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INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES / LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

Centre Bloomfield de recherche sur le vieillissement

The Bloomfield Centre for Research in Aging

Cancer and Aging: Two Faces of the Same Coin

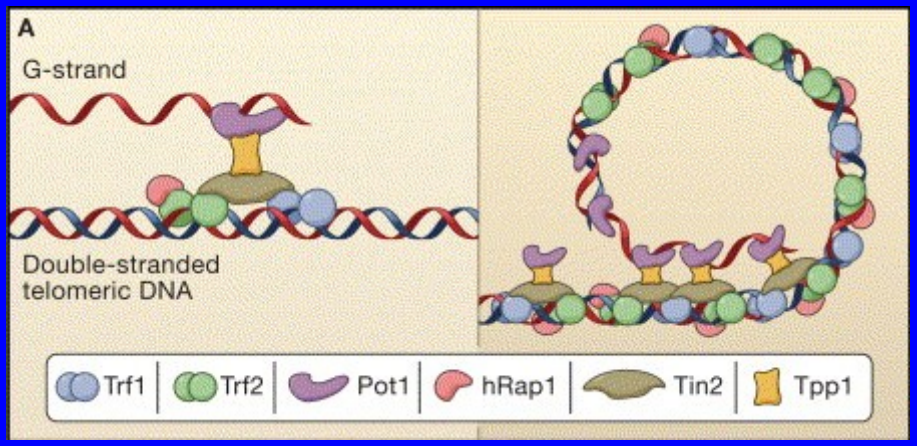
(2) Telomere Biology and Aging

TELOMERE BIOLOGY AND AGING

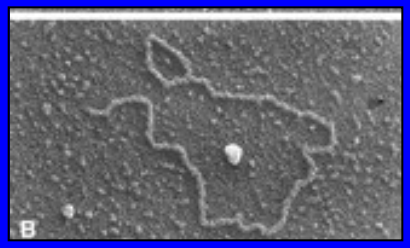
1. Telomeres: composition & function
2. Consequences of telomere shortening
3. Telomere shortening and human aging
4. Telomerase
5. Telomere hypothesis of aging and immortalization
6. Telomere-dependent and independent cellular senescence
7. Cellular senescence, aging, tumor suppression, and tumor promotion
8. Telomerase knockout and transgenic mice

Telomere structure

- Coated with telomeric proteins
- Form a non-linear structure that sequesters/hides the DNA end (T-loop)



Baumann, P. Cell 2006



Griffith, JD et al., Cell 1999

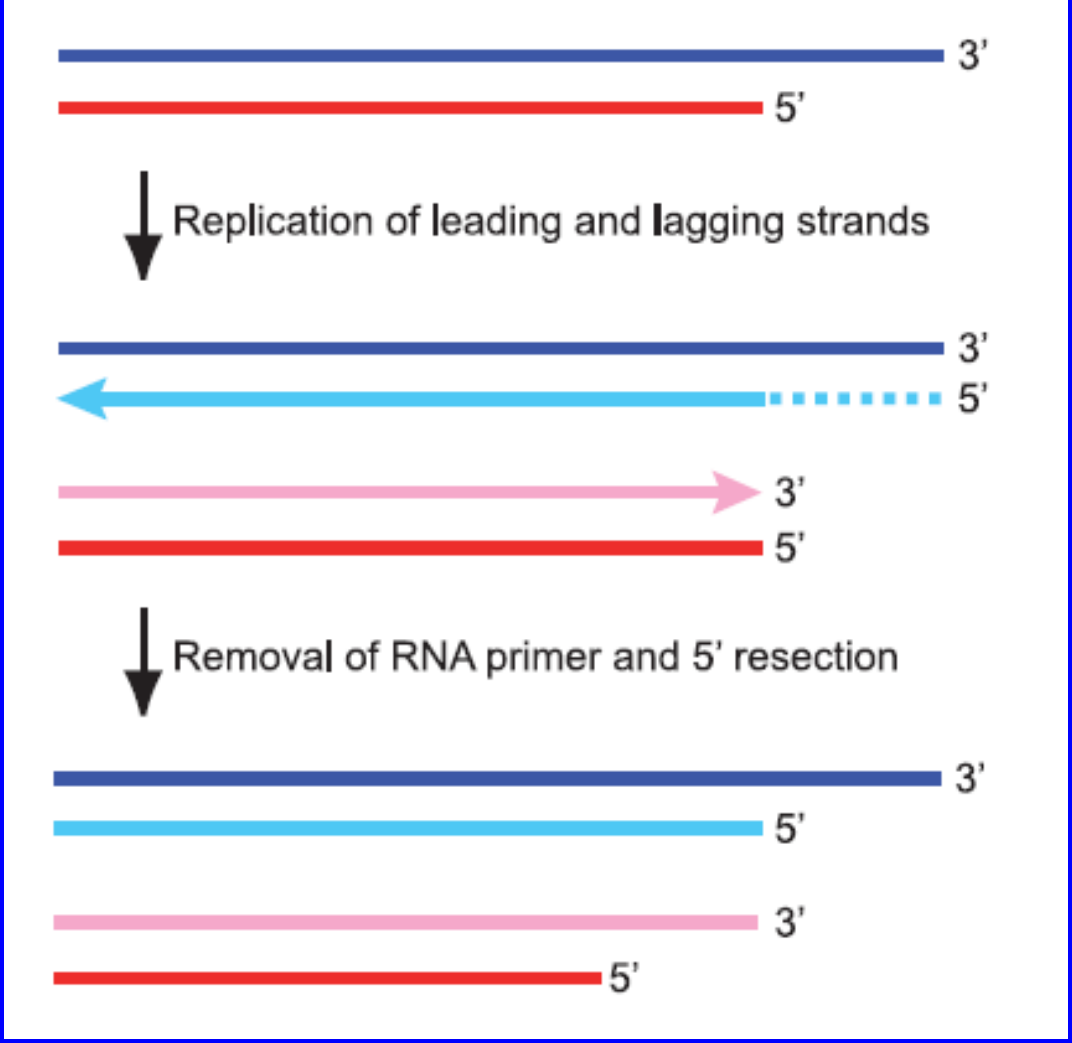
Telomere interference :

- telomeric chromosome fusions
- chromosome instability
- replicative senescence
- cell death

Telomere integrity:

- essential for replicative immortality

End-replication problem



Lagging strand
Leading strand

DNA replication

Lagging strand

Leading strand

RNA primer removal,
Okazaki fragment ligation

Lagging strand

Leading strand

Osterhage and Friedman. 2009. JBC, 284, 16061-16065

Functions of telomeres

- **Ensure complete replication of DNA at chromosome ends** (via telomerase, a ribonucleoprotein and reverse transcriptase which synthesizes the telomeric repeats on the G-rich strand)

- **'Cap' natural chromosome ends to make them stable structures:**
  - Shield chromosome ends from degradation and end-to-end fusions
  - Prevent activation of DNA damage checkpoints

Nobelprize.org

The Nobel Prize in Physiology or Medicine 2009

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

Photo: Gerbil, Licensed by Attribution Share Alike 3.0

Elizabeth H. Blackburn

Photo: Gerbil, Licensed by Attribution Share Alike 3.0

Carol W. Greider

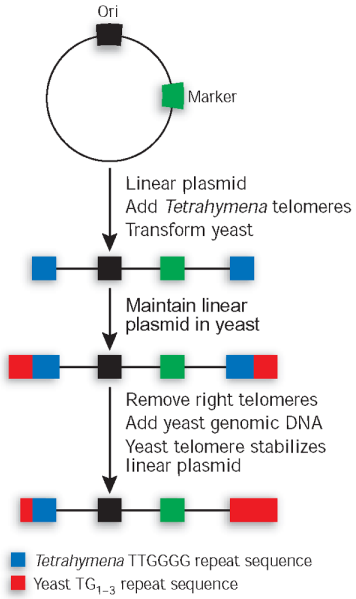
Photo: Jussi Puikkonen

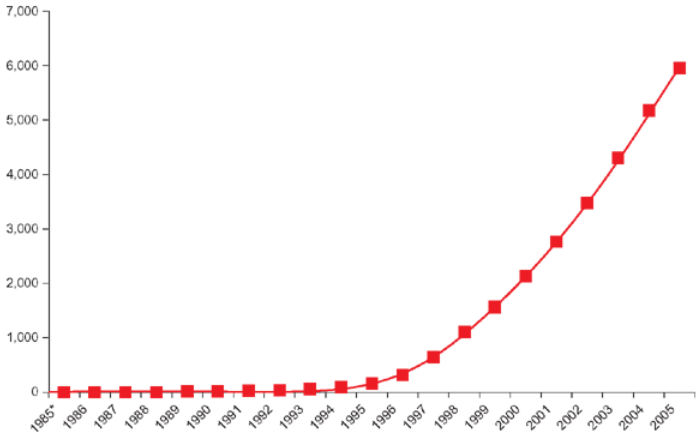
Jack W. Szostak

nature.com/naturemedicine

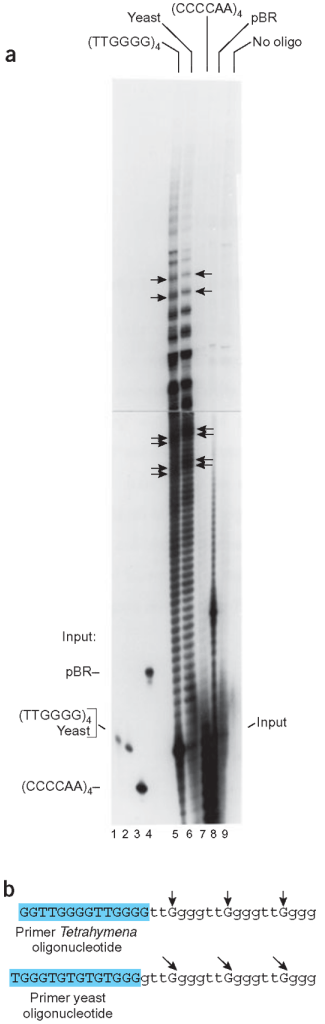
Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging

Elizabeth H Blackburn, Carol W Greider & Jack W Szostak





Cumulative citations for telomerase in Medline



Yeast sequences are added to *Tetrahymena* telomeres *in vivo*.

Tetrahymena sequences are added to yeast telomeres *in vitro*.

Non-shelterin proteins associated with vertebrate telomere maintenance

Table 1 – DNA damage response proteins involved in telomere maintenance

Protein	Cell origin	Function	Sensitivity	Telomere dysfunction			Shelterin interaction	Reference
				Length	Fusions	Other		
ATM	Human Mouse	Damage signaling	IR	Shorter	Yes	Yes	Yes	[2–4]
Ku	Mouse	NHEJ	IR	Shorter/ longer	Yes	ND	Yes	[5–8]
	Human	Shorter						
DNA-PKcs	Mouse (scid)	NHEJ	IR	Longer	Yes	ND	Yes	[9,10]
	Mouse KO	Normal						
RAD54	Mouse	HR	IR	Shorter	Yes	ND	ND	[11]
RAD51D	Mouse Human	HR	IR	Shorter	Yes	ND	Yes	[12]
NBS1	Human	Damage sensing?	IR	Shorter	No	ND	Yes	[13]
MRE11	Human	Damage sensing?	IR	ND	ND	ND	Yes	[14]
PARP-2	Human	BER	IR	Normal	No	ND	Yes	[15]
ERCC1	Human	NER	UV	ND	No	Yes	Yes	[16]
XPF	Human	NER	UV	ND	No	Yes	Yes	[16]
WRN	Human	Helicase	Topoisomerase inhibitors	Shorter	ND	ND	Yes	[17]
BLM	Human	Helicase	Topoisomerase inhibitors	Shorter	ND	ND	Yes	[17]
FANCA	Human	Damage sensing?	MMC	Shorter	Yes	Yes	ND	[18]
RAD50	Human	Damage sensing?	IR	ND	ND	ND	Yes	[14,19]
	Mouse	IR	ND	Yes				
BRCA1	Human	HR	IR	Longer	Yes	ND	Yes[a]	[20–24]
	Mouse	Shorter	Yes	ND				
Rad9	Human Mouse	Damage sensing?	IR	ND	Yes	ND	Yes	[25]
PARP-1	Human	BER	IR	Normal	Yes	ND	Yes	[26]

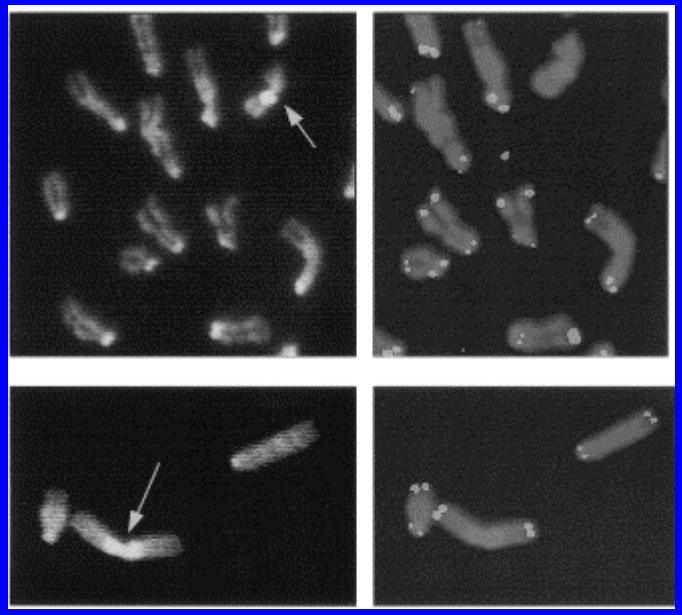
Two inclusion criteria were used to compile Table 1: (a) loss of telomere function in the form of either accelerated loss of telomeric DNA or appearance of cytological signs of telomere dysfunction in affected cells and (b) interaction of a particular DNA damage response protein with shelterin in normal cells. IR, ionizing radiation; NHEJ, non-homologous end-joining; HR, homologous recombination; ND, not determined.

[a] Partial interaction.

Slijepcevic, P. 2006. DNA repair 5,1299-306

Consequences of telomere shortening

End-to-end chromosome fusions



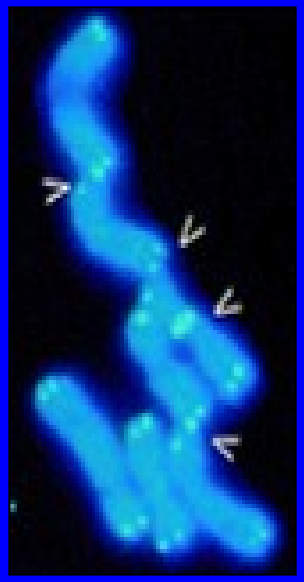
DAPI

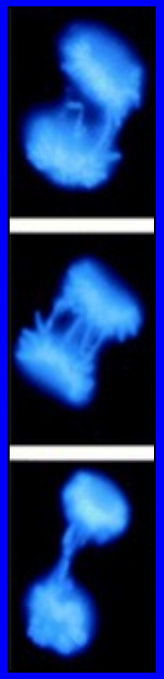
Telomere probe

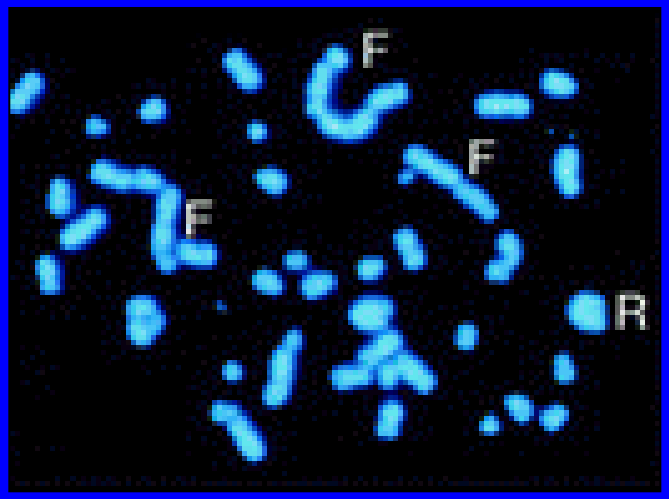
Latre et al., 2003

Cytogenetic abnormalities resulting from telomere shortening/telomere dysfunction

Fusion-bridge-breakage cycles







Telomeric fusion → ‘Bridge’ chromosomes → Chromosome breakage/ missegregation → Fusion of broken chromosomes

vanSteensel et al., 1998

Cytogenetic consequences of fusion-bridge-breakage cycle

- **Chromosome and gene deletions**
- **Complex non-reciprocal translocations**
  (hallmark of human carcinomas)

DNA DAMAGE

Cellular consequences of telomere shortening-induced DNA damage

- **Replicative senescence** (permanent growth arrest)
- **Apoptosis** (programmed cell death)
- **Carcinogenesis** (in the absence of functional DNA damage checkpoints such as p53)

Cellular senescence and apoptosis as major tumor suppressor mechanisms

Carcinogenesis can occur when important DNA damage checkpoint-regulating genes and pathways (eg. p53, pRb, $p16^{INK4A}$) are absent or defective.

The products of these tumor suppressor genes ensure that cells with irreparably damaged genomes die (apoptosis) or stop dividing permanently (replicative senescence).

Cells with damaged genomes can only continue to proliferate if they accumulate genetic mutations that inhibit the major tumor suppressor pathways.

Major regulators of replicative senescence: p53

'guardian of the genome'

- Tumor suppressor gene at the hub of many different signaling pathways that provide information about cellular stress states—DNA damage strongly upregulates p53 activity
- Transcriptional regulator —downregulates many genes; upregulates some others
- p53 signaling elicits cell cycle arrest (in G1, S or G2/M) and/or cell death or senescence
- p53 is inactivated by MDM2, which binds to p53 and inhibits its ability to regulate transcription
- p53 is specifically targeted by many important oncogenic, transforming viruses (e.g. SV40, HPV)
- p53 is mutated or deleted in at least 50% of human cancers, and is dysregulated in many more

Major regulators of replicative senescence: $p16^{INK4A}$ and $p19^{ARF}$

- **INK4A locus**
  - Codes for both the $p16^{INK4A}$ and $p19^{ARF}$ tumor suppressor gene products (in alternative reading frames)
  - Frequently deleted or silenced in human cancers (eliminating expression of both $p16^{INK4A}$ and $p19^{ARF}$)

- **$p16^{INK4A}$**
  - Important for replicative senescence in human cells
  - Increased expression in primary human fibroblasts with increasing population doubling number
  - Mouse primary fibroblasts that bypass senescence lose expression of $p16^{INK4A}$
  - Regulates the pRB (retinoblastoma) pathway via cdk4 and cdk6 (inhibits cellular proliferation)
  - Does not require p53 for antiproliferative function (alternative senescence pathway)
  - Frequently targeted by oncogenic viruses

- **$p19^{ARF}$ (also called $p14^{INK4A}$ in human cells)**
  - Important for replicative senescence in mouse cells
  - Binds and sequesters MDM2 (prevents it from inactivating p53)

Telomere shortening and human aging

- HUMAN AGING
  - CANCER!!! (especially epithelial cancers)
  - decline of the immune system
  - reduced skin thickness and wound healing capacity
  - changes in the morphology and function of epithelial tissues in the digestive and cardiovascular systems
  - reduced fertility

- All tissues in the adult body are renewed by cellular replication
  - exception: terminally differentiated (post-mitotic) cells such as neurons and cardiac muscle cells

- Apoptosis and replicative senescence in cells with short telomeres could slow or prevent tissue self renewal

- Tissues that undergo the highest rates of cell division and self-renewal would be most affected by replicative senescence and apoptosis (immune system, epithelial tissues)
- …..these are the tissues that are most profoundly affected during aging, and commonly give rise to cancers in adult humans

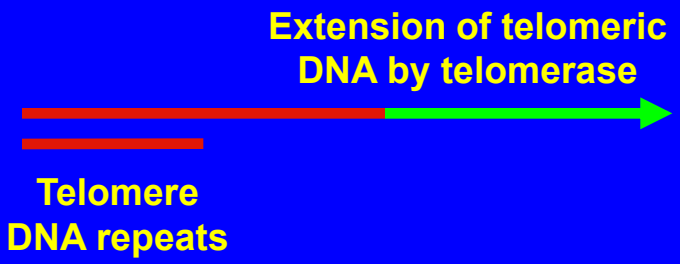
Solutions to the end replication problem

- Circular chromosomes (bacteria)
- Terminal hairpin structures (vaccinia virus, some bacteria with linear chromosomes)
- Terminal proteins (adenovirus, Φ29)
- Telomerase (most eukaryotes)
- Retrotransposition (drosophila)
- Alternative mechanisms (ALT) (recombination-based: yeast, 15% human cancer cells)

What is telomerase?

- Essential for replicative immortality of most eukaryotic cells
- DNA polymerase
- Caps linear DNA molecules with telomere DNA repeats

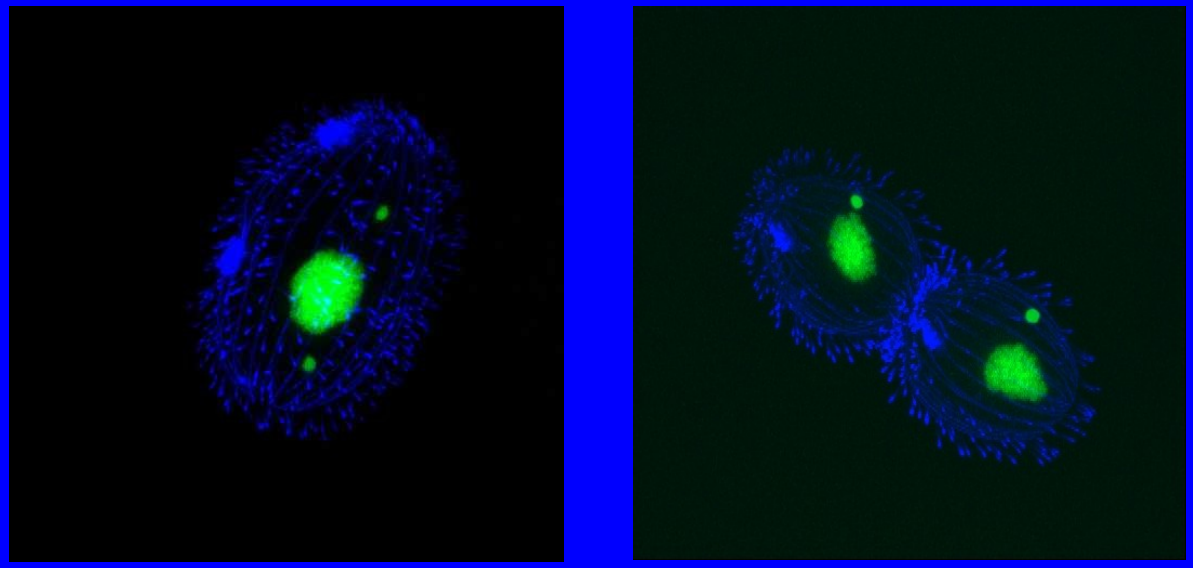
Scanning electron micrograph



Uniciliates
Yeasts
Plants
Vertebrates

TETRAHYMENA

- Unicellular protist
- Two nuclei: micronucleus is a conventional germline precursor
macronucleus is the somatic or transcriptionally active nucleus
- Highly developed unicell with features characteristic of metazoans with highly differentiated tissues



Vegetative cell undergoing micronuclear
or macronuclear division

Tetrahymena as a model system for the study of telomerase

Telomerase activity is abundant in *Tetrahymena* compared to human (during conjugation and macronuclear development there is extensive chromosome fragmentation, DNA rearrangement and DNA deletion and amplification creating >10000 chromosome end compared to 92 in humans)

Telomerase activity and the telomerase RNA component were first identified in *Tetrahymena*

Tetrahymena Telomerase RNA

AUACCCGCUUAAUUCAUUCAGAUCUGUAAUAGAACUGUCAUU

CAACCCCAAAAAUCUAGUGCUGAUAUAACCUUCACCAAUUAG

GUUCAAAUAAGUGGUAAUGCGGGACAAAAGACUAUCGACAUU

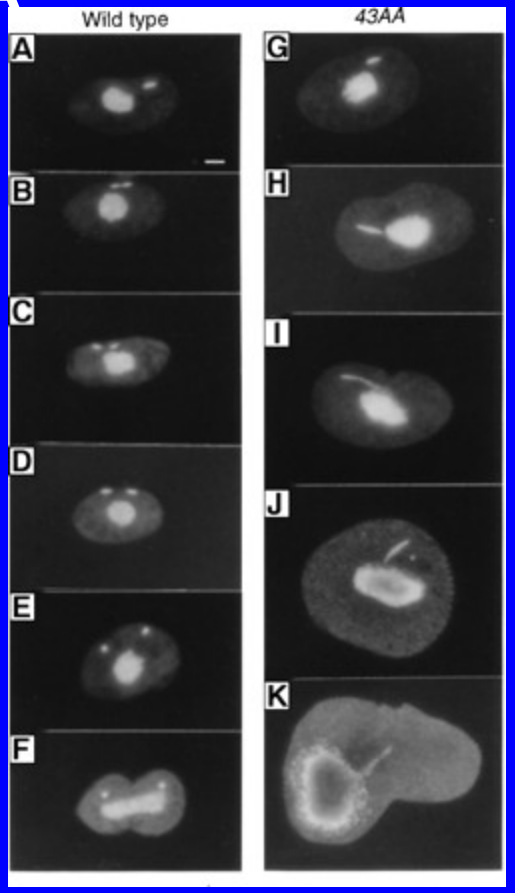
UGAUACACUAUUUAUCAAUGGAUGUCUUAUUUUU

Greider and Blackburn, 1989

Telomere Dysfunction

Consequence of altered telomerase RNA template *in vivo* first demonstrated in *Tetrahymena* (Yu et al., 1990; Kirk et al., 1997)

- Altered telomere sequences
- Altered telomere lengths
- Impaired cell division
- Severe delay or block in completing mitotic anaphase
- Senesence phenotype

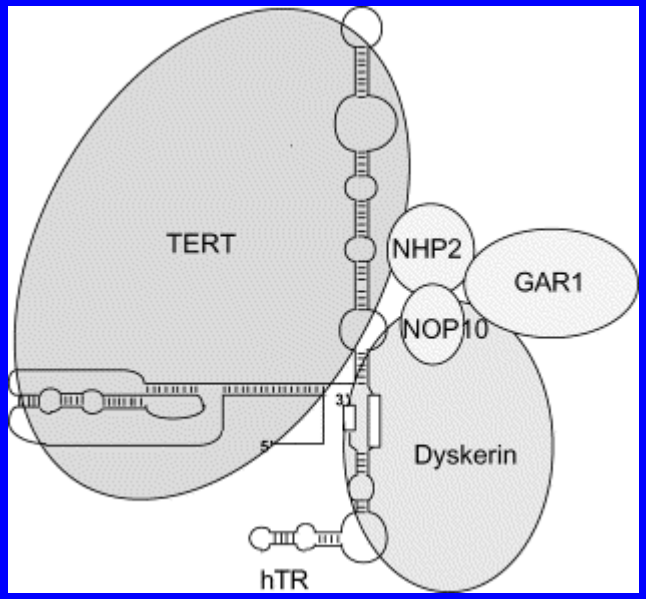


Kirk et al., 1997

Human telomerase complex

hTERT
(telomerase reverse transcriptase)

hTR
(telomerase RNA)
***aka* hTERC**



Dokal I. And Vulliamy T. 2003. Blood Rev. 17, 217-225

Other telomerase-interacting proteins:

RNA processing and ribonucleoprotein assembly (snoRNA-associated proteins)
Dyskerin, NHP2, NOP10, GAR1

Molecular chaperones (Hsp90, p23)

Localization (TCAB1)

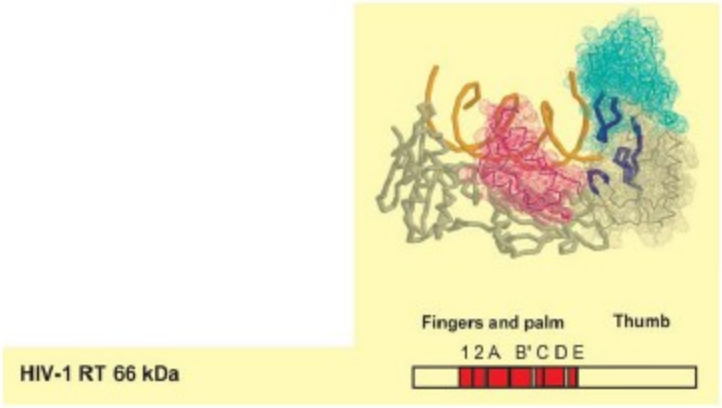
Post-translational modification

Recruitment of telomerase to telomeres TPP1, Pot1

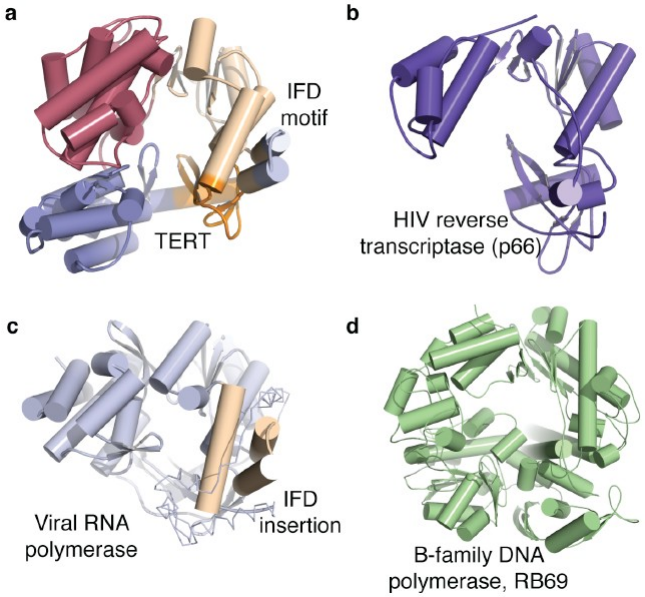
DNA replication machinery

Minimal telomerase components (RRL reconstitution) = hTR + hTERT

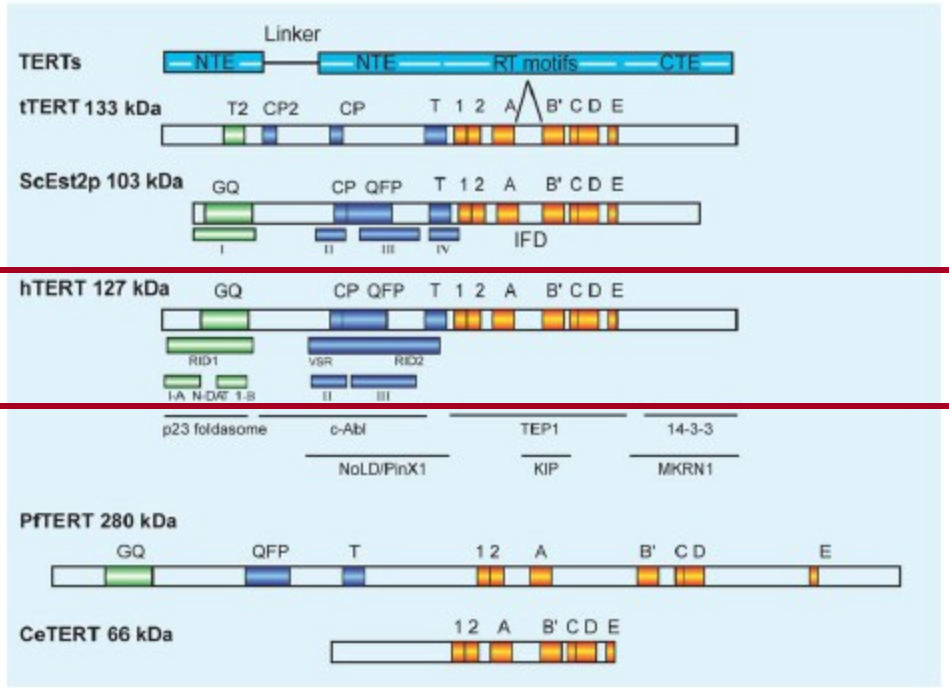
Organization of the reverse transcriptase (RT) motifs in the telomerase reverse transcriptase (TERT) from different organisms and HIV-1 RT



Structure of the Tribolium castaneum telomerase catalytic subunit TERT

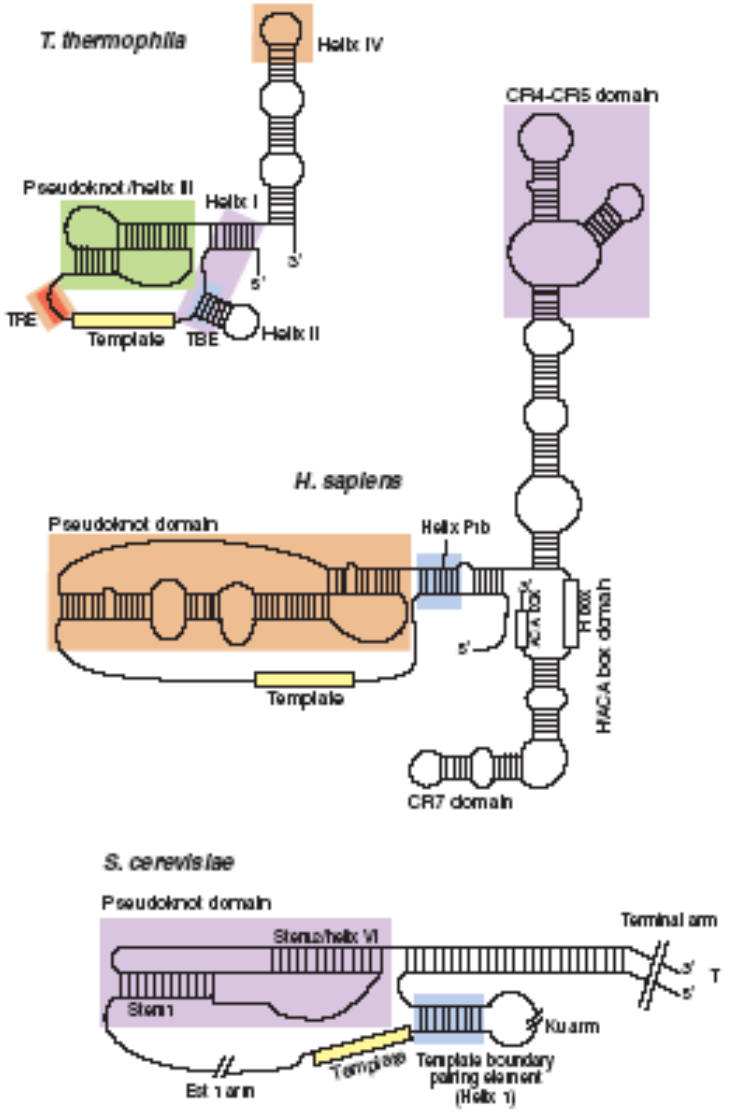


Gillis et al. Nature, 455(7213), 633-7, 2008



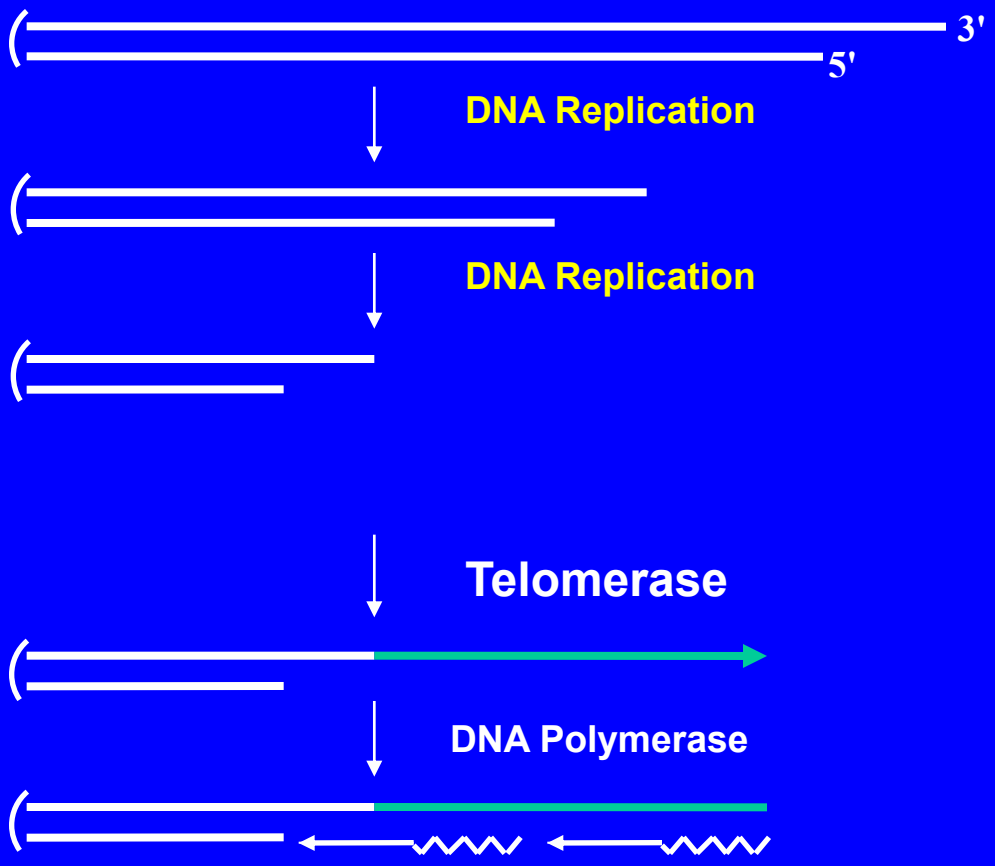
Autexier and Lue, 2006 Ann. Rev. Biochem.

Phylogenetically conserved telomerase RNA structure



Autexier and Lue, 2006 Ann. Rev. Biochem.

Telomerase prevents telomere shortening



Telomere shortening:

- telomeric chromosome fusions
- chromosome instability
- replicative senescence
- cell death

Telomere length maintenance:

- essential for replicative immortality

Synthesis of telomeric sequences

1) Recognition

DNA substrate binding to hTERT and RNA template

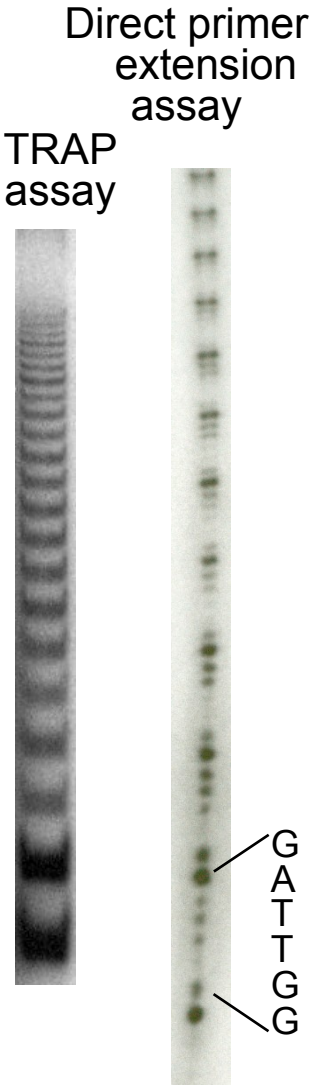
2) Elongation

Addition of nucleotides

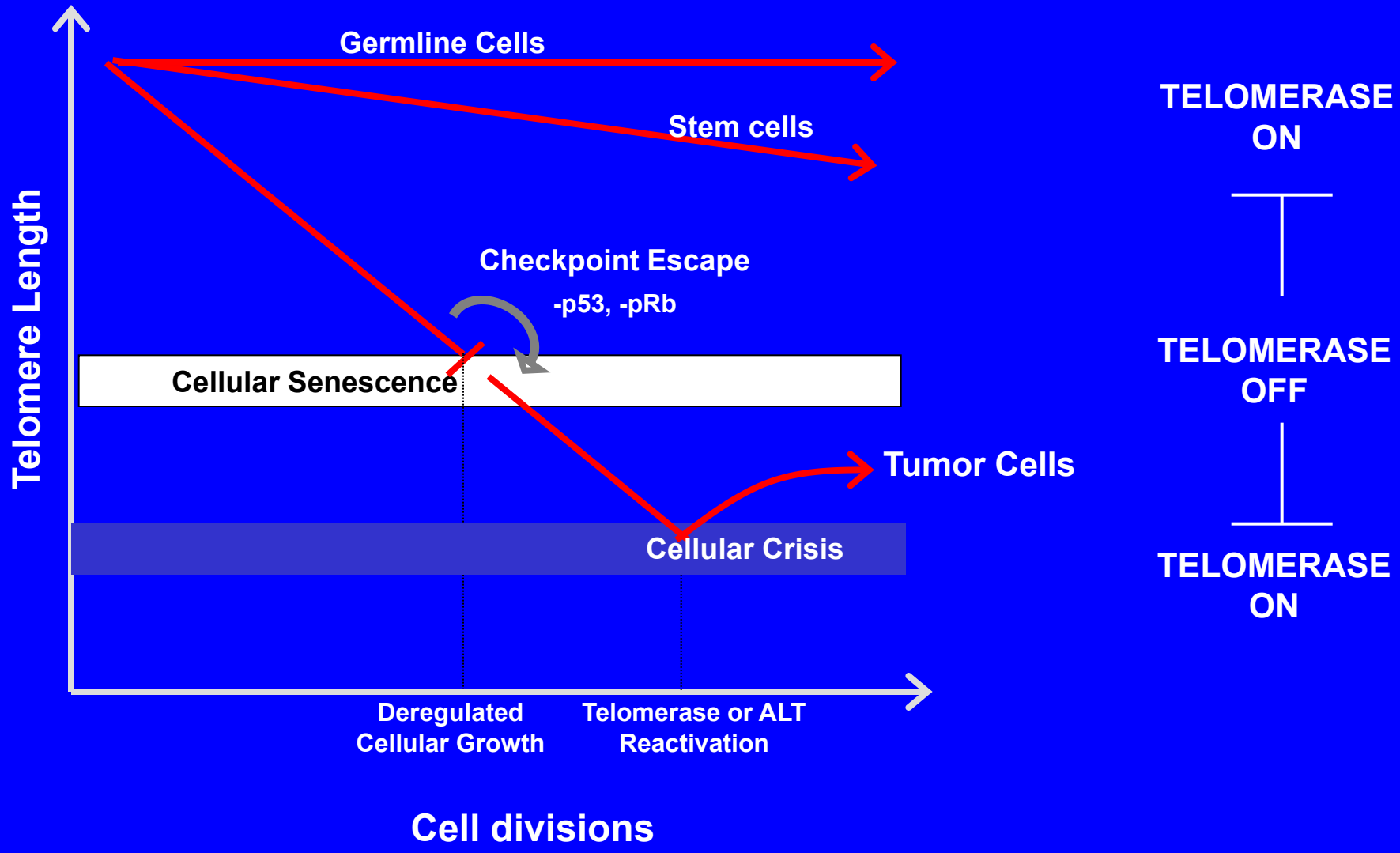
3) Translocation

DNA substrate and enzyme repositioning

4) Repeated translocation and elongation=repeat addition processivity



Telomere Hypothesis of Cellular Aging and Immortalization



Testing the telomere hypothesis of cellular aging and immortalization

- **Many studies found a CORRELATION between:**
  - **Telomere shortening and cell death or replicative senescence**
  - **Telomere length maintenance, telomerase activity and cellular immortalization**

How could you test these correlations?

Testing the telomere hypothesis of cellular aging and immortalization

- Many studies found a CORRELATION between:
  - Telomere shortening and cell death or replicative senescence
  - Telomere length maintenance, telomerase activity and cellular immortalization

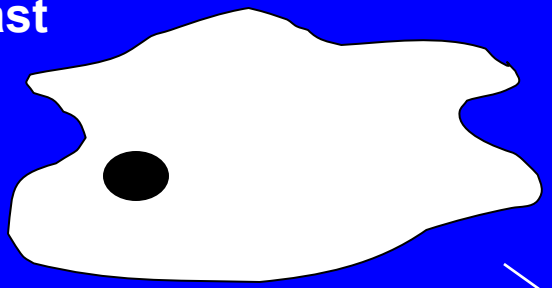
- Test the telomere hypothesis directly by manipulating telomere length via telomerase inhibition or activation

Question 1

Is telomere shortening a cell division clock that limits cellular lifespan?

Telomerase activation immortalizes normal human cells

Normal human fibroblast



hTERT

- Telomerase activity induced
- Telomere maintenance or elongation occurs
- Cells have an extended lifespan
- Cells do not have characteristics of cancer cells

Telomere shortening/senescence
"Tumour suppressor mechanism"

Bodnar et al., 1998

Telomerase activation is not sufficient for immortalization of some human cell types

e.g. express hTERT in keratinocytes and mammary epithelial cells

Result:

- cells senesce
- $p16^{INK4A}$ expression must be downregulated in these cells for immortalization to occur

Conclusion:

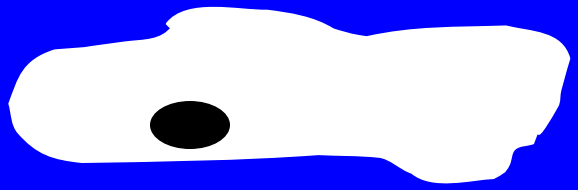
- other factors besides telomere length contribute to replicative senescence in some cell types

Question 2

Does telomerase activation transform human cells?

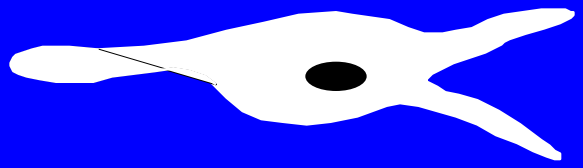
Telomerase activation is essential but not sufficient for transformation of human cells

Normal skin cells



hTERT

Alterations in other key cellular genes:
Expression of SV40LTAg (pRB, p53),
SV40 sTAg (protein phosphatase 2A)
and mutant Ras



Tumor cells

Hahn et al., 1999; 2002

Mouse models: Differences in the biology of telomeres, telomerase and replicative senescence in mice and humans

❖Telomere erosion is unlikely to be a primary tumor suppressor mechanism in rodents

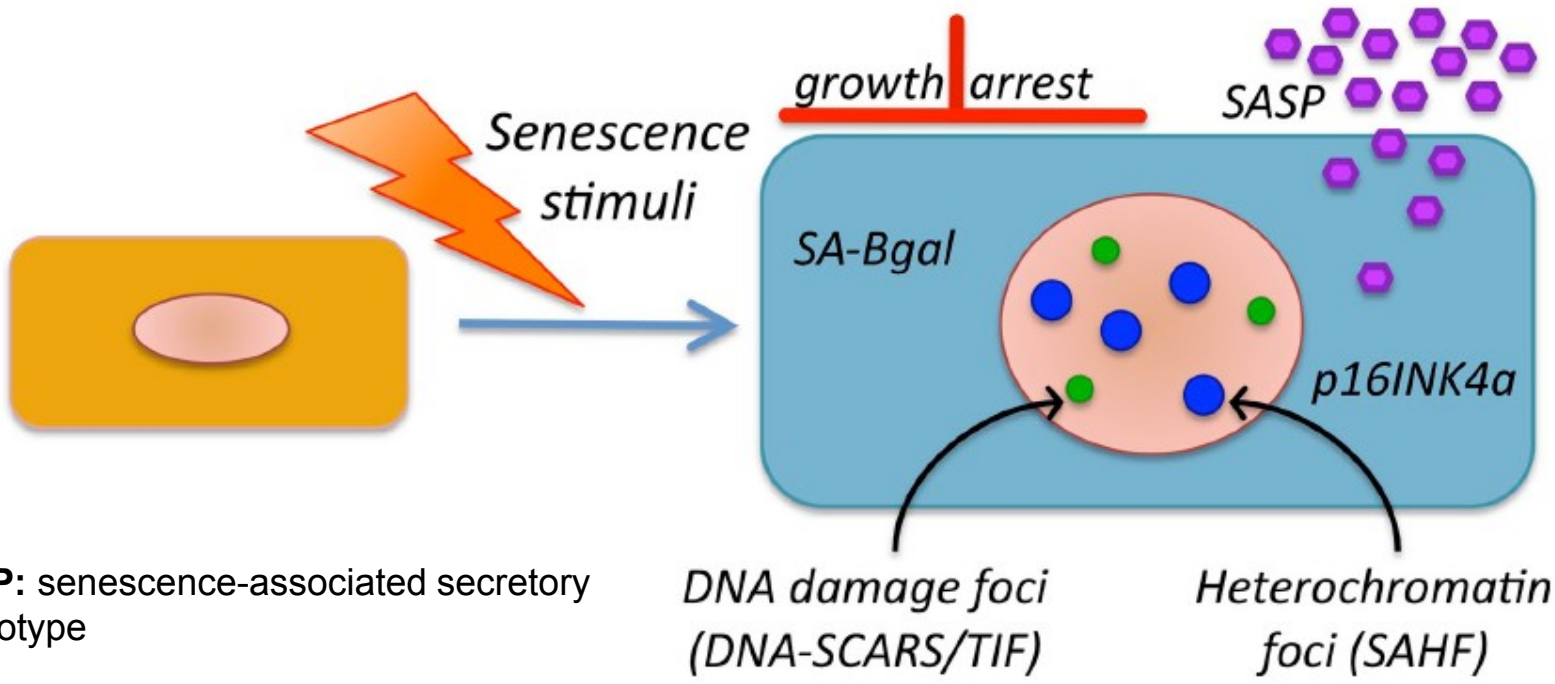
Mouse telomeres ~ 20 KB longer than human telomeres
Telomerase activity is not stringently repressed in the somatic tissues of mice

❖Replicative senescence is different in rodent and human cells

Replicative senescence occurs in rodent cells with long telomeres

Rodent cells can spontaneously immortalize in culture at detectable frequencies without the aid of oncogenes (unlike human cells)

Hallmarks of senescent cells



SASP: senescence-associated secretory phenotype

Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

What defines a senescent cell?

(i) Permanent growth arrest that can't be reversed by known physiological stimuli

(i) Cell size increase

(i) Senescence-associated β-galactosidase, partly reflects the increased lysosomal mass

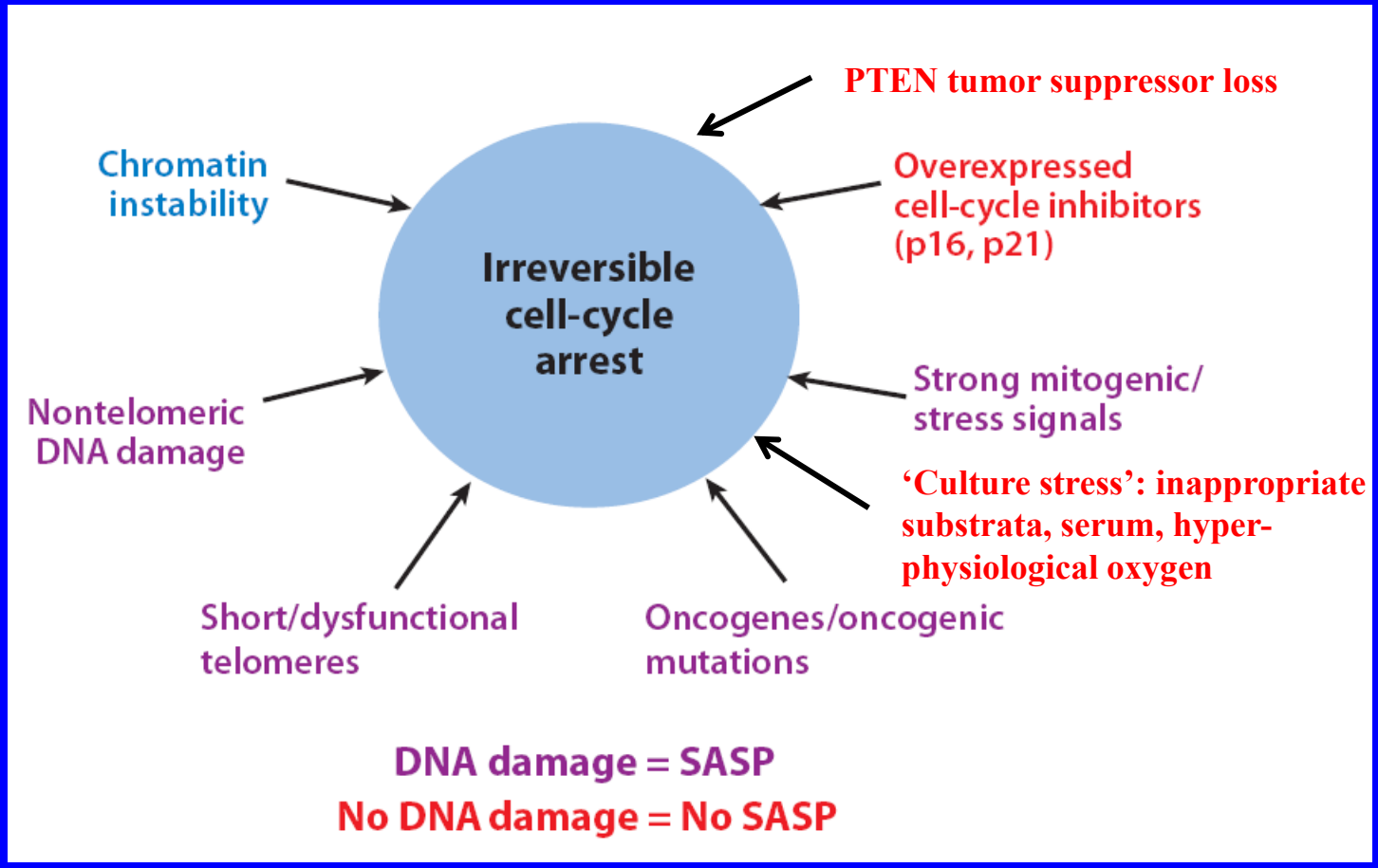
(i) p16INK4a expression causes formation of senescence-associated heterochromatin foci
- -p16INK4a expression increases with age in mice and humans
- -p16INK4a activity linked to decreased progenitor cell number in aging tissues

(v) Cells that senesce with persistent DNA damage signaling harbor persistent nuclear foci
termed DNA segments with chromatin alterations reinforcing senescence
DNA-SCARS (include TIFs-telomere dysfunction-induced foci)

(vi) Senescent cells with persistent DNA damage signaling secrete growth factors, proteases, cytokines, and other factors that have potent autocrine and paracrine activities (senescence-associated secretory phenotype:SASP)

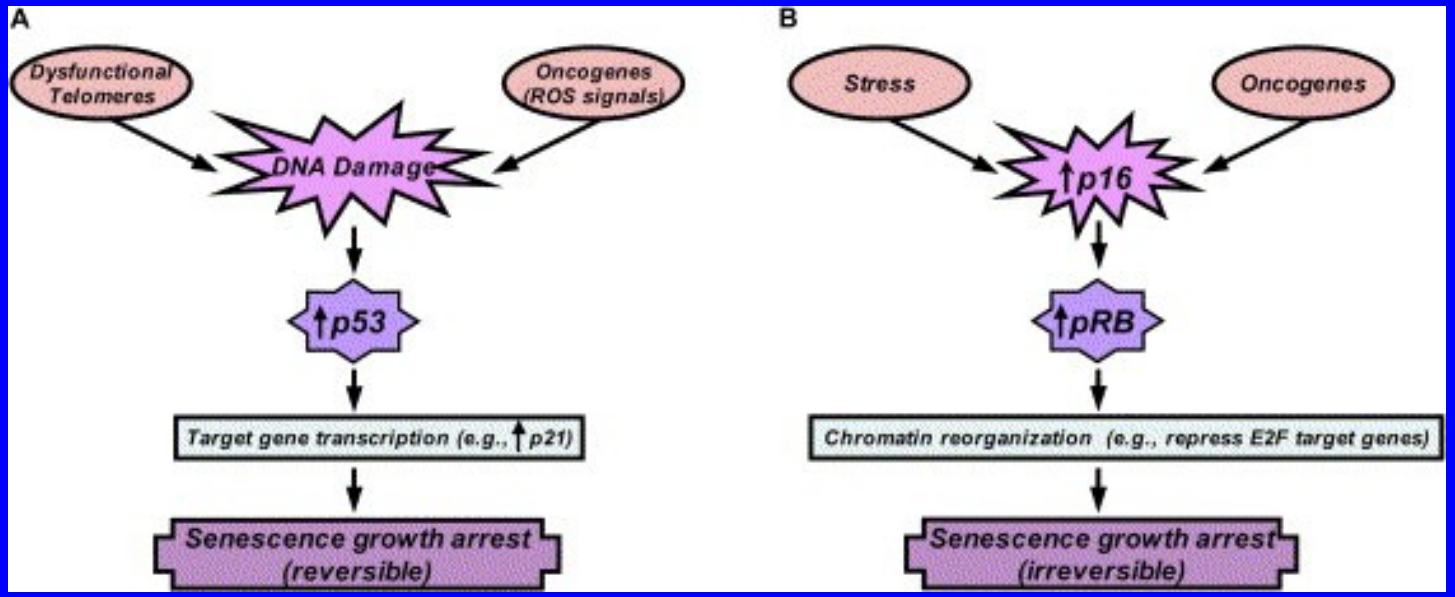
Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

Causes of cellular senescence



Coppé, J.-P. et al. 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu. Rev. Pathol. Mech. Dis. 2010. 5, 99-118.

p53 and p16/pRb Pathways in the Senescence Response



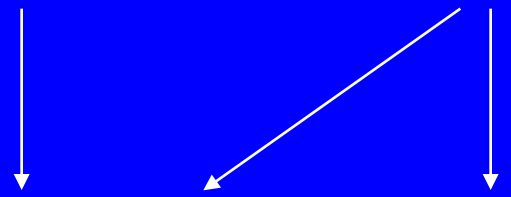
By inactivation of p53, but not by physiological mitogens

Campisi, J. 2005. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120, 513-522.

Tumor Suppressors

Caretaker tumor suppressors prevent cancer by protecting the genome from mutation

Gatekeeper tumor suppressors, prevent cancer by acting on intact cells through the induction of apoptosis or cellular senescence



Deplete nonrenewable/renewable tissues of proliferating or stem cell pools

Dysfunctional senescent cells may actively disrupt normal tissues as they accumulate

Gatekeeper tumor suppressors may be antagonistically pleitropic, beneficial early in life by suppressing cancer but detrimental later in life by compromising tissue function

Campisi, J. 2005. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120, 513-522.

Cellular Senescence as a Tumor Suppressor

- Senescent markers accumulate in premalignant cells but not in the cancers that can develop from these cells
- Tumor progression can be inhibited by senescence
- Some tumor cells retain the ability to senesce and regress (e.g. upon p53 reactivation or inactivation of apoptosis)
- Imposes a cell-autonomous block to the proliferation of oncogenically damaged/stressed cells

Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

Cellular Senescence as a Tumor Suppressor

Short Telomeres Limit Tumor Progression In Vivo by Inducing Senescence

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DOI 10.1016/j.ccr.2007.02.026

Restoration of p53 function leads to tumour regression *in vivo*

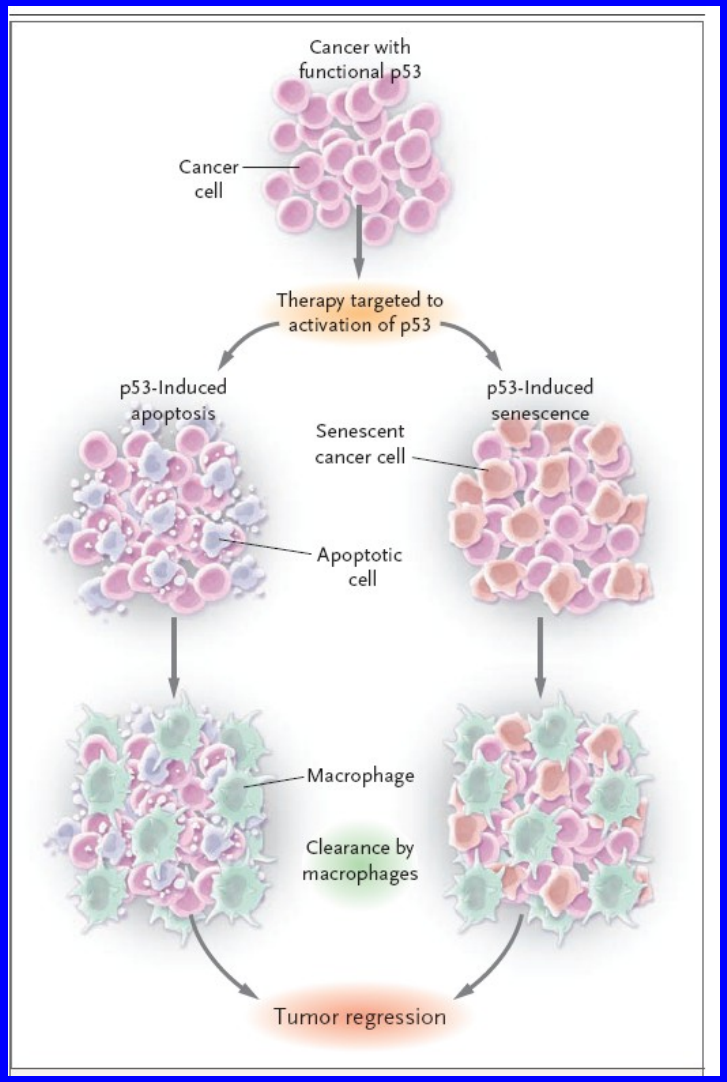
Andrea Ventura[1*], David G. Kirsch[1,2*], Margaret E. McLaughlin[1], David A. Tuveson[1], Jan Grimm[3], Laura Lintault[1], Jamie Newman[1], Elizabeth E. Reczek[1], Ralph Weissleder[3] & Tyler Jacks[1,4]

Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas

Wen Xue[1*], Lars Zender[1*], Cornelius Miething[1], Ross A. Dickins[1,2], Eva Hernando[3], Valery Krizhanovsky[1], Carlos Cordon-Cardo[3] & Scott W. Lowe[1,2]

Telomere dysfunction suppresses spontaneous tumorigenesis *in vivo* by initiating p53-dependent cellular senescence

Wilfredo Cosme-Blanco[1], Mei-Feng Shen[1], Alexander J.F. Lazar[2], Sen Pathak[1], Guillermina Lozano[1], Asha S. Multani[1] & Sandy Chang[1,3,+]



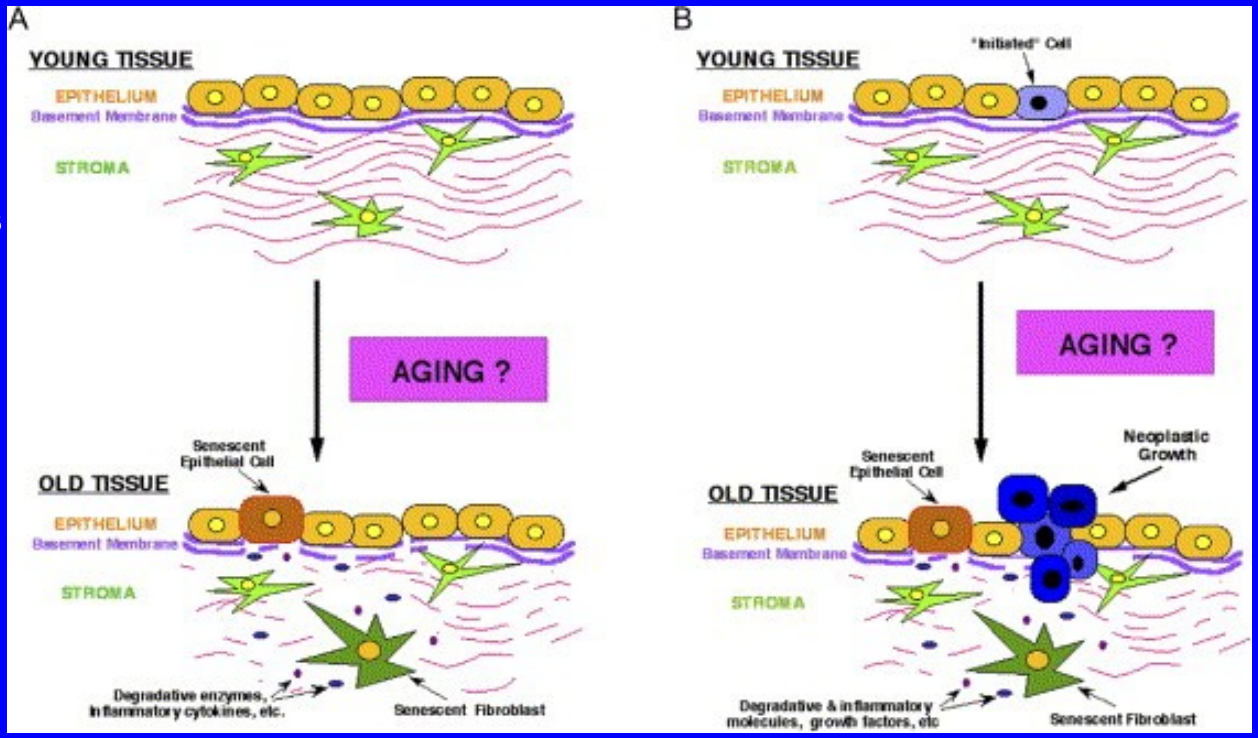
Serrano, M. 2007. Cancer regression by senescence. NEJM 356, 1996-1997.

Cellular Senescence and Aging

Extensive evidence that senescent cells (as defined by high levels of p16 and SA-β-gal) accumulate with age in multiple tissues from both human and rodents; present at sites of age-related pathologies.

Fibroblasts maintain the stromal support for virtually all renewable epithelial tissues

Stimulate chronic tissue remodeling and/or local inflammation



Stimulate the proliferation of cells that harbor pre-neoplastic mutations

Campisi, J. 2005. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120, 513-522.

Cellular Senescence and tumor promotion

- Senescent cells increase with age

- SASP factors
  stimulate the proliferation of premalignant epithelial cells (growth related oncogene, IL-6, IL-8)
  stimulate endothelial cell migration (VEGF)
  facilitate tumor cell invasiveness (matrix metalloproteinases)

- In xenografts, senescent cells can promote malignant progression of precancerous and established cancer cells

Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

Cellular Senescence and Aging

Constitutive expression of artificially ($p53^{+/m}$) or naturally truncated p53 (p44 isoform) in mice leads to p53 activation

- **Cancer-free**
- **Shortened life span and premature aging (can extend lifespan depending on physiological context-discussed later)**
- **Tissues accumulated senescent cells**

Table 2 Ageing-related phenotypes in $p53^{+/+}$, $p53^{+/m}$ and pL53 mice

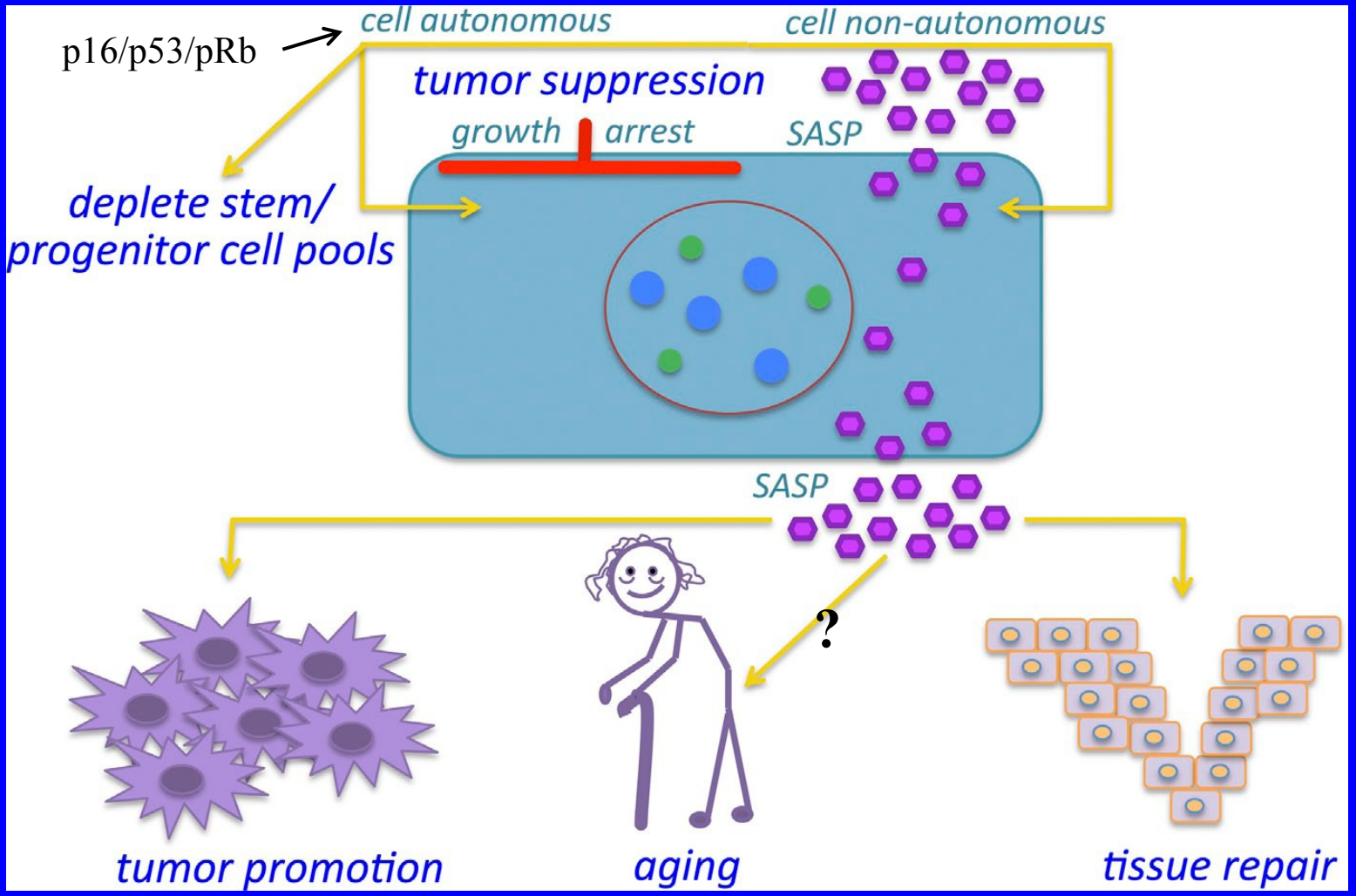
Phenotype	$p53^{+/+}$	$p53^{+/m}$	pL53
Median lifespan	118 weeks	96 weeks	ND
Maximum lifespan	164 weeks	136 weeks	ND
Cancer incidence	>45%	<6%	20% (18 months)
Body weight	Reduced at 30 months	Reduced at 18 months	Slightly reduced
Liver, spleen, kidney mass	Minimal loss of mass	25–40% reduced at 24 months	Minimal loss
Lymphoid atrophy	Moderate	Pronounced	Moderate
Lordokyphosis	Modest	Pronounced	Pronounced
Osteoporosis	Minimal	Pronounced	Pronounced
Blood chemistry	Normal	Normal	Normal
Urinalysis	Normal	Normal	ND
Peripheral WBC, RBC	Normal	Normal	Normal
Hair-greying and alopecia	Minimal	Minimal	Some alopecia
Hair regrowth	Modestly reduced	Greatly reduced	Greatly reduced
Dermal thickness	Moderately reduced	Greatly reduced	Moderately reduced
Subcutaneous adipose	Moderately reduced	Greatly reduced	Greatly reduced
Wound-healing	Normal	Retarded	Retarded
Muscle atrophy	Minimal	Pronounced	Minimal
Anaesthetic stress tolerance	Well tolerated	Poorly tolerated	Poorly tolerated
5-FU myeloablation	Robust WBC replenishment	Reduced WBC replenishment	ND

Phenotypes of $p53^{+/+}$ and $p53^{+/m}$ mice were assessed at 24 months of age; phenotypes of pL53 mice were assessed at 16–20 months of age. ND, not done; WBC, white blood cell; RBC, red blood cell.

mutant p53 transgenic (pL53) mice containing roughly 20 copies of a mutation at codon 135

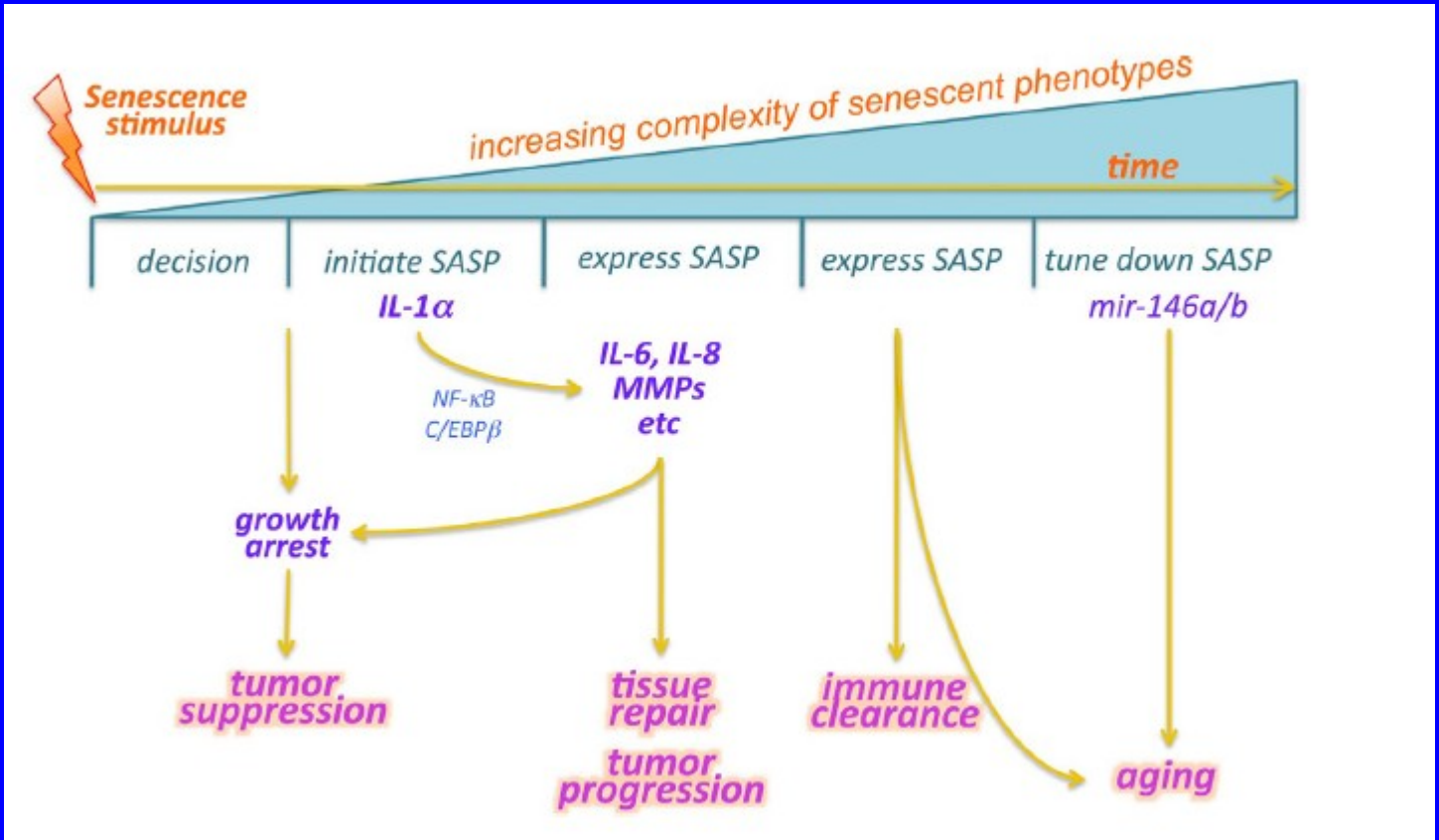
Tyner, S.D. et al. 2002. p53 mutant mice that display early ageing-associated phenotypes. Nature 415, 45-53.

Biological activities of cellular senescence



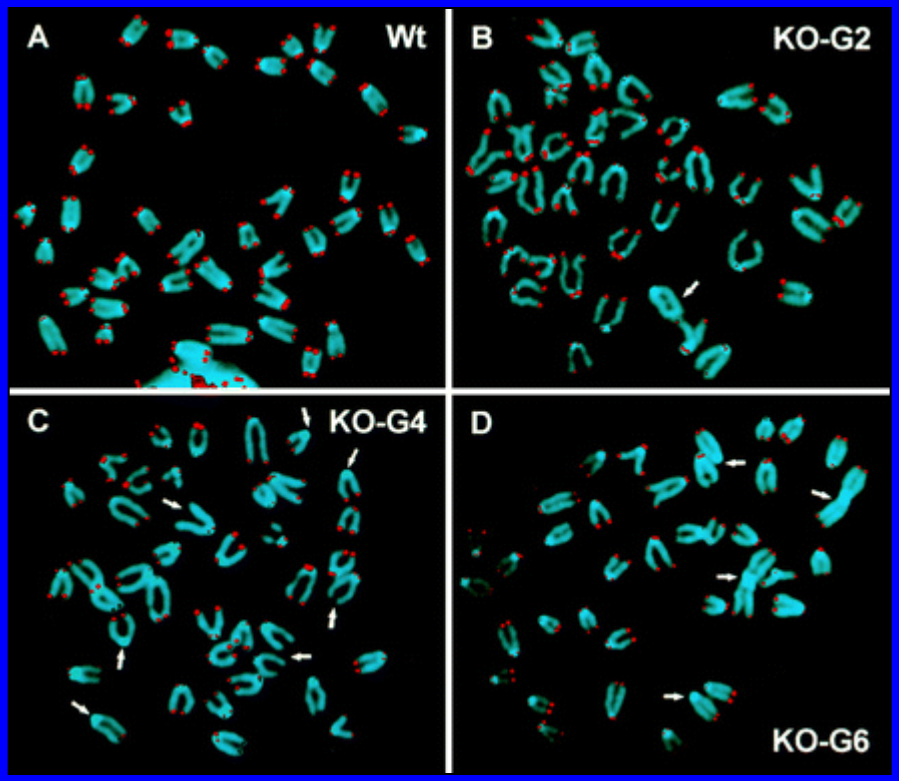
Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

Four Faces of Cellular Senescence



Rodier, F. and Campisi, J. 2011. Four faces of cellular senescence. JCB 192, 547-556

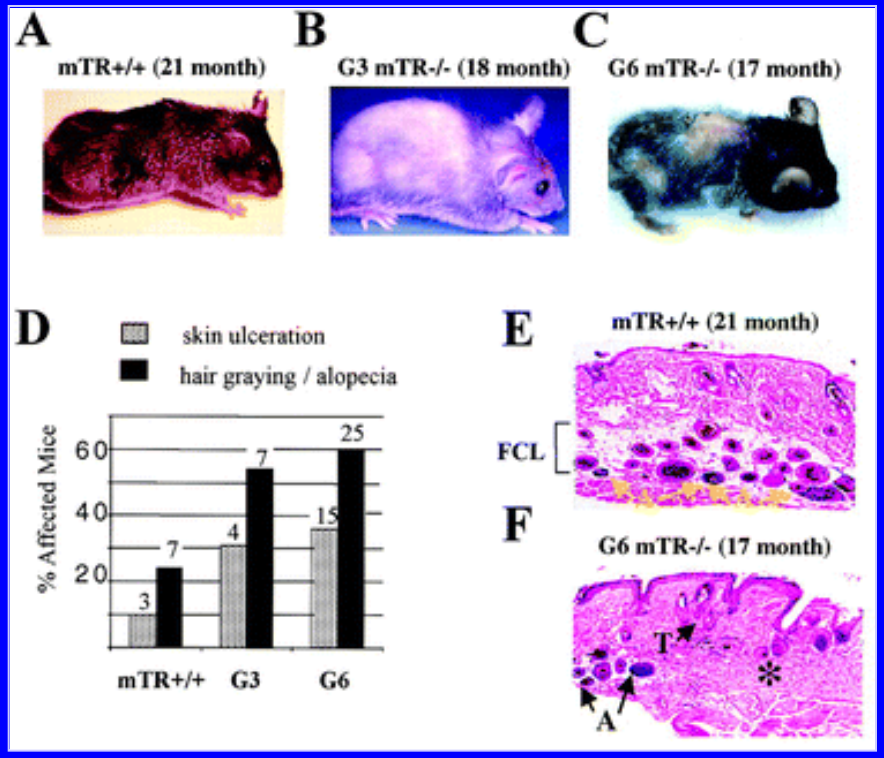
mTR knockout mouse: model for aging?

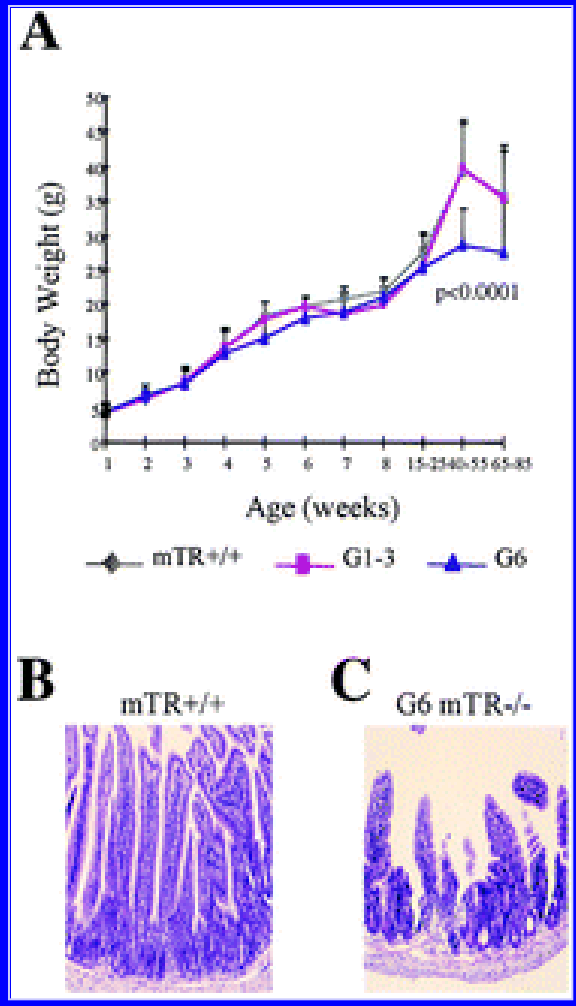


Progressive telomere shortening over successive generations

Blasco et al., 1997

mTR knockout mouse phenotypes

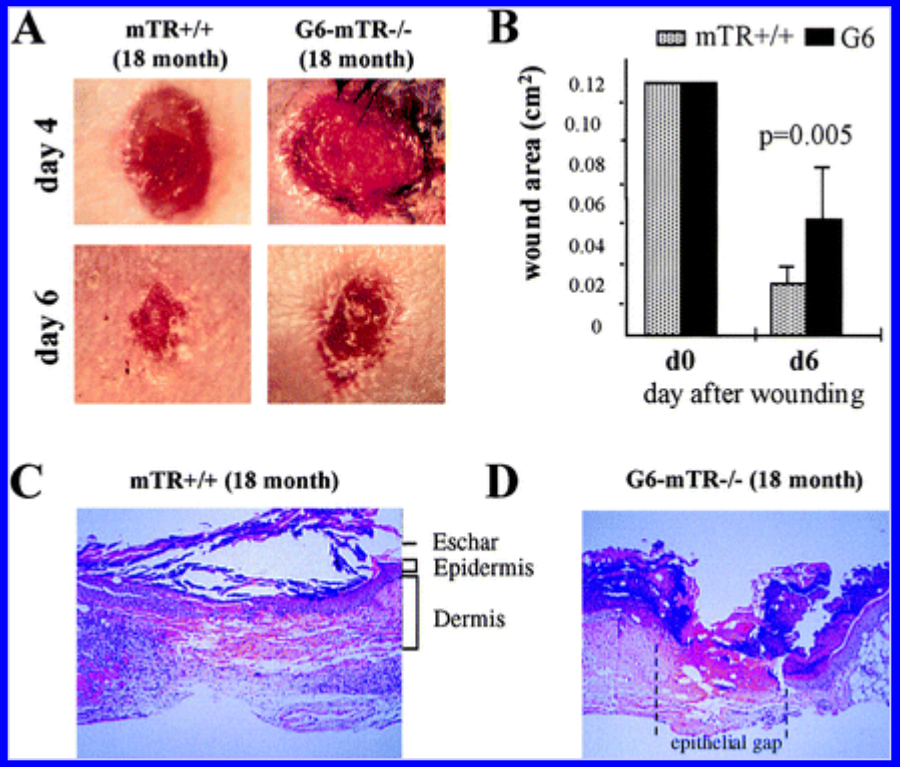


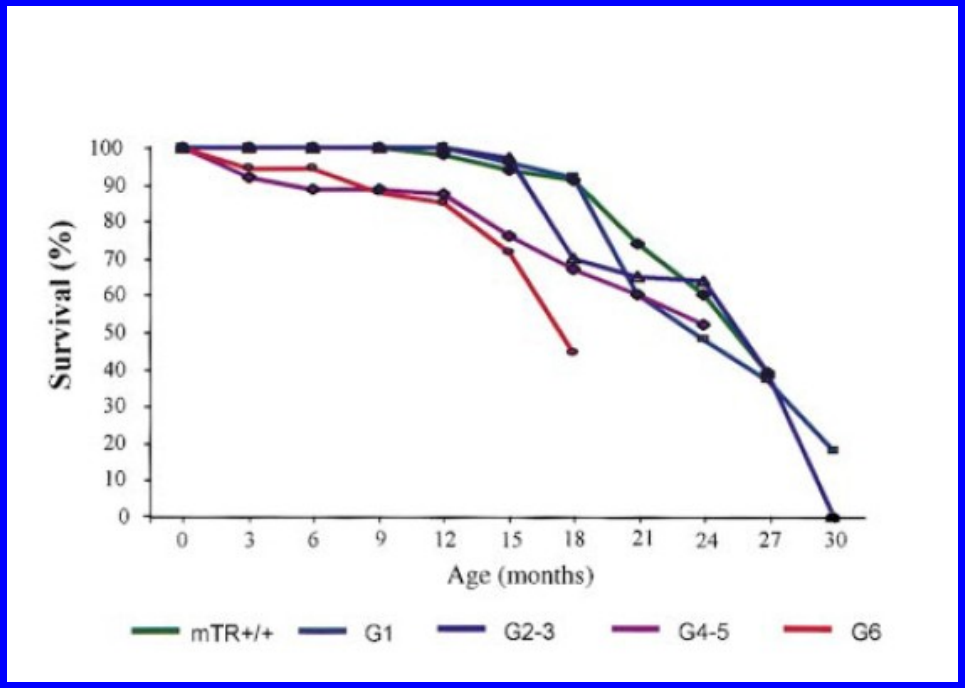


- Hair graying, hair loss
- Decreased skin thickness
- Reduced body weight in old age
- Atrophied intestinal villi

Rudolph et al., 1999

mTR knockout mouse phenotypes

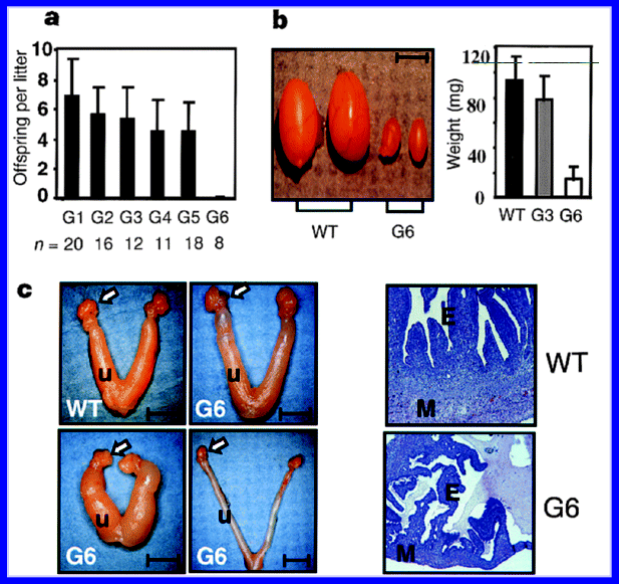


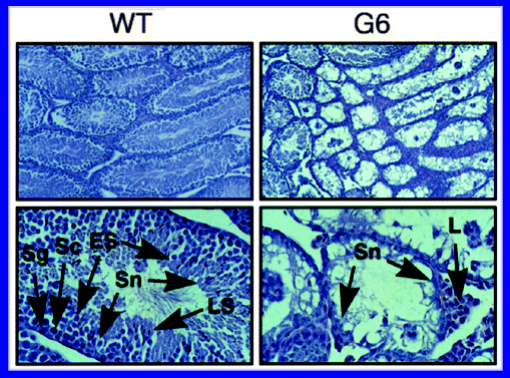


- Delayed wound healing
- Reduced regenerative capacity
- Decreased peripheral white blood cells and haemoglobin
- Reduced longevity

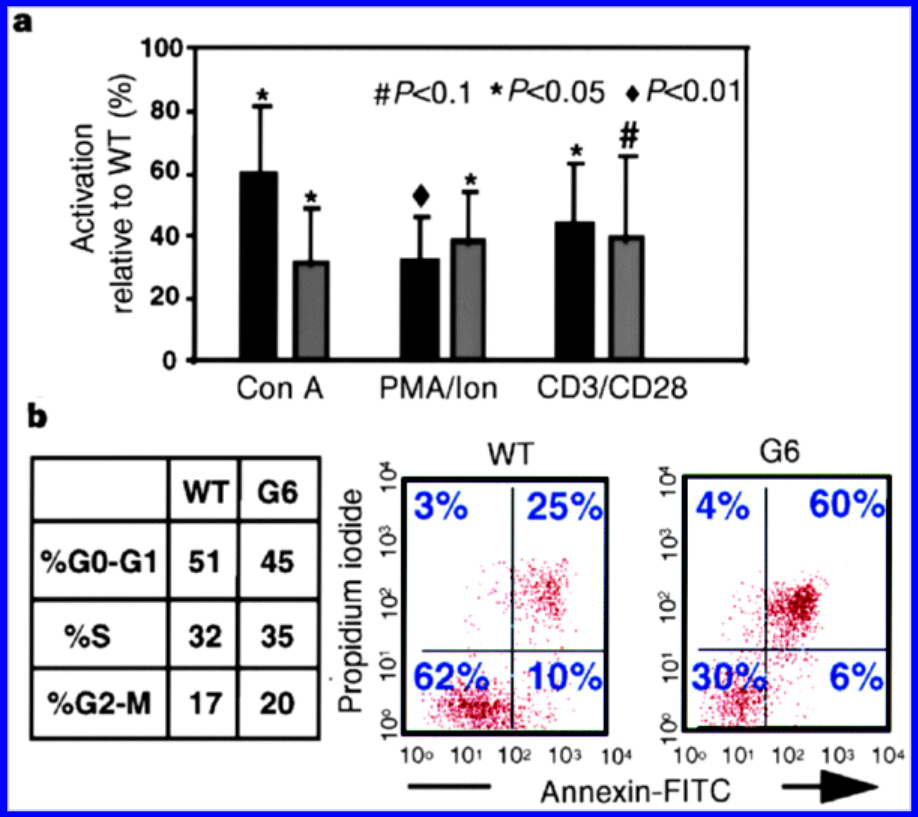
Rudolph et al., 1999

mTR knockout mouse phenotypes





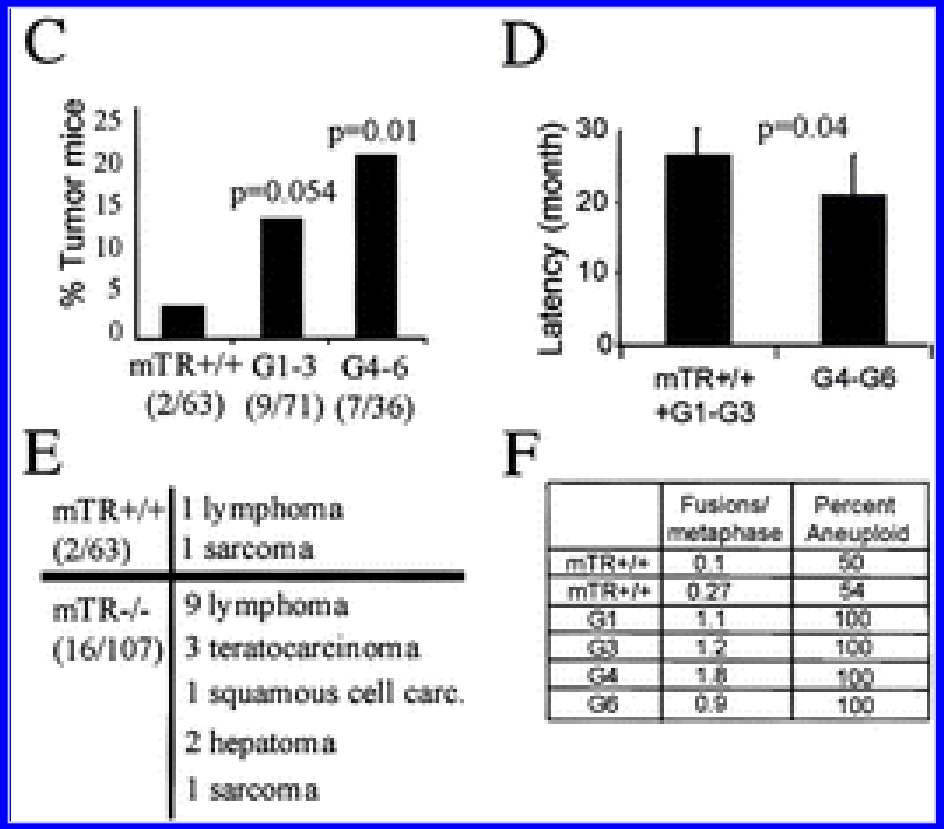
Seminiferous tubules



- Reduction in size of reproductive organs
- Reduced cellularity in seminiferous tubules
- Decreased proliferation of splenocytes following mitogenic stimulation

Lee et al., 1998

mTR knockout mouse phenotypes



	Fusions/ metaphase	Percent Aneuploid
mTR+/+	0.1	50
mTR+/+	0.27	54
G1	1.1	100
G3	1.2	100
G4	1.8	100
G6	0.9	100

Moderate increased incidence of spontaneous tumors in highly proliferative epithelial cell types lymphomas and teratocarcinomas typically much less frequent in mice

Rudolph et al., 1999

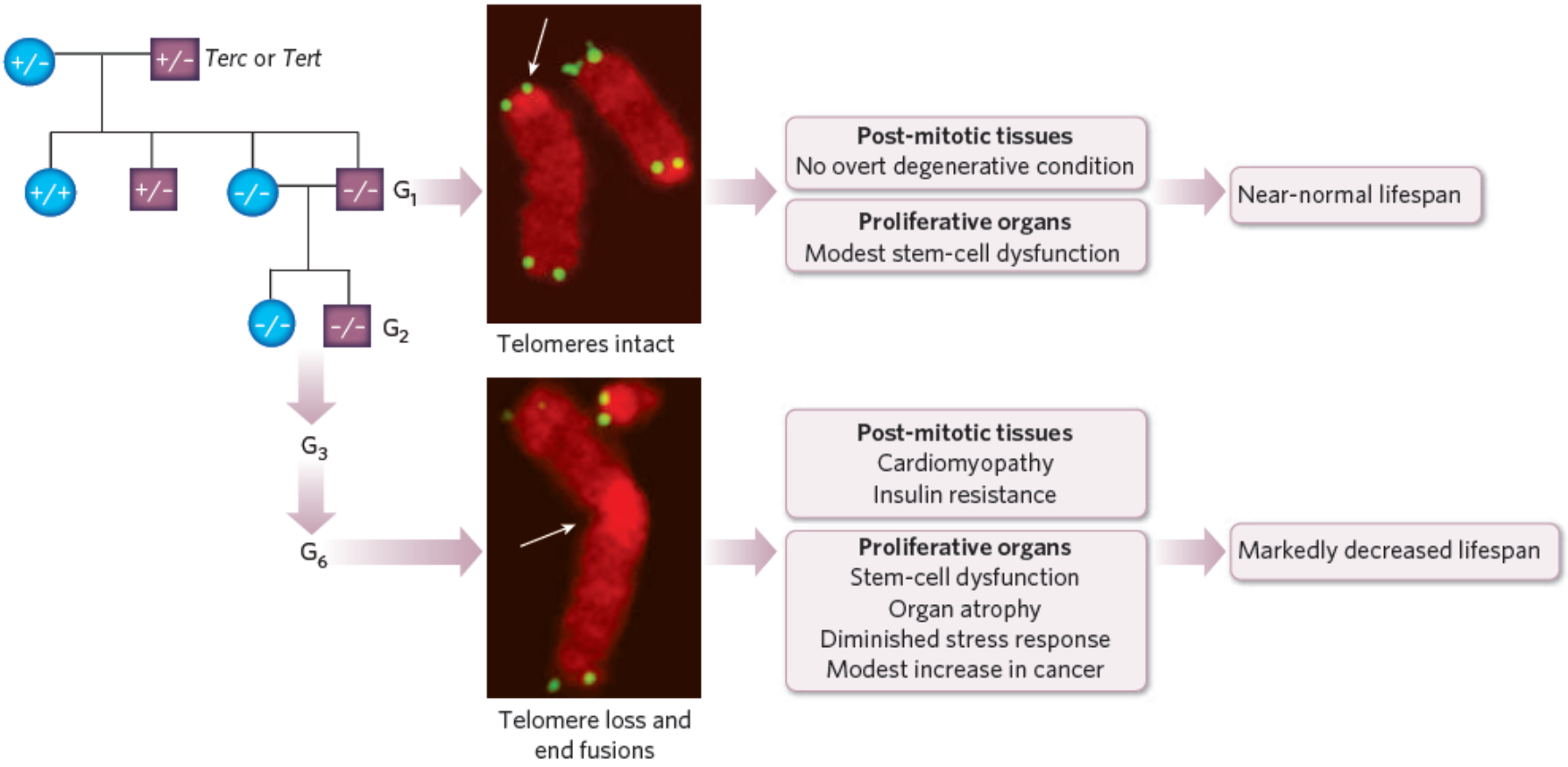
Summary of phenotypes of mTR$^{-/-}$ mice

Table 1. Summary of Phenotypic Analysis in Aging Mice

	mTR$^{+/+}$	G3 mTR$^{-/-}$	G6 mTR$^{-/-}$
Body weight	Normal	Normal	20%–25% decreased in >6-month-old mice
Diabetes	Normal GTT	Normal GTT	Normal GTT
Osteoporosis	Normal X-ray	Normal X-ray	Normal X-ray
Artherosclerosis	None	None	None
Peripheral RBC & WBC counts	Normal	Normal	Normal
Blood chemistry	Normal profile	Normal profile	Normal profile
Cataract	15%	20%	10%
Male fecundity	12–15 months	6.5 months	Normally infertile, rarely successful in generating offspring
Hair graying and alopecia	25%	54%	60%
Skin histology	Normal	Decrease of hair follicles in anagen, increase in telogen	Decrease of hair follicles in anagen, increase in telogen, loss of subcutaneous fat
Ulcerative skin lesions	10%	31%	37%
Wound healing	Normal	Delayed reepithelilization	Delayed reepithelialization
Cancer incidence	3.3%	13%	19%
Life span (50% mortality mark)	24 months	24 months	18 months

Rudolph et al., 1999

Dysfunctional telomeres and premature aging



Sahin, E. And DePinho, R.A. 2010. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature, 464, 520-528.

Conclusion

Late generation mTR knockout mice exhibit a phenotype similar to some features of human aging

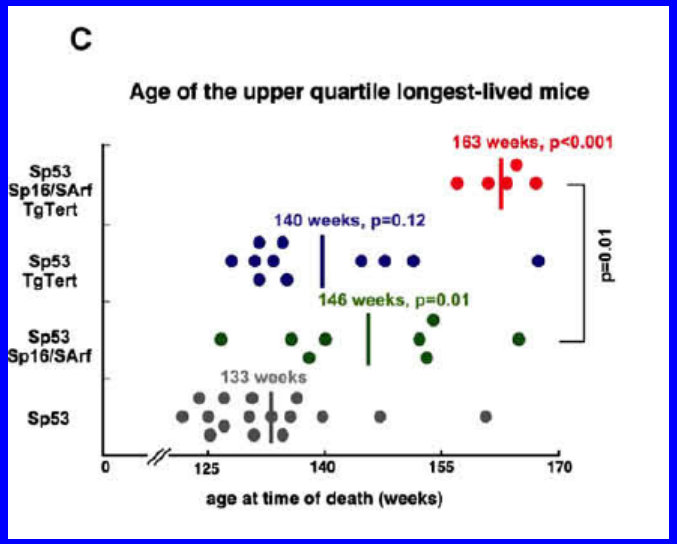
Can telomerase overexpression extend lifespan?

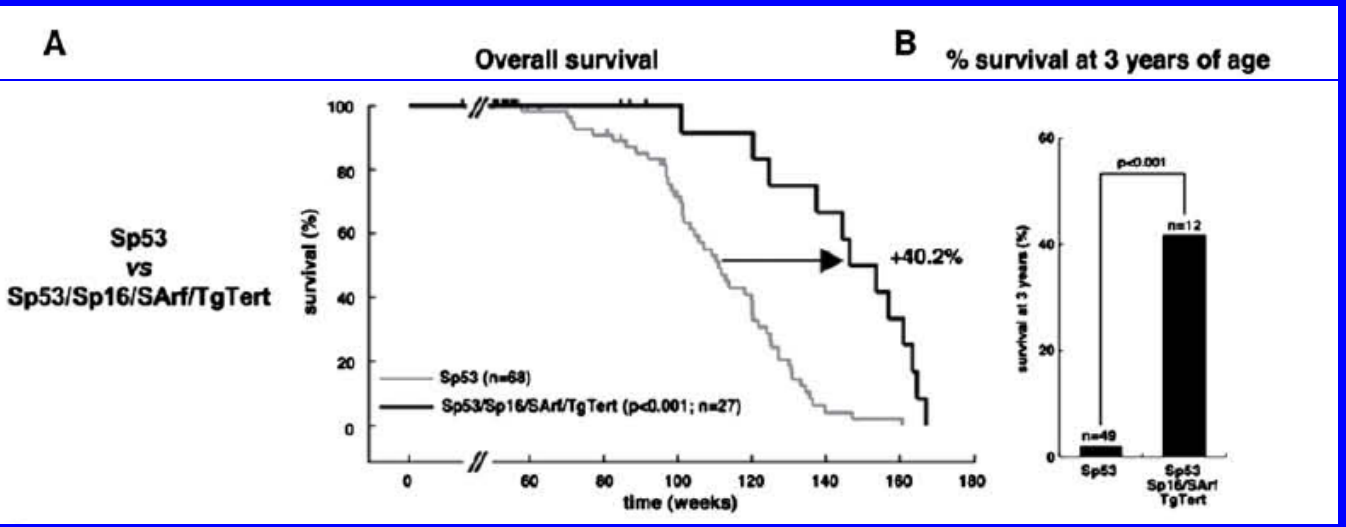
Telomerase Reverse Transcriptase Delays Aging in Cancer-Resistant Mice

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,*]

In mice with enhanced expression of p53, p16 and p19ARF

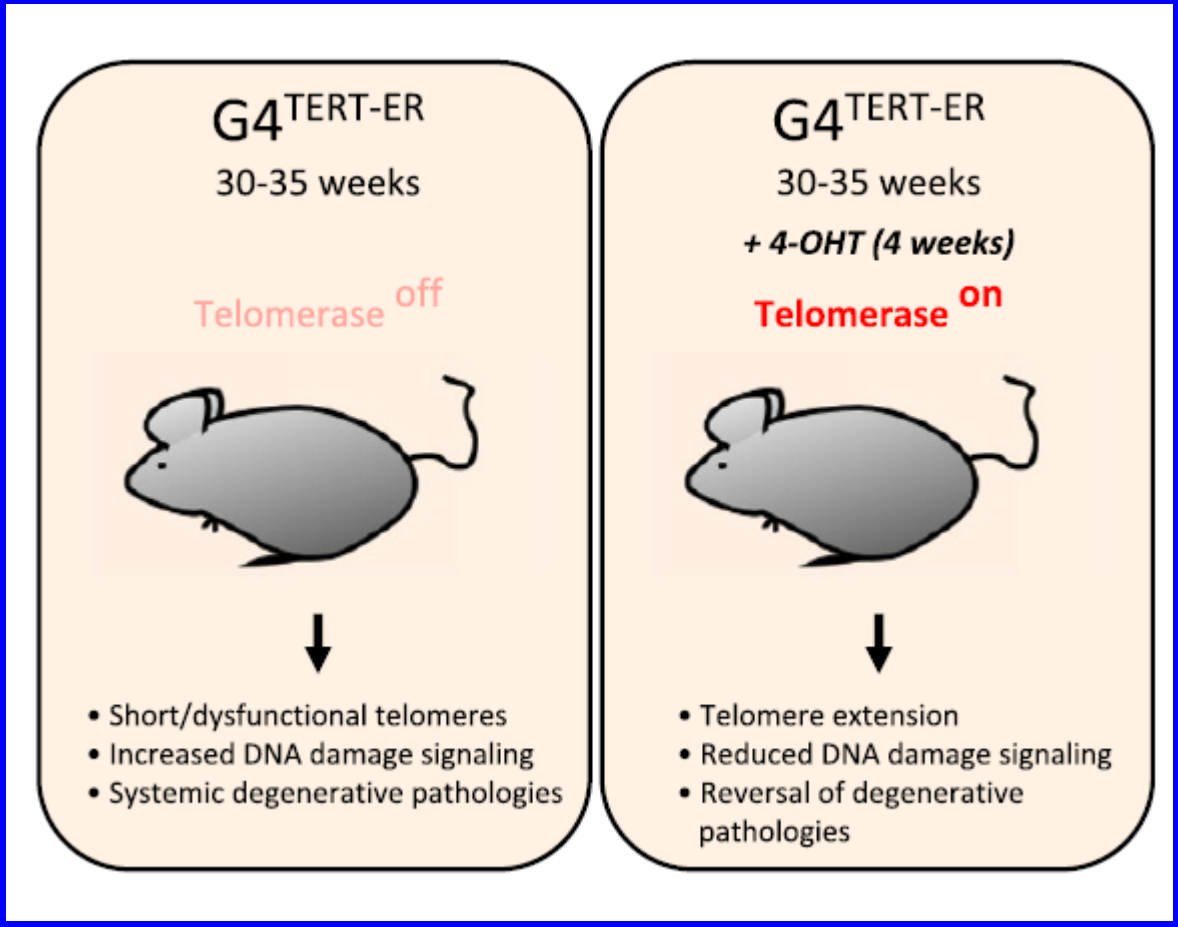
- Improved GI tract epithelial barrier function
- Decreased biomarkers of aging
- Decreased molecular markers of aging
- Increased median life span and longevity
- Delayed telomere loss





Aging by Telomere loss can be reversed!

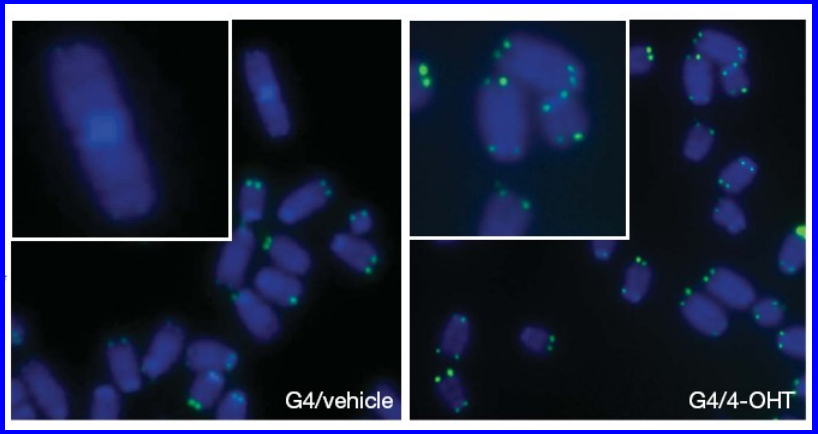
Telomerase reactivation in adult mice after establishment of telomere-induced aging
Use of a knock-in allele encoding a tamoxifen responsive TERT under control of endogenous promoter

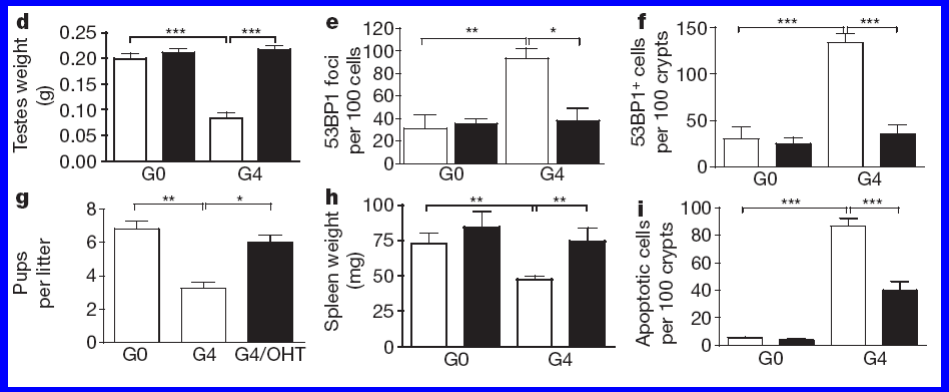


Jaskelioff, M. et al. 2011. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature 469, 102-106.

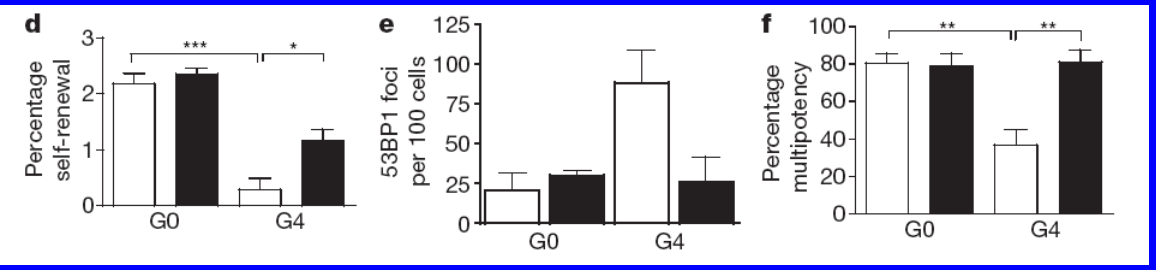
Reversal of Degenerative Pathologies

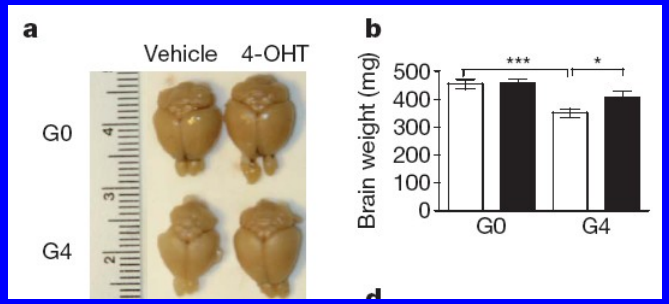
Telomere function

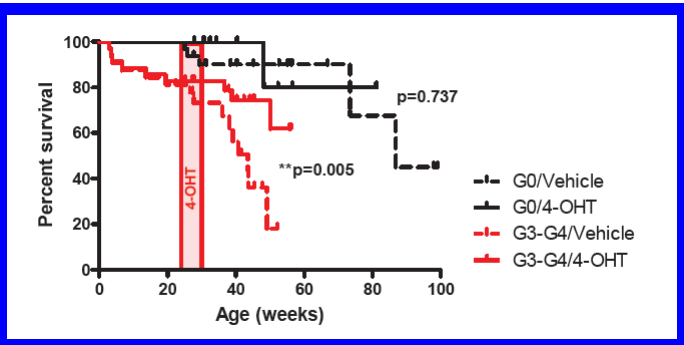


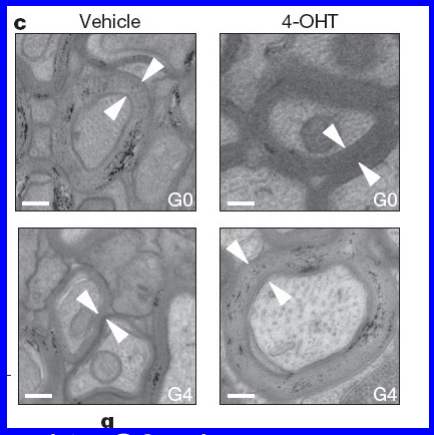


Neural stem cell function









Ameliorate decreased survival of TERT-ER mice but lifespan not extended compared to G0 mice

The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence

Bruno Bernardes de Jesus,[1] Kerstin Schneeberger,[1] Elsa Vera,[1,2] Agueda Tejera,[1] Calvin B. Harley[3] and Maria A. Blasco[1]

Summary

Here, we show that a small-molecule activator of telomerase (TA-65) purified from the root of *Astragalus membranaceus* is capable of increasing average telomere length and decreasing the percentage of critically short telomeres and of DNA damage in haploinsufficient mouse embryonic fibroblasts (MEFs) that harbor critically short telomeres and a single copy of the telomerase RNA *Terc* gene (G3 $Terc^{+/-}$ MEFs). Importantly, TA-65 does not cause telomere elongation or rescue DNA damage in similarly treated telomerase-deficient G3 $Terc^{-/-}$ littermate MEFs. These results indicate that TA-65 treatment results in telomerase-dependent elongation of short telomeres and rescue of associated DNA damage, thus demonstrating that TA-65 mechanism of action is through the telomerase pathway. In addition, we demonstrate that TA-65 is capable of increasing mouse telomerase reverse transcriptase levels in some mouse tissues and elongating critically short telomeres when supplemented as part of a standard diet in mice. Finally, TA-65 dietary supplementation in female mice leads to an improvement of certain health-span indicators including glucose tolerance, osteoporosis and skin fitness, without significantly increasing global cancer incidence.