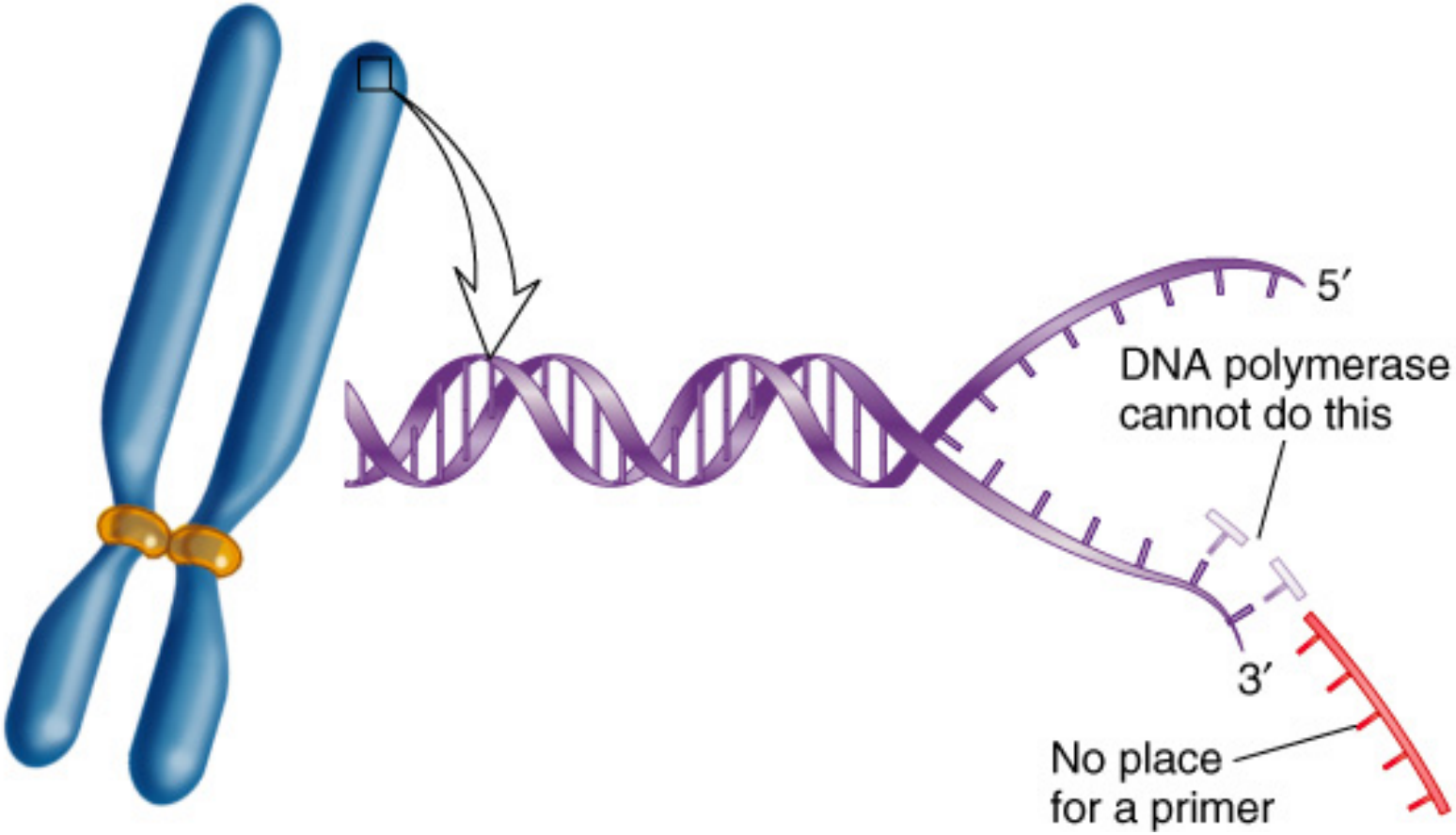
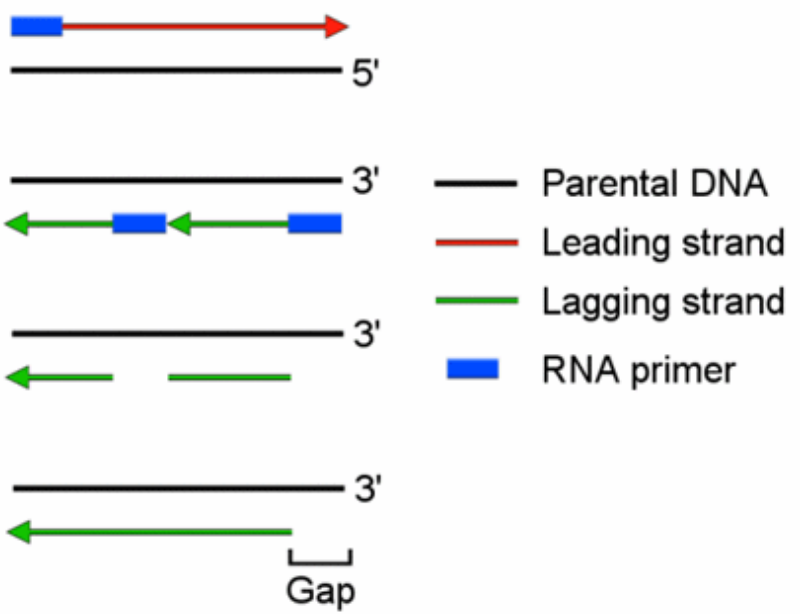
# The Problem at the ends of eukaryotic linear Chromosomes




- DNA polymerases can only synthesize DNA only in the 5’ to 3’ direction and cannot initiate DNA synthesis
- These two features pose a problem at the 3’ end of linear chromosomes

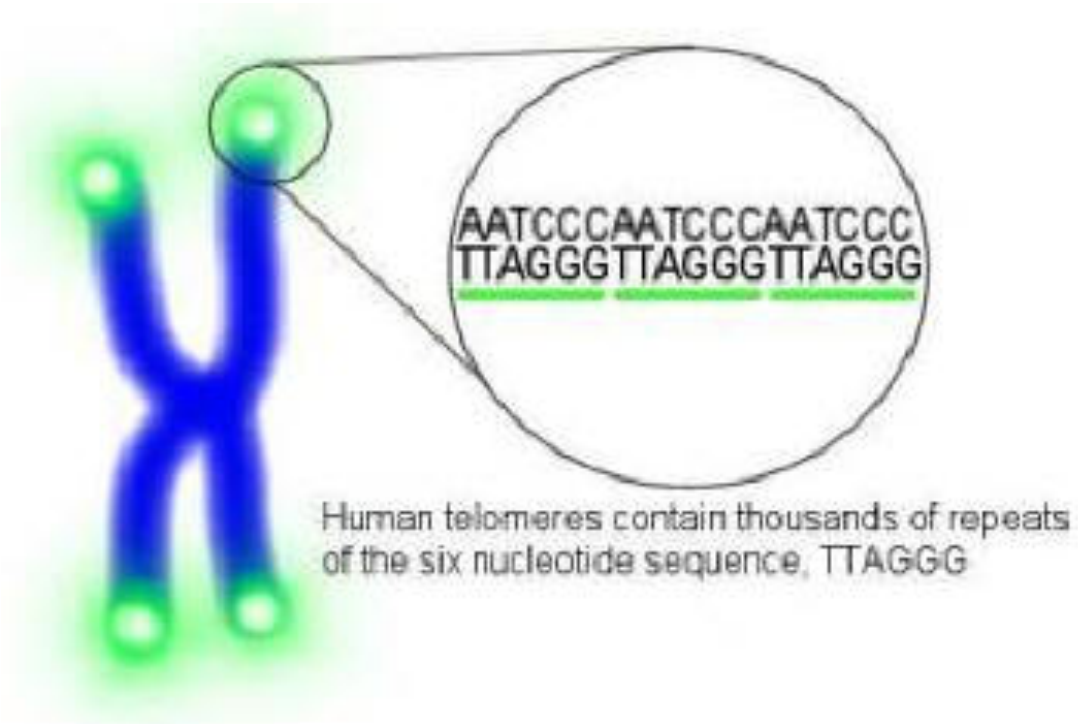
# The Problem at the ends of eukaryotic linear Chromosomes

This mechanism encounters a special problem when the replication fork reaches an end of a linear chromosome: there is no place to produce the RNA primer needed to start the last Okazaki fragment at the very tip of a linear DNA molecule (the **end replication problem**)




- Bacteria solve this "end-replication" problem by having circular DNA molecules as chromosomes
- Eucaryotes have special nucleotide sequences at the ends of their chromosomes, which are incorporated into telomeres, and attract an enzyme called telomerase.

# Telomeres




Telomere DNA sequences are similar in organisms as diverse as protozoa, fungi, plants, and mammals.

They consist of many **tandem repeats** of a short sequence that contains a block of neighboring G nucleotides.

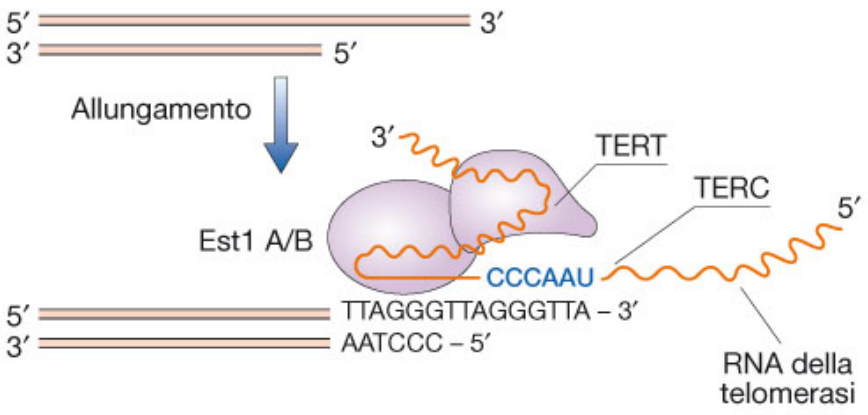
In humans, this sequence is **TTAGGG**, extending for about 10,000 nucleotides.

# La Telomerasi

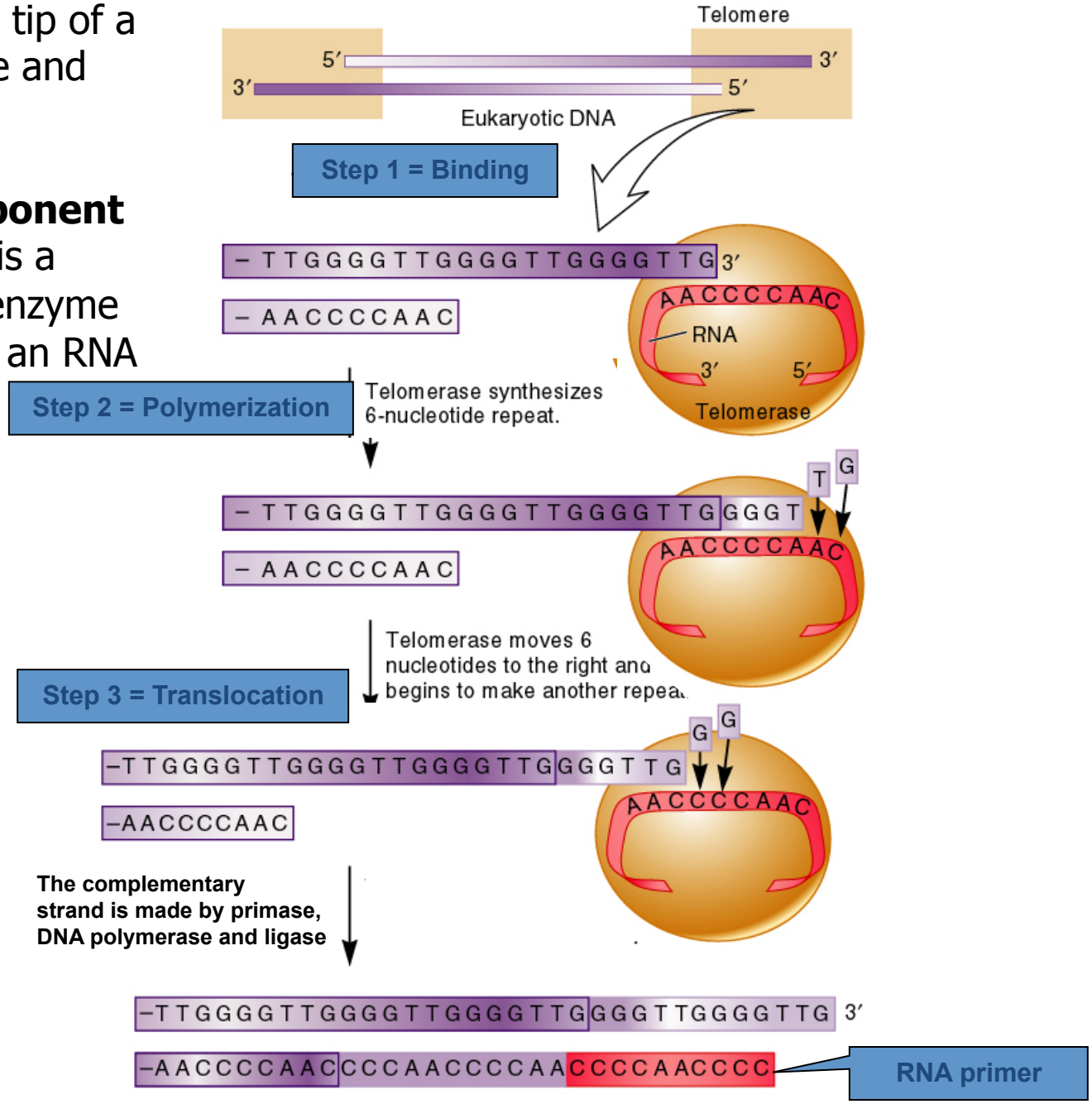
E’ una ribonucleoproteina costituita da due componenti principali:

1. Una proteina (TERT: *Telomerase Reverse Transcriptase*) che agisce come una trascrittasi inversa, essendo capace di sintetizzare DNA copiando uno stampo di RNA;
2. Una molecola di RNA stampo, chiamata TERC (*Telomerase RNA Component*)

La subunita’ catalitica TERT si associa con altre proteine accessorie a formare la macchina proteica coinvolta nel mantenimento dei telomeri.



Telomerase recognizes the tip of a G-rich strand of a telomere and elongates it in the 5′-to-3′ direction, using an **RNA template that is a component of the enzyme itself.** It is a **reverse transcriptase**, enzyme that synthesize DNA using an RNA template



# Three Scientists Win Nobel Prize in Medicine

Their work involved the health of cells and the aging process. *Transcript of*

2009: Elisabeth Blackburn, Carol Greider e Jack Szostak

Three scientists based in the United States have won the two thousand nine Nobel Prize for Physiology or Medicine. They are being honored for their work in the nineteen eighties about the health of cells and the aging process.

The winners are Elizabeth Blackburn from the University of California, San Francisco; Jack Szostak from Harvard Medical School in Massachusetts and Carol Greider from Johns Hopkins University in Maryland. They will share the one million four hundred thousand dollar prize.

**Elizabeth Blackburn, left, and Carol Greider after receiving a science prize in Frankfurt, Germany, earlier this year**

The scientists' work begins with telomeres. These are like protective coverings on the ends of chromosomes. Elizabeth Blackburn compares them to the plastic tips on the ends of shoelaces. She says without telomeres the chromosome and the genes it holds would come apart.

Telomeres are necessary for a cell to divide. They also are involved in directing the number of divisions.

**Jack Szostak**

Mizz Blackburn and Mister Szostak discovered the special system of genetic information in the telomeres that protects the chromosomes from ruin. Later, Mizz Blackburn and Mizz Greider discovered the substance in the body that builds telomeres. The scientists named the enzyme telomerase.

Their research showed that cells age if telomeres are shortened. But, cell death is delayed if a lot of the enzyme telomerase is produced.

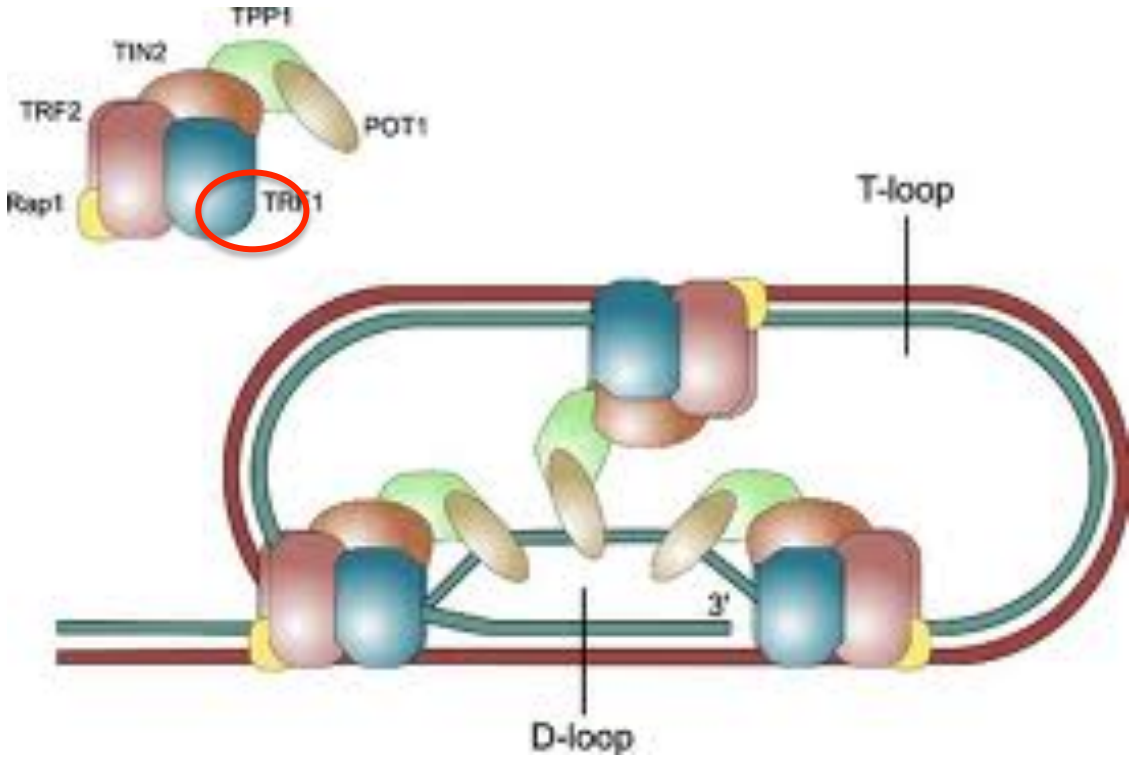
Rune Toftgard is a Nobel Committee member from Sweden's Karolinska Institute. He says the work of telomeres is important to the understanding of how genetic material is copied and saved.

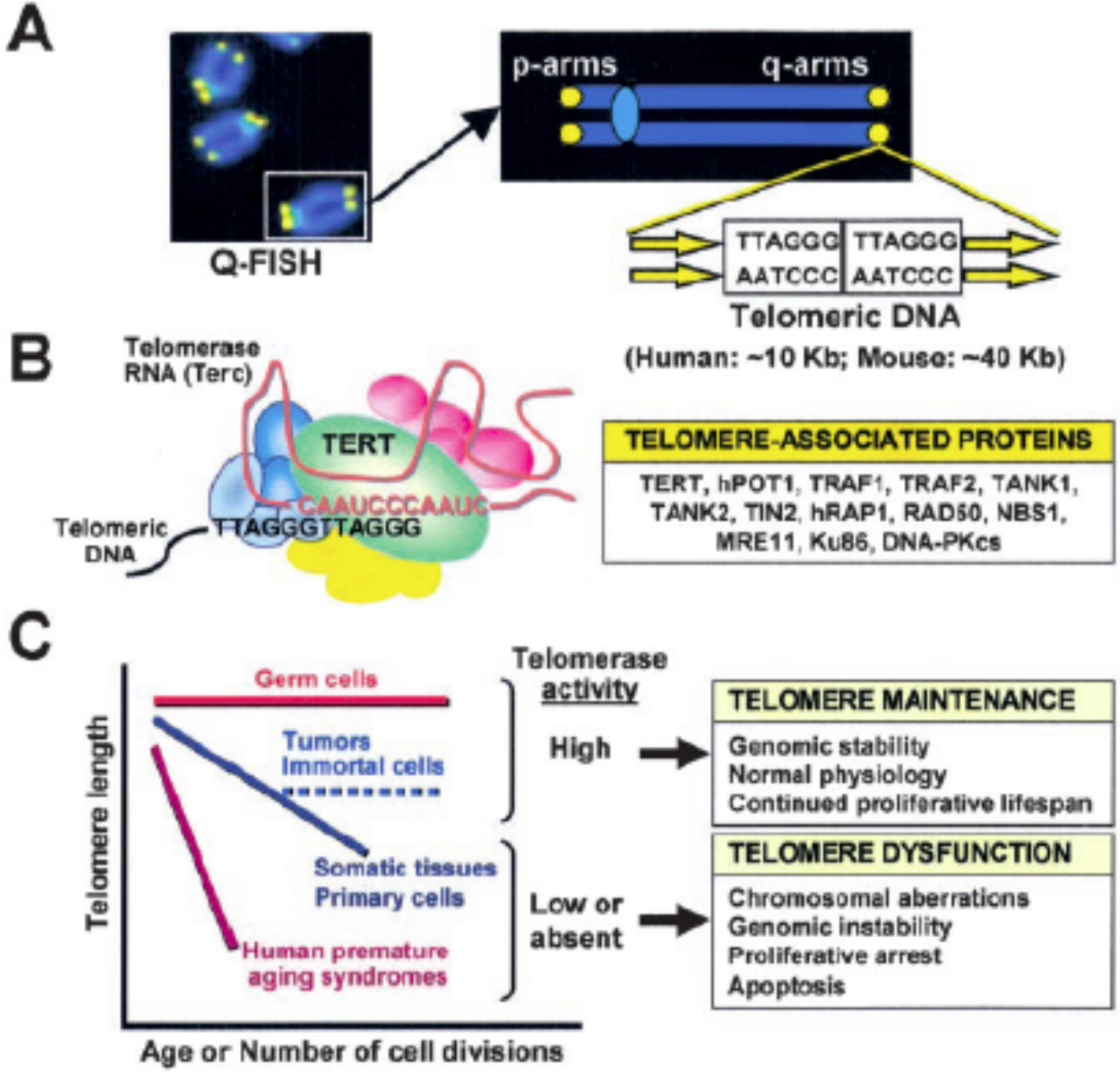
# Protezione dei telomeri

La lunghezza dei telomeri condiziona l'accesso della telomerasi ed è quindi CONTROLLATA:

a) Proteina **POT1**: quando il numero delle ripetizioni ricche di G è elevato si lega ad altri fattori proteici sul telomero, fa ripiegare la cromatina ed impedisce l'accesso della telomerasi

The extended telomeric cap helps to maintain the stability of the genome




A
p-arms
q-arms
Q-FISH
TTAGGG
TTAGGG
AATCCC
AATCCC
Telomeric DNA
(Human: ~10 Kb; Mouse: ~40 Kb)
B
Telomerase
RNA (Terc)
TERT
CAAUCCCAAUC
Telomeric
DNA
TTAGGGTTAGGG
TELOMERE-ASSOCIATED PROTEINS
TERT, hPOT1, TRAF1, TRAF2, TANK1,
TANK2, TIN2, hRAP1, RAD50, NBS1,
MRE11, Ku86, DNA-PKcs
C
Telomere length
Germ cells
Tumors
Immortal cells
Somatic tissues
Primary cells
Human premature
aging syndromes
Age or Number of cell divisions
Telomerase
activity
High
Low or
absent
TELOMERE MAINTENANCE
Genomic stability
Normal physiology
Continued proliferative lifespan
TELOMERE DYSFUNCTION
Chromosomal aberrations
Genomic instability
Proliferative arrest
Apoptosis

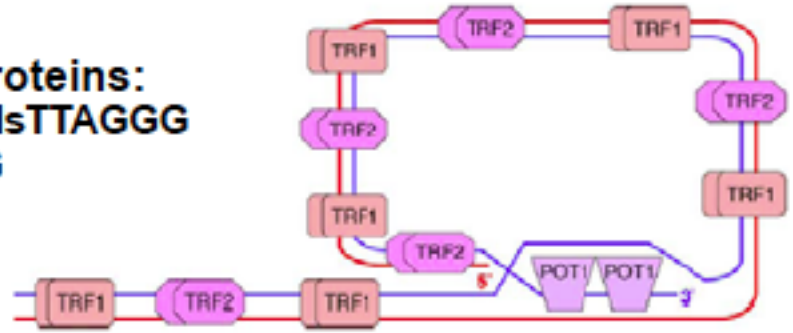
# Telomere structure

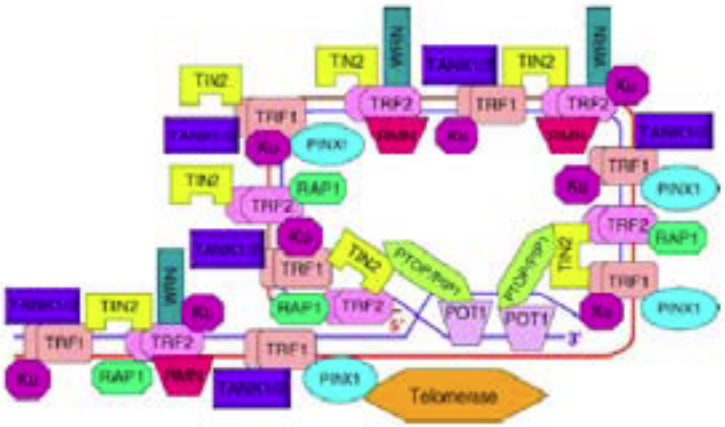
Vertebrate telomeres are long stretches (1-50 kb) of dsDNA containing the repetitive sequence TTAGGG, which terminate in 100-200 bases of ss TTAGGG at the 3' end. This 3' overhang circles back end embed in the duplex DNA

Terminal 3' ssDNA tail (G strand overhang) buried into adjacent ds repetitive telomeric DNA, forming a protective "t loop" structure. The "t loop" is stabilized by a "D loop" (displacement) loop. The G strand overhang is the substrate of telomerase (Terc), which employs an RNA template.

**3 telomere-associated proteins:**

- **TRF1 and TRF2 bind dsTTAGGG**
- **POT1 bind ssTTAGGG**




**The extended telomeric cap helps maintain the stability of the genome**

In molti tipi cellulari ci sono bassi livelli di telomerasi e ciascuna cellula nasce con un ben definito numero di unità ripetitive a livello dei telomeri.

Nella maggior parte delle cellule somatiche umane ad ogni divisione cellulare vengono persi 50-100 nucleotidi (accorciamento dei telomeri) e dopo varie generazioni le cellule iniziano ad avere cromosomi difettivi e vanno in **senescenza** (cioè cessano di dividersi).

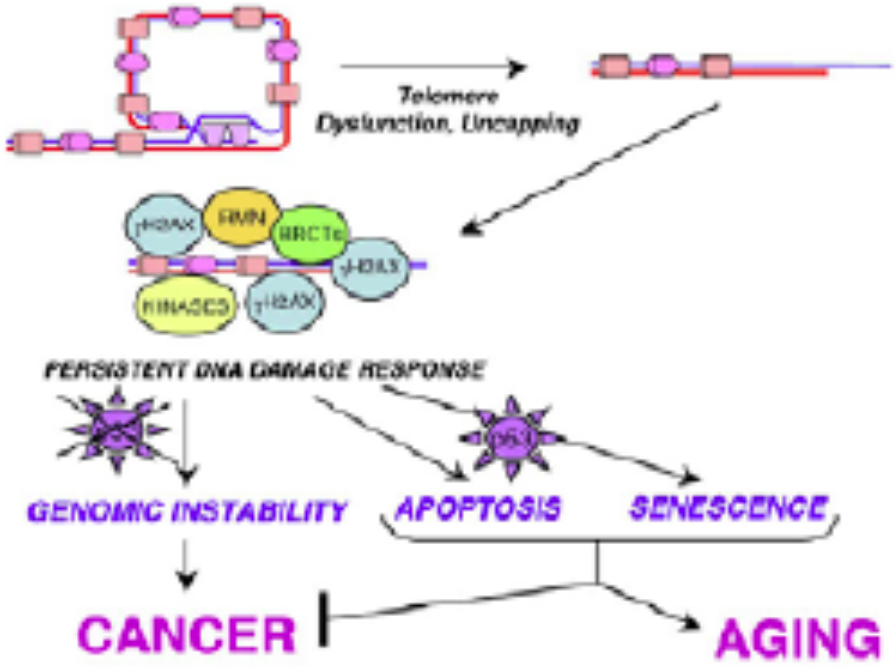
**Eccezioni**: cellule in attiva divisione: cellule ovaio, testicolo, cellule epiteliali proliferanti, linfociti, cellule embrione

# Telomeres uncapping

During the proliferation of human cells, a gradual shortening of the average length of telomeres is observed because of the "**end-replication problem**" - the inability of the DNA replication to complete DNA synthesis at the very beginning of the replicated lagging strand.

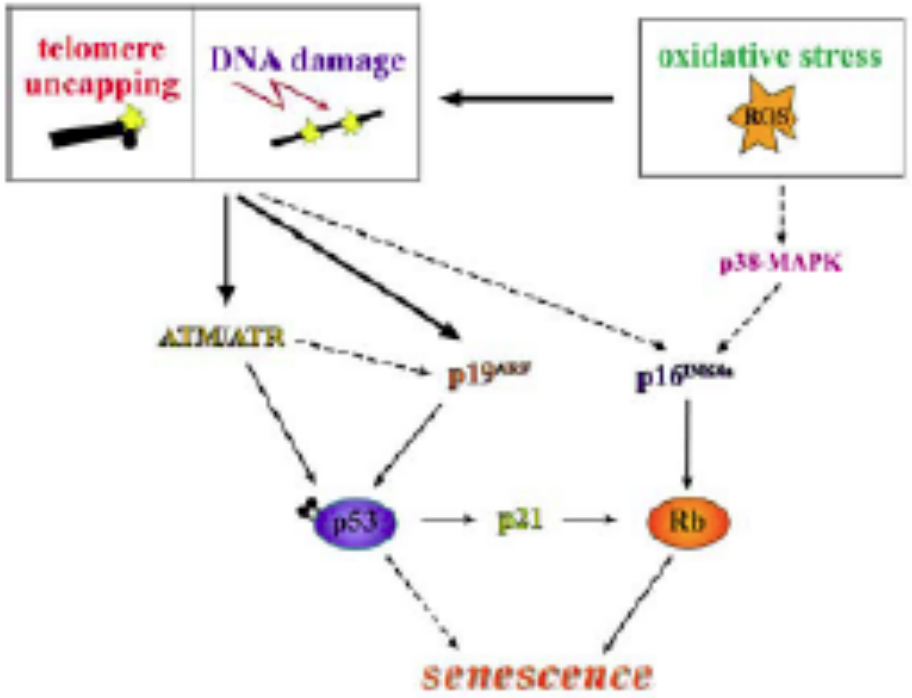
*Senescent telomeres lose some of their single-stranded portion - the telomeric overhang - which is crucial for the maintenance of the T-loop and the subsequent formation of the cap*

*Telomere uncapping (disruption of the proper structure of the protective cap) seems to be recognized as a **dsDNA break**, activating the DNA damage machinery.*



# Telomeres uncapping causes a DNA damage response

DNA damage foci appear at the telomeres of senescent cells, containing many DNA-damage-response proteins, including γ-H2AX, 53BP1, MDC1, NBS1, MRE11 and RAD17




How do cells choose between senescence and apoptosis upon DNA damage-induced p53 activation?

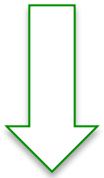
*-Different post-translational modifications of p53 in response to different stimuli?*

*-Binding of different proteins to p53?*

*-Activation of different sets of transcriptional targets?*

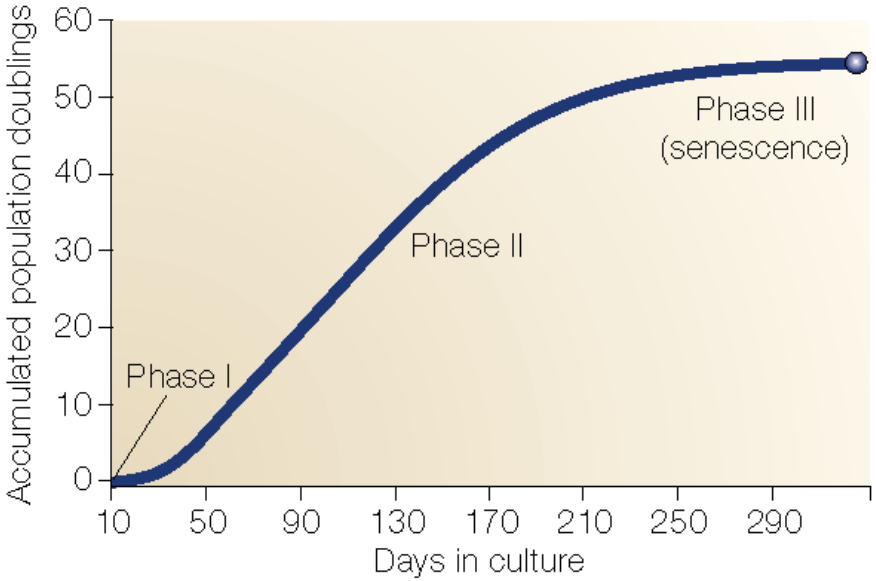
# Regolazione lunghezza telomeri

La maggior parte delle cellule somatiche umane non esprime abbastanza telomerasi per mantenere una lunghezza costante dei telomeri:
ACCORCIAMENTO DEI TELOMERI
(50-100 nt/divisione cell.)



Orologio cellulare per l'invecchiamento?

# TEORIA DI HAYFLICK (1965)

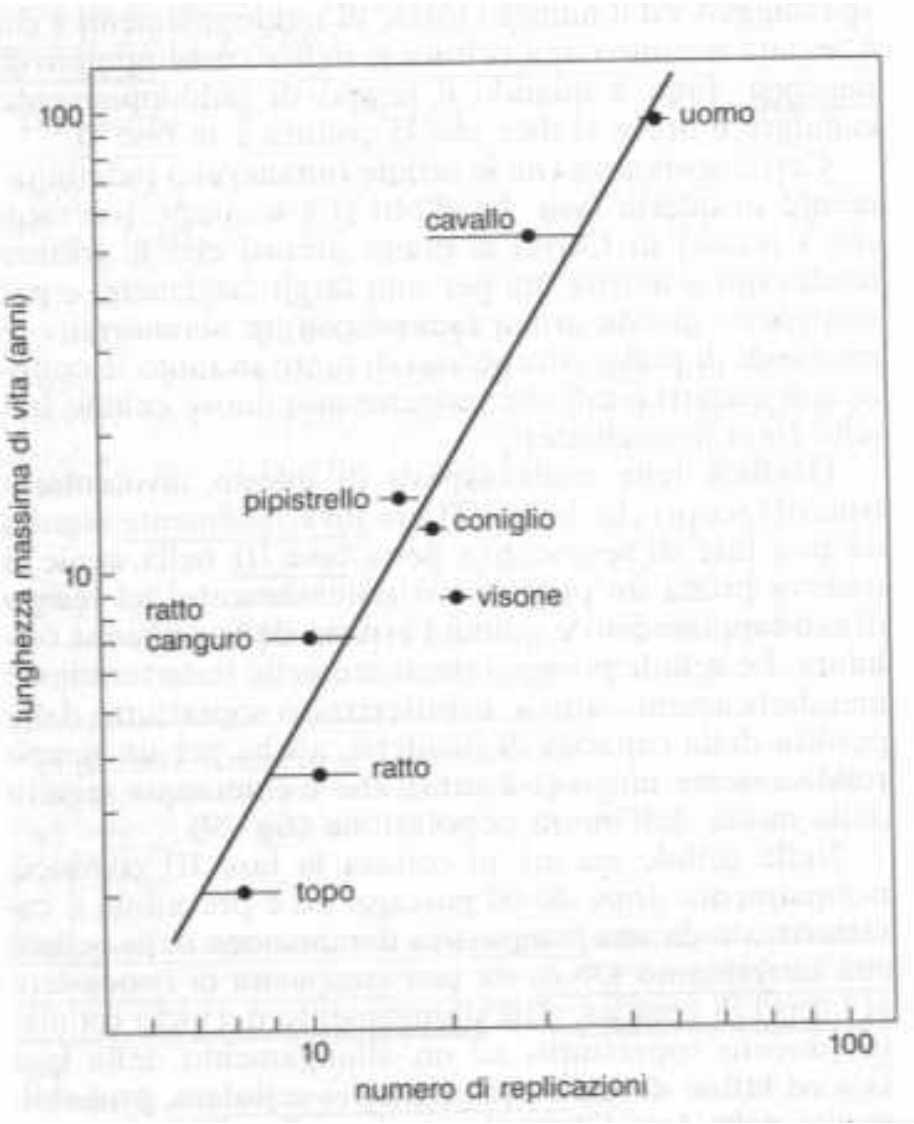



Nature Reviews | Molecular Cell Biology

Hayflick ha basato la sua teoria dell'invecchiamento su esperimenti di coltura "in vitro" di fibroblasti dermici, ma anche in molti altri tessuti.
Hayflick notò che il numero delle replicazioni, cioè del raddoppio delle cellule della coltura, in presenza di adeguate sostanze nutritive, nei fibroblasti umani era circa di 50; subentrava quindi una fase di senescenza, che, dopo circa altre 10 suddivisioni, portava alla estinzione della colonia, vale a dire alla morte di tutte le cellule.
**Il numero delle replicazioni dei fibroblasti diminuiva proporzionalmente all'eta' dell'organismo**.

# TEORIA DI HAYFLICK

Successivamente Hayflick fu in grado di dimostrare che il numero delle replicazioni di fibroblasti appartenenti a varie specie animali era proporzionale alla lunghezza massima della vita dell'animale stesso (figura).
Ne trasse la conclusione che la durata della vita, per cui ciascuna specie, era legata a fattori genetici, e un individuo possiede come un "orologio interno", che è programmato per una durata di vita prefissata.
(Teoria genetica dell' invecchiamento)



# Cellular senescence

- Normal human cells have a limited ability to proliferate in vitro (Hayflick, 1965)
- Growth potential of a primary cell declines 0.2 population doublings per year of life
- Correlation between the number of senescent cells in vivo and age of donor
- Cells from progerie syndrome patients have limited doubling potential
- Association with several molecular changes
- Overexpression of telomerase overcomes senescence; overexpression of ras induces senescence

**TABLE 1.** Selected Alterations in Cell Phenotype with the Onset of Senescence

| Phenotypic alteration in senescence[a] | Cell type | Ref. |
|---|---|---|
| Permanent growth arrest | All | 13, 19, 64, 65 |
| Repression of c-fos | Fibroblasts, T lymphocytes | 66, 67 |
| Repression of cyclins A and B | Fibroblasts | 68 |
| $G_1$ arrest on restimulation without division | Fibroblasts, T lymphocytes | 69, 37 |
| Elevated collagenase | Fibroblasts | 42 |
| Elevated TIMP-2 | Fibroblasts, endothelial cells | 44, 70 |
| Elevated PAI-1 | Fibroblasts, endothelial cells | 44 |
| Elevated ceramide | Fibroblasts | 71 |
| Transcriptional repression of IGF-1 | Fibroblasts | 72 |
| Induction of Ws3.10 inhibitor of $Ca^{2+}$-dependent membrane currents | Fibroblasts | 73 |
| Elevated IL-1α expression | Fibroblasts | 56 |
| Decreased IL-6 expression | Fibroblasts | 74 |
| Senescence-associated β-galactosidase | Fibroblasts, keratinocytes, mammary epithelial cells, endothelial cells, neonatal melanocytes | 23 |
| Induction of SAG gene | Fibroblasts | 75 |
| Repression of 17α-hydroxylase | Adrenocortical cells | 11 |
| Elevation of cytochrome *b* and NADH 4/4L subunit | Fibroblasts | 76 |
| Elevated *hic-5* expression | Fibroblasts | 77 |

[a]TIMP-2, tissue inhibitor of metalloprotein 2; PAI-1, plasminogen activator inhibitor 1; IGF-1, insulin-like growth factor 1; IL, interleukin.

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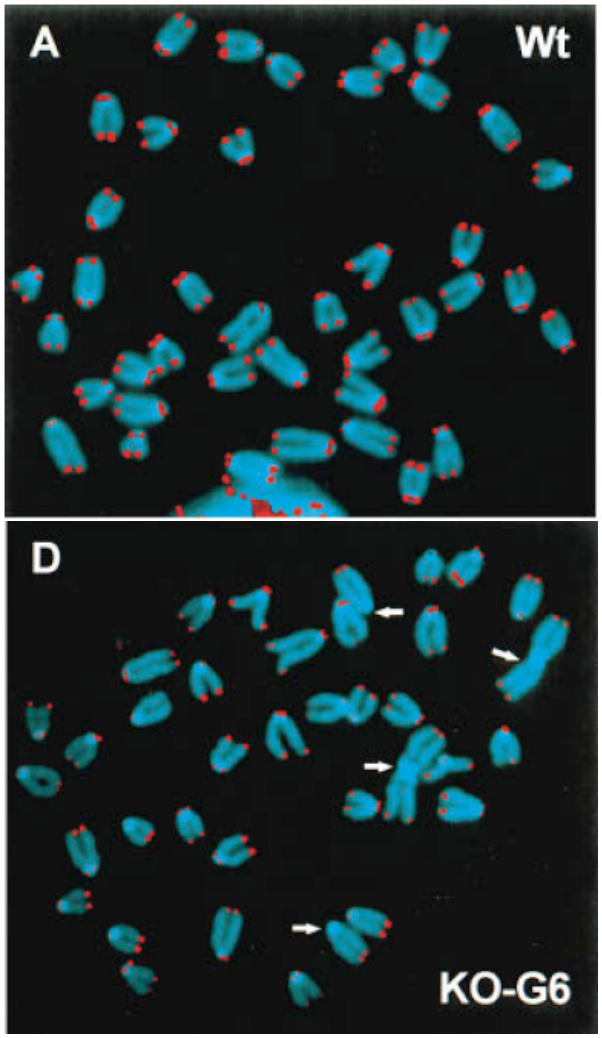
Cell, Vol. 91, 25–34, October 3, 1997, Copyright ©1997 by Cell Press

# Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA

María A. Blasco,[1,3,7] Han-Woong Lee,[2,7]
M. Prakash Hande,[4] Enrique Samper,[3]
Peter M. Lansdorp,[4,5] Ronald A. DePinho,[2,8]
and Carol W. Greider[1,6,8]

To examine the role of telomerase in normal and neoplastic growth, the telomerase RNA component (*mTR*) was deleted from the mouse germline.
Telomeres were shown to shorten at a rate of 4.8± 2.4 kb/ generation.
Cells from the fourth generation onward possessed chromosome ends lacking detectable telomere repeats, aneuploidy, and chromosomal abnormalities, including end-to-end fusions.

These results indicate that telomerase is essential for telomere length maintenance.



# Extension of Life-Span by Introduction of Telomerase into Normal Human Cells

Andrea G. Bodnar,* Michel Ouellette,* Maria Frolkis, Shawn E. Holt, Choy-Pik Chiu, Gregg B. Morin, Calvin B. Harley, Jerry W. Shay, Serge Lichtsteiner,† Woodring E. Wright†

Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence. To test this hypothesis, two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced staining for β-galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life-span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine.

**Constitutive** telomerase expression in several independent Tert-transgenic mouse models resulted in increased incidence of spontaneous tumors
(Gonzalez-Suarez et al., 2001; Gonzalez-Suarez et al., 2002; Artandi et al., 2002; Canela et al., 2004).

- Together, these evidences strongly suggests that telomerase activity and telomere length are rate limiting for mammalian life span and supports a model in which short telomeres actively contribute to aging by limiting tissue renewal.

- An important prediction of this model is that slowing the rate of telomere shortening should delay aging.

- However, to address experimentally this prediction, it is necessary to take into account the role of telomere biology in cancer.

LETTER

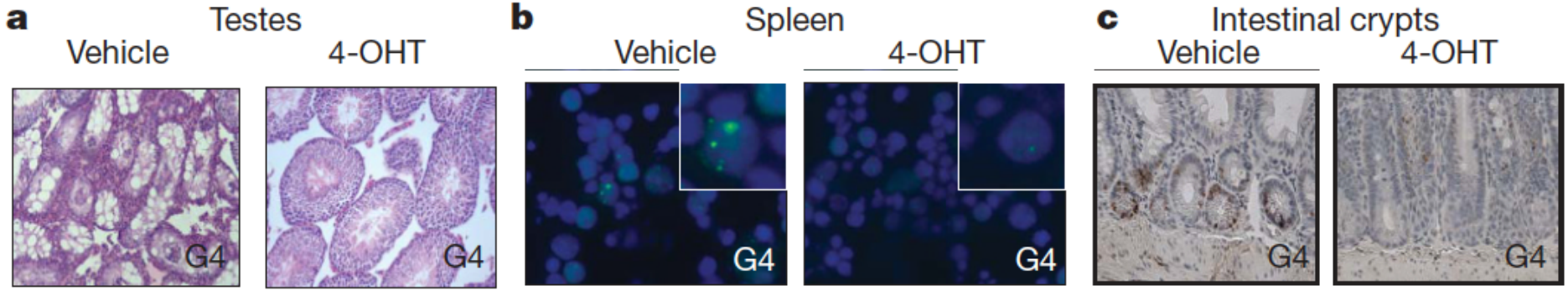
102 | NATURE | VOL 469 | 6 JANUARY 2011

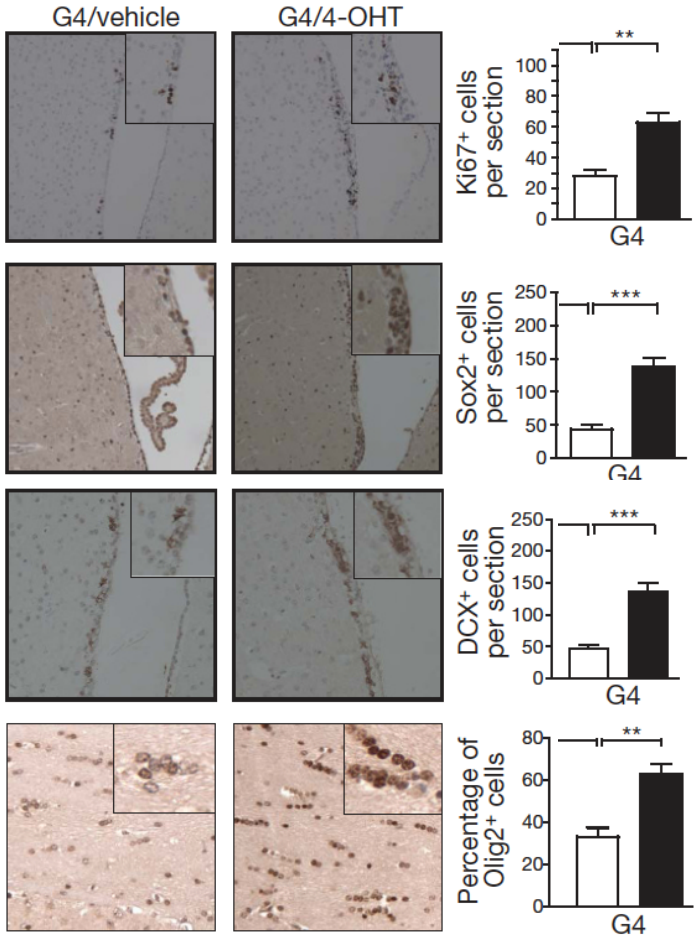
doi:10.1038/nature09603

# Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice

Mariela Jaskelioff[1], Florian L. Muller[1], Ji-Hye Paik[1], Emily Thomas[1], Shan Jiang[1], Andrew C. Adams[2], Ergun Sahin[1], Maria Kost-Alimova[1], Alexei Protopopov[1], Juan Cadiñanos[1], James W. Horner[1], Eleftheria Maratos-Flier[2] & Ronald A. DePinho[1]

- A novel mouse model to explore the impact of physiological telomerase reactivation across diverse adult cell types and organ systems.
- Notably, the mice enlisted into this study are adults exhibiting significant **progeroid phenotypes** (mice null for mTerc or mTert).
- In TERT-ER mice with advanced degenerative phenotypes, short-term telomerase reactivation restored telomere reserves, quelled DNA damage signalling, and alleviated cellular checkpoint responses in several high-turnover organ systems with significant functional impact including increased fecundity….




Telomerase reactivation extends telomeres, reduces DNA damage signalling and associated cellular checkpoint responses, allows resumption of proliferation in quiescent cultures, and eliminates degenerative phenotypes across multiple organs including testes, spleens and intestines.

Notably, somatic telomerase reactivation reversed neurodegeneration with restoration of proliferating neural progenitors.

The brief course of telomerase reactivation was not sufficient to promote carcinogenesis.
However, it remains possible that more prolonged telomerase reactivation schedules or applications in later life may provoke carcinogenesis.

Cell *135*, 609–622, November 14, 2008 ©2008 Elsevier Inc.

# Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice

Antonia Tomás-Loba,[1,5] Ignacio Flores,[1,5] Pablo J. Fernández-Marcos,[2] María L. Cayuela,[1,6] Antonio Maraver,[2] Agueda Tejera,[1] Consuelo Borrás,[3] Ander Matheu,[2] Peter Klatt,[1,2] Juana M. Flores,[4] José Viña,[3] Manuel Serrano,[2] and Maria A. Blasco[1,*]

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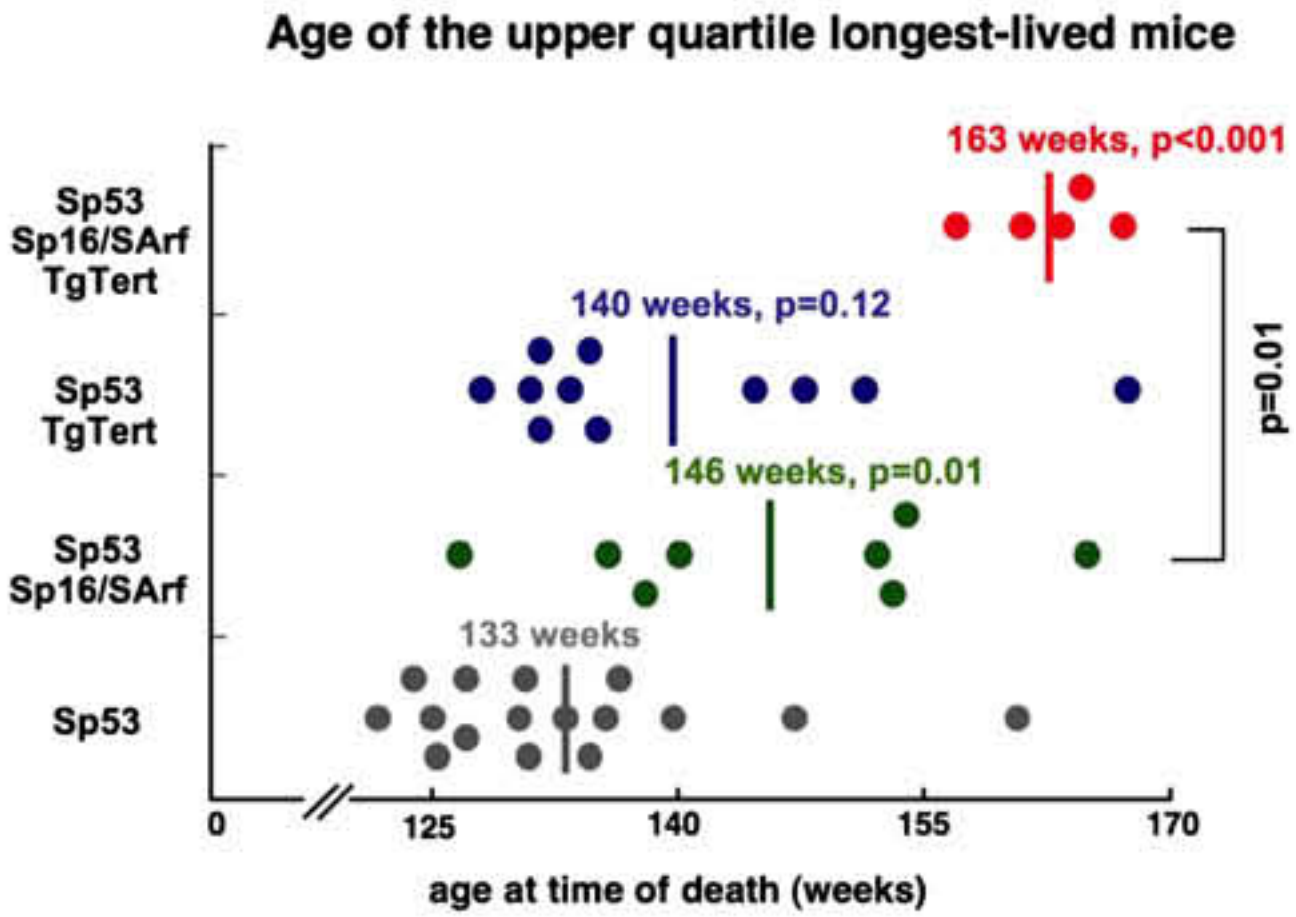
DOI 10.1016/j.cell.2008.09.034

Telomerase confers limitless proliferative potential to most human cells through its ability to elongate telomeres, the natural ends of chromosomes, which otherwise would undergo progressive attrition and eventually compromise cell viability. However, the role of telomerase in organismal aging has remained unaddressed, in part because of the cancer-promoting activity of telomerase. To circumvent this problem, we have constitutively expressed telomerase reverse transcriptase (TERT), one of the components of telomerase, in mice engineered to be cancer resistant by means of enhanced expression of the tumor suppressors p53, p16, and p19ARF (these three tumor suppressors are involved in protection against a large variety of cancers-Collado et al., 2007). In this context TERToverexpression improves the fitness of epithelial barriers, particularly the skin and the intestine, and produces a systemic delay in aging accompanied by extension of the median life span. These results demonstrate that constitutive expression of Tert provides anti-aging activity in the context of a mammalian organism.


Age of the upper quartile longest-lived mice
163 weeks, p<0.001
140 weeks, p=0.12
146 weeks, p=0.01
133 weeks
p=0.01
Sp53 Sp16/SArf TgTert
Sp53 TgTert
Sp53 Sp16/SArf
Sp53
0
125
140
155
170
age at time of death (weeks)