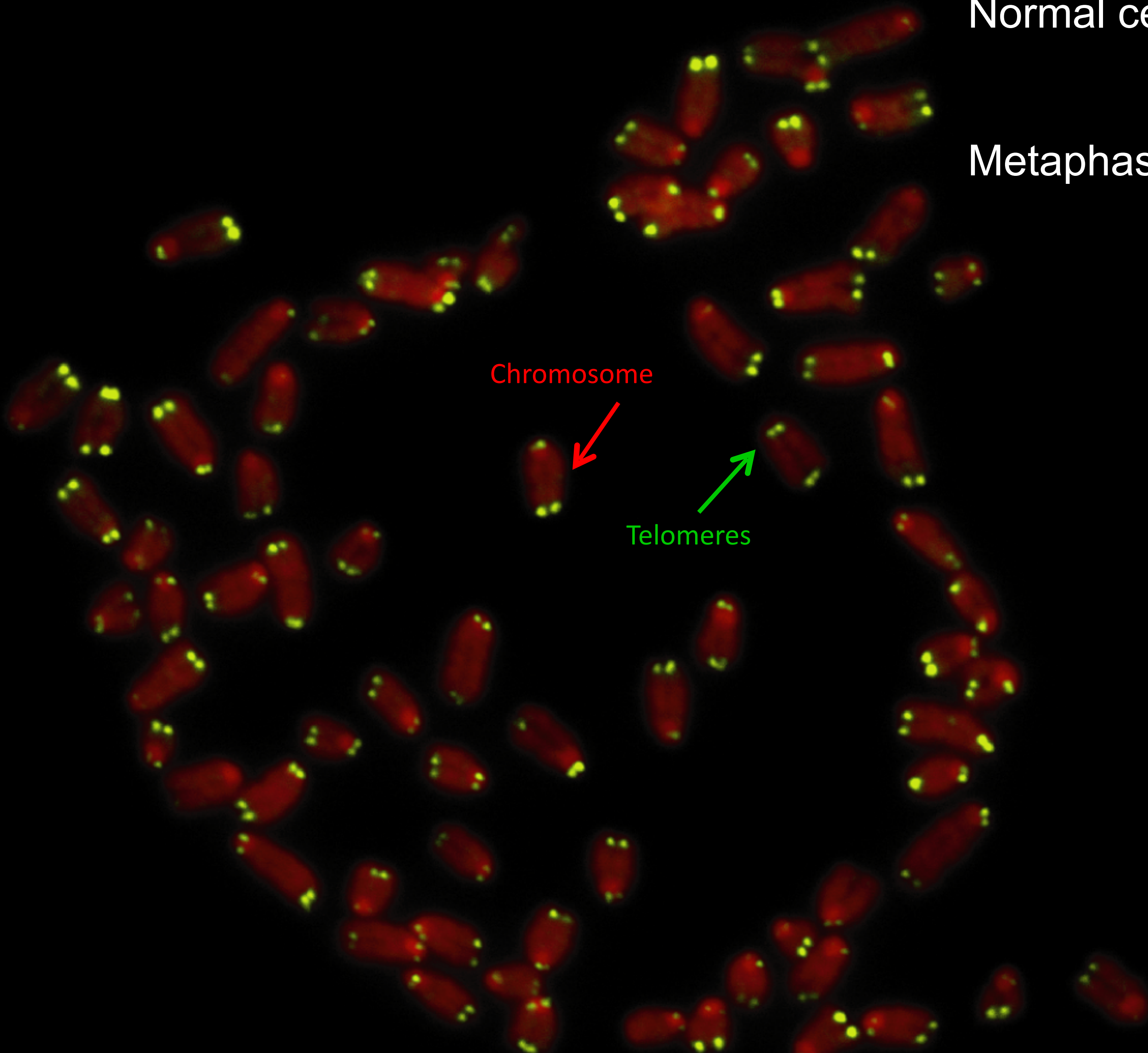


Sfeir et al., Science 2012



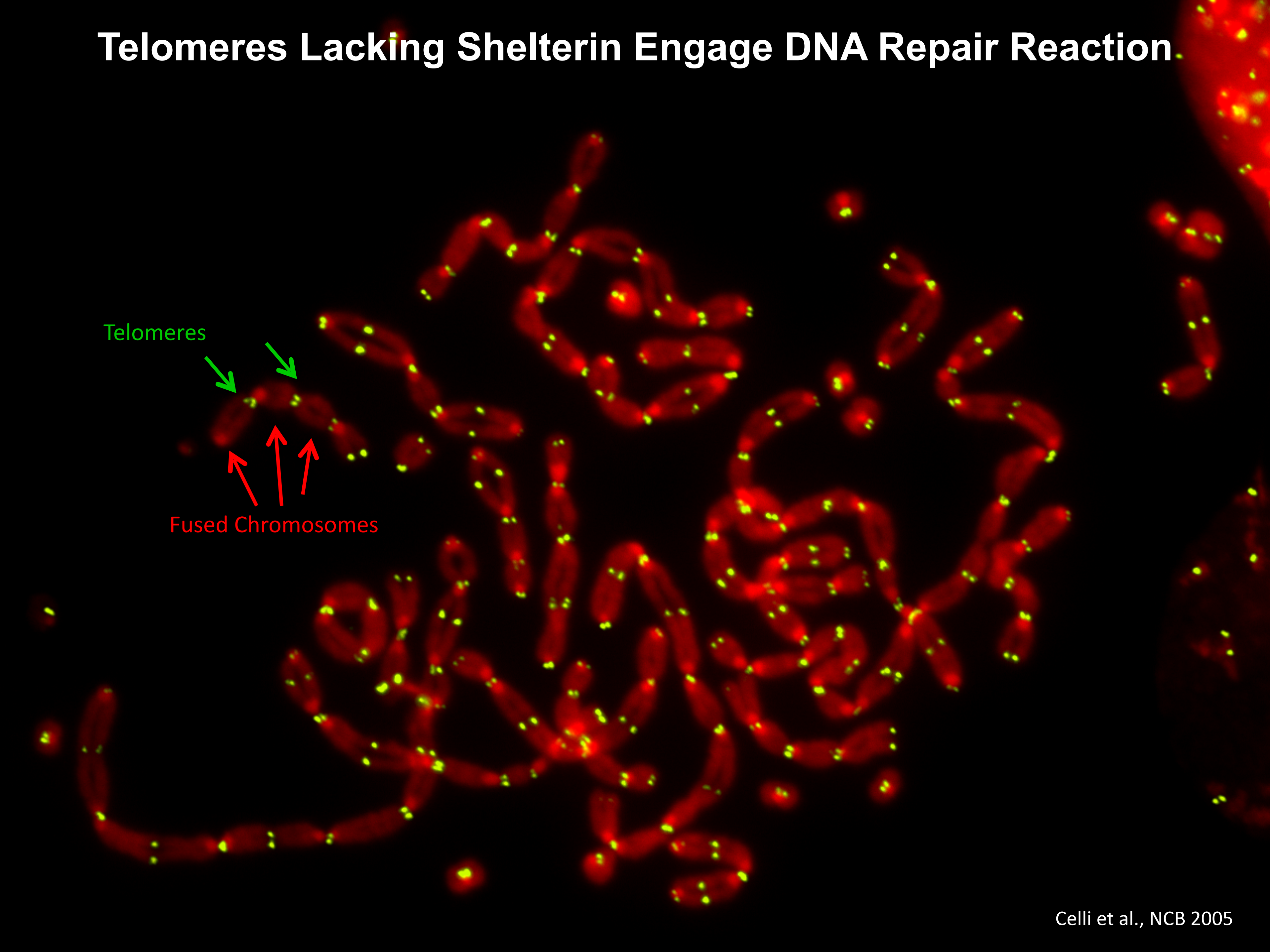
Normal cells

Metaphase spread



Celli et al., NCB 2005

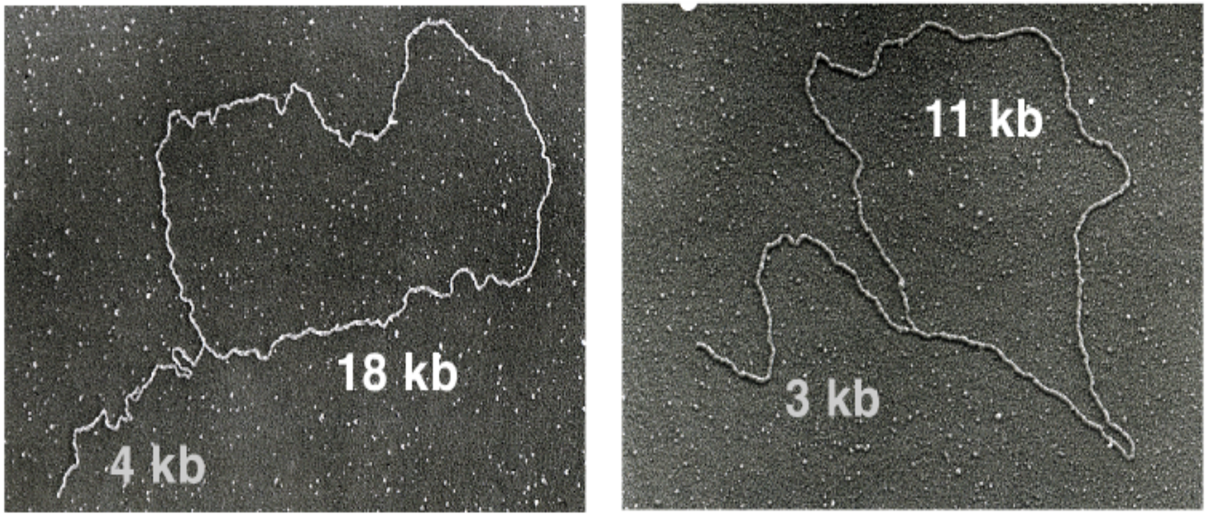
Telomeres Lacking Shelterin Engage DNA Repair Reaction



Celli et al., NCB 2005

How does Shelterin Mask the Ends from the DNA Damage Response?

T-loop

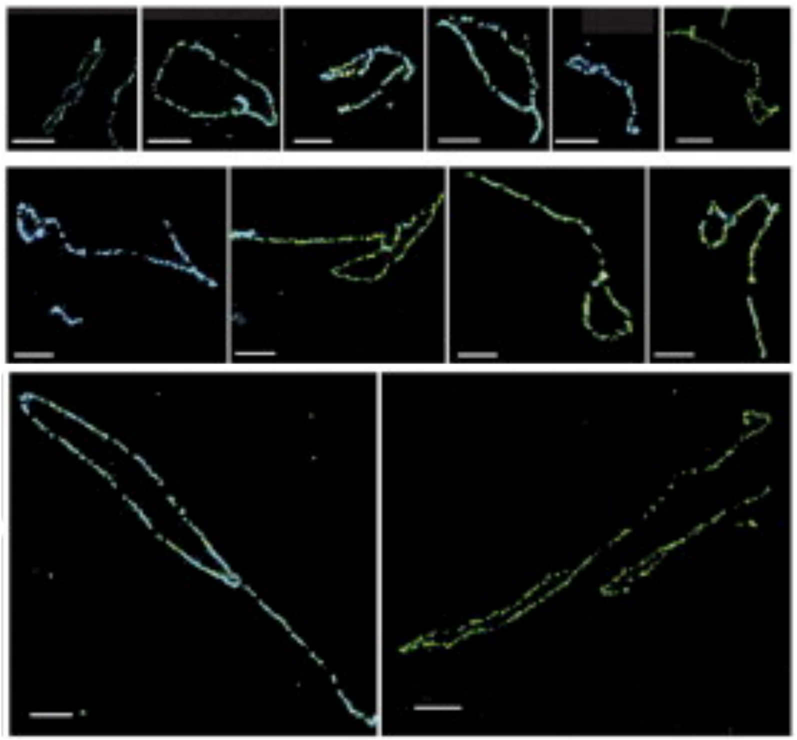


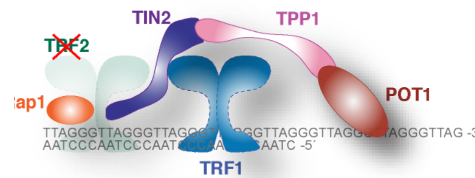
Electron Microscopy

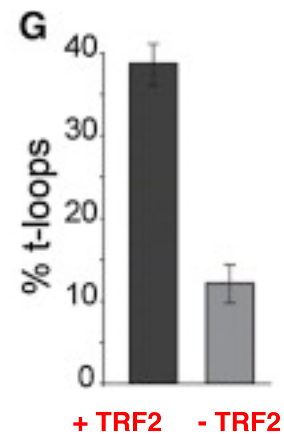
Griffith et al., Cell 1999

TRF2 is the Shelterin Subunit Required for T-Loop Formation

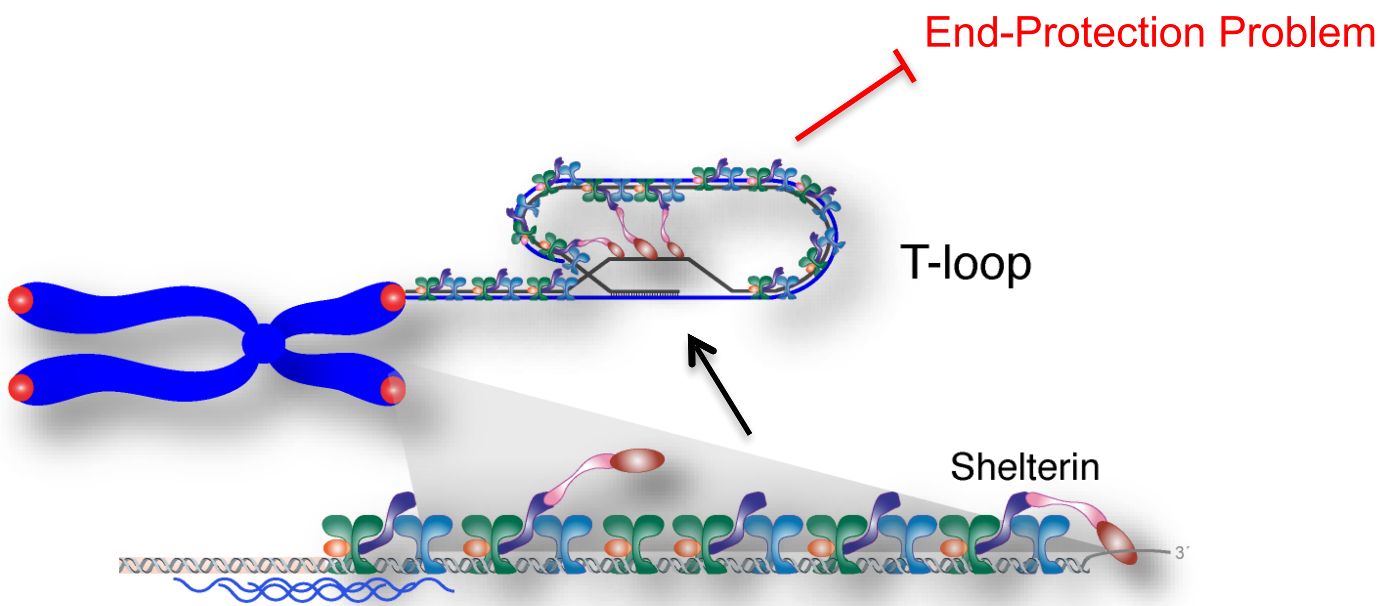
Super Resolution Microscopy
(STORM)



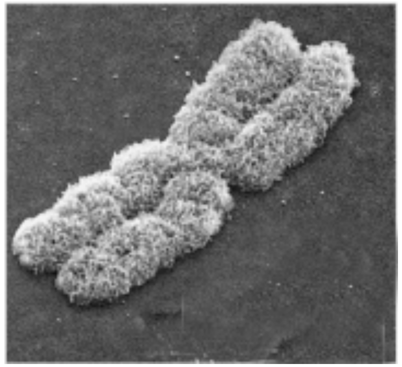




Doksani et al, Cell 2013


End-Protection Problem
T-loop
Shelterin
3´

The End Replication Problem



vs.

The end-replication problem

James Watson
(1928 -)

Alexei Olovnikov
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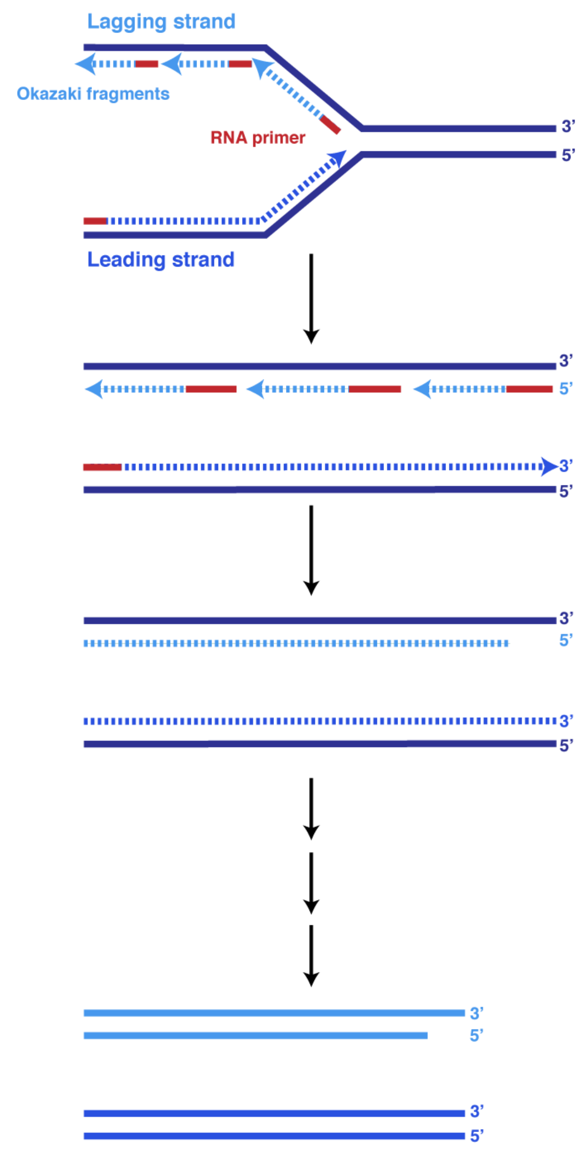
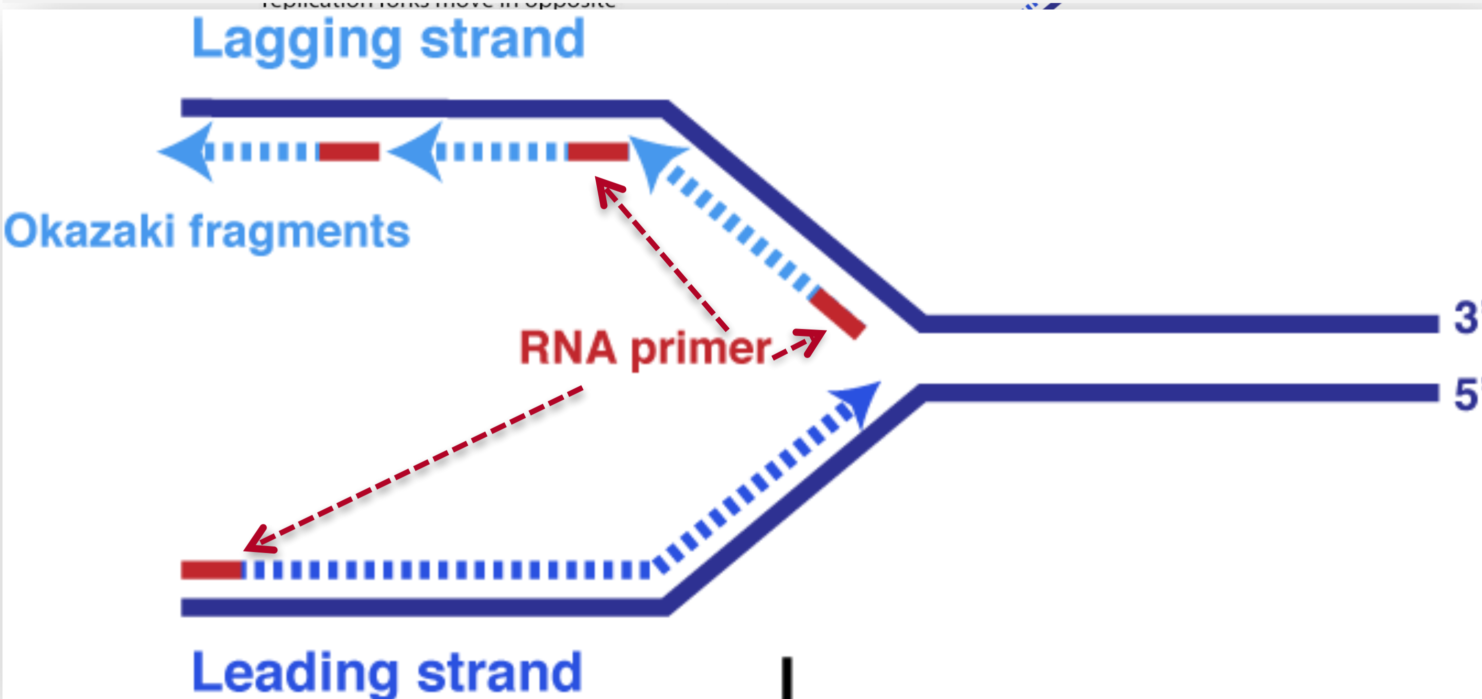
Inherent to the DNA Replication Machinery

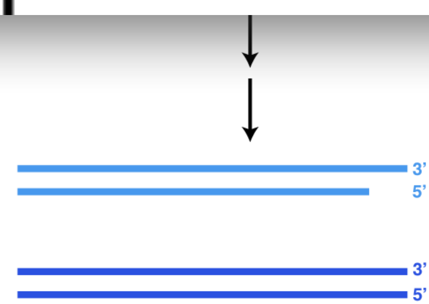


End Replication Problem

Every new DNA strand starts with RNA

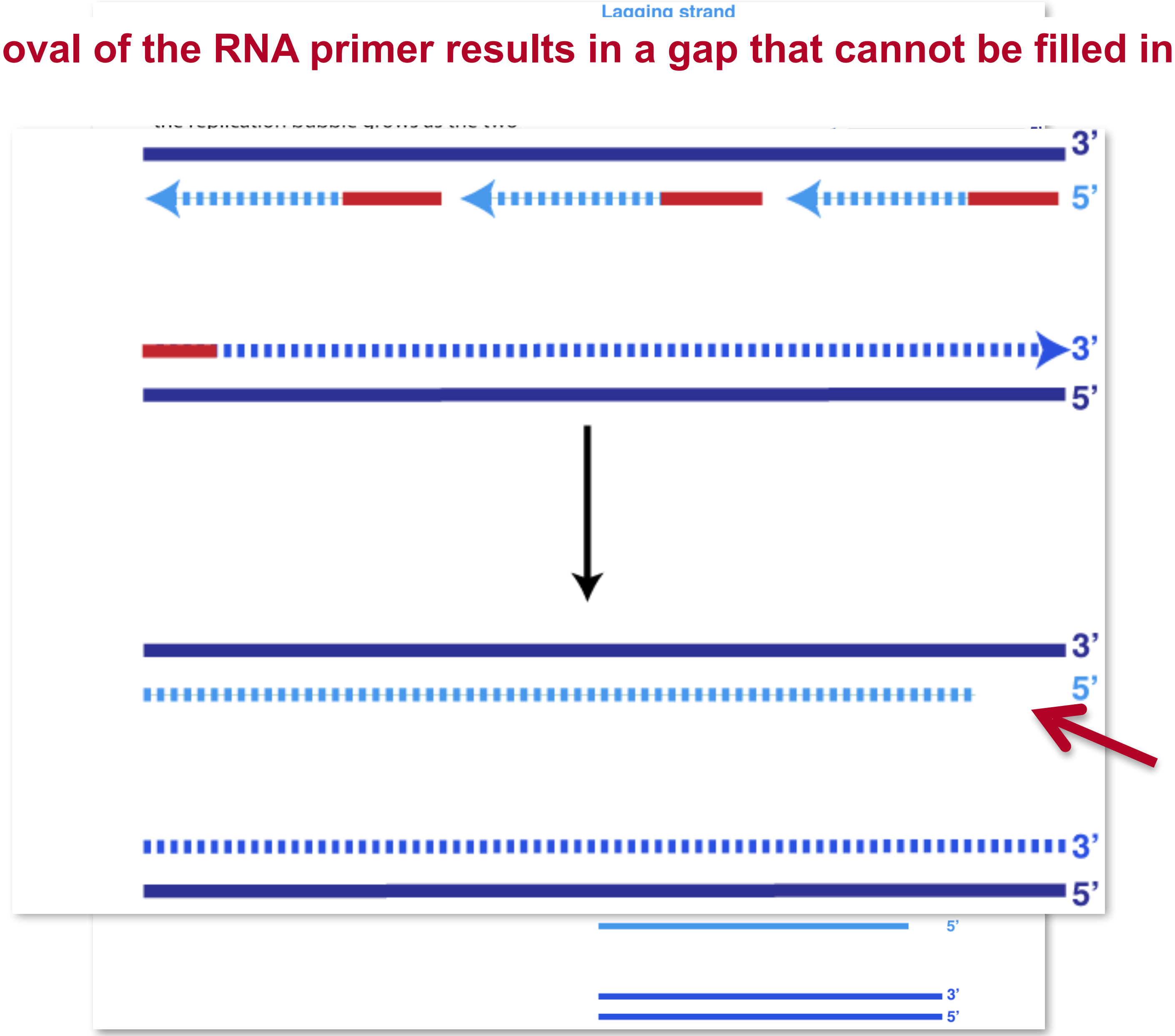


4 Each round of replication generates shorter and shorter DNA molecules.

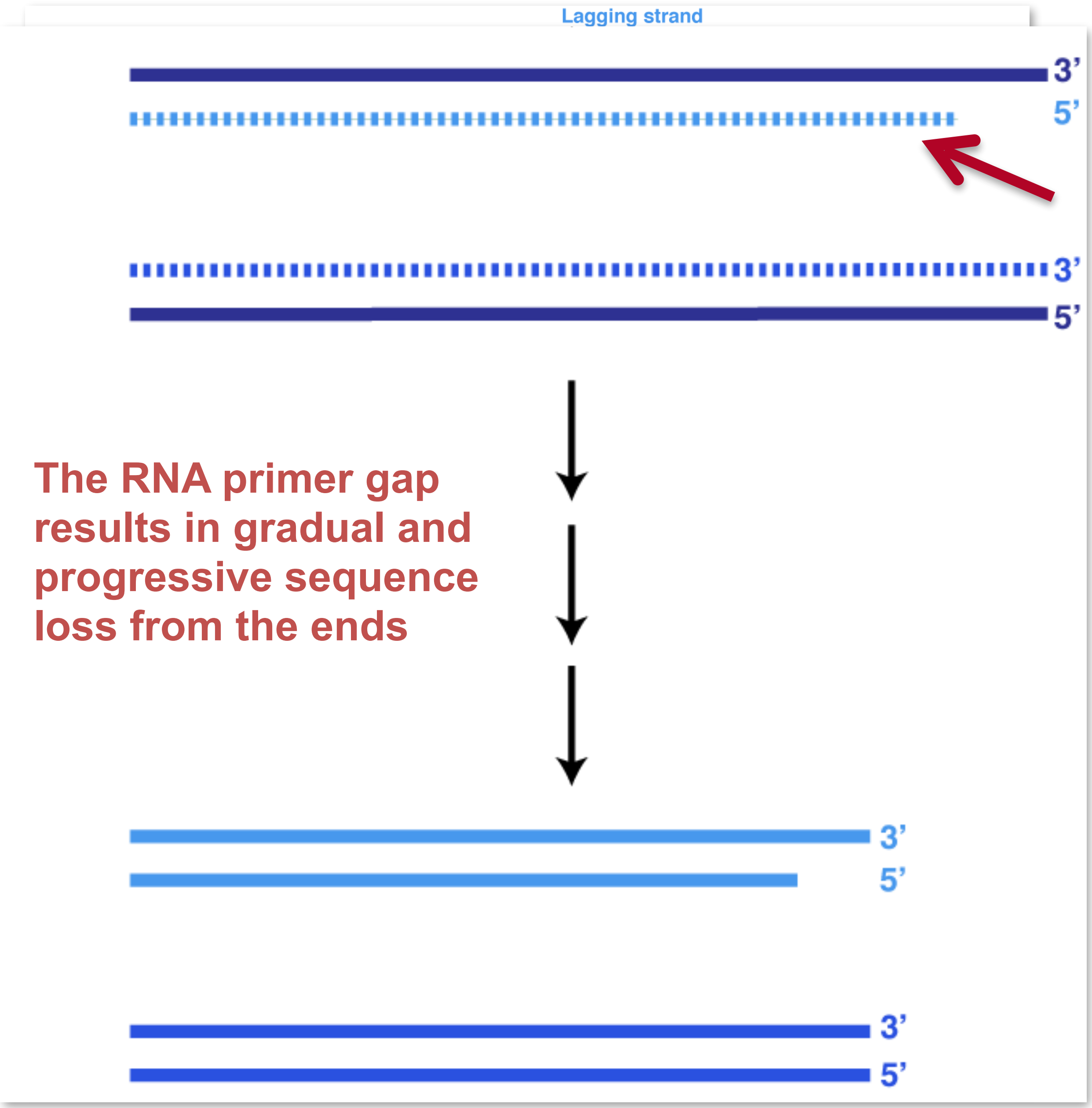


End Replication Problem

Removal of the RNA primer results in a gap that cannot be filled in.

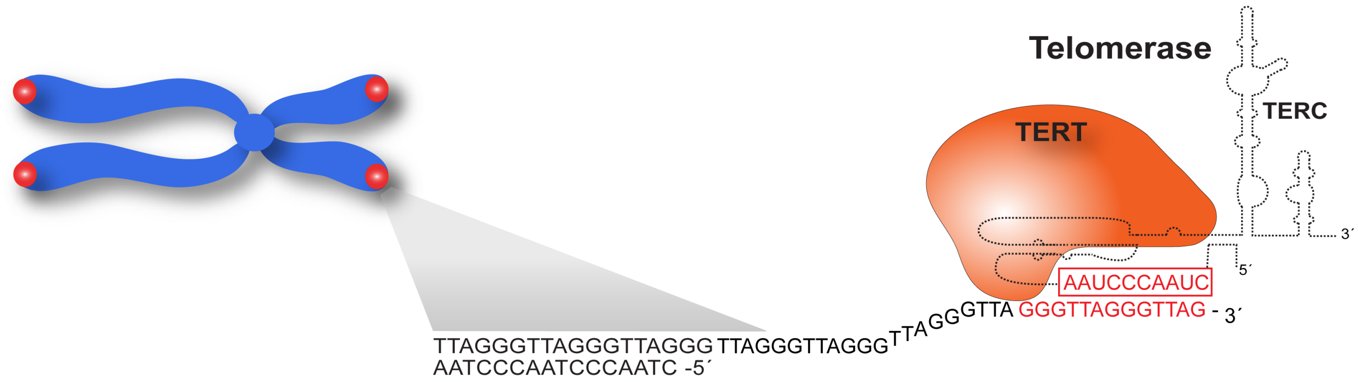


End Replication Problem



The RNA primer gap results in gradual and progressive sequence loss from the ends

End Replication Problem Solved by Telomerase



Telomerase elongates the 3' end of the chromosome end, thereby balancing sequence loss with DNA replication

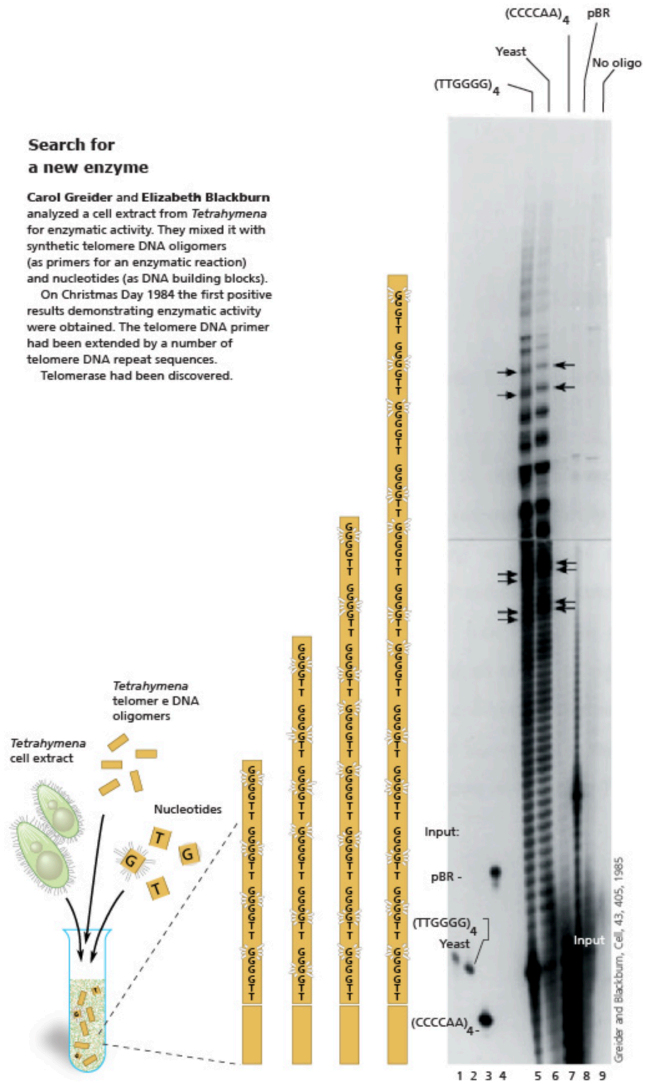
The breakthrough experiment – worth a Nobel prize

Search for a new enzyme

Carol Greider and **Elizabeth Blackburn** analyzed a cell extract from *Tetrahymena* for enzymatic activity. They mixed it with synthetic telomere DNA oligomers (as primers for an enzymatic reaction) and nucleotides (as DNA building blocks).

On Christmas Day 1984 the first positive results demonstrating enzymatic activity were obtained. The telomere DNA primer had been extended by a number of telomere DNA repeat sequences.

Telomerase had been discovered.



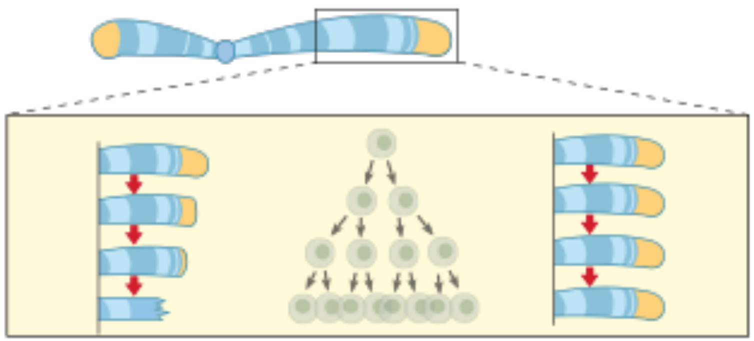
1. Assay for telomere elongation

Different synthetic single-stranded telomere DNA oligomers were added to a *Tetrahymena* cell extract along with radioactively labeled nucleotides allowing visualization of the reaction product.

2. Telomerase synthesizes telomeres

The experiment showed that an unknown enzyme extends telomere DNA. A ladder of bands was obtained when either *Tetrahymena* or yeast telomere oligomers were used as primers (lanes 5 and 6) but not when unrelated DNA sequences were used.

Telomerase is Switched off in Somatic Cells

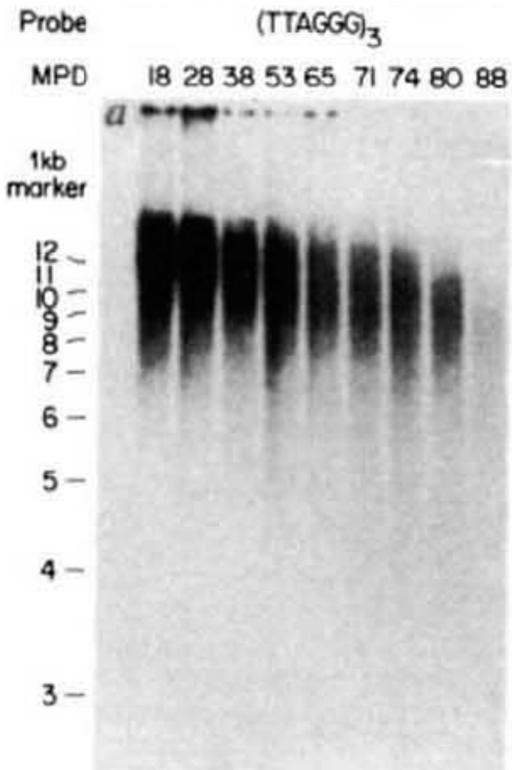


Soma

Germline
(stem cells)

- hTERT strongly suppressed in most somatic cells except for highly proliferative cells (B-, T-cells)
- Human cells show progressive telomere shortening at a rate of 50-100 bp/cell division in vitro

Telomere length decreases with cell division and human aging



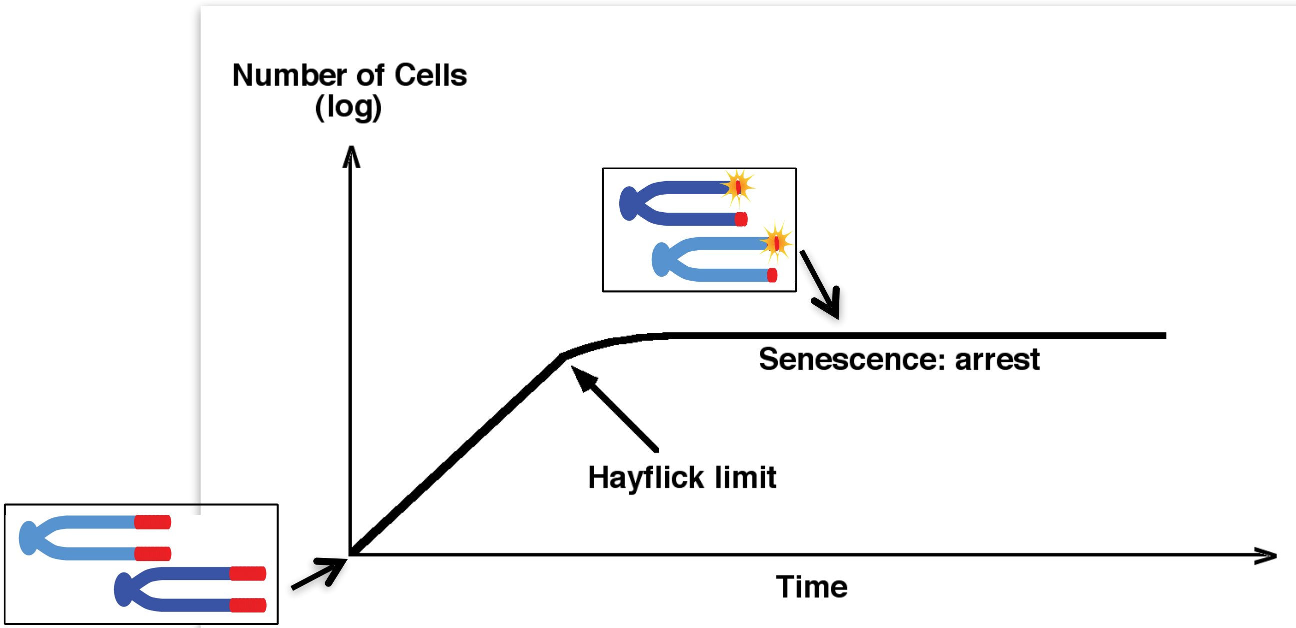
HSC172 (fetal fibroblasts)
MPD = mean population doublings

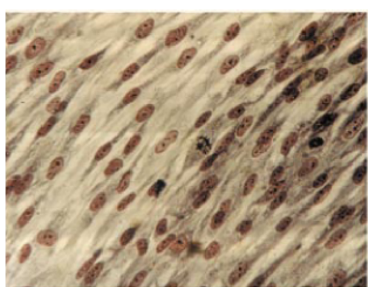
TABLE 1 Effect of donor age on telomere length in human fibroblasts

Cell strain	Age: *in vivo* years	Age: *in vitro* MPD (MPD max)	Mean telomere length kb ± s.d. (*n*)
HSC172	Fetal	18–28 (88)	8.6 ± 0.5 (3)
A30S	0	33 (58)	7.3 (1)
A38	24	31–33 (68)	6.9 ± 0.3 (2)
A35	70	19 (41)	6.7 (1)
F001	71	21–29 (40)	6.5 ± 0.4 (5)
F002	91	18–20 (45)	6.2 ± 0.1 (3)

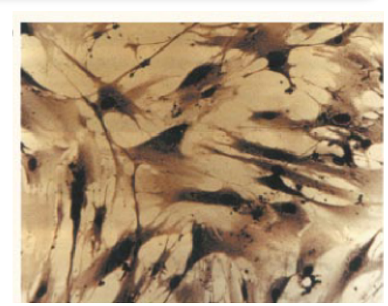
human fibroblasts have a typical maximum of 40-60 PDs, after which they stop dividing but remain viable (Hayflick limit)

Telomere Shortening Limits the Replicative Lifespan of Human Cells





Young Fibroblasts



Senescent Fibroblasts

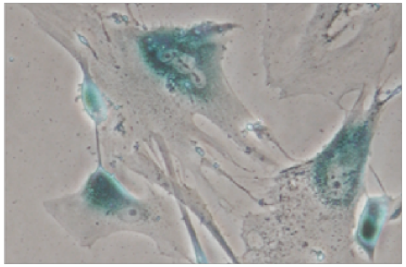
Features of Replicative Senescence

- Irreversible growth inhibition

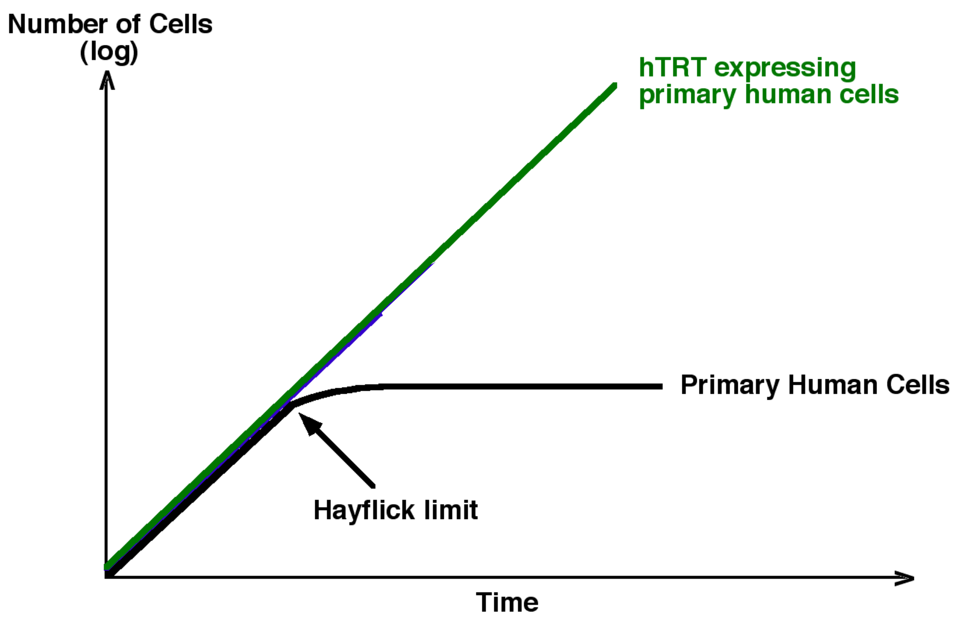
- Cells remain thermodynamically alive for years

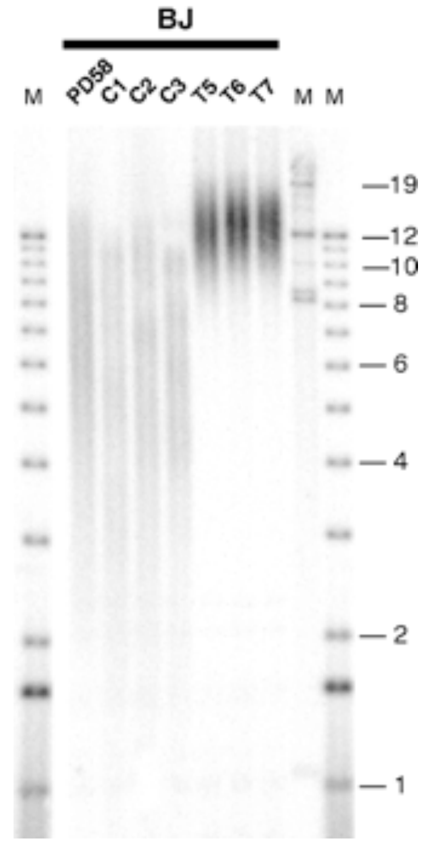
- Markers:
  - Flat morphology
  - Senescence associated-beta-galactosidase (stress marker)
  - RB hypophosphorylated
  - p53 and p21 high
  - p16 high
  - SA-heterochromatin foci (SAHFs)

- Genetic requirements: p53 and/or Rb pathways (human)



Telomerase Bypasses Senescence Leading to Immortalization

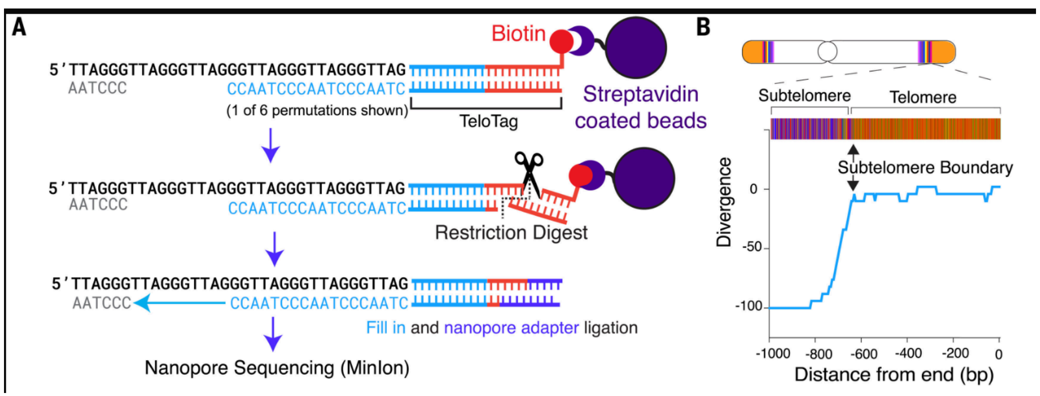


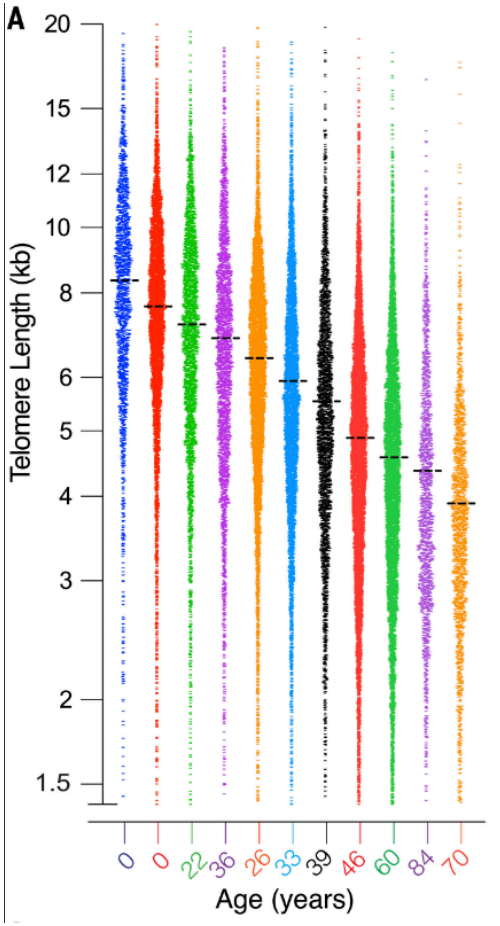


Immortal but not transformed

Bodnar et al., Science 1998

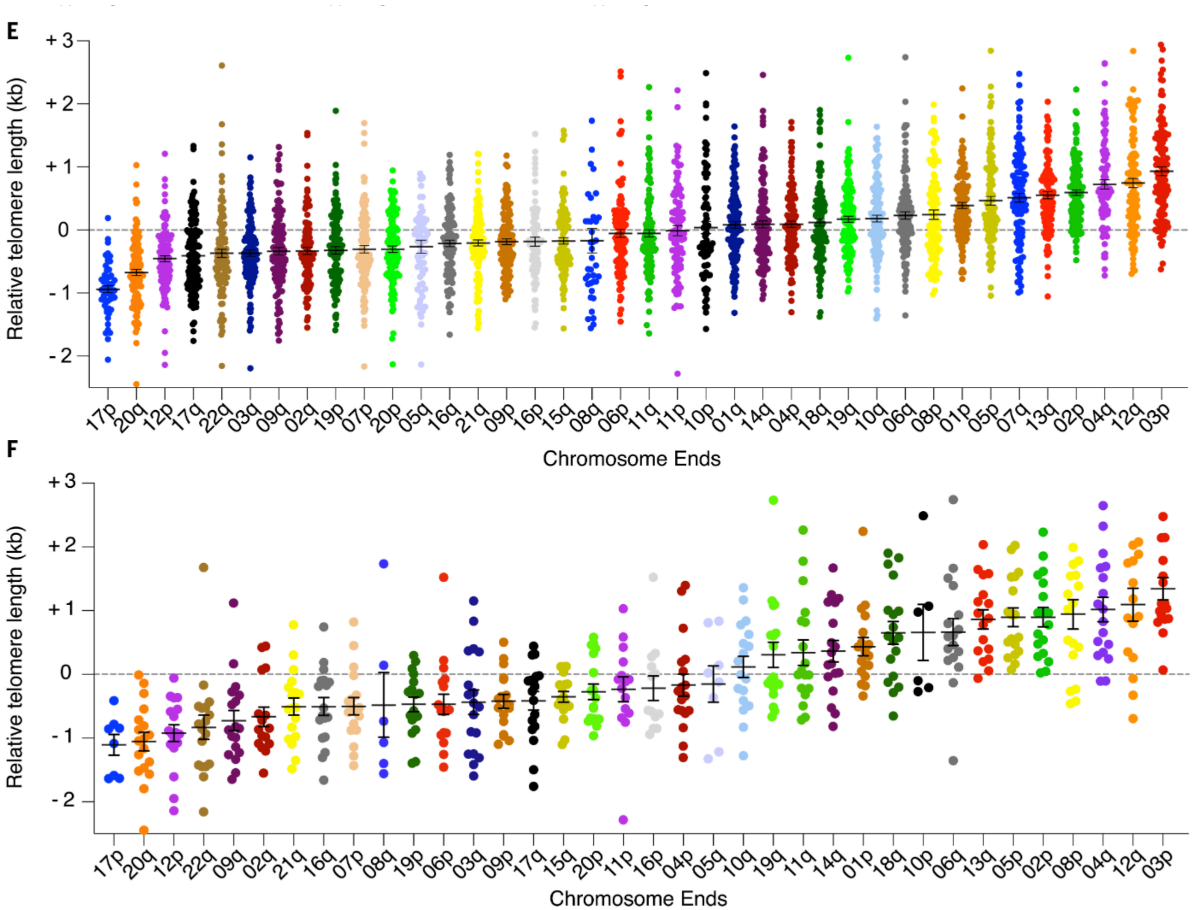
Oxford Nanopore Sequencing to assess telomere length





Karimian et al., Science 2024

Human telomere length is chromosome end–specific and conserved across individuals



Karimian et al., Science 2024

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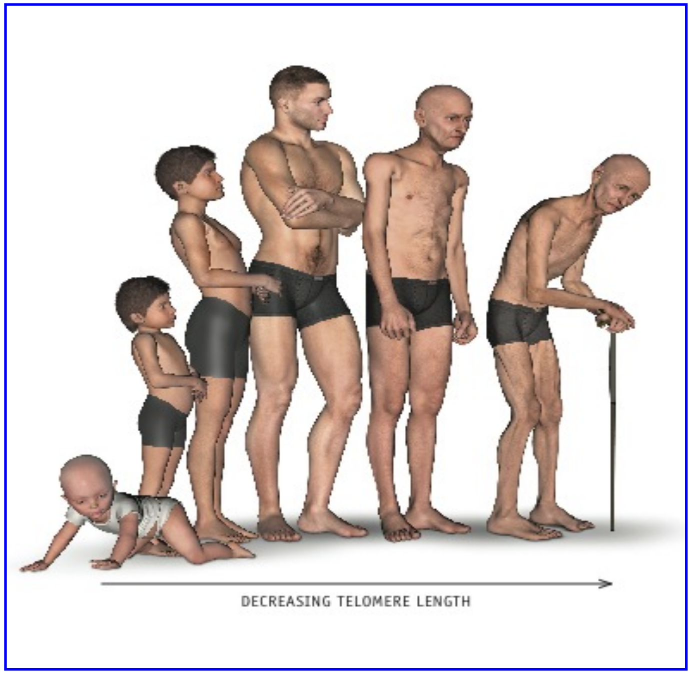
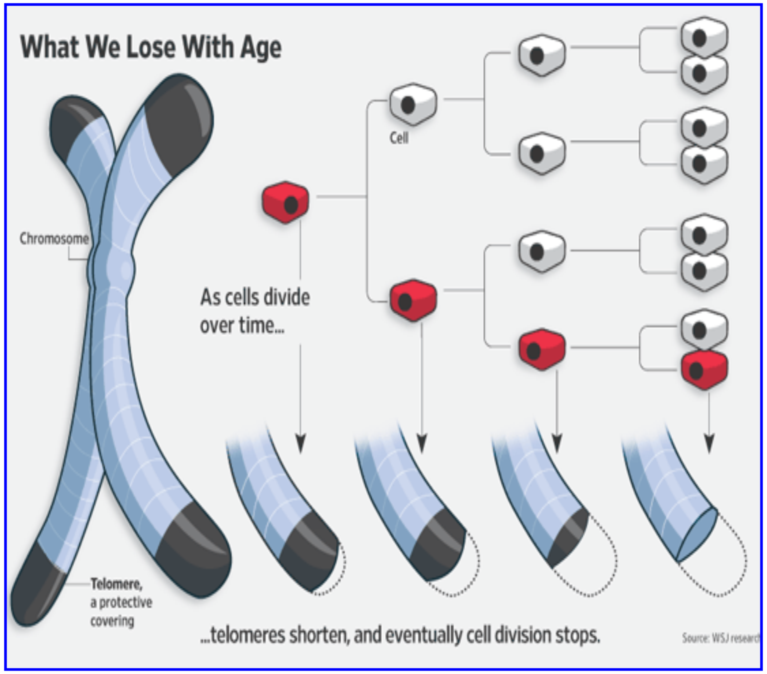
- ★Telomere in Embryonic Stem cells

Telomere length Change with Human Aging

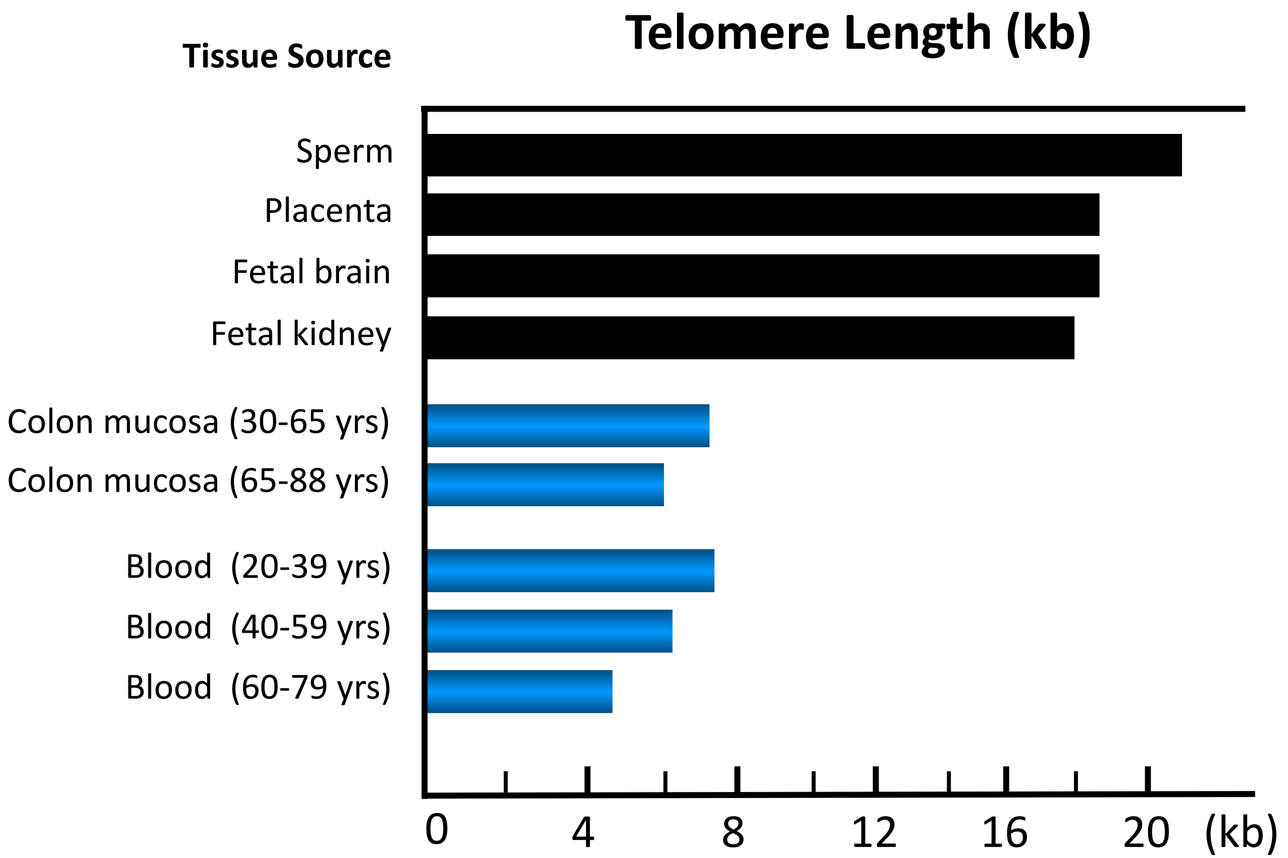




In newborn humans, telomeres are approximately 15-20kb in length

Telomeres shorten gradually through life, suggesting that telomere length may serve as a surrogate marker for aging.

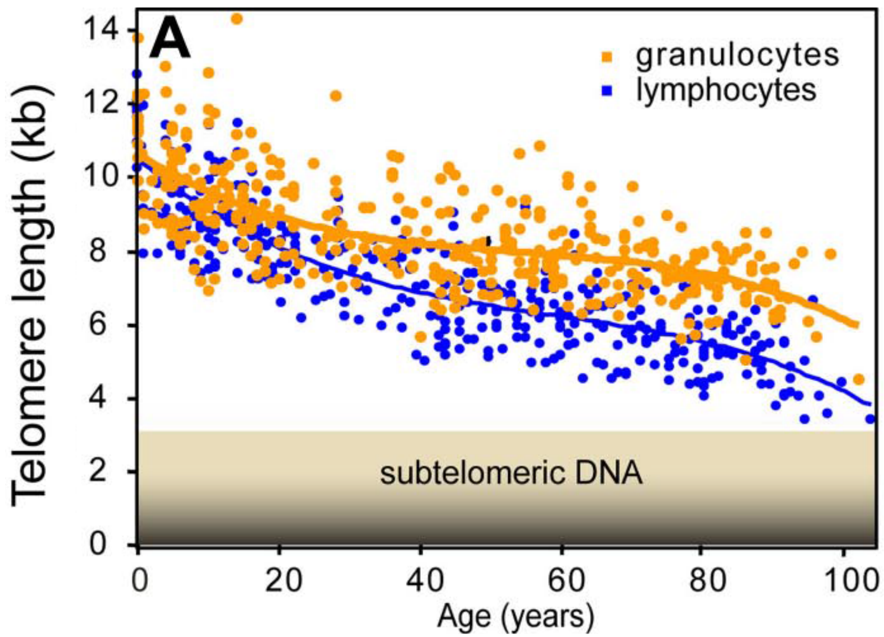
Telomeres Shorten with Increased Age



Short telomeres correlate with increased age

Hastie et al, Nature, 346: 866

Telomeres Shorten with Increased Age

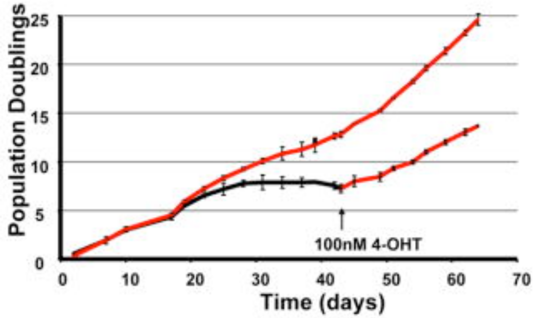


A number of recent reports examining telomere length in peripheral blood (PBMCs) show an association of short telomeres with:

- myocardial infarction
- vascular dementia
- atherosclerosis
- Alzheimer's disease
- liver cirrhosis
- Barrett's esophagus
- ulcerative colitis
- myeloproliferative disorders

Aubert et al, Plos genetics , 2012

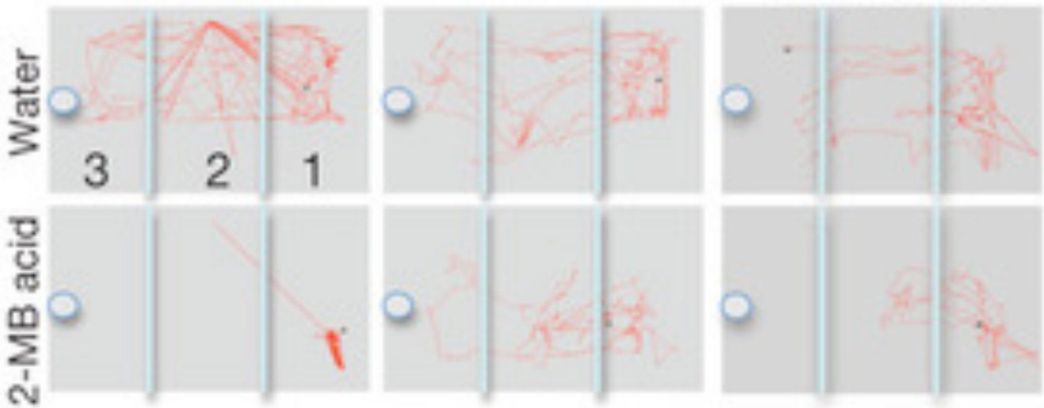
Telomerase Reactivation Reverses Brain Tissue Degeneration in Aged Mice

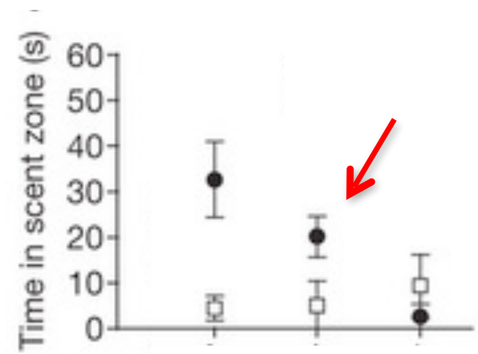


old

+ telomerase

young old old + telomerase

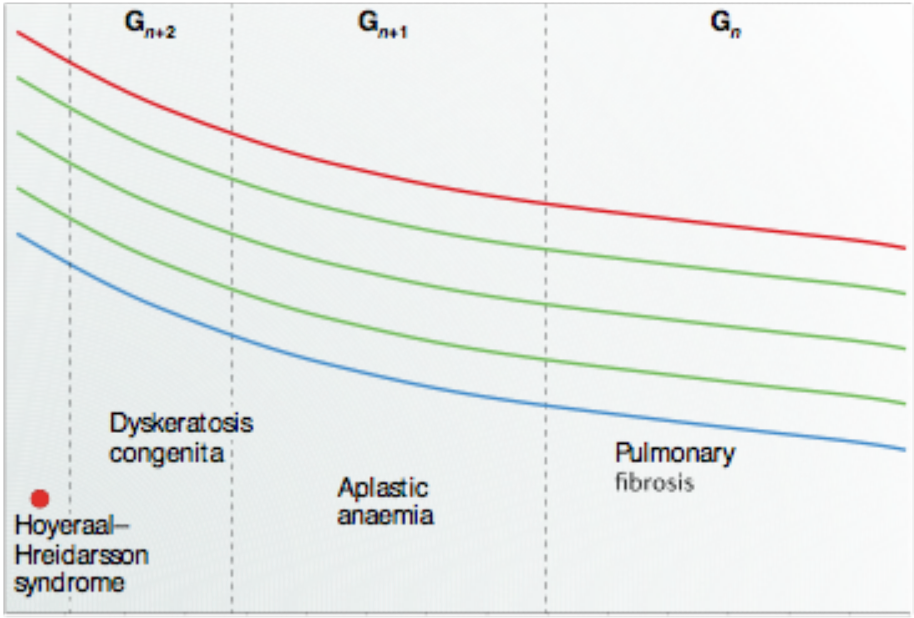




old + telomerase young

Jaskelioff et al., Nature 2011

Telomeropathies – Syndromes of short telomeres



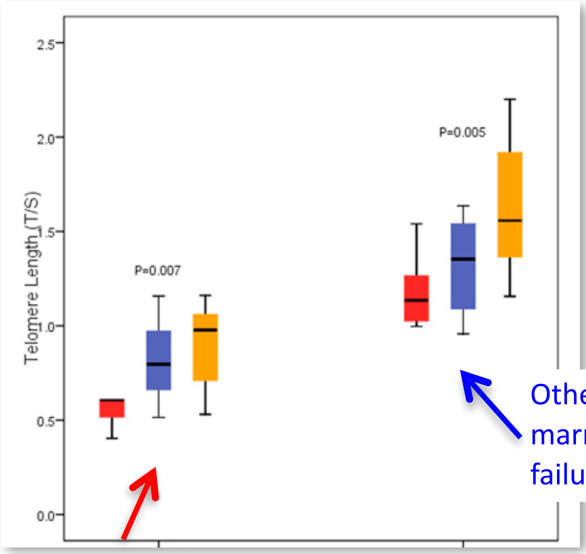
Armanios and Blackburn 2010

Telomere Disease: Dyskeratosis Congenita

Rodrigo T et al., . N Engl J Med 2009; 361:2353-2365

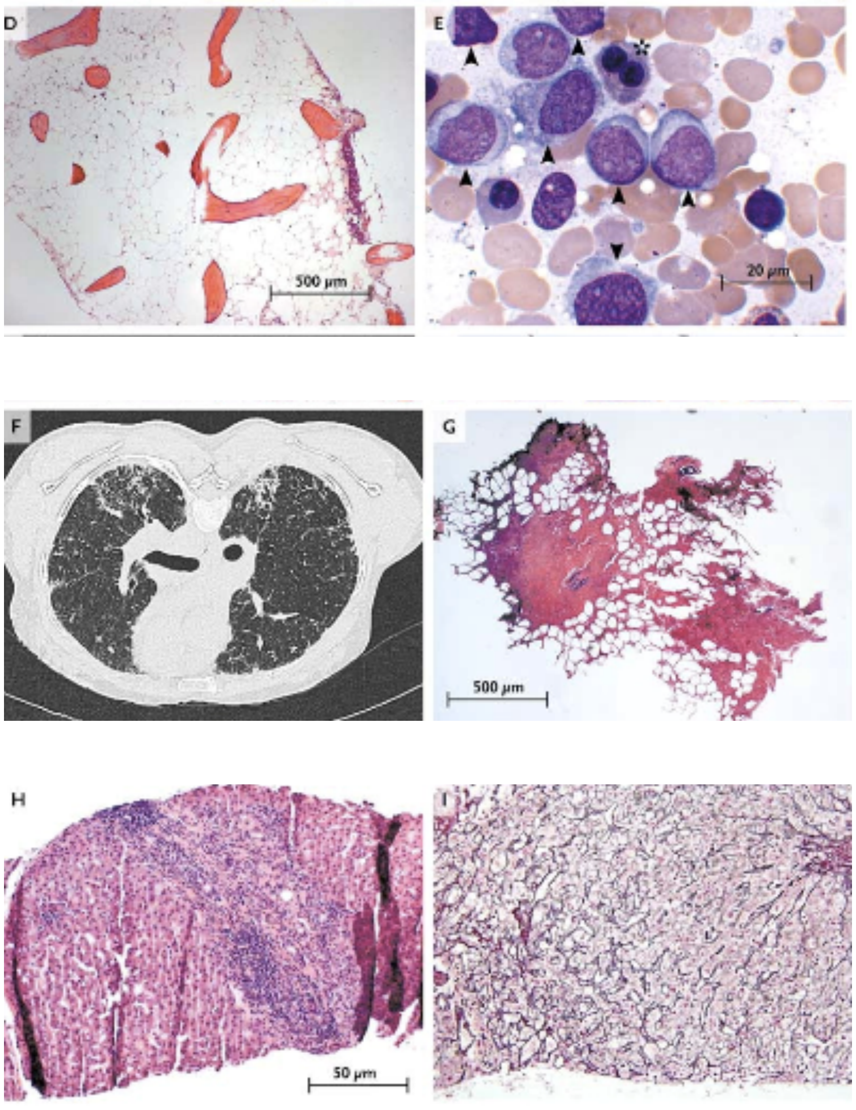
incidence: 1/1,000,000

Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes:



Gadalla. Aging Cell Dec. 2010

Telomere Disease: Dyskeratosis Congenita



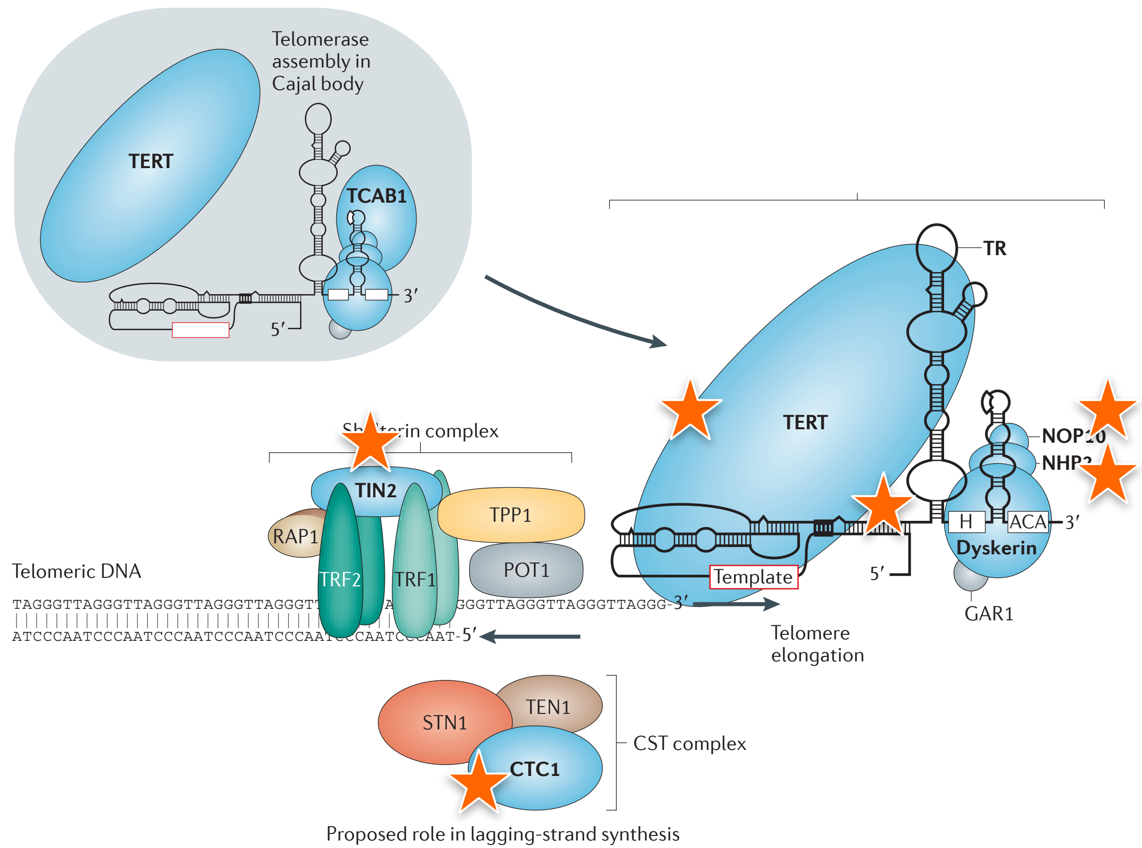
Hypoplastic bone marrow
Opportunistic infections
Anaplastic anemia
Progressive bone marrow failure
Acute myeloid leukemia

Lung fibrosis

Liver cirrhosis

Rodrigo T et al., . N Engl J Med 2009; 361:2353-2365

Mutations in Several Telomere Maintenance Genes



★ Dyskeratosis congenita mutations

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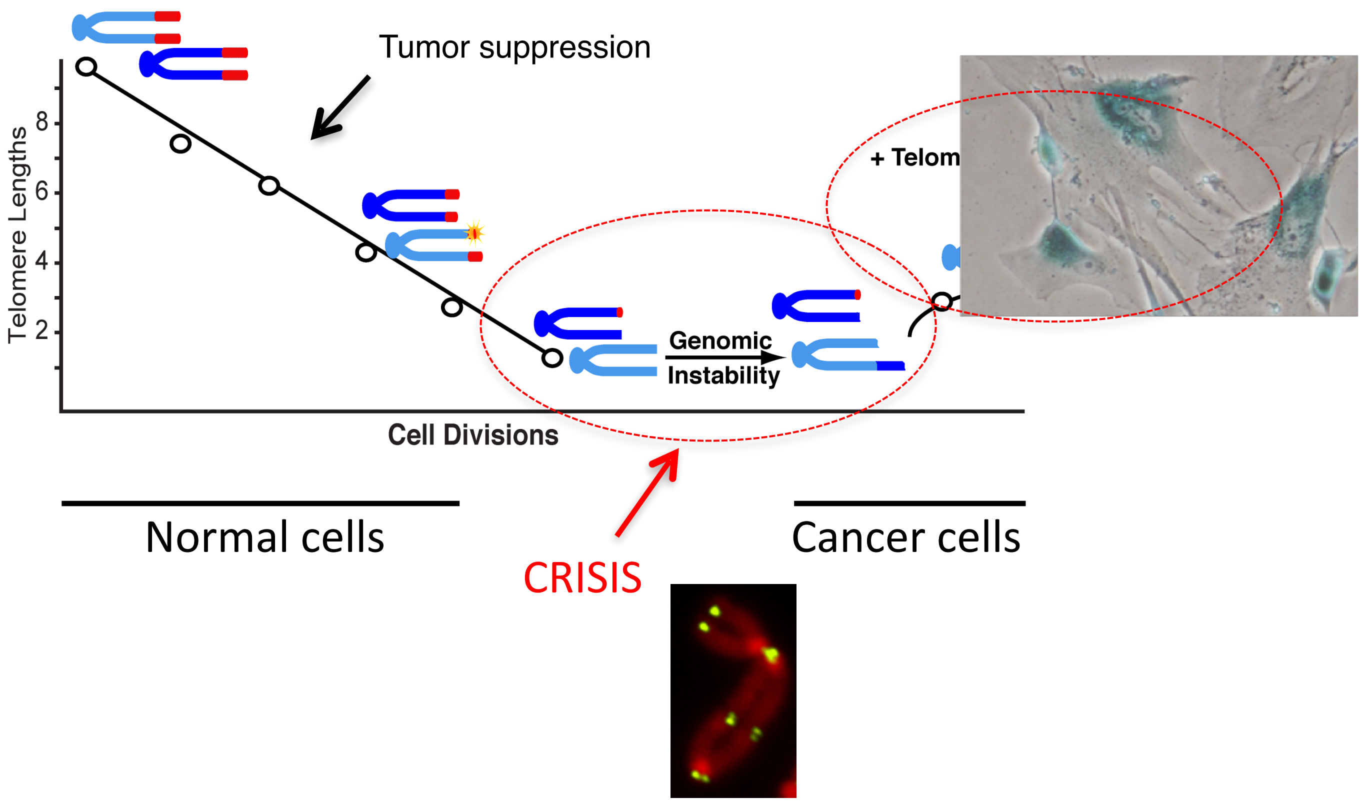
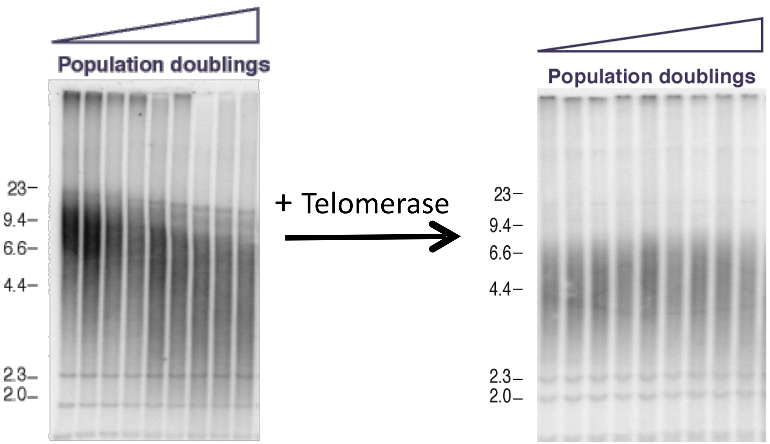
…. Now to the Good Cop / Bad Cop
Dysfunction Telomeres and Telomerase Reactivation



hTERT is Activated in Almost All Tumors …. Key for Unlimited Proliferative Capacity



Normal cells

Cancer cells

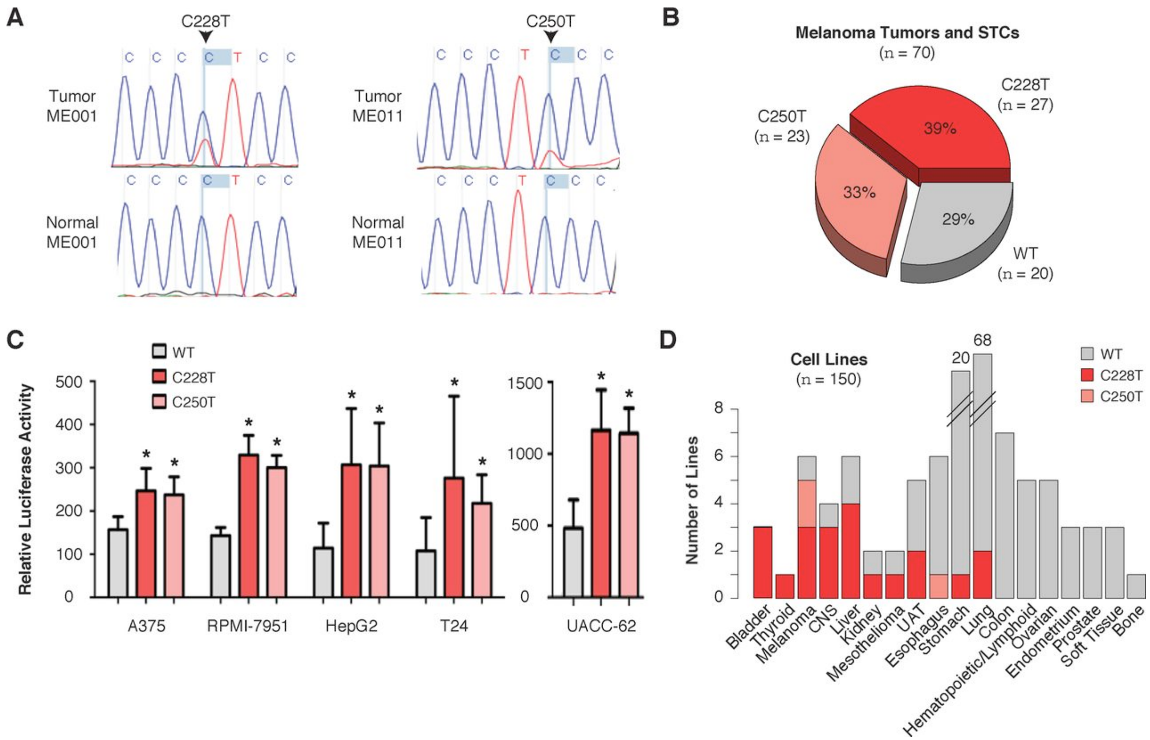
Pathology	# Positive/# Tested	% Positive
Normal*	1/ 196	0.5%
Preinvasive	123/ 410	30%
Malignant	1934/ 2031	95%
Adjacent to malignant	77/ 690	11%

Kim et al., Science 1998

Mechanism of hTERT Reactivation

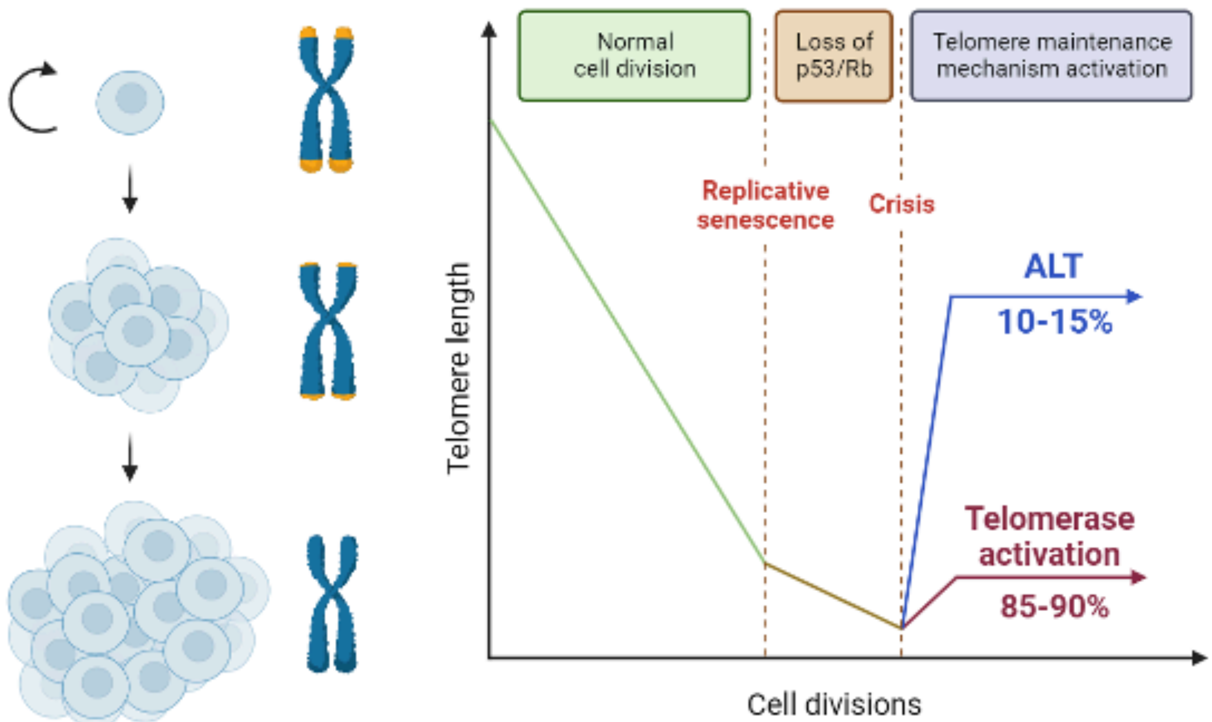
Highly recurrent TERT Promoter Mutations in:

- Familial and Sporadic Melanoma
- Adrenal Tumors
- Basal Cell carcinoma
- Squamous Cell Carcinoma
- Meduloblastoma
- Glioma

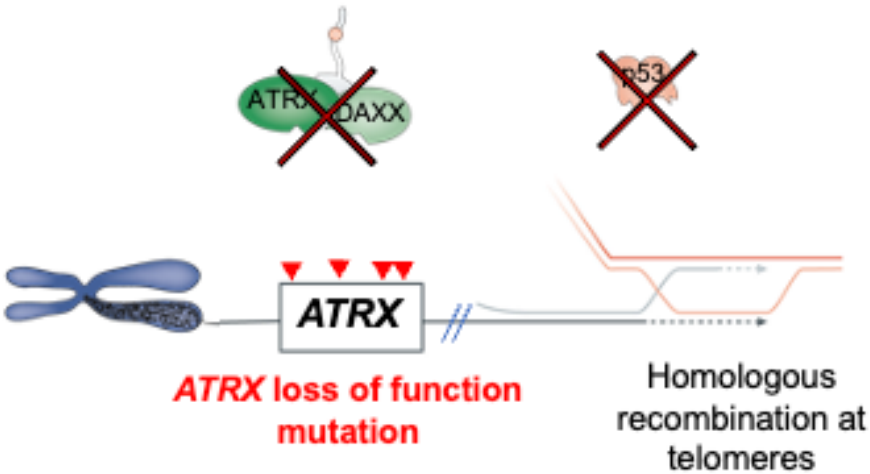


Horn et al., and Huang et al., Science 2013

Another major telomere maintenance pathway: ALT (Alternative Lengthening of Telomeres)



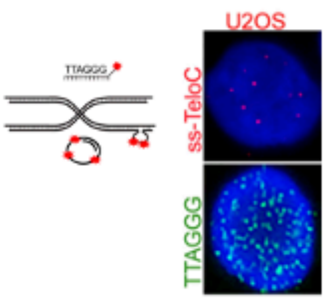
Loss of function mutations in *ATRX/DAXX*



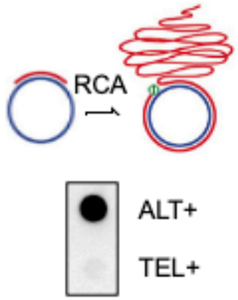
80% of IDH-mutant astrocytoma
30% of sarcoma
30% of neuroblastoma

Characteristics of ALT

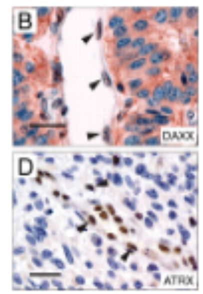
Single-stranded telomeric C-rich DNA (ss-TeloC)



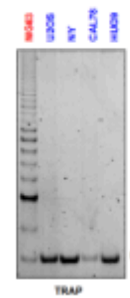
Extrachromosomal C-circles



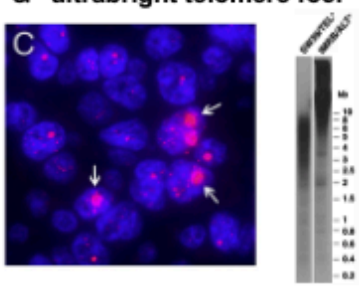
Loss of function mutations in *ATRX, DAXX* or *SMARCAL1*



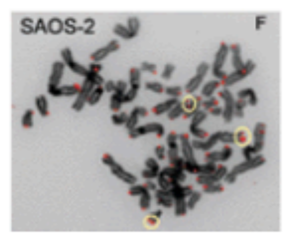
Lack telomerase activity/TERT expression



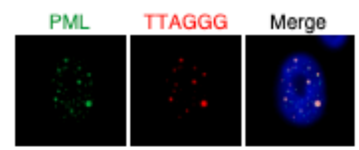
Heterogeneous telomere lengths & "ultrabright telomere foci"



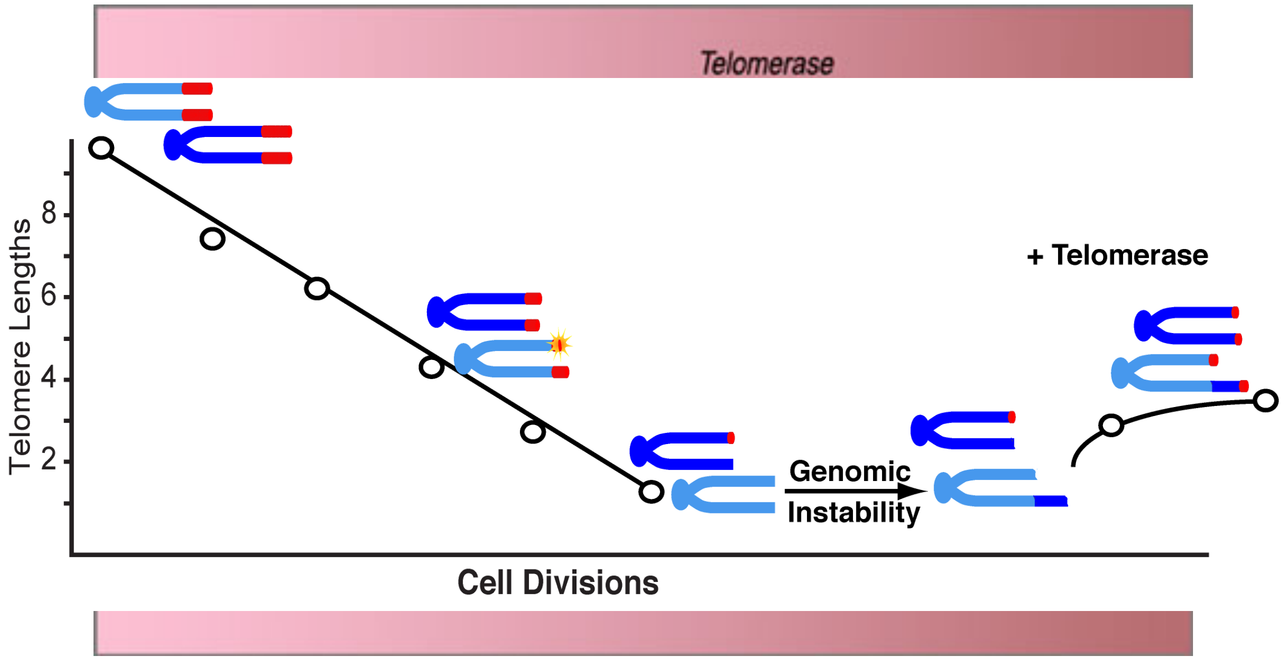
Telomere sister chromatid exchange (T-SCEs)



ALT-associated PML bodies (APBs)



Telomeres Shorten in The Early Stages of Tumorigenesis

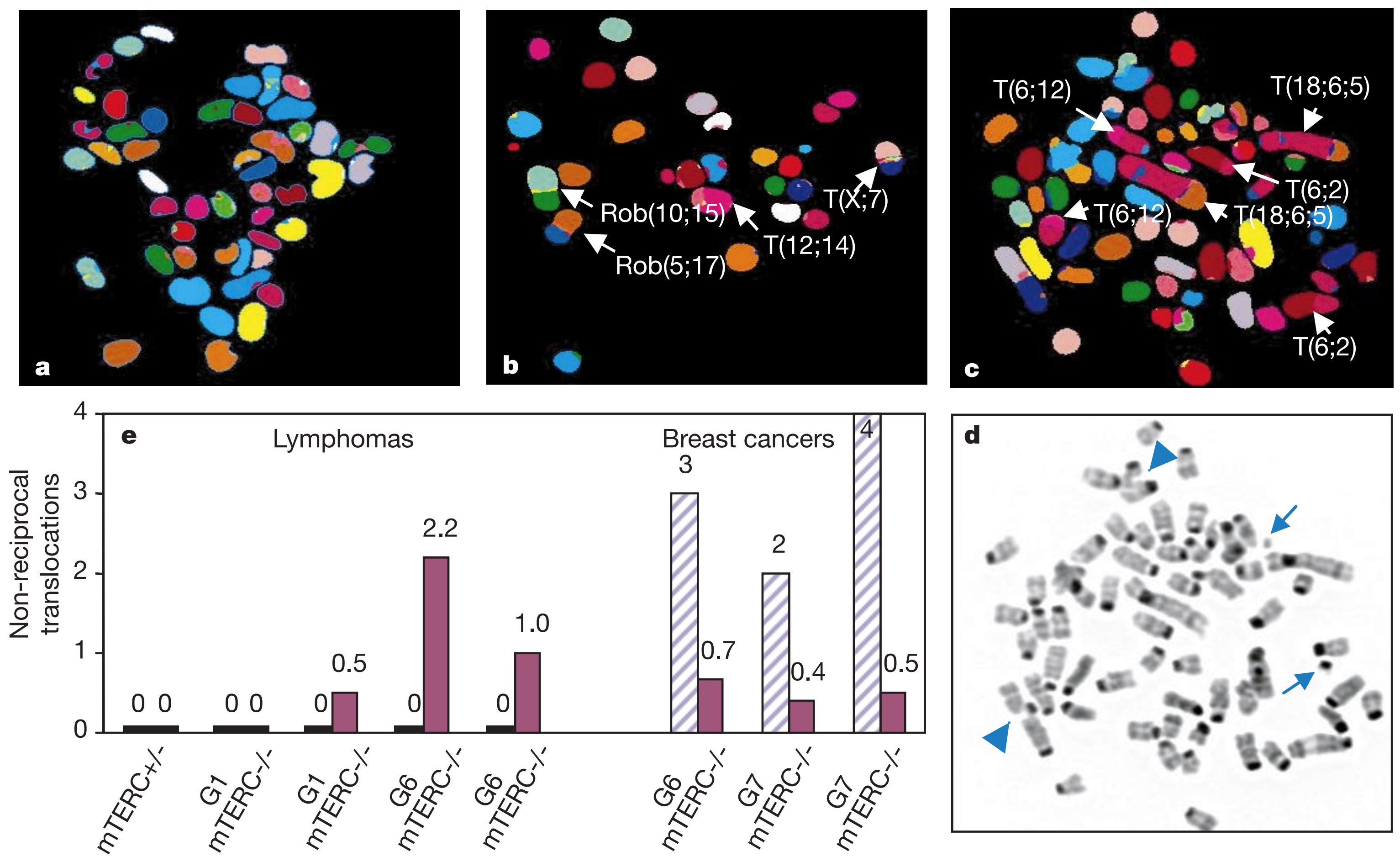


Breast cancer

Chin et al. Nat. Genet. 2004

Same applies for oral cavity cancer, intestinal, prostate and pancreatic preinvasion neoplasia

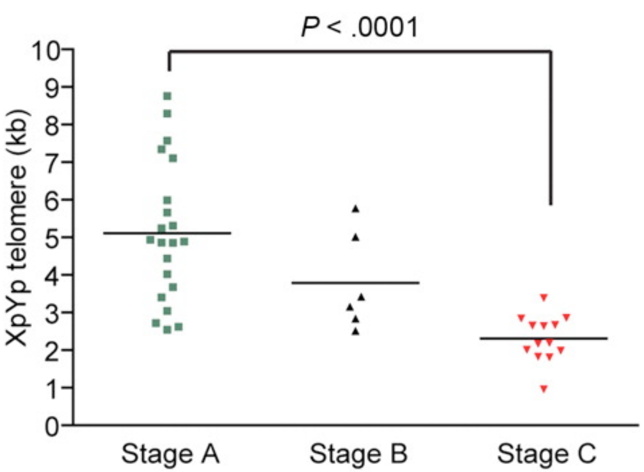
Tumors that develop in TERC / P53 KO mice exhibit Highly rearranged chromosomes



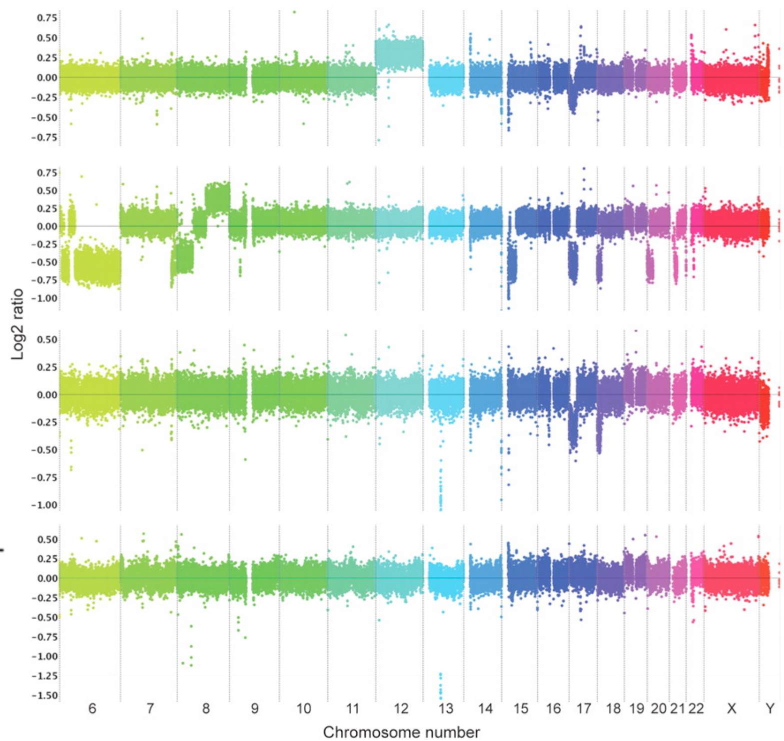
Artandi et al., 2000

Evidence of Telomere Crisis During CLL (Chronic Lymphocytic Leukemia) Progression

Telomere Length



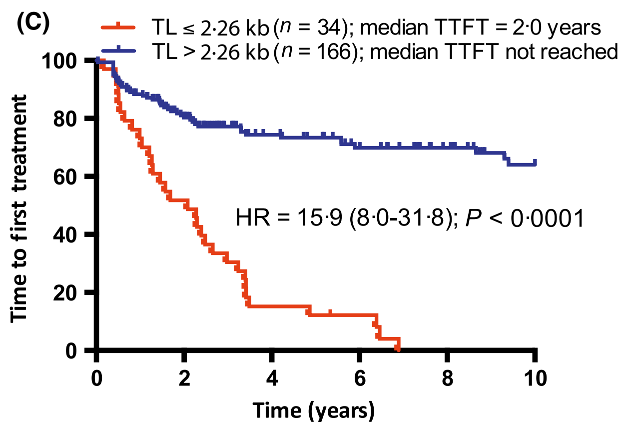
Array CGH

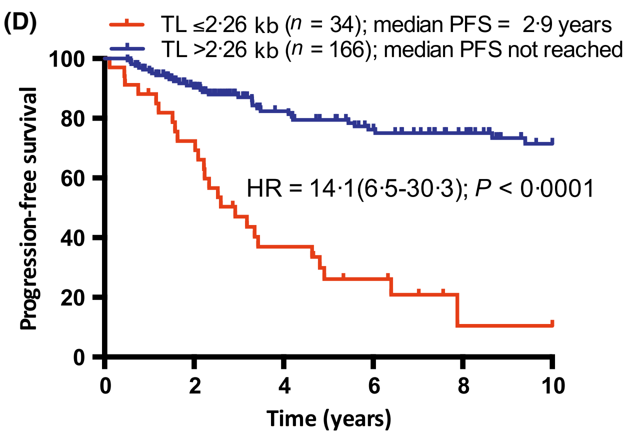


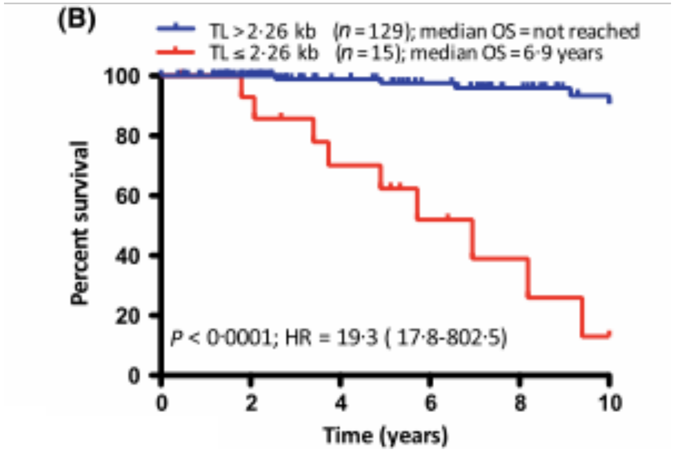
		XpYp telomere		
Id	Binet stage	Mean ± SD (kb)	Lower 20th% (kb)	Telomer dysfunctic
#36	C	1.378 ±1.30	0.445	Yes
#35	C	1.522 ±0.64	0.994	Yes
#21	A	1.955 ±2.13	0.480	Yes
#17	A	4.716 ±1.70	3.467	No

Lin TT et al., Blood 2010

Telomere Dysfunction Accurately Predicts Clinical Outcome in CLL

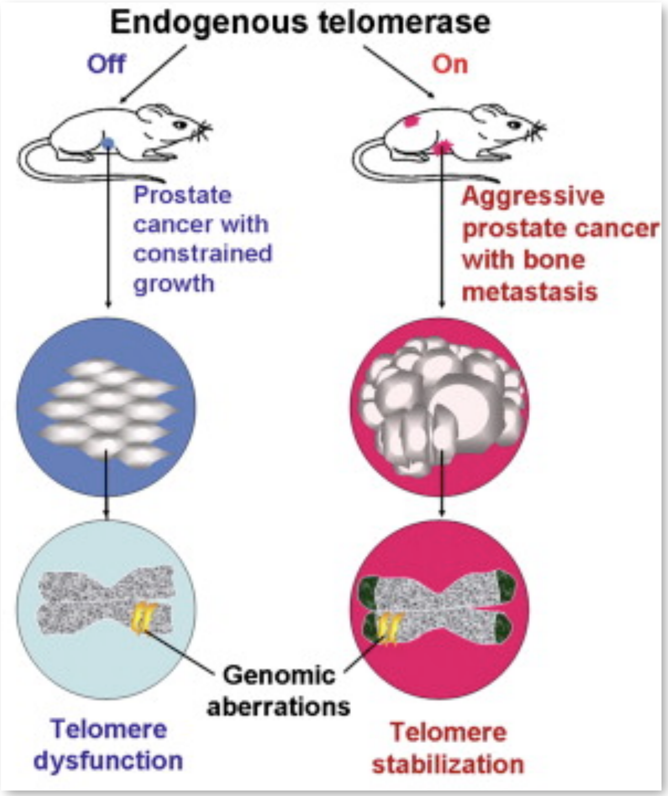






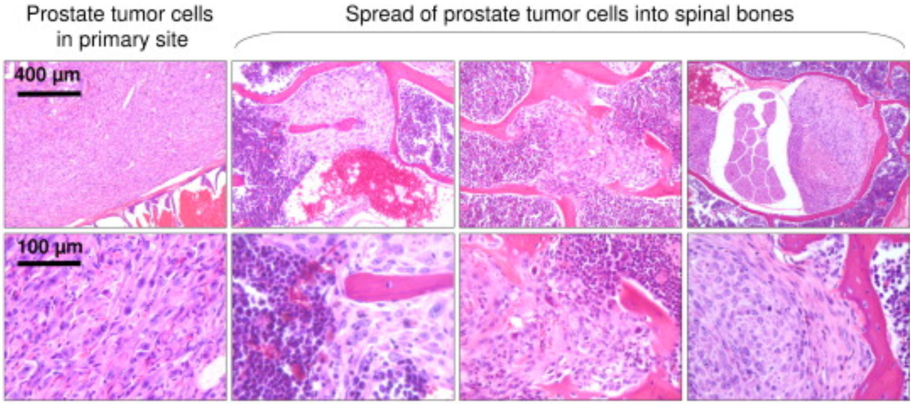
Lin TT et al., BJH 2014

The cleanest Experiment was Done using Prostate Cancer Mouse Model (PTEN/P53)



- p53/PTEN conditional knockout mice characteristically develop locally invasive non-metastatic prostate adenocarcinoma.

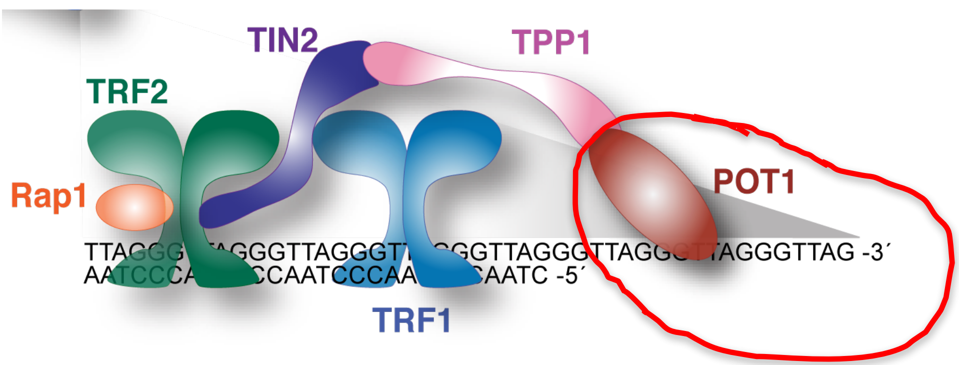
- Activating telomerase follow a period of telomere dysfunction drives metastatic progression of the tumor to the bone.

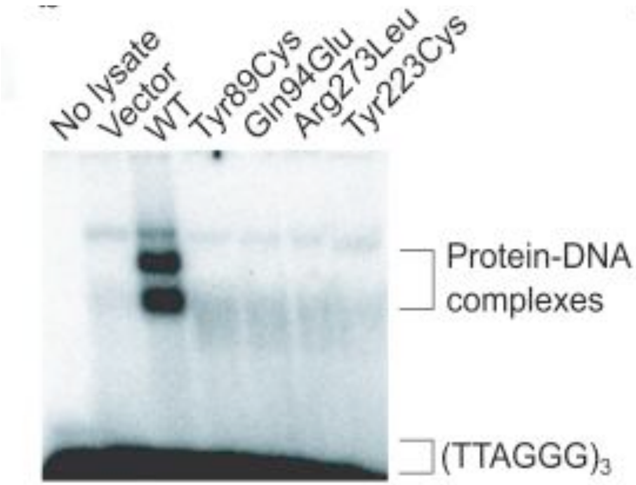


Ding et al., Cell 2012

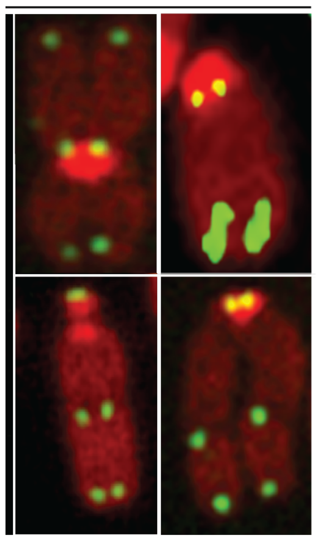
A Novel Pathway that Induced Telomere Dysfunction

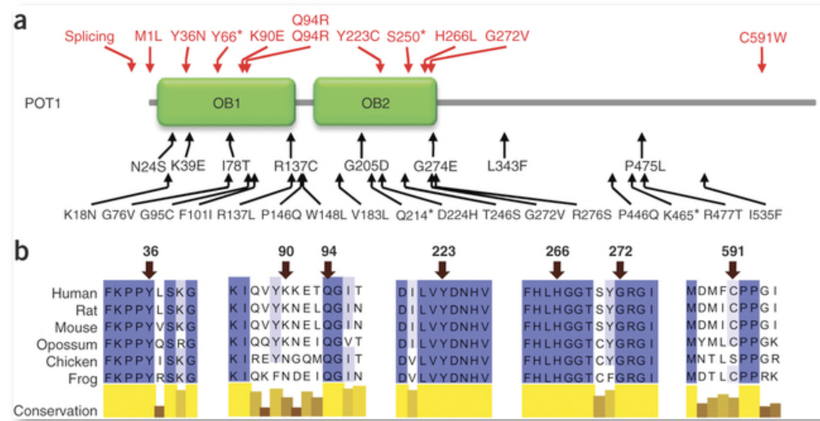
Mutations in the Shelterin Subunit – POT1





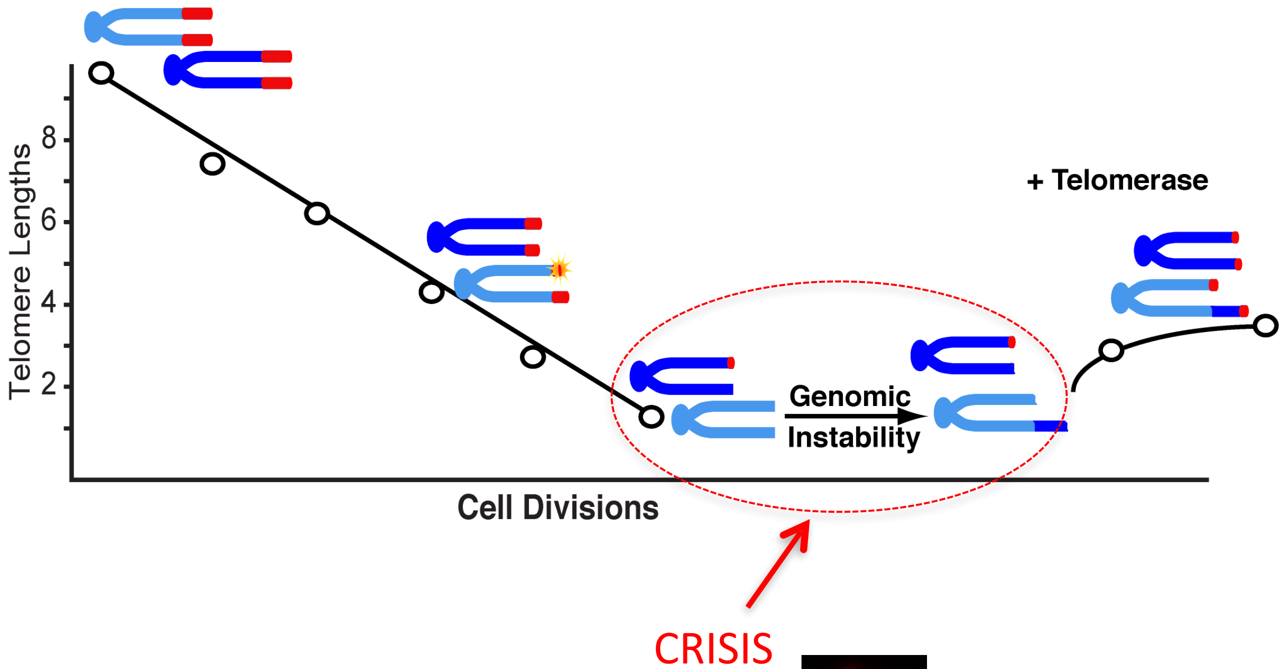
POT1 mutations cause telomere dysfunction in Lymphoid Tumors (chronic lymphocytic leukemia) and in Familial Melanoma

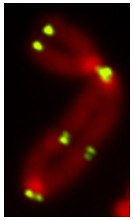




Ramsey et al., 2013 nature genetics
Shi et al., nature genetics 2014
Espinosa et al., nature genetics 2014

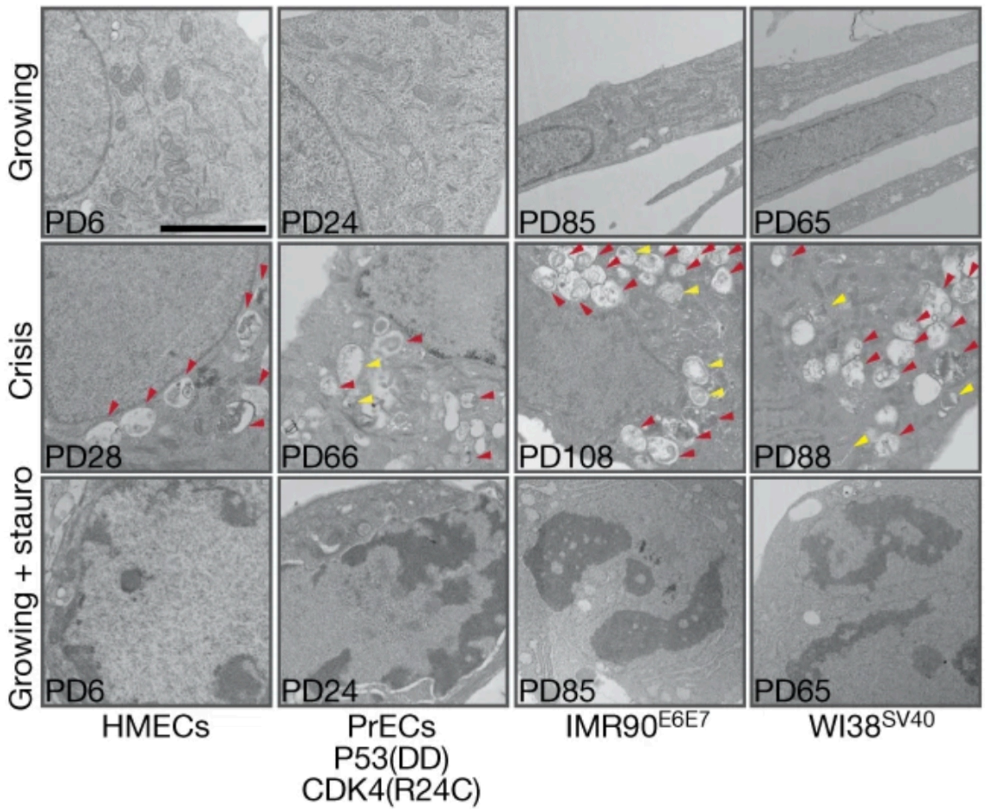
Let's dig deeper into ....**Telomere Crisis**



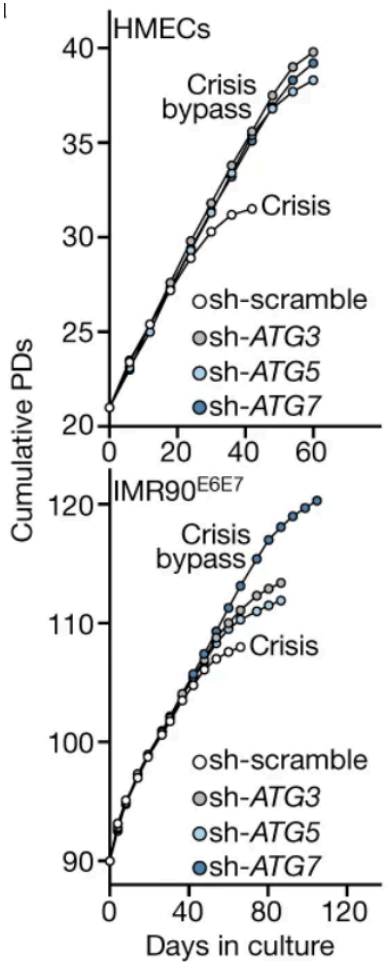


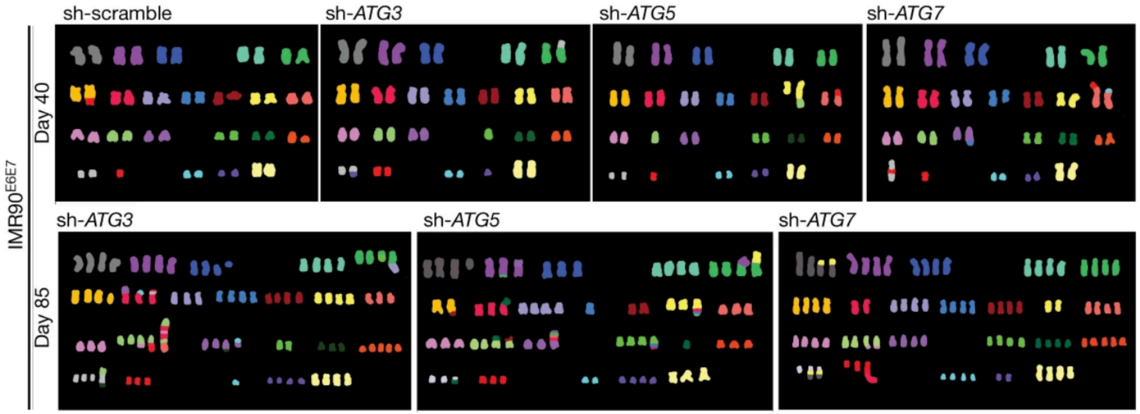
Cell Death
BFB: breakage fusion bridge

Crisis cells exhibit features of active autophagy



Autophagy inhibition promotes crisis bypass.





★ End-replication problem

★ End-protection problem

★ Telomeres and Telomerase

★ Cellular aging (Senescence) and Telomeres

★ Telomere Length Changes in Human Aging

★ Telomeropathies – Dyskeretosis Congenita

★ Telomere/Telomerase dynamics in Cancer

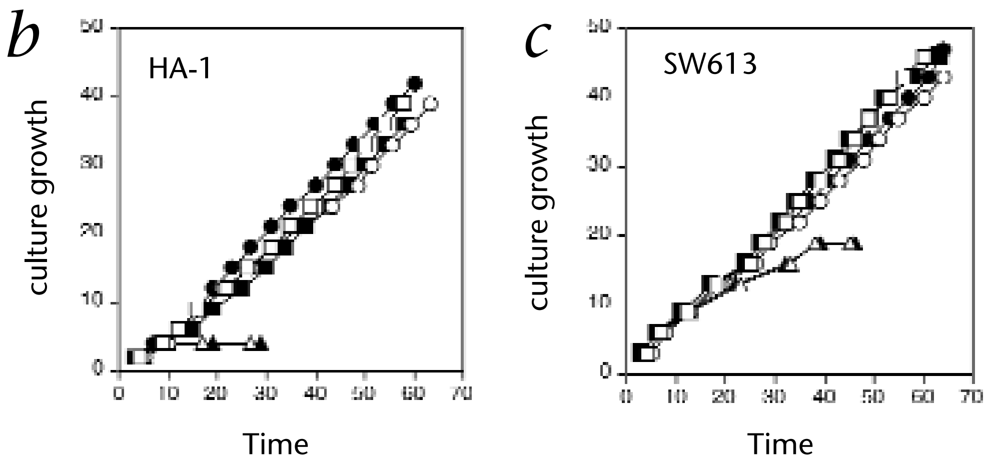
★Telomerase Inhibition in the Clinic

★Telomere dynamics in Embryonic Stem cells

Can Telomerase Inhibition be Used to Treat Cancer?

Proof of principle experiment

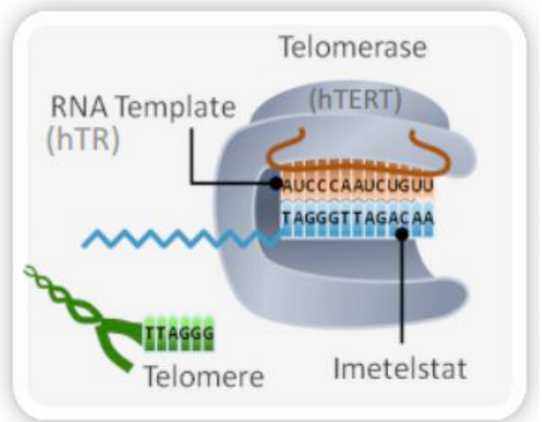
Weinberg lab: Introduced catalytically dead hTERT into human tumor lines



Hahn and Weinberg et al., Nature Medicine 1999

Telomerase Inhibitors in Clinical Trials

Class I - Oligonucleotide-based therapeutics small molecule inhibitors – GRN163L / Imetelstat (GERON)



N3' → P5' - *thio* – Phosphoramidate

- 14 clinical trials: 4 Phase II and 10 Phase I

- Phase II
  - Pediatric solid tumors
  - Non small cell lung cancer (NSCLC)
  - Myelofibrosis
  - Multiple Myeloma

- Phase III: Myelodisplastic syndromes

- Imetelstat is currently used to Treat Hematologic Myeloid Malignancies and Thrombocytopenia

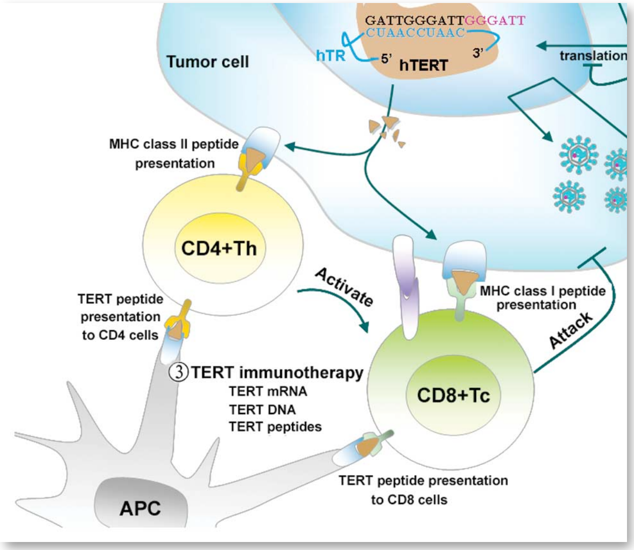
Major challenges: toxicity and time for efficacy to show

Telomerase-directed immunotherapy

Class II: hTERT-based Immunotherapy: vaccines targeting telomerase

Peptide vaccines•

- GV1001 and INO-1400 are peptide vaccine consisting of a small amino acid epitope of hTERT
- UV1 combined with checkpoint inhibitors is being tested across solid tumors (melanoma, mesothelioma); multiple phase II studies report favorable safety and signals of benefit. (Fast Track in mesothelioma.)
- • GV1001: a large phase III in pancreatic cancer (TeloVac) was negative overall; biomarker-enriched follow-ups (eotaxin-high) are being explored.



TCR-engineered T cells recognizing hTERT peptides are in early clinical development; feasibility shown, with the broader TCR-T field advancing in solid tumors.

Telomerase-vaccines

Vaccine	Type	Target	Status	Key Findings
GV1001	Peptide	hTERT (611–626)	Phase III (pancreatic)	Safe; limited survival benefit
GX301	Multi-peptide	hTERT	Phase II	Induced T-cell responses
UV1	Long peptide	hTERT (mutliple epitopes)	Phase II–III	Promising synergy with PD-1 blockade
INO-1400	DNA	Full-length hTERT	Phase I–II	Durable immune memory, good safety
V934/ V935	Viral vector	hTERT	Phase I	Induced $CD8^+$ T cells

UV1 + pembrolizumab (melanoma): Durable responses; phase II data show improved survival compared to PD-1 blockade alone (NCT03538314).

UV1 + nivolumab/ipilimumab: Under evaluation in mesothelioma, prostate, and head & neck cancers.

INO-1400 + IL-12 DNA: Evaluated as maintenance therapy post-resection in solid tumors (NCT02960594).

ARTICLE

https://doi.org/10.1038/s41467-019-10179-z OPEN

FANCM limits ALT activity by restricting telomeric replication stress induced by deregulated BLM and R-loops

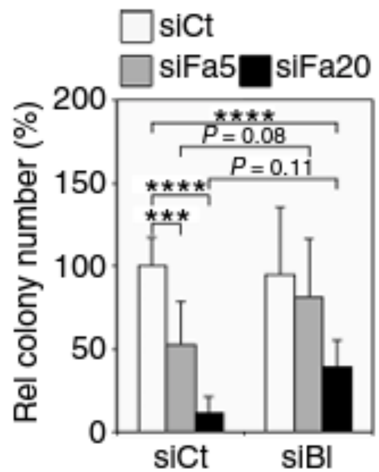
Bruno Silva[1], Richard Pentz[1], Ana Margarida Figueira[1], Rajika Arora[1], Yong Woo Lee[1], Charlotte Hodson[2], Harry Wischnewski[3], Andrew J. Deans[2,4] & Claus M. Azzalin[1]

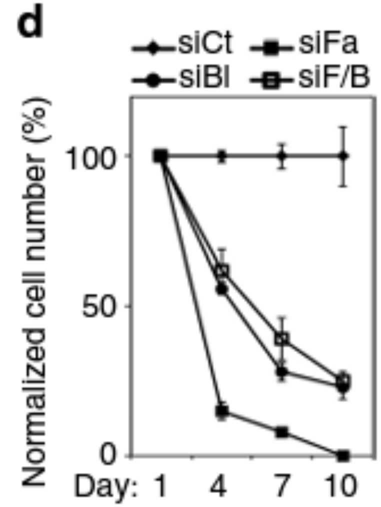
ARTICLE

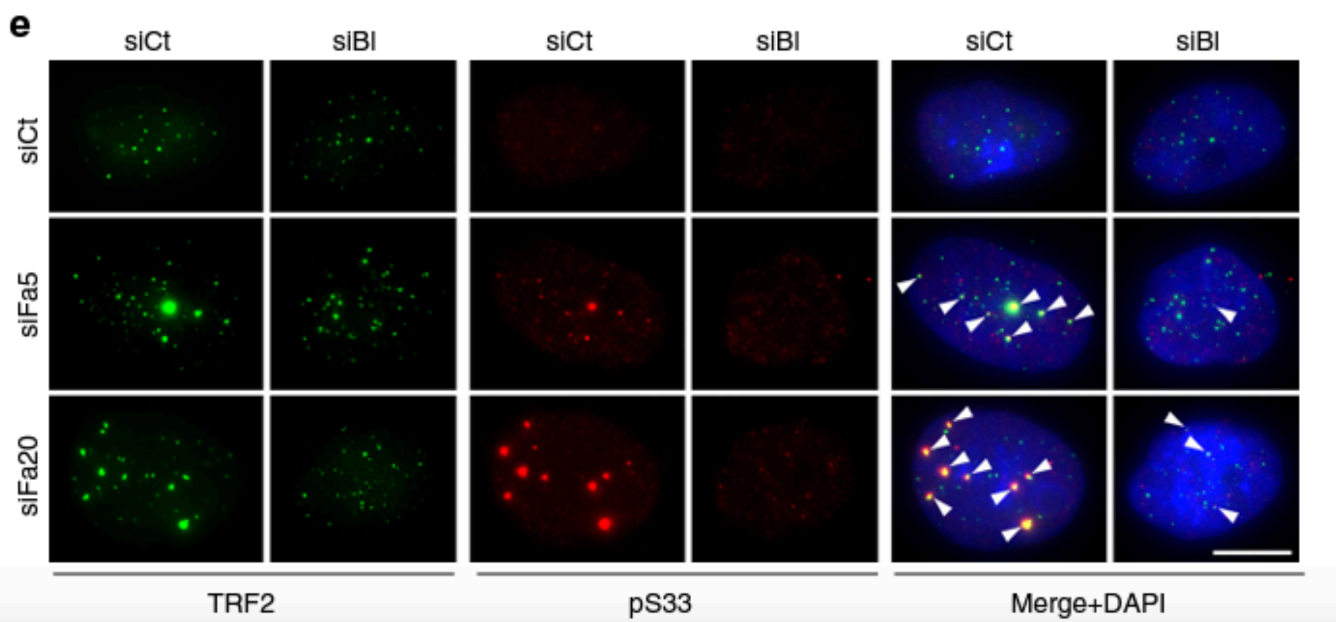
https://doi.org/10.1038/s41467-019-10180-6 OPEN

The FANCM-BLM-TOP3A-RMI complex suppresses alternative lengthening of telomeres (ALT)

Robert Lu[1,5], Julienne J. O'Rourke[2,3,5], Alexander P. Sobinoff[1,5], Joshua A.M. Allen[1], Christopher B. Nelson[1], Christopher G. Tomlinson[1], Michael Lee[1], Roger R. Reddel[4], Andrew J. Deans[2,3] & Hilda A. Pickett[1]

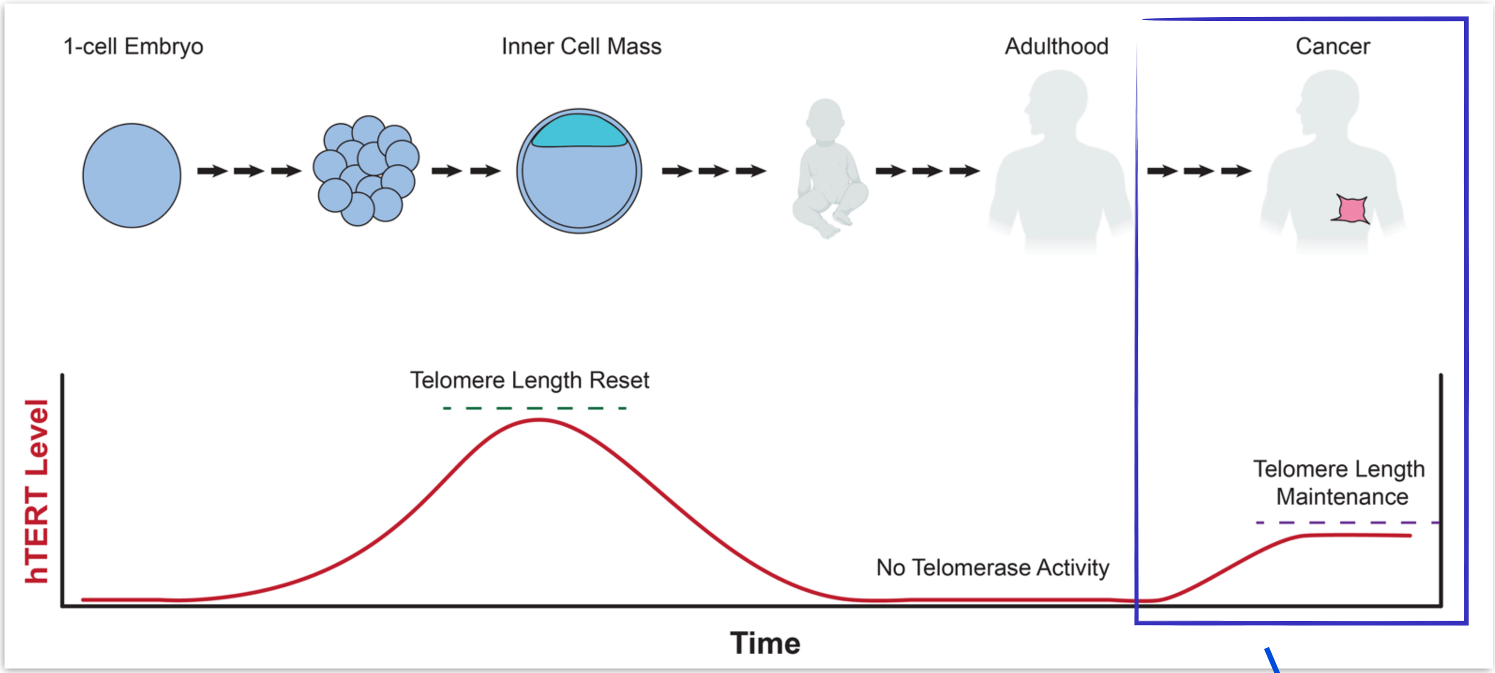

siCt
siFa5
siFa20
Rel colony number (%)
200
150
100
50
0
****
P = 0.08
P = 0.11
****
***
siCt
siBl

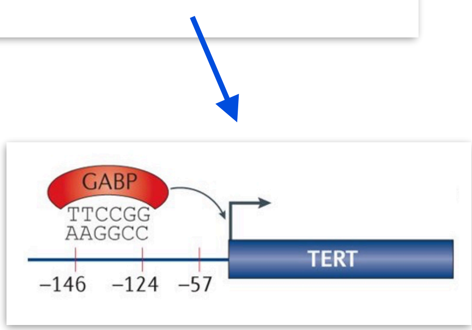

d
siCt
siFa
siBl
siF/B
Normalized cell number (%)
100
50
0
Day: 1 4 7 10


e
siCt
siBl
siCt
siBl
siCt
siBl
siCt
siFa5
siFa20
TRF2
pS33
Merge+DAPI

- ★ End-replication problem
- ★ End-protection problem
- ★ Telomeres and Telomerase
- ★ Cellular aging (Senescence) and Telomeres
- ★ Telomere Length Changes in Human Aging
- ★ Telomeropathies – Dyskeretosis Congenita
- ★ Telomere/Telomerase dynamics in Cancer
- ★ Telomerase Inhibition in the Clinic
- ★ **Telomere dynamics in Embryonic Stem cells**

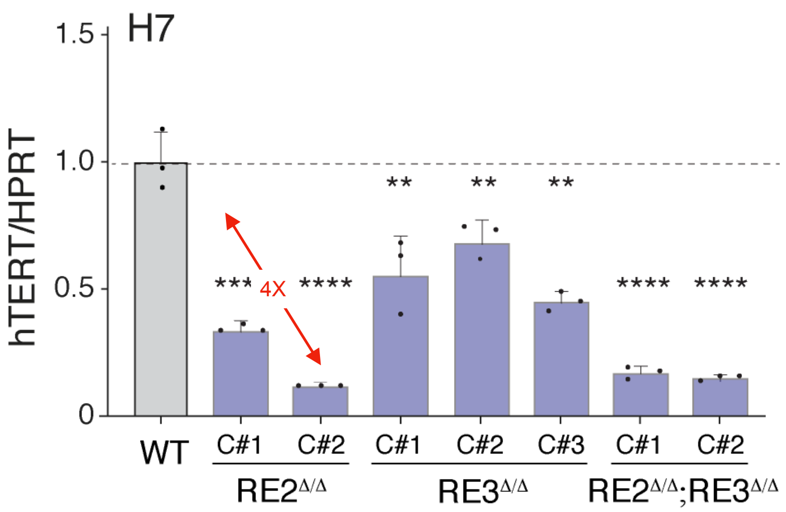
hTERT expression/telomere lengthening are tightly regulated during human development

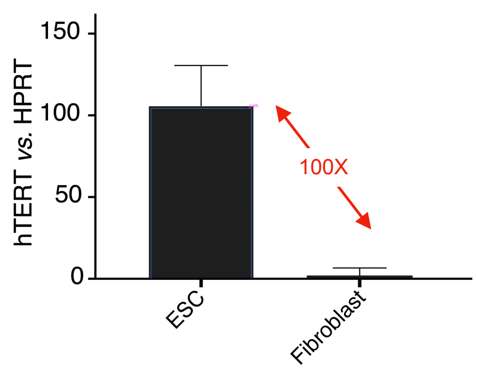




Horn et al., 2013. Huang et al., 2013

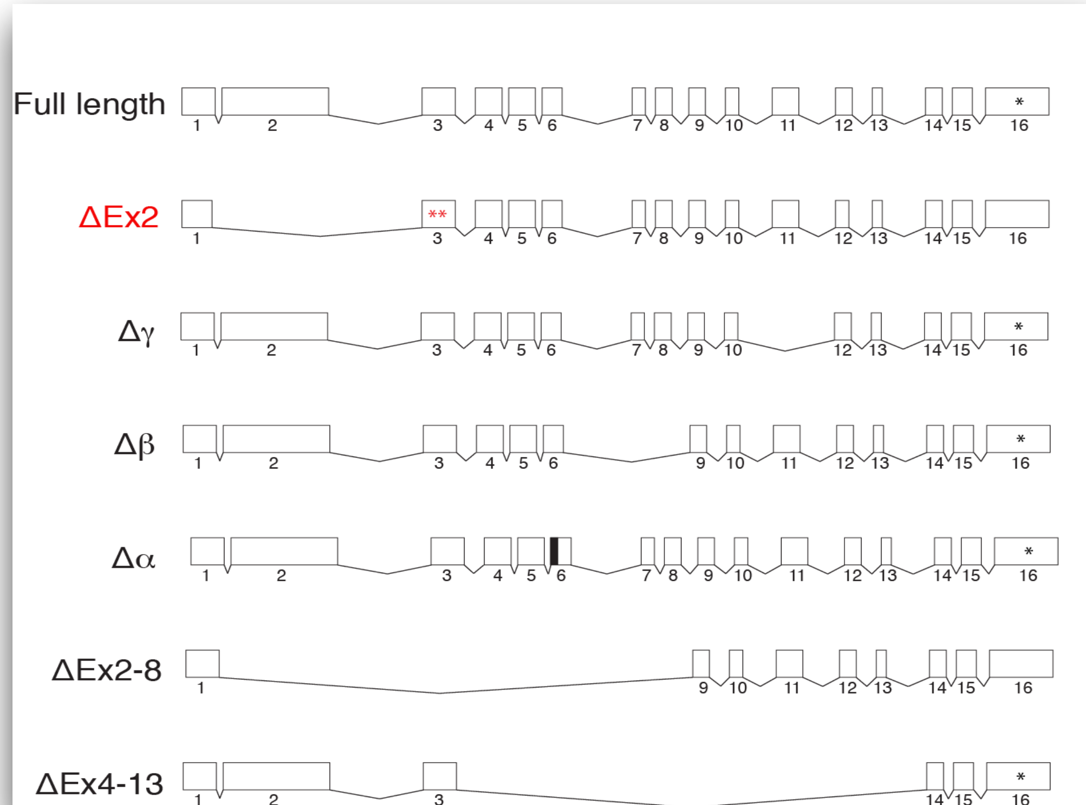
Mapping the landscape of hTERT enhancers





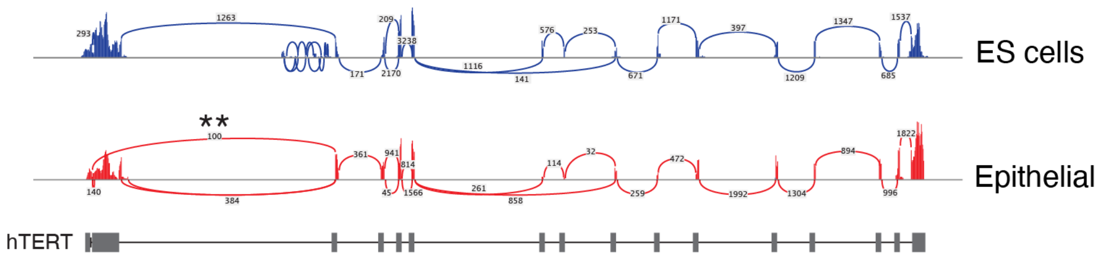
Penev et al., 2021.

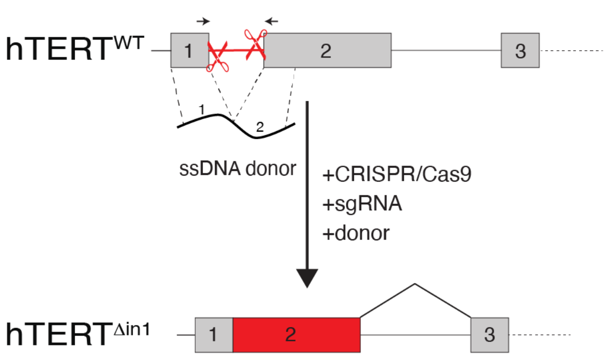
hTERT mRNA is extensively alternatively spliced

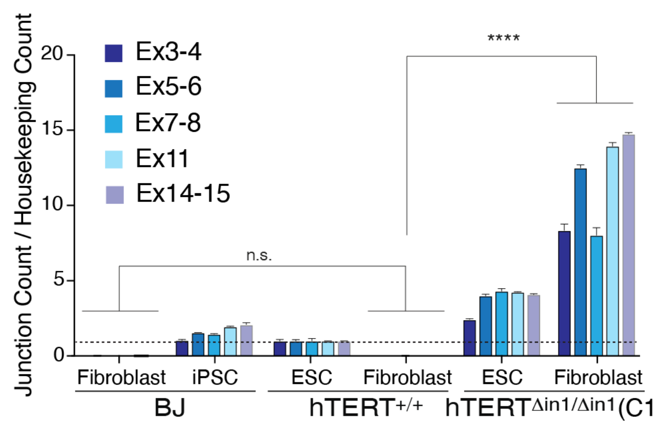


Alternative splicing of hTERT Exon-2 regulates telomerase activity

RNA Capture-seq

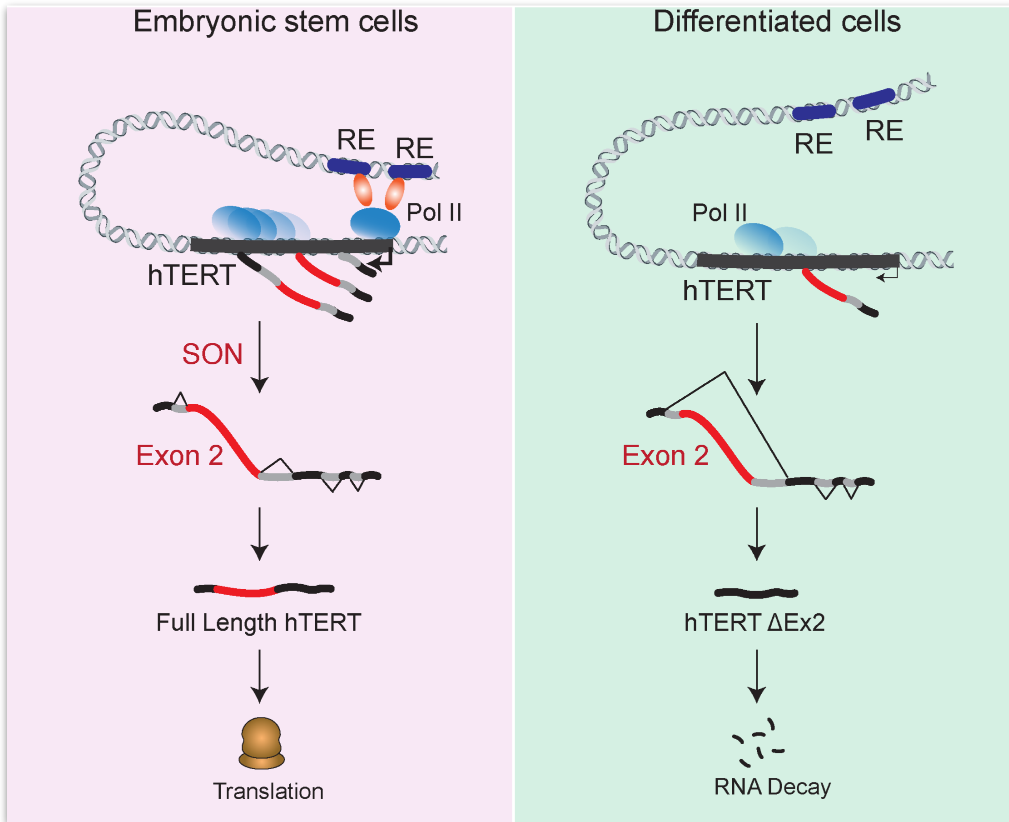






Penev et al., 2021.

Alternative splicing is a developmental switch for hTERT expression



Penev et al., 2021.PMID: 33852895

….. This was the telomere path from maize, Tetrahymena and yeast, all the way to **human cancer…**