# Cancer Biology I :

**Topics covered**

**Week 5:**
**Lecture 5/Exercises-paper: Telomeres and cellular senescence**
**(Chapters 10 (Weinberg))**

Weeks 6:
Lecture 6/Exercises-Q&A: CDKs and G1/S control
(Chapter 8 (Weinberg book): pRb and control of the cell cycle clock)

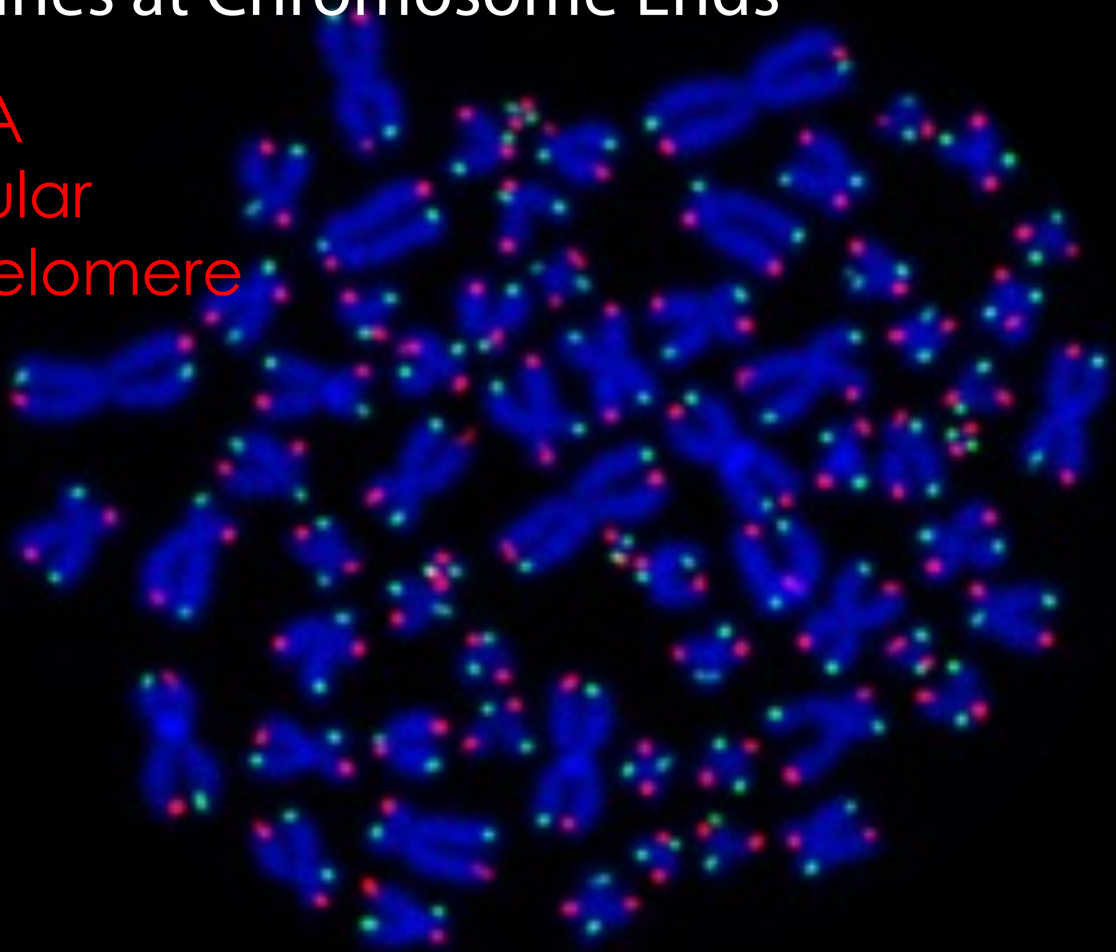
1 week break

Week 7, Monday: Q & A session: discussion of your questions
(to be submitted via email to me in advance during week 6!!!)
Wednesday October 30st 2024: exam in **CM 1 121**(contrôle continu)

Today:

# Telomeres and Telomerase:
# RNA/protein-dependent Machines at Chromosome Ends

- Telomerase, telomerase RNA
- Telomeres and cancer: cellular senescence and cell crisis/telomere crisis
- ALT pathway, TERRA



# Telomeres: Two Fundamental Functions

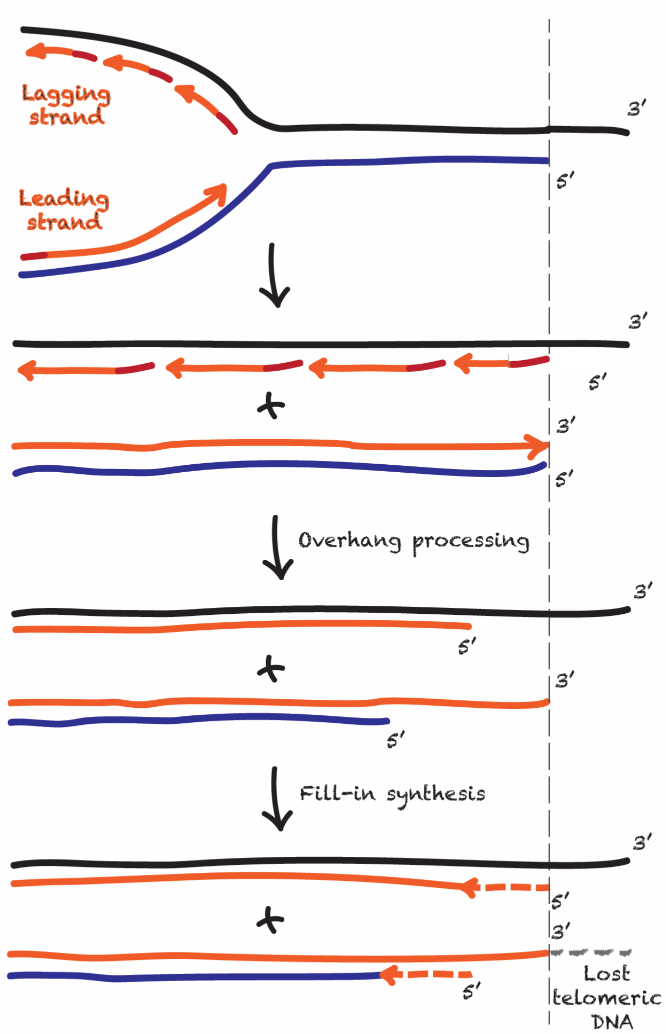
1. **Regulation of cellular lifespan**

→ **Tumor suppression**

2. **Repression of chromosome end-to-end fusions**

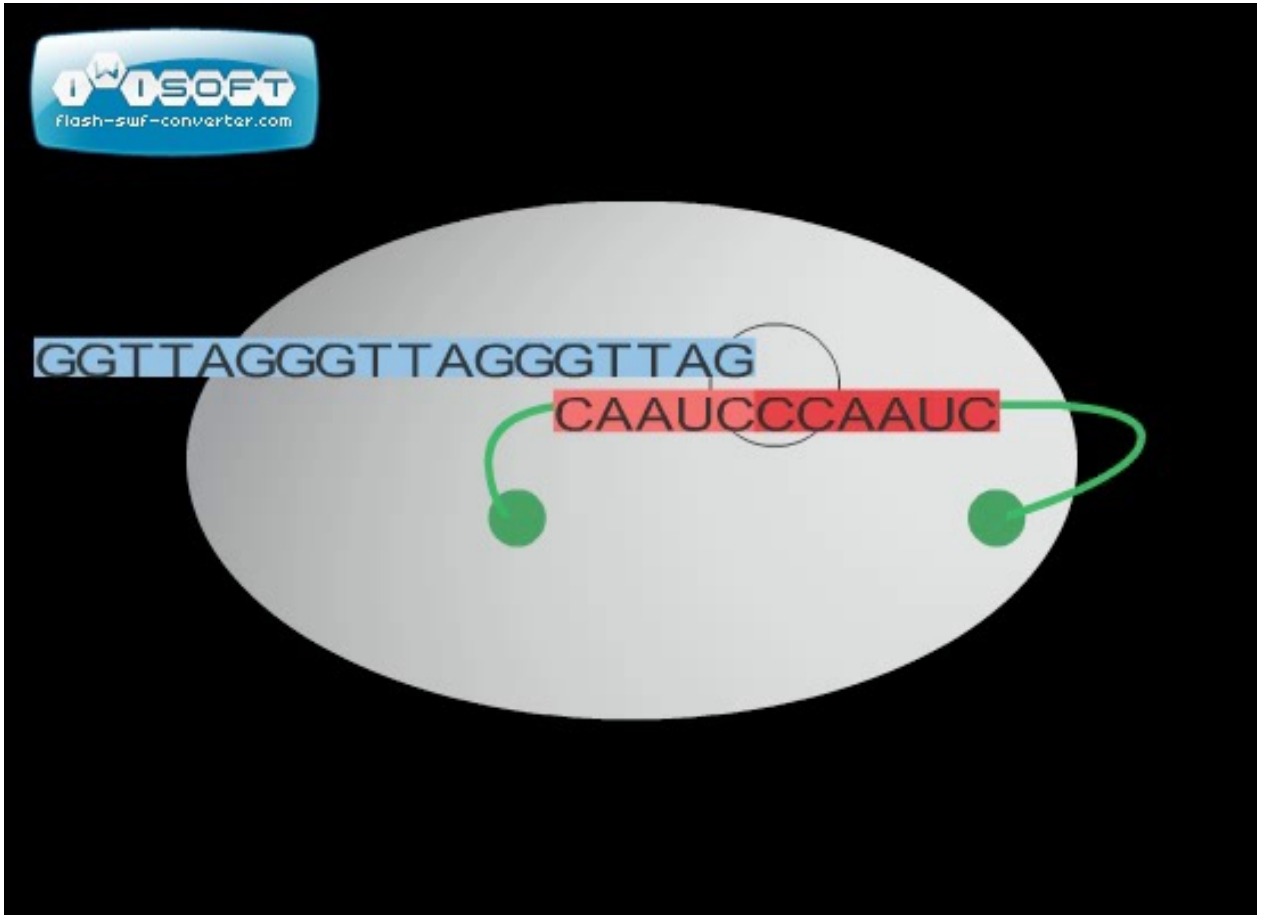
→ **Genome stability**

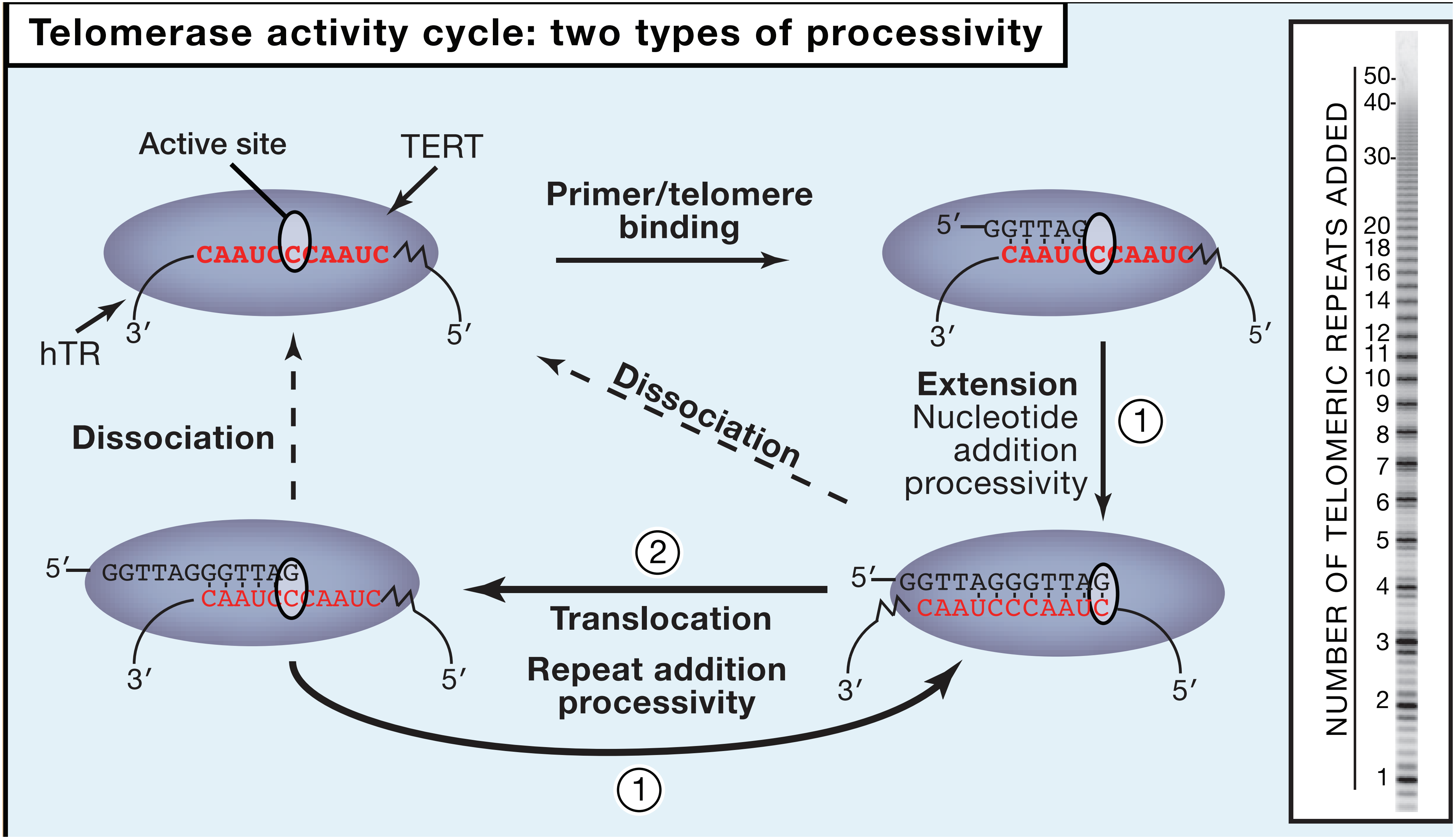
## The Telomere End Replication Problem



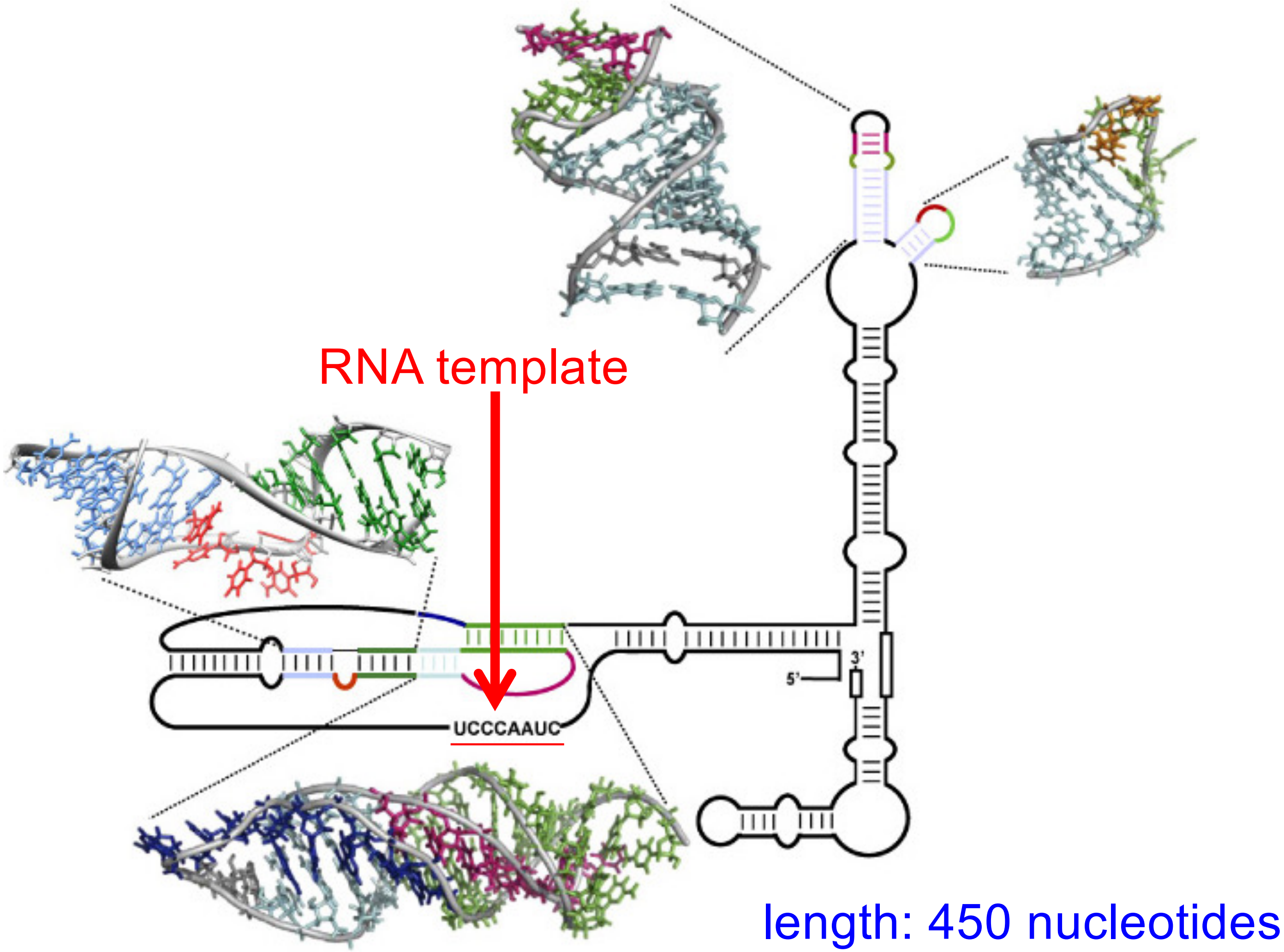

solved by **telomerase**

# Movie: Telomerase Reaction Cycle

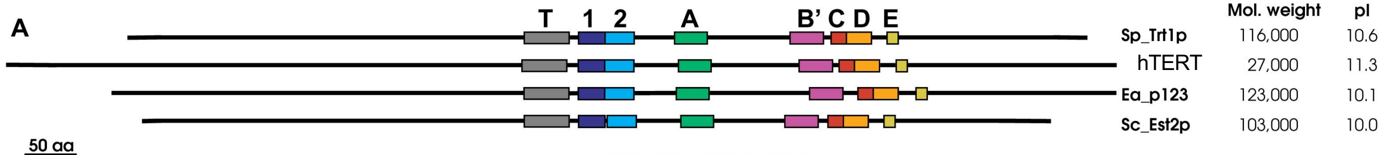


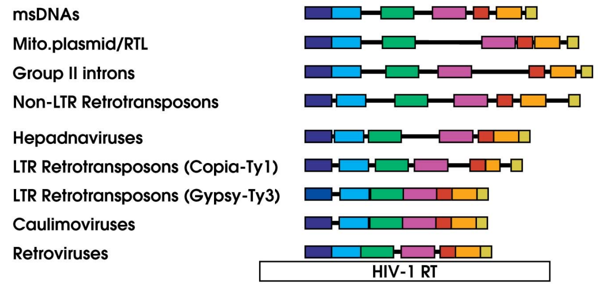

Telomerase activity cycle: two types of processivity
Active site
TERT
hTR
CAAUCCCAAUC
3′
5′
Primer/telomere binding
5′
GGTTAG
CAAUCCCAAUC
3′
5′
Extension
Nucleotide addition processivity
1
Dissociation
Dissociation
5′
GGTTAGGGTTAG
CAAUCCCAAUC
3′
5′
2
Translocation
Repeat addition processivity
1
5′
GGTTAGGGTTAG
CAAUCCCAAUC
3′
5′
NUMBER OF TELOMERIC REPEATS ADDED
50-
40-
30-
20
18
16
14
12
11
10
9
8
7
6
5
4
3
2
1

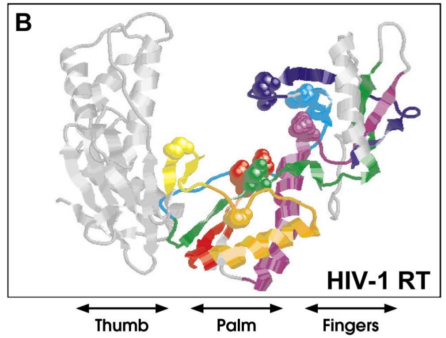
## Human Telomerase RNA Secondary Structure



# RT-motifs in Telomerase Reverse Transcriptases (TERT)




C

```
                         Motif T
TRT con          WL    hh hh pFFY TE p   p   Y RK  W  L  h I    K
Sp_Trt1p  429 WLYNSFIIPILQSFFYITESSDLRNRTVYFRKDIWKLLCRPFITSMKM   8
hTRT      546 WLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLK 10
Ea_p123   441 WIFEDLVVSLIRCFFYVTEQQKSYSKTYYYRKNIWDVIMKMSIADLKK  8
Sc_Est2p  366 WLFRQLIPKIIQTFFYCTEISSTVT-IVYFRHDTWNKLITPFIVEYFK  8

                       Motif 1     Motif 2                                    Motif A
TRT con              h    hRhIPKK p    FRhI  h   h        K             PcLYFh hDh  CYD I       hhK  K
Sp_Trt1p  NNVRMDTQKTTLPPAVIRLLPKKNT- 0 FRLITNLRKRFLIKMGSNKKMLVSTNQTL 40 FGRKKYFVRIDIKSCYDRIKQDLMFRIVKKKLKD  82
hTRT      EVRQHREARPALLTSRLRFIPKPDG- 0 LRPIVNMDYVVGARTFRREKRAERLTSRV 45 PPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKP  87
Ea_p123   KEVEEWKKSLGFAPGKLRLIPKKTT- 0 FRPIMTFNKKIVNSDRKTTKLTTNTKLLN 41 GQPKLFFATMDIEKCYDSVNREKLSTFLKTTKLL 100
Sc_Est2p  CRNHNSYTLSNFNHSKMRIIPKKSNN 1 FRIIAIPCRGADEEEFTIYKENHKNAIQP 42 VLPELYFMKFDVKSCYDSIPRMECMRILKDALKN  68
                                                      R                                 AF
RT con                  p hh h K      hR h         K                      h  hDh  GY  h
Sc_a1     LSNELGTGKFKFKPMRIVNIPKPKGG 0 IRPLSVGNPRDKIVQEVMRMILDTIFDKK 27 FGGSNWFIEVDLKKCFDTISHDLIIKELKRYISD  20
Dm_TART   SILRIGYYPDAWKHAQVKMILKPGKS 6 YRPISLLSGLSKMFERLLLKRLFRVDLFK 32 RKEYCSAVFLDISEAFDRVWHEGLLLKLAKILPY  25
HIV-1     EGKISKIGPENPYNTPVFAIKKKDST 1 WRKLVDFRELNKRTQDFWEVQLGIPHPAG  0 LKKKKSVTVLDVGDAYFSVPLDEDFRKYTAFTIP   7

                Motif B’                      Motif C                      Motif D            Motif E
TRT con   K Y Q  GIPQGS LS hL  h Y DL      F        LLRL DDFLhIT      A  F    h G  c p   N cK          W G S
Sp_Trt1p  SQYLQKVGIPQGSILSSFLCHFYMEDLIDEYLSFT  6 LLRVVDDFLFITVNKKD 0 AKKFLNLSLRGFEKHNFSTSLEKTVI 17 KKRMPFFGFSV 181
hTRT      KSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGI  5 LLRLVDDFLLVTPHLTH 0 AKTFLRTLVRGVPEYGCVVNLRKTVV 19 HGLFPWCGLLL 197
Ea_p123   KFYKQTKGIPQGLCVSSILSSFYYATLEESSLGFL 14 LMRLTDDYLLITTQENN 0 AVLFIEKLINVSRENGFKFNMKKLQT 23 QDYCDWIGISI 179
Sc_Est2p  KCYIREDGLFQGSSLSAPIVDLVYDDLLEFYSEFK  8 ILKLADDFLIISTDQQQ 0 VINIKKLAMGGFQKYNAKANRDKILA 20 KELEVWKHSST 146
                                                  F
RT con             hPQG   pP hh   h             h  Y DDhhh                        Gh h  cK  h       hLG  h
Sc_a1     TYHKPMLGLPQGSLISPILCNIVMTLVDNWLEDYI 55 YVRYADDILIGVLGSKN 2 KMIKRDLNNFLNS-LGLTMNEEKTLI  4 ETPARFLGYNI
Dm_TART   RAGQIGAGVPQGSNLGPILYSIFSSDMPLPHIYHP  7 LSTYADDTIVLSSDILA 6 NENYLKTFSDWADKWGISVNAAKTGH 25 ESKQSYLGVIL
HIV-1     GIRYQYNVLPQGWKGSPAIFQSSMTKILEPFKKQN  4 IYQYMDDLYVGSDLEIG 1 HRTKIEELRQHLLRWGLTTPDKKHQK  0 EPPFLWMGITL
```

# Telomerase Structure

TCAB1: Important for telomerase assembly in Cajal bodies.
Dyskerin, GAR1, NHP2: hTR stability and telomerase RNA 3′ end formation.

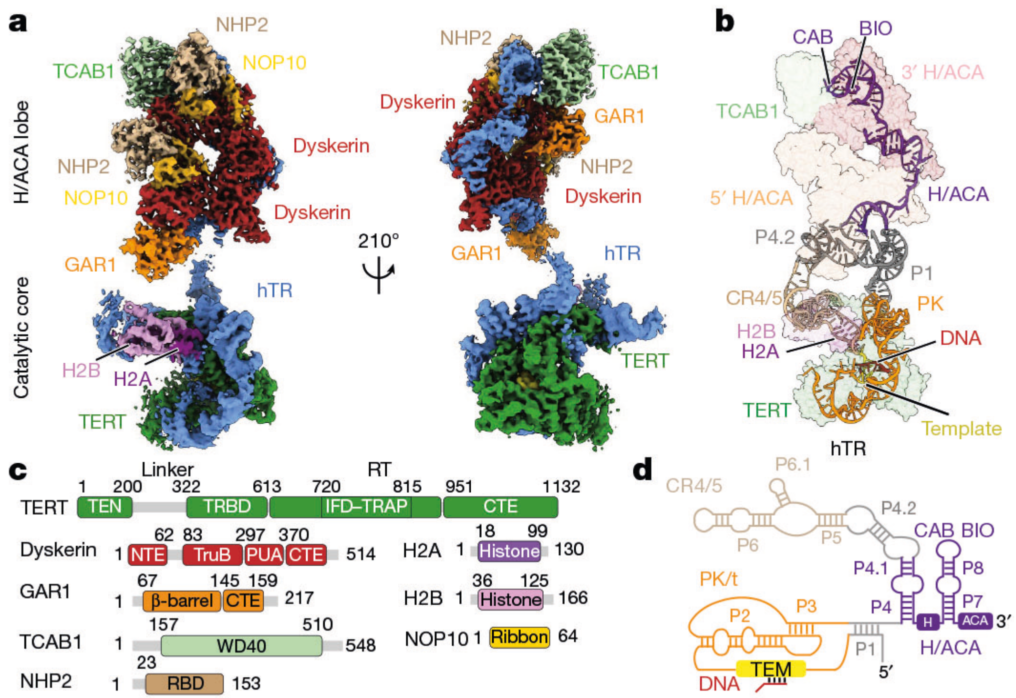
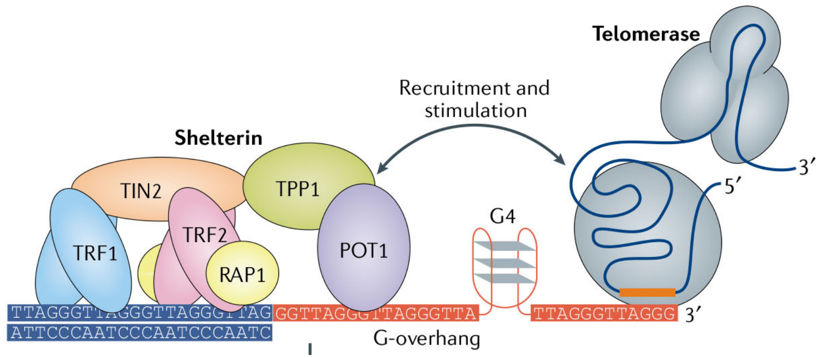



**Fig. 1 | Cryo-EM structure of human telomerase holoenzyme.** **a**, Front (left) and back (right) views of the composite cryo-EM maps of the catalytic core and the H/ACA lobe at 3.8 Å and 3.4 Å, respectively, with subunits coloured as indicated. **b**, Front view of the structure in which hTR and the DNA substrate are highlighted. **c**, Domain architectures of protein subunits. TEN, telomerase essential N-terminal domain; TRBD, telomerase RNA-binding domain; CTE, C-terminal extension; NTE, N-terminal extension; RT, reverse transcriptase; TruB, tRNA pseudouridine synthase B-like domain; WD40, Trp-Asp 40 repeat domain; RBD, RNA-binding domain. **d**, Secondary structure of hTR and the DNA substrate (Extended Data Fig. 4a). TEM, template.

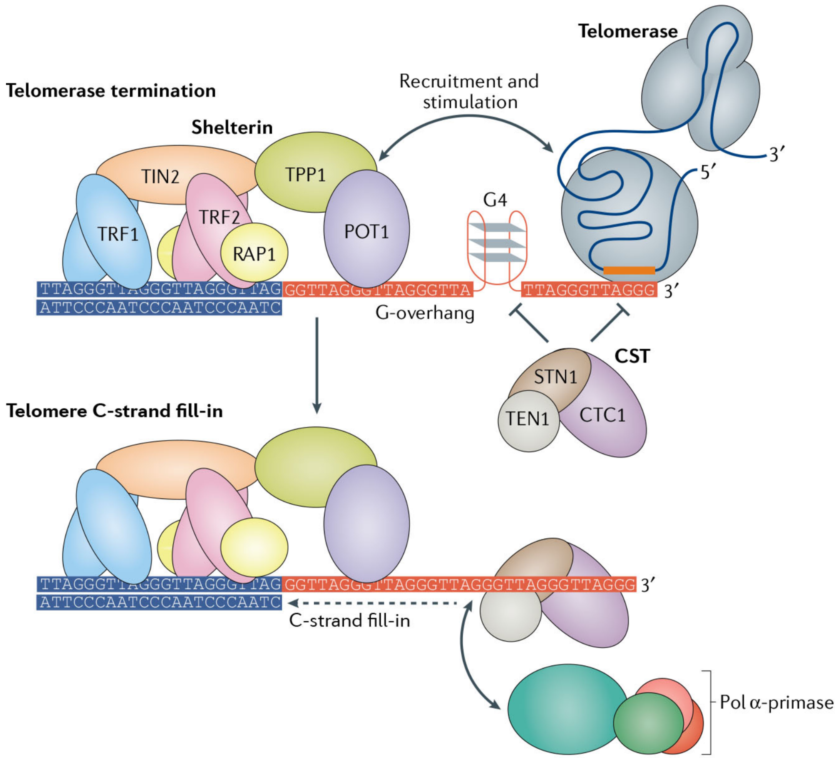
From:Ghanim et al., Nature 593, 449 (2021)

# Telomerase Recruitment to Telomeres?

→ TPP1 in human cells: recruits telomerase and stimulates the processivity of the telomerase enzyme (i.e. the propensity to add multiple telomeric repeats prior to dissociation).



# Termination of Telomere Elongation and Fill-in Synthesis




- TPP1 recruits telomerase.
- Upon telomere extension by telomerase, the complementary C-rich strand is filled in by conventional DNA polymerases (DNA pol $\alpha$ primase).
- Fill in synthesis requires the CST complex, an RPA-related trimeric complex which binds the TTAGGG-repeats. CST competes with telomerase for DNA binding thus limiting telomere elongation.

CST-assisted fill-in synthesis limits the length of the 3' overhang to roughly 100 nucleotides

From: NatRevMolBiol **22**, 283 (2021)

Nobelprize.org

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

Elizabeth H. Blackburn
1/3 of the prize
USA
University of California
San Francisco, CA

Carol W. Greider
1/3 of the prize
USA
Johns Hopkins
University
Baltimore, MD

Jack W. Szostak
1/3 of the prize
USA
Harvard Medical School;
Massachusetts
General Hospital Boston, MA
Howard Hughes Medical Institute

# Telomeres: 2 Fundamental Functions

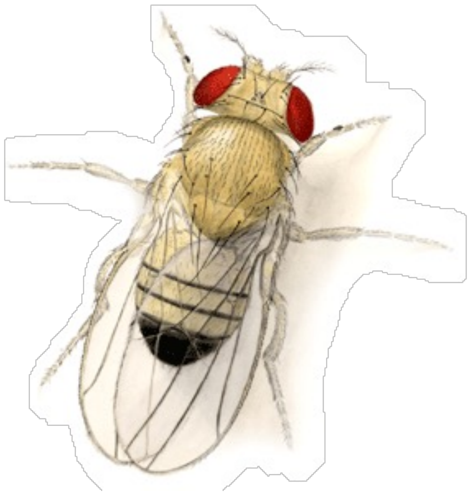
1. Regulation of cellular lifespan

**2. Repression of chromosome end-to-end fusions**

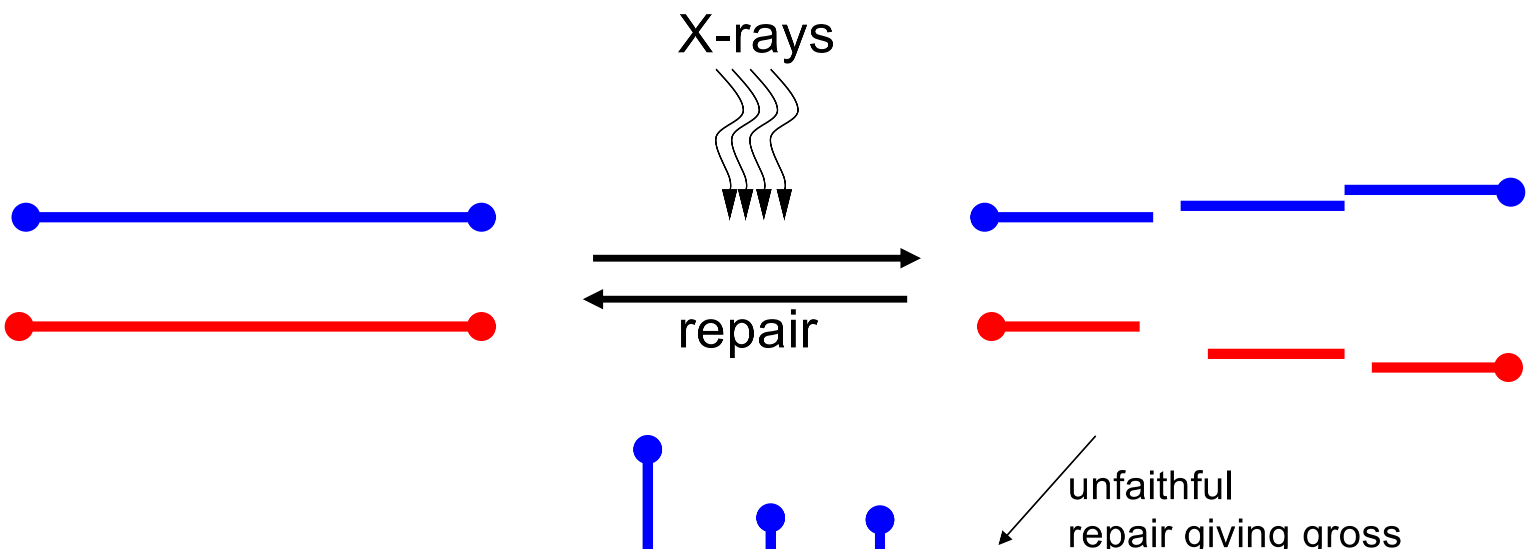
# Historical Perspective: Chromosome Ends Are Different

Hermann Muller

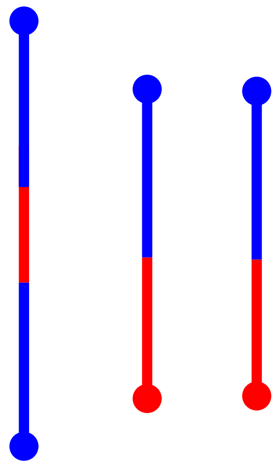
Barbara McClintock



# Chromosome Ends Are Different

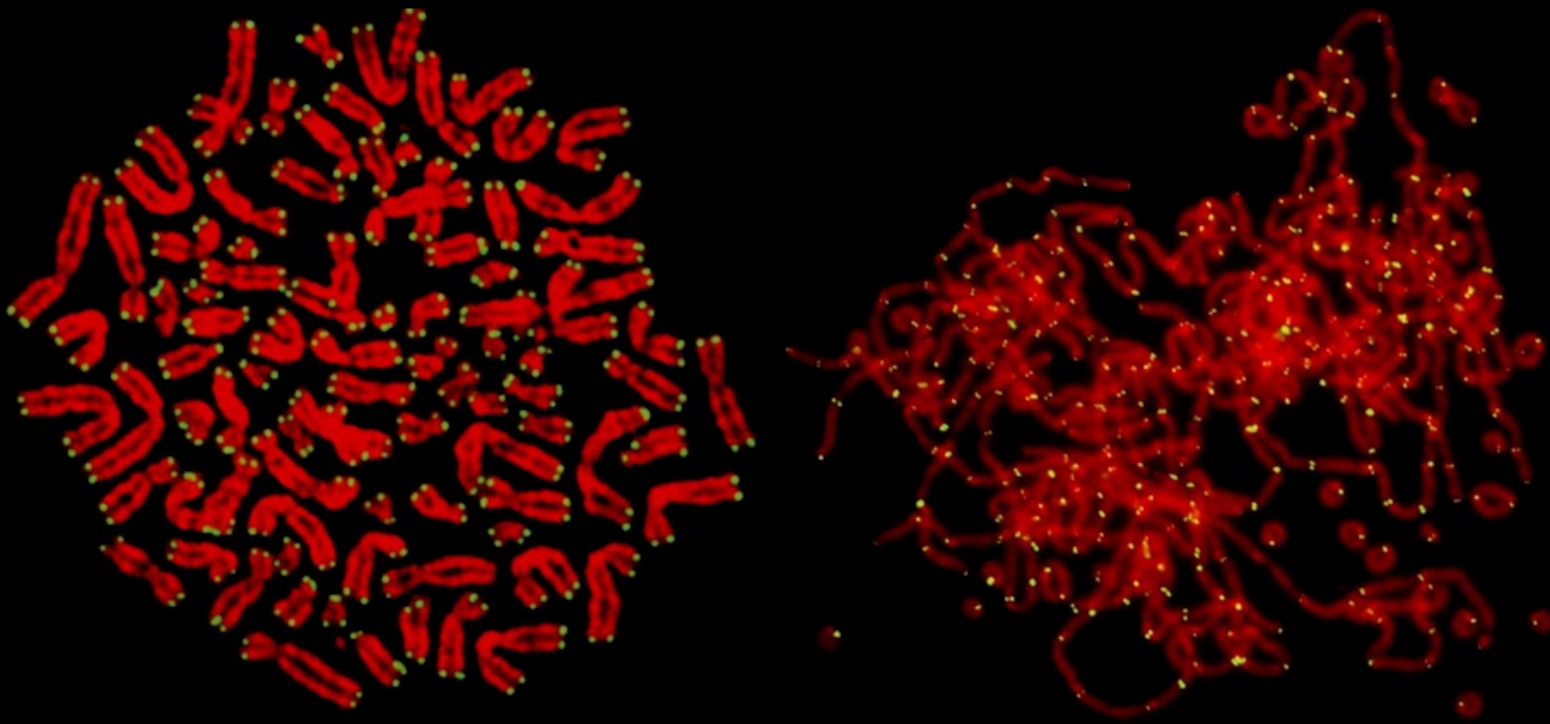



Telomeres
are refractory to fusion
events



Telomere = “end part”

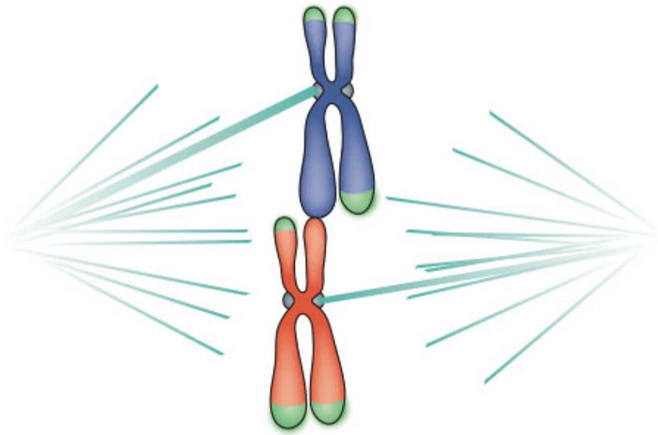
# Telomere Function: Protection from End Fusions



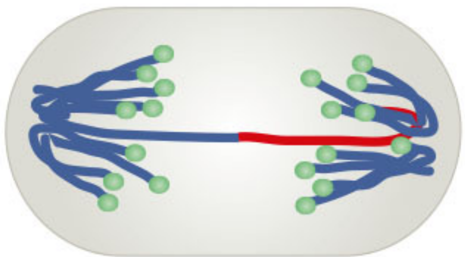
Wild type　　　　Loss of TRF2

Celli and de Lange, Nat. Cell Biol. (2005)

# Fused Chromosomes Cannot be Correctly Partitioned in Anaphase



Dicentric chromosome



Anaphase bridges

# Telomere Structure and Composition

# Telomeric DNA

| Group | Organism | Telomeric repeat (5' to 3' toward the end) |
|---|---|---|
| Vertebrates | Human, mouse, *Xenopus* | TTAGGG |
| Ciliate protozoa | *Tetrahymena*, *Glaucoma* | TTGGGG |
| | *Paramecium* | TTGGG(T/G) |
| | *Oxytricha*, *Stylonychia*, *Euplotes* | TTTTGGGG |
| Apicomplexan protozoa | *Plasmodium* | TTAGGG(T/C) |
| Higher plants | *Arabidopsis thaliana* | TTTAGGG |
| Green algae | *Chlamydomonas* | TTTTAGGG |
| Roundworms | *Ascaris lumbricoides* | TTAGGC |
| Fission yeasts | *Schizosaccharomyces pombe* | TTAC(A)(C)$G_{(1\text{-}8)}$ |
| Budding yeasts | *Saccharomyces cerevisiae* | TGTGGGTGTGGTG (from RNA template)<br>or $G_{(2\text{-}3)}(TG)_{(1\text{-}6)}T$ (consensus) |

Telomeric DNA length:

~8,000 bp in humans at birth; ~100 nucleotide 3’ overhang in humans

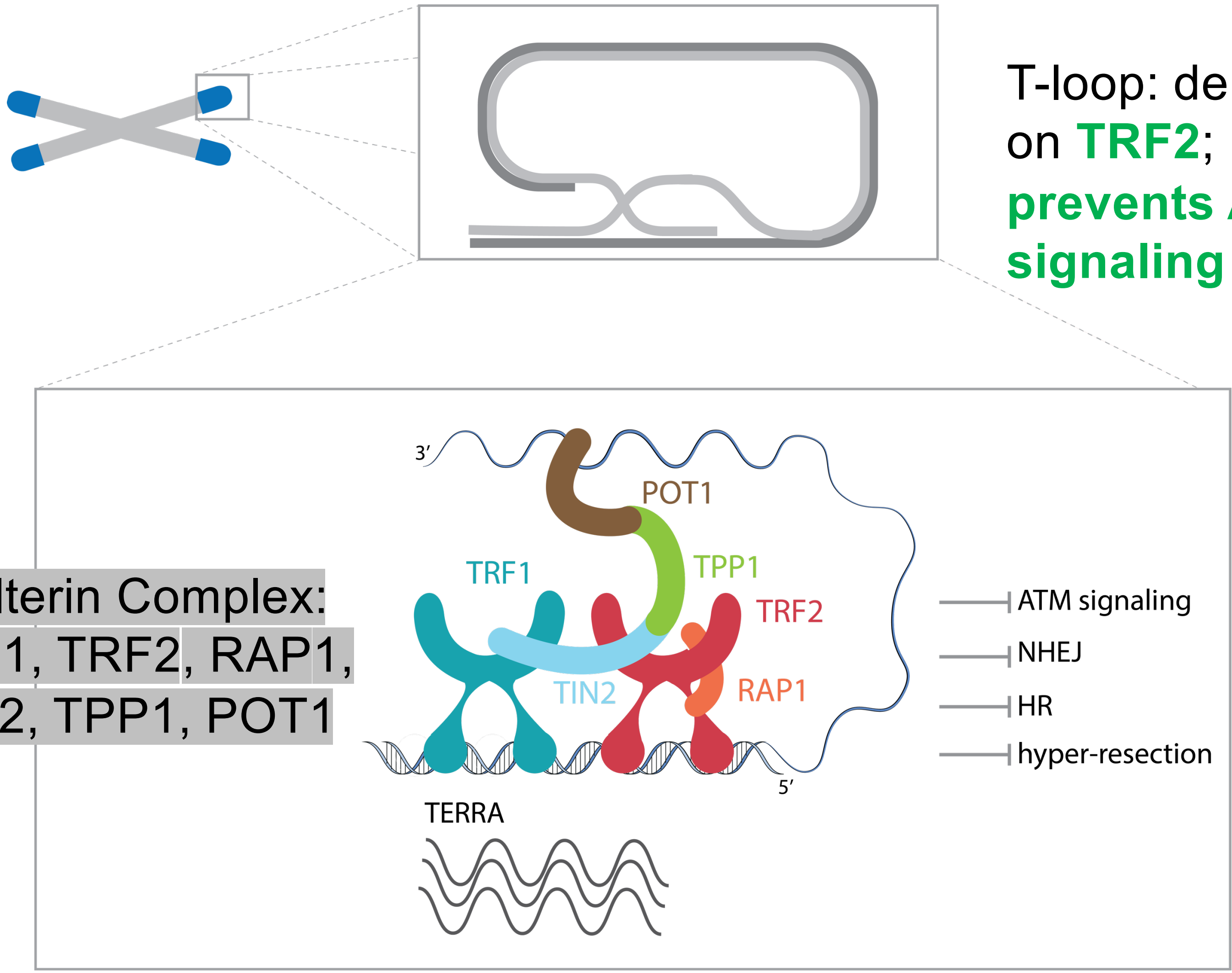
~300 bp in *S. cerevisiae*; 3-5 nucleotide 3’ overhang

# Telomere Structure and Composition

T-loop:

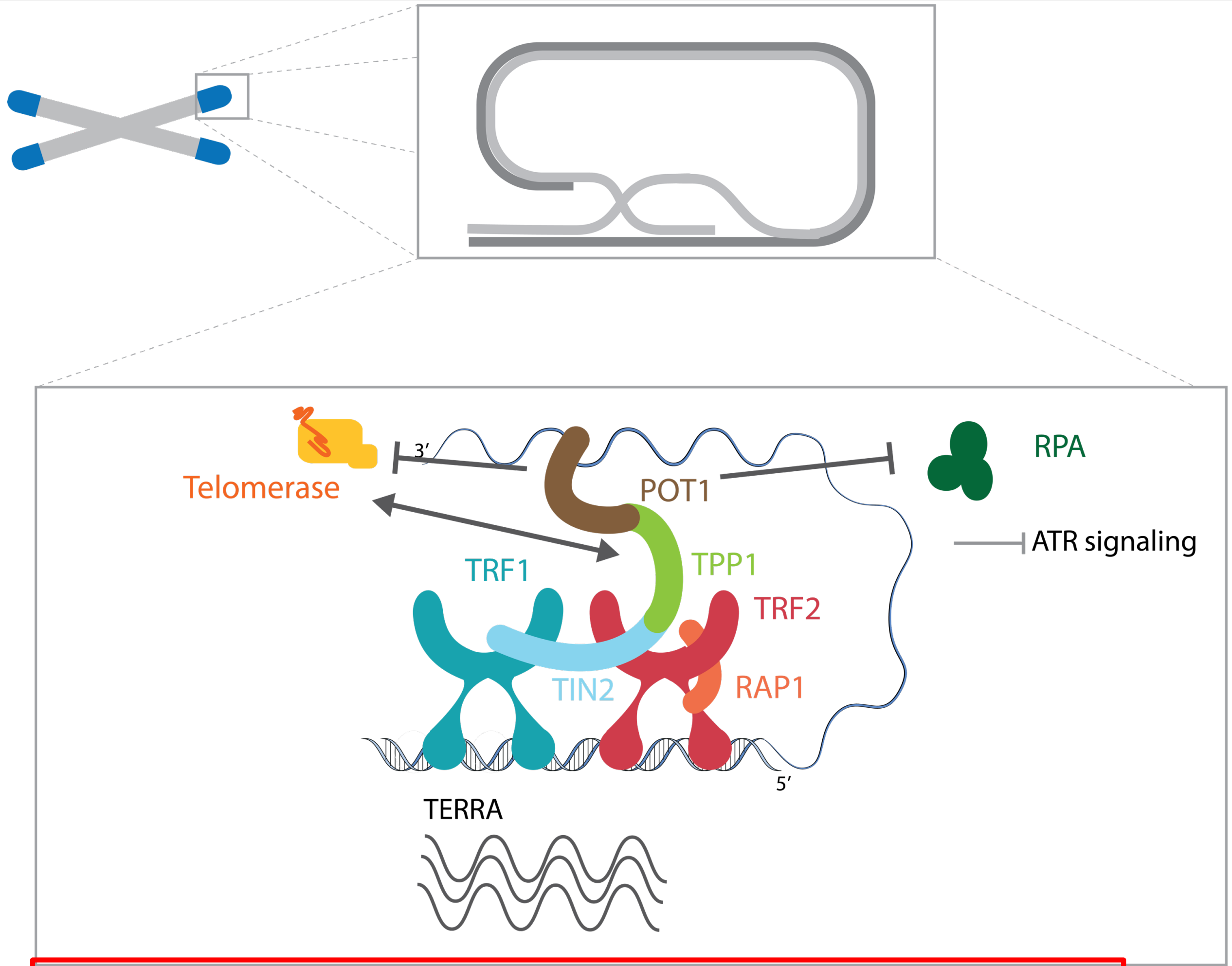
# Introduction Telomeres

T-loop: depends on **TRF2**; **prevents ATM signaling**




Shelterin Complex:
TRF1, TRF2, RAP1,
TIN2, TPP1, POT1

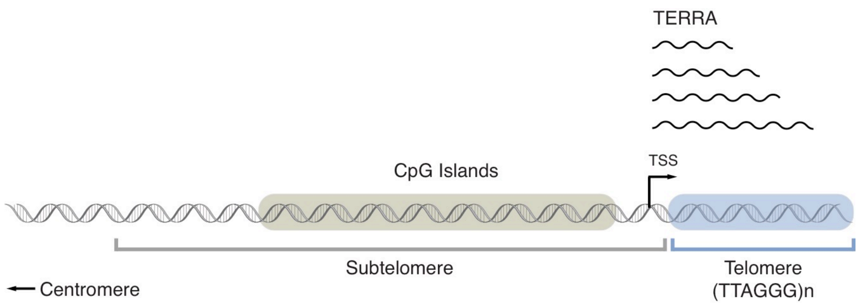
# Introduction Telomeres

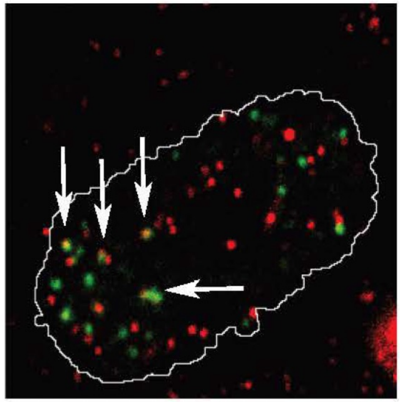



**POT1:**
**prevents RPA binding and ATR signaling**

Telomeric proteome: >100 different proteins? Functions?
Telomeres are transcribed: Long noncoding RNA TERRA

# TERRA: **Te**lomeric **R**epeat containing **R**N**A**



Telomeres
TERRA
Merge

Characteristics:

- conserved in all eukaryotes
- transcribed at a large number of chromosome ends
- 100-9000 nt long
- nuclear localization, partially co-localizing with telomeres
- Associates with telomeres forming DNA:RNA hybrid structures known as R-loops

→ Roles at telomeres when very short or damaged:

**→Stimulation of HR in ALT (discussed below) and pre-senescent cells (shown in *S. cerevisiae*)**

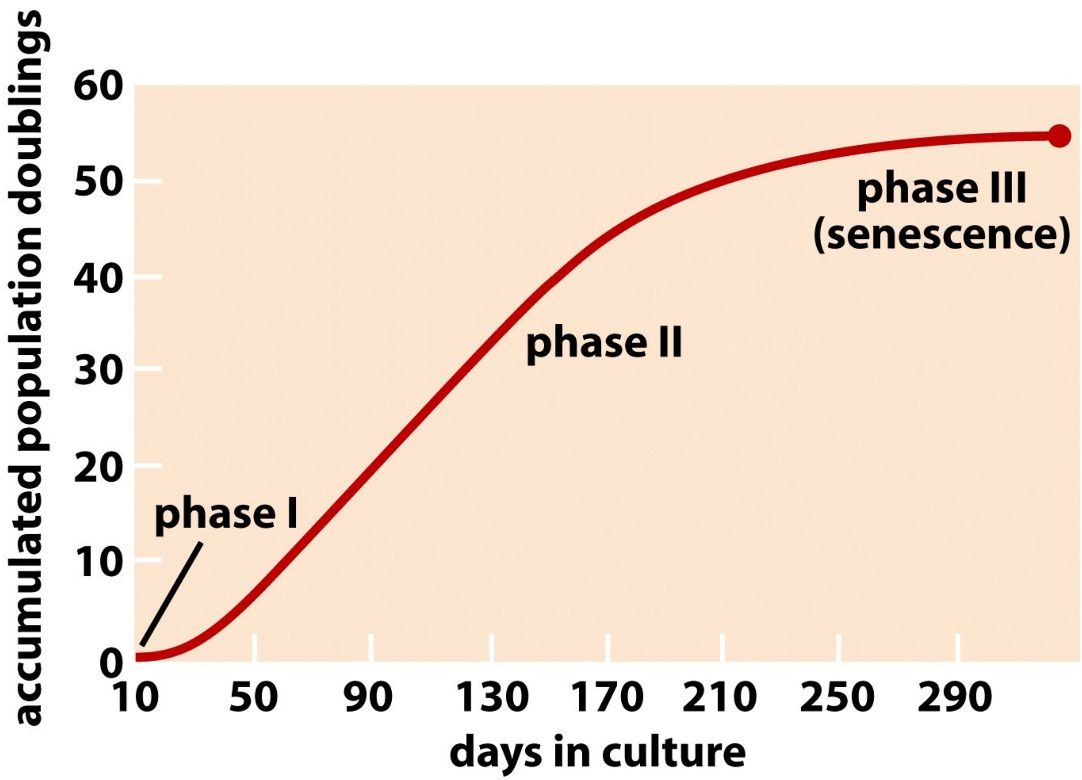
**→Stimulating cell death during cell crisis (discussed below).**

**…**

# What Roles do Telomeres Play in Cancer?

# Normal Human Somatic Cells Undergo Cellular Senescence

Adult human body: $10^{13}$ - $10^{14}$ cells
Life span: $10^{16}$ cell divisions



In contrast to primary cells, many cancer cells are immortal in culture (immortal cell lineage).

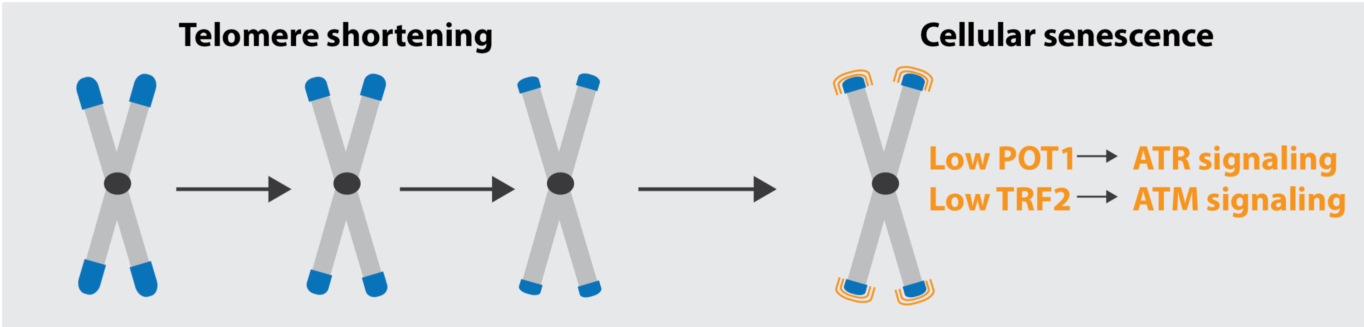
Example: HeLa cells: derived in 1951 from a cervical carcinoma of Henrietta Lacks

## The Regulation of Telomerase and Telomeres Shortening Regulate Cellular Lifespan

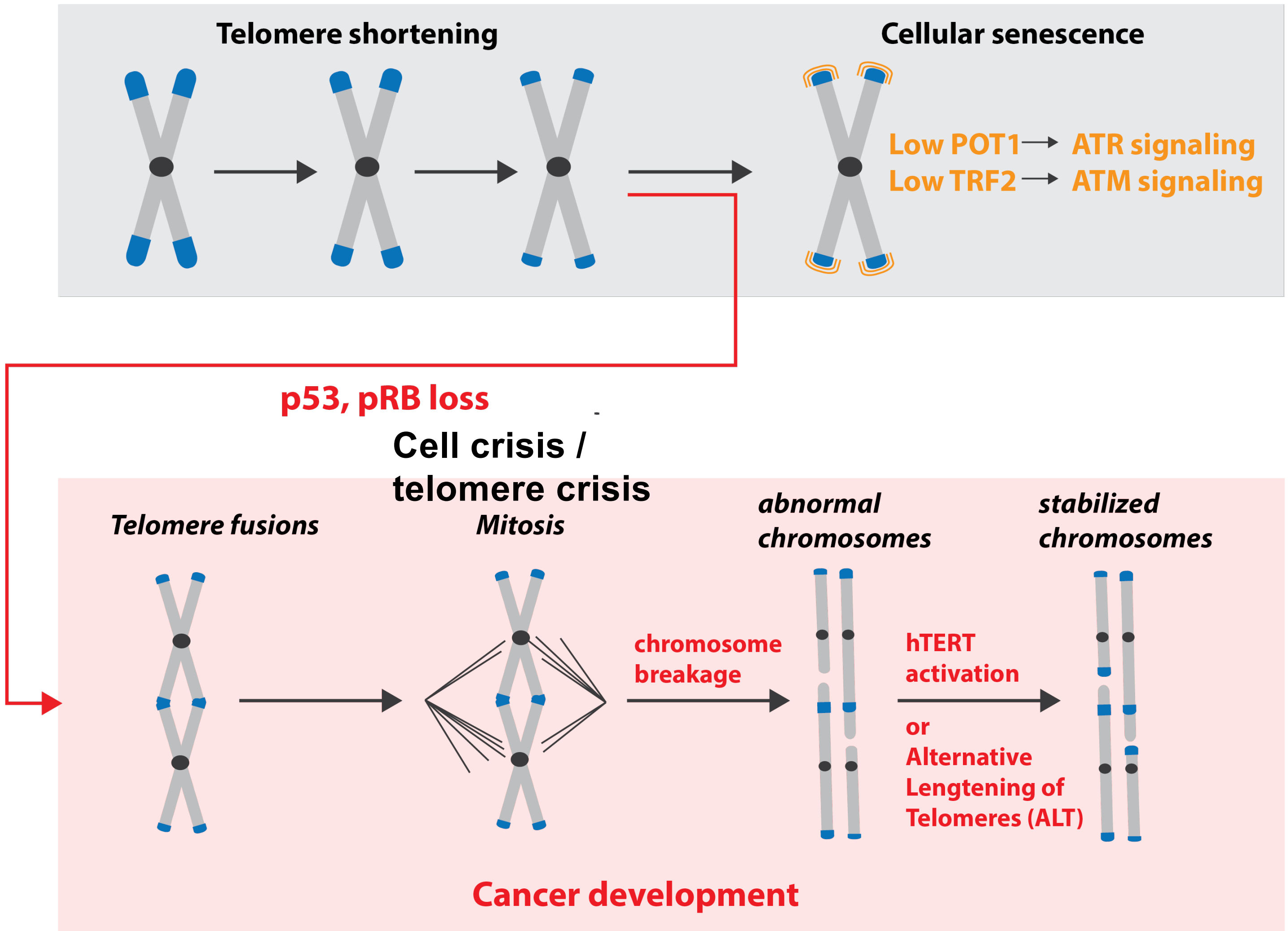
- Most normal human somatic cells do not express telomerase. **hTERT transcription is repressed**.

  But: Telomerase is expressed in the germ line, during early embryogenesis, and in stem cells in the adult.

# Cellular Clock: Telomeres during Tumorigenesis



# Cellular Clock: Telomeres during Tumorigenesis

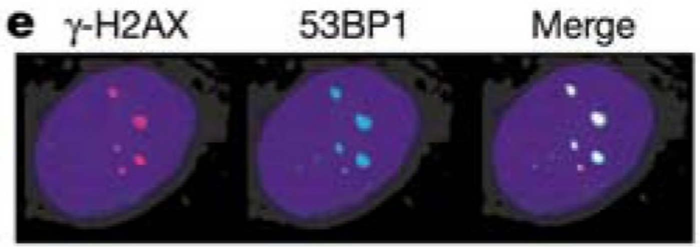


## Two Critical Telomeric States During Tumorigenesis

- **Cellular senescence:**
Permanent cell cycle arrest with a G1 DNA content.

- **Cell crisis:**
Active cell cycling, chromosome end-to-end fusions, chromosome fragmentation and missegregation, frequent cell death.

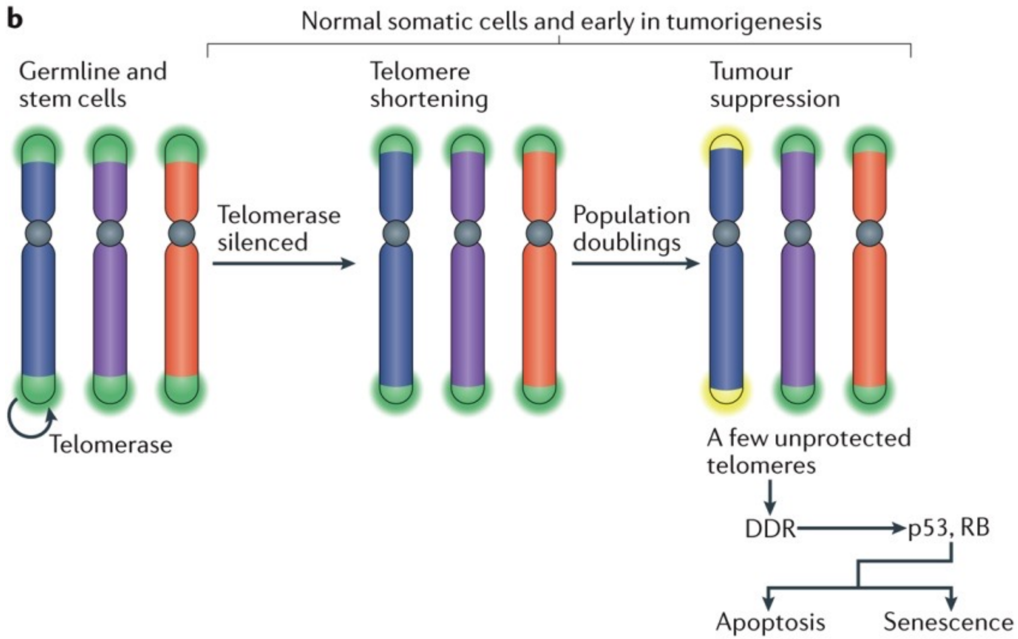
Senescent cells: permanent cell cycle arrest. Telomeres shorten in cells towards their progression to senescence. DNA damage signaling from short telomeres prevents cell cycle progression.




γ-HA2X, 53BP1: DNA damage markers at short telomeres;
these markers are also found at DNA double strand breaks.

From *Nature.* 426:194 (2003)

# Telomere Shortening as a Barrier to Tumorigenesis




During development, TERT is silenced. As a result, telomeres shorten gradually. After numerous population doublings, a few telomeres become too short (yellow) and lose their protective function. The kinases ATM and ATR are activated at the unprotected chromosome ends and this DNA damage response (DDR) induces senescence or apoptosis. This limits the proliferative capacity of incipient cancer cells, thus functioning as a tumour suppressor pathway. Cells lacking p53 and RB function can avoid this replicative arrest.

From Nat Rev Mol Cell Biol 18, 175 (2017)

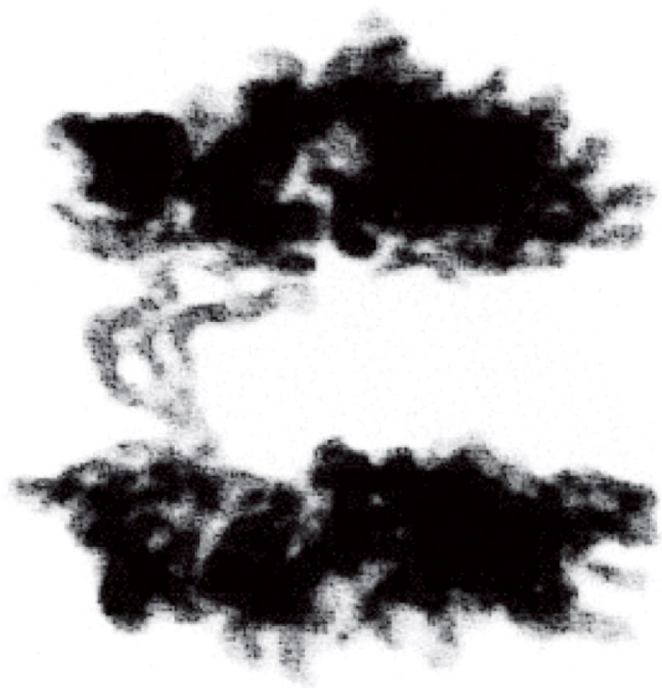
## Two Critical Telomeric States During Tumorigenesis

- Cellular senescence

- **Cell crisis:**
  Active cell cycling, chromosome end-to-end fusions by MMEJ (alt-NHEJ), chromosome fragmentation and missegregation, frequent cell death (by autophagy).

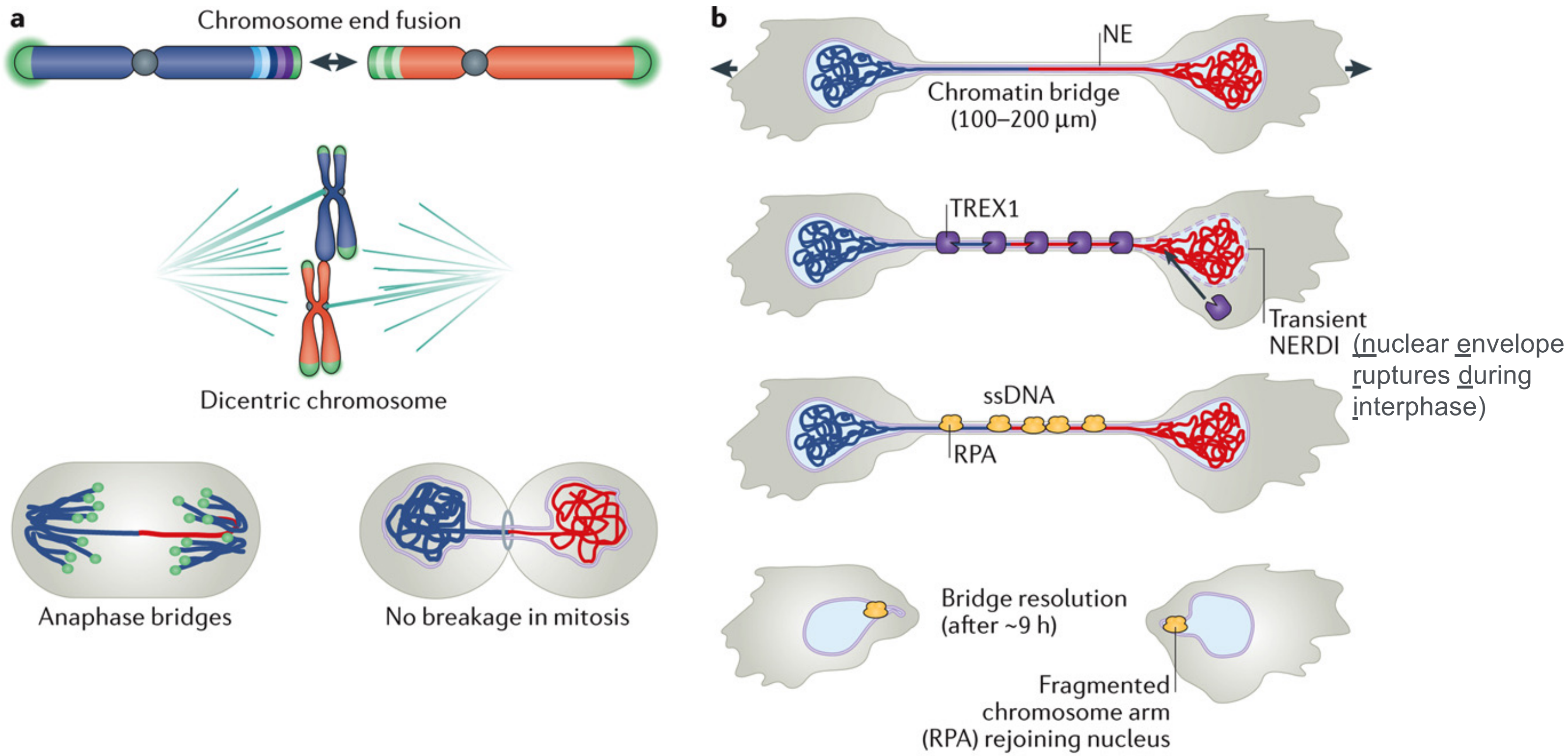
Dicentric Chromosomes cannot be correctly Segregated at Anaphase

Dicentric chromosome



Anaphase bridge

# Breakage of Dicentric Chromosomes in Cell Crisis




Dicentric chromosomes formed by telomere fusion rarely break during mitosis. After cell division, TREX1 3' nuclease resolves the bridges between daughter cells.

From Nat Rev Mol Cell Biol 18, 175 (2017)

**Recent Discovery:**

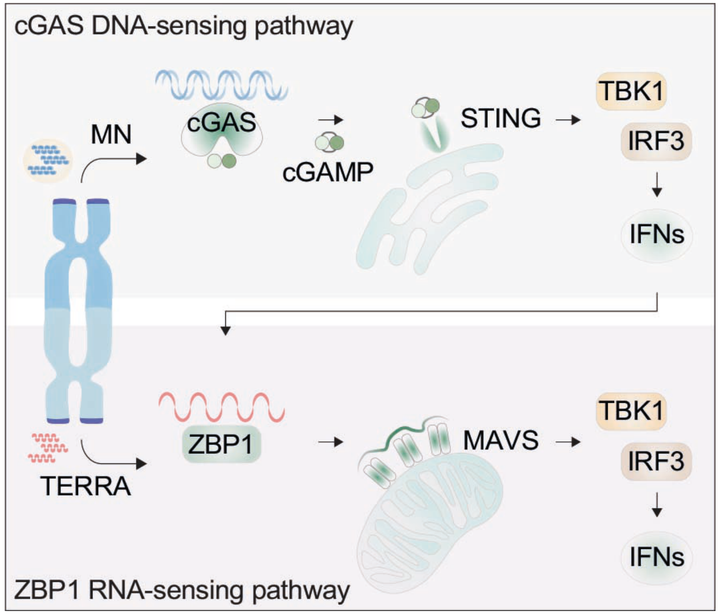
# Telomere-to-mitochondria signalling by ZBP1 mediates replicative crisis

https://doi.org/10.1038/s41586-023-05710-8

Received: 30 September 2021

Accepted: 5 January 2023

Joe Nassour[1], Lucia Gutierrez Aguiar[1], Adriana Correia[1,2], Tobias T. Schmidt[1], Laura Mainz[1], Sara Przetocka[1], Candy Haggblom[1], Nimesha Tadepalle[1], April Williams[1], Maxim N. Shokhirev[1], Semih C. Akincilar[3,4], Vinay Tergaonkar[3,4,5,6], Gerald S. Shadel[1]✉ & Jan Karlseder[1]✉



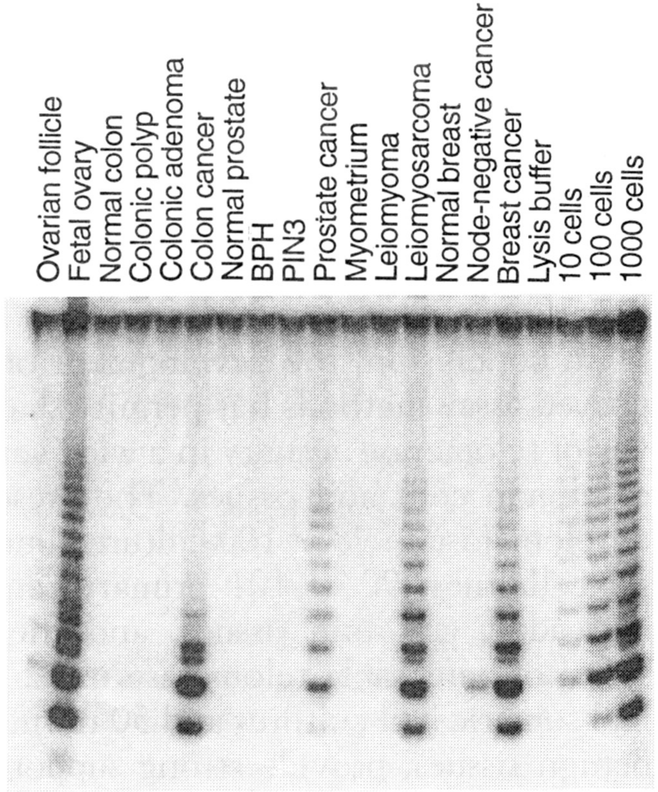

During replicative crisis breakage of chromosomes with critically short telomeres leads to accumulation of cytoplasmic DNA, such as micronuclei (MN). These DNA species activate the cGAS/STING pathway, which leads to interferon signaling (IFN). One of the interferon-stimulated genes is ZBP1. By itself, however, ZBP1 is inactive. Only when it associates with TERRA transcripts from short telomeres does it gain the ability to form filaments at mitochondria, where it activates MAVS (mitochondrial antiviral signaling protein). The result is an amplification of the inflammation response that causes cell death, thereby removing cells with critically short telomeres from the population.

This pathway still needs to be elucidated. However, the new data indicate that during cell crisis TERRA-mediated signaling from telomeres induces cell death, rather than simply chromosome mis-segregation and breakage events as asssumed previously.

# How Can Telomere Crisis be Overcome?

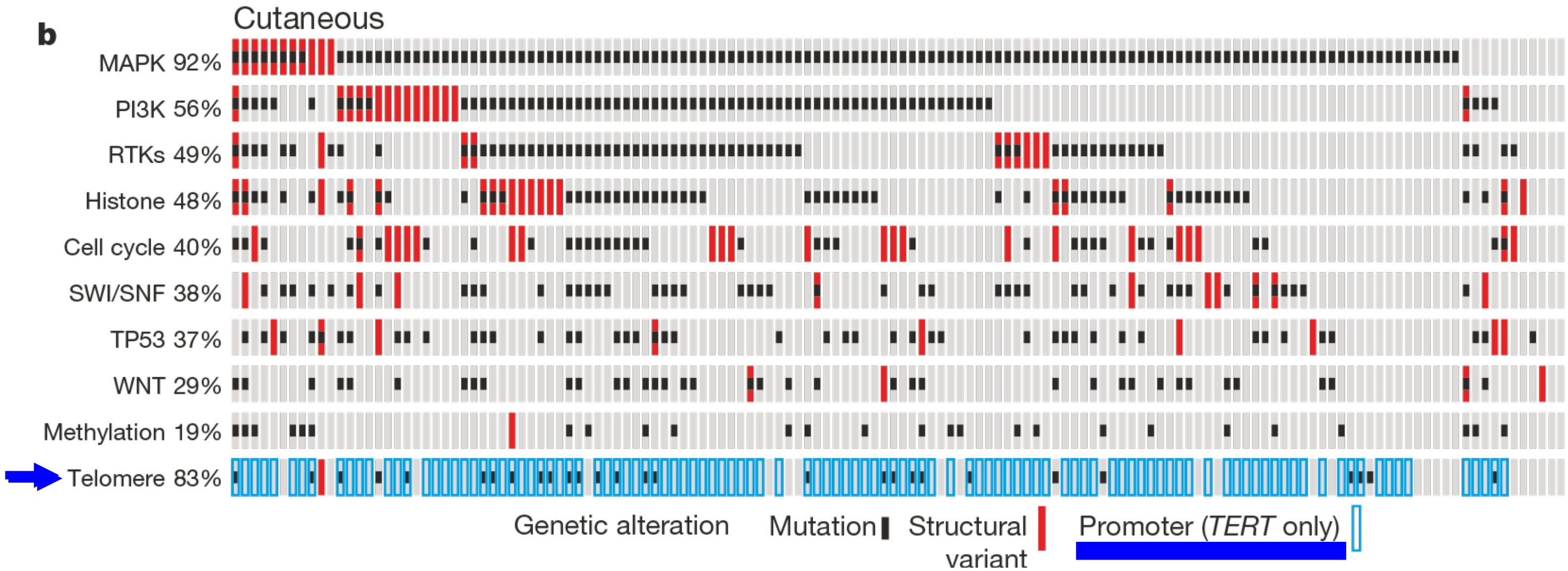
# Telomerase is Reactivated in Cancer Cells

Telomerase activity assay (TRAP):




(from Kim et al., *Science 266*, p. 2011-2015, 1994)

## Whole Genome Sequencing of 140 Cutaneous Melanomas: Frequency of Aberrations in Pathways




From: Nature (2017), 545:175-180. doi: 10.1038/nature22071

**Point mutations in the promoter of telomerase reverse transcriptase (TERT) are the most frequent non-coding mutation in human cancer.** These TERT promoter mutations create de novo binding sites for a family of transcription factors known as ETS (identified through a highly conserved DNA binding domain, the ETS domain, which is a winged helix-turn-helix structure.)

nature cell biology

SERIES | LETTERS

https://doi.org/10.1038/s41556-020-0471-6

Nat Cell Biol 22, 282–288 (2020). https://doi.org/10.1038/s41556-020-0471-6

Check for updates

# Programmable base editing of mutated *TERT* promoter inhibits brain tumour growth

**Xinjian Li[1,2,11], Xu Qian[3,4,11], Bin Wang[1,2], Yan Xia[5,6], Yanhua Zheng[5], Linyong Du[7], Daqian Xu[5], Dongming Xing[8,9], Ronald A. DePinho[6] and Zhimin Lu[10]**

**Clustered regularly interspaced short palindromic repeats (CRISPR), CRISPR interference and programmable base editing have transformed the manipulation of eukaryotic genomes for potential therapeutic applications[1-4]. Here, we exploited CRISPR interference and programmable base editing to determine their potential in editing a *TERT* gene promoter-activating mutation, which occurs in many diverse cancer types, particularly glioblastoma[5-8]. Correction of the −124C>T *TERT* promoter mutation to −124C was achieved using a single guide RNA (sgRNA)-guided and catalytically impaired *Campylobacter jejuni* CRISPR-associated protein 9-fused adenine base editor (CjABE). This modification blocked the binding of members of the E26 transcription factor family to the *TERT* promoter, reduced *TERT* transcription and TERT protein expression, and induced cancer-cell senescence and proliferative arrest. Local injection of adeno-associated viruses expressing sgRNA-guided CjABE inhibited the growth of gliomas harbouring *TERT*-promoter mutations. These preclinical proof-of-concept studies establish the feasibility of gene editing as a therapeutic approach for cancer and validate activated *TERT*-promoter mutations as a cancer-specific therapeutic target.**

Clustered regularly interspaced short palindromic repeats (CRISPR) prokaryotic adaptive immune systems have been widely than a current Cas9 nuclease-based method (CORRECT) without double-stranded-DNA cleavage and with fewer off-target genome modifications[3,4]. In addition, recent studies displayed continuous evolution of cytosine base editors, which convert cytosine in the GC context to thymine, as well as ABEs with expanded target compatibility and improved activity[4,13–15]. Determining the potential of gene editing in correcting cancer-specific mutational drivers is an area of intensive investigation.

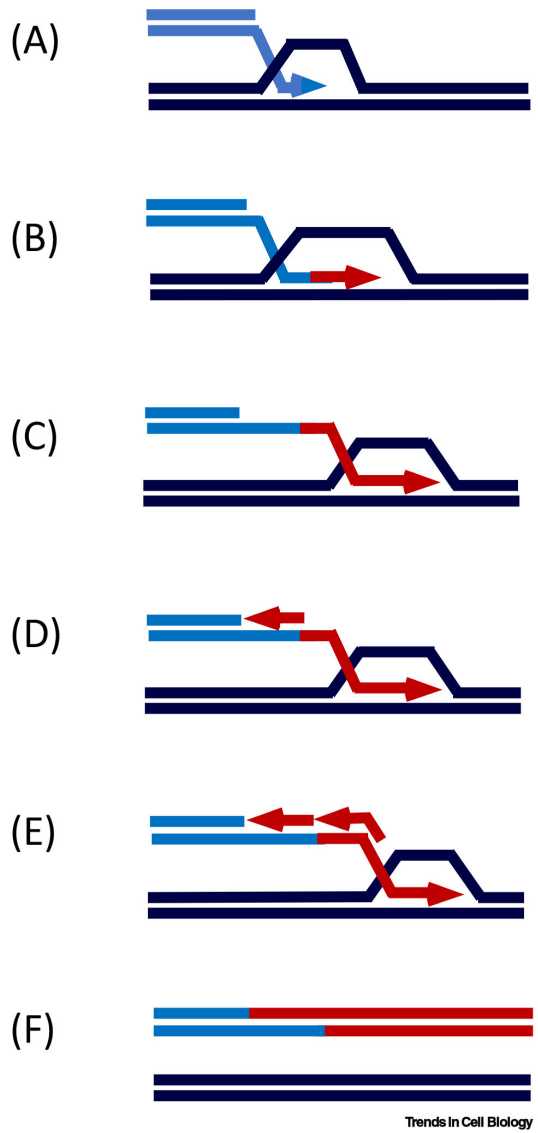
The *TERT* gene encodes a highly specialized reverse transcriptase that adds hexamer repeats to the 3′ ends of chromosomes[16]. Although somatic mutations in the *TERT* coding region are not common in human tumours, germline and somatic mutations of the *TERT* promoter are present at high percentages in many human cancers, including 83% of primary glioblastomas (GBM)[5–8]. Such mutations have occurred in two hotspot positions located −124 and −146 base pairs upstream of the ATG start site (−124G>A and −146G>A, respectively; −124C>T and −146C>T on the opposite strand). These mutations generate a de novo consensus binding site (GGAA) for members of the E26 (ETS) transcription factor family—including ETS1 and the multimeric GA-binding protein A (GABPA)—and confer increased *TERT* promoter activity[5,6,17]. These mutations have been demonstrated to be critical for increased telomerase promoter activity

## The ALT (**A**lternative **L**engthening of **T**elomeres) Pathway and TERRA

- Most cancers rely on telomerase for maintaining telomeres but roughly 10-15% rely on ALT (mostly of mesenchymal origin; some brain tumors).

# ALT: Homologous Recombination (HR) Promoting Telomere Synthesis

**Break-induced replication (BIR). BIR begins by strand invasion (A) to create a D-loop that can migrate as new DNA synthesis (red) is initiated at the invading 3′ end (B**). Unlike normal DNA replication, BIR creates a long single-stranded intermediate (C) that becomes copied to form double-stranded DNA by delayed lagging-strand synthesis (E), such that all **the newly copied DNA is conservatively inherited** (F).

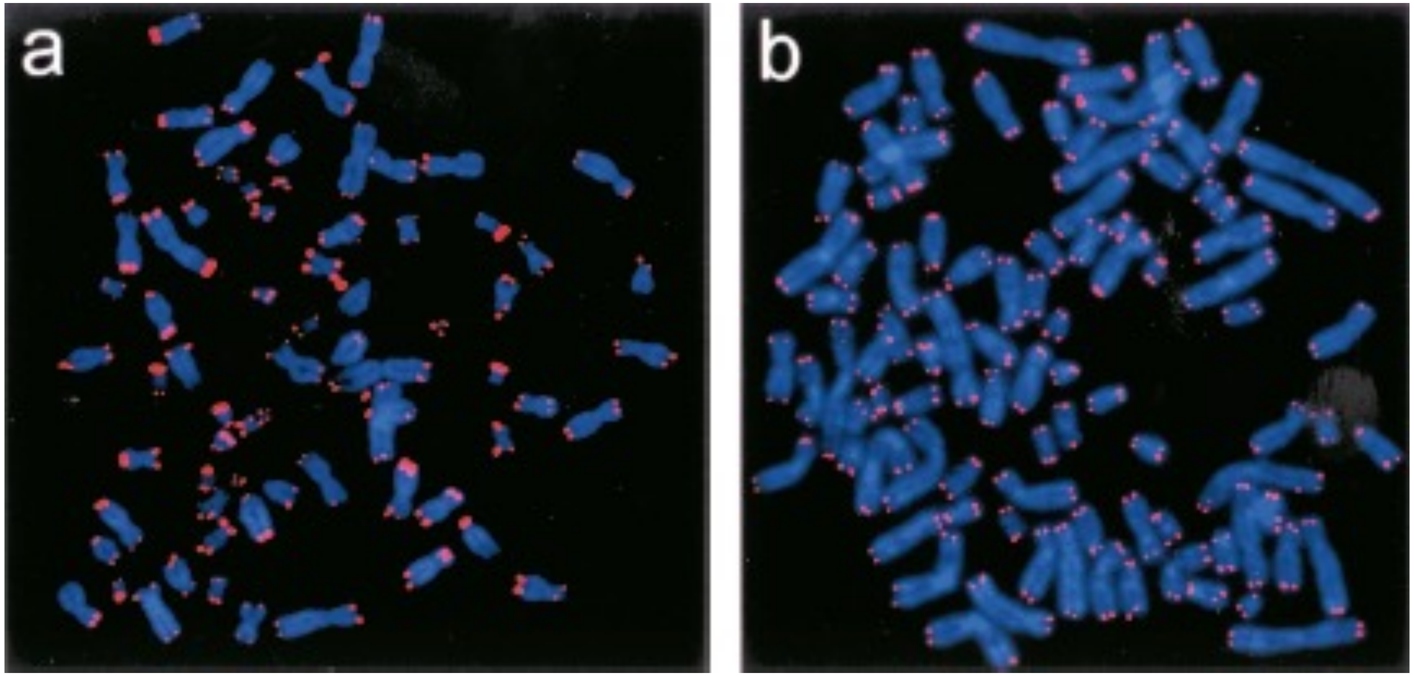


## Some Features of ALT

- Changes in telomeric chromatin: mutations in ATRX/DAXX (deposits H3.3 at telomeres) and the histone H3.3 variant.
- Replication stress at telomeres
- TERRA upregulation and TERRA R-loops
- Heterogeneous telomere length
- Extrachromosomal telomere repeats
- ALT-associated PML bodies (APBs). Membrane less nuclear structures. Contain promyeolytic leukemia protein (PML), telomeric DNA, proteins involved in DNA repair, recombination and replication and TERRA.

…Required for ALT.

# Telomerase-Independent Telomere Maintenance: The ALT-Pathway




ALT

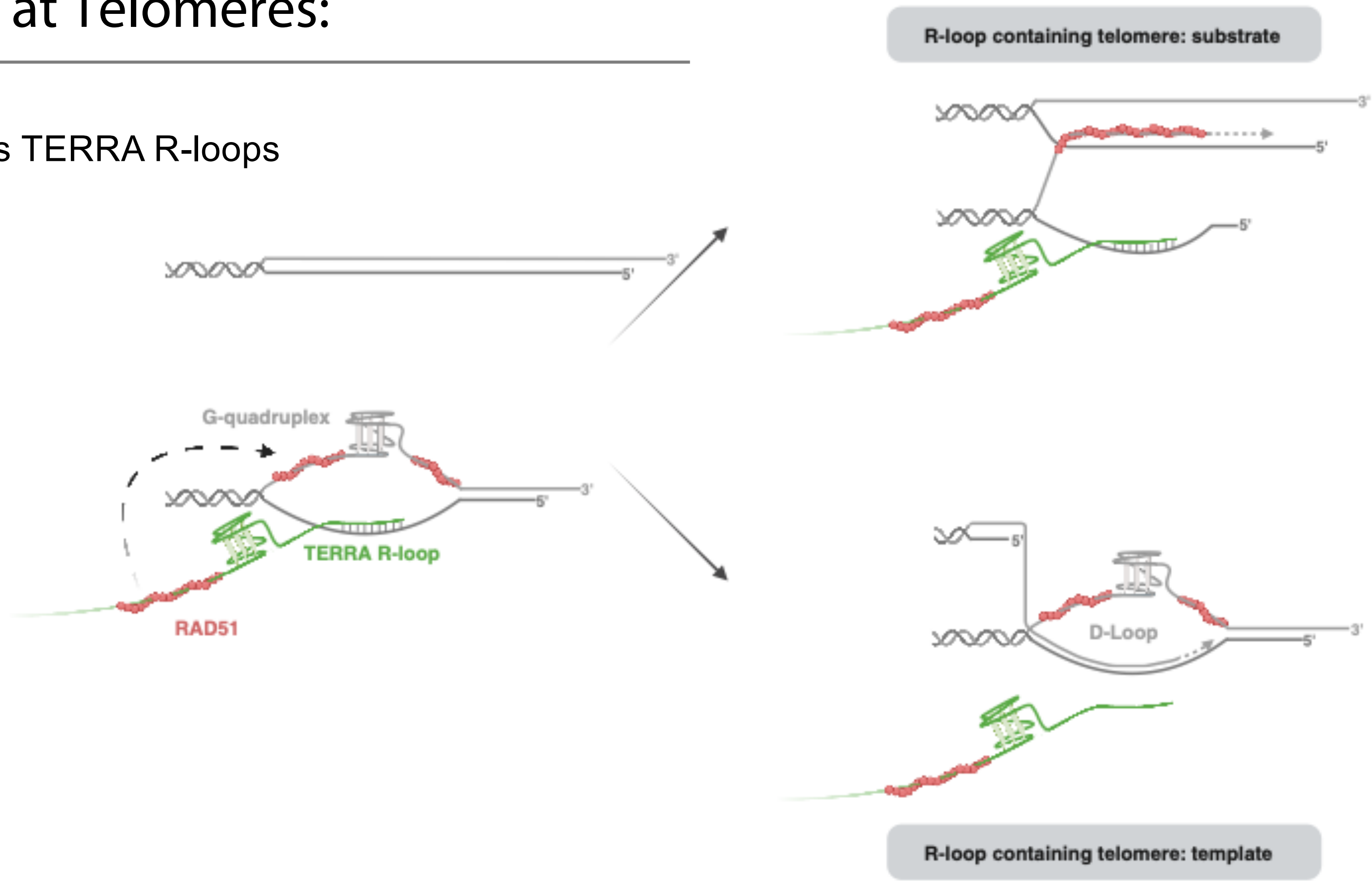
Telomerase

Telomere-specific fluorescence *in situ* hybridization (FISH) on metaphase chromosomes of ALT and telomerase-positive cells, illustrating the highly heterogeneous telomere lengths within individual ALT cells (red, telomere-specific probe; blue, DAPI-stained metaphase chromosomes).

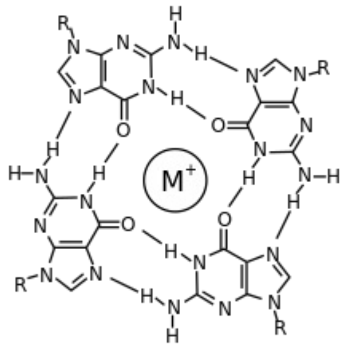
ALT occurs in a smaller fraction of sarcomas (cancer of connective or supportive tissue; bone, cartilage, muscle etc.), many glioblastomas

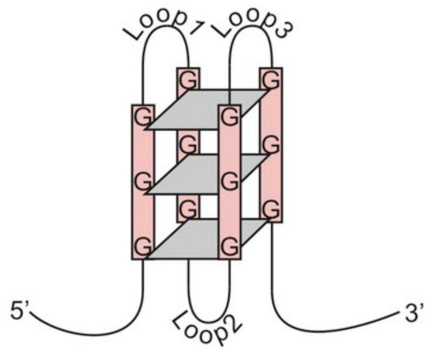
# Speculative Models on how TERRA may Stimulate BIR at Telomeres:

- RAD51 promotes TERRA R-loops



The single stranded TTAGGG- repeats of telomeric DNA (and UUAGGG-repeats of TERRA) can form so-called G-quadruplex (G4) structures, which are highly stable:



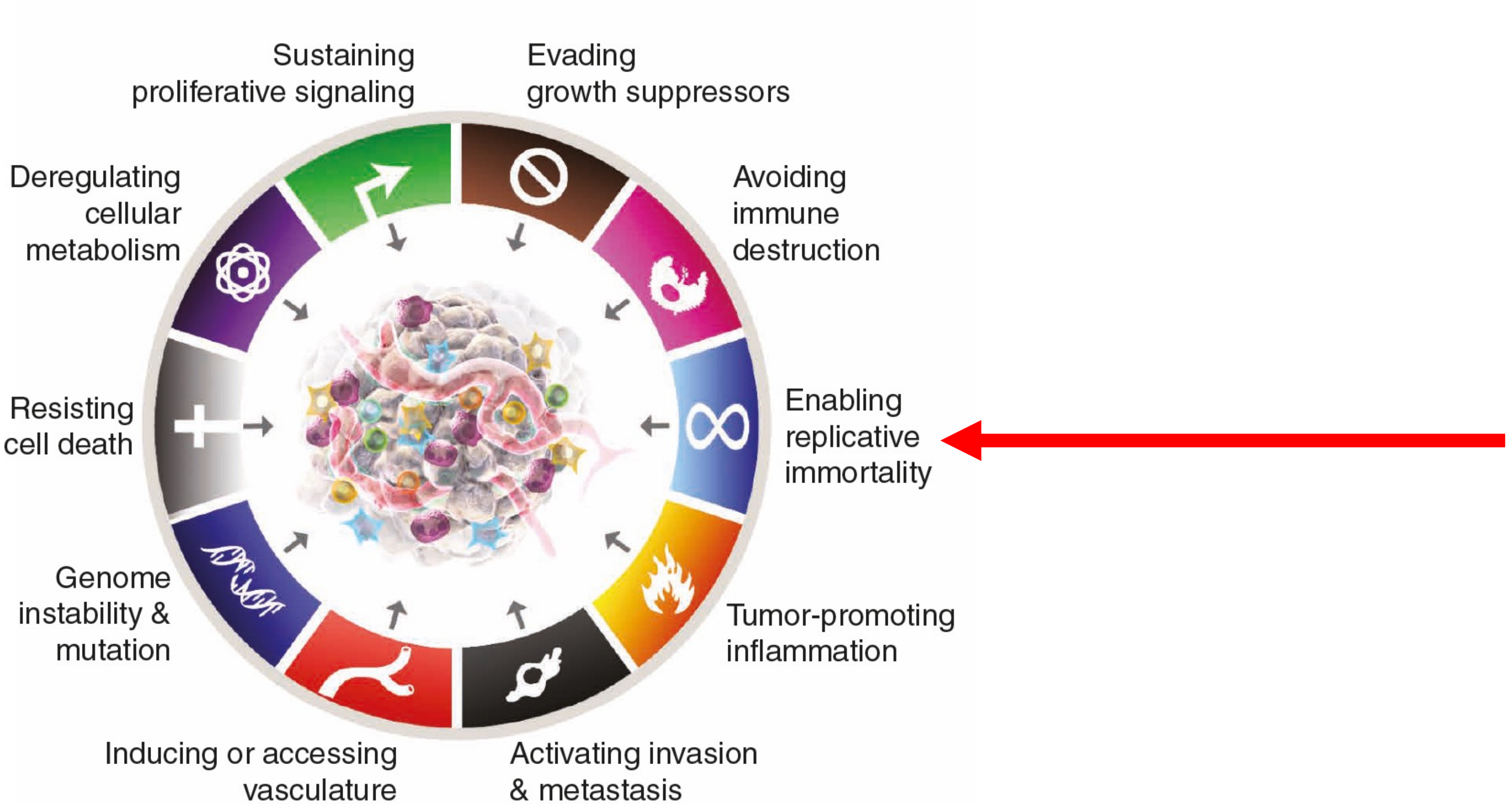


# What Happens to Normal Cells in which Telomerase is Reactivated ?

# Requirements for Immortalization and Transformation of Human Cells

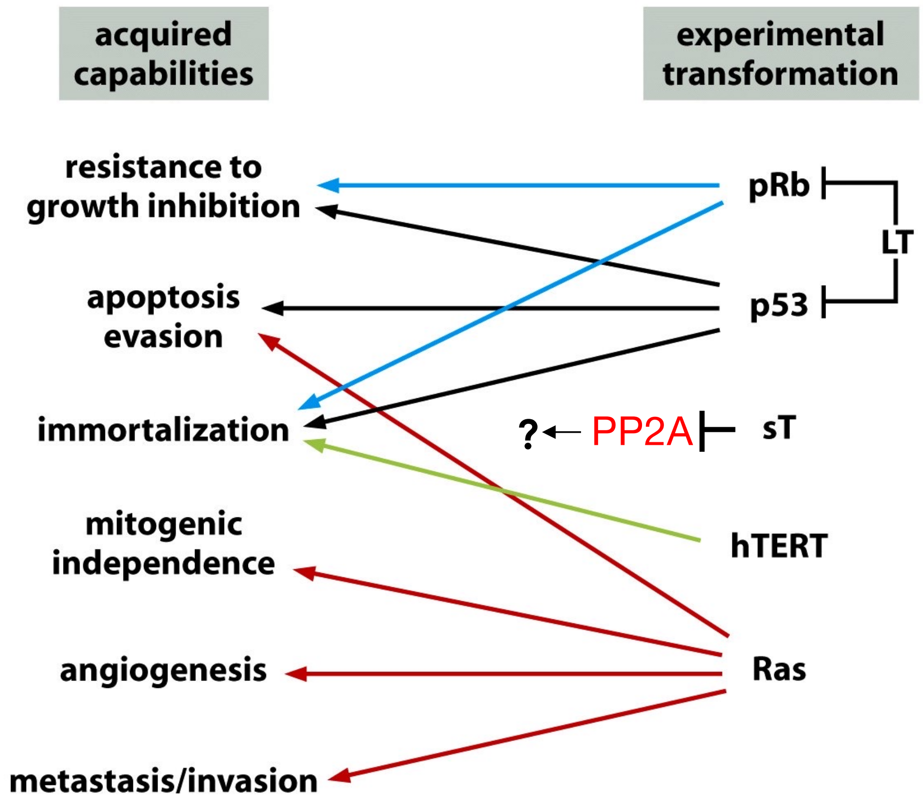
- In tissue culture, normal human cells undergo cellular senescence.
- **Human cells (fibroblasts, endothelial cells, epithelial cells) can be immortalized by ectopic expression of hTERT if cultured under appropriate conditions!**
- hTERT-immortalized cells remain karyotypically and functionally normal.
- hTERT-immortalized cells do not spontaneously acquire the characteristics of cancer cells.

# **From Week 1:** Acquired Capabilities of Cancer



Weinberg and Colleagues (Mol. Cell Biol. 22, 2111 (2002); Nature 400, 464 (1999)):

Tumorigenic Phenotype through Defined Genetic Alterations in Human Fibroblasts and Kidney Epithelial Cells:




→ Tumors in immunocompromised animals
(invasivness and metastatic ability not addressed)

# Key Concepts

- Intact telomeres protect from chromosomes from end-to-end fusions (suppression of NHEJ and MMEJ).
- TRF2 suppresses NHEJ and ATM; TRF2 also mediates formation of t-loops which prevent ATM activation.
- POT1 suppresses ATR.
- Cellular senescence: permanent cell cycle arrest with a G1 DNA content; active ATM and ATR signaling.
- Cell crisis: frequent telomere end-to-end fusions mediated by MMEJ; chromosome missegregation and breakage events; active cell cycling accompanied by frequent autophagy-dependent cell death.
- Cell crisis: ZBP1 induced by cGAS-STING pathway binds TERRA →MAVS-dependent innate immune response.
- Telomerase: cellular reverse transcriptase which uses an internal RNA template. TERT is tightly regulated in humans (but not in mice). hTERT is re-expressed in 90% of cancer (often due to TERT-promoter mutations).
- ALT pathway: recombination-based pathway to maintain chromosome ends. TERRA stimulates homologous recombination through formation of R-loops, which form post transcription.