Role of telomere function in aging and cancer

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Conflict of interest

A scientific advisor of Life Length.

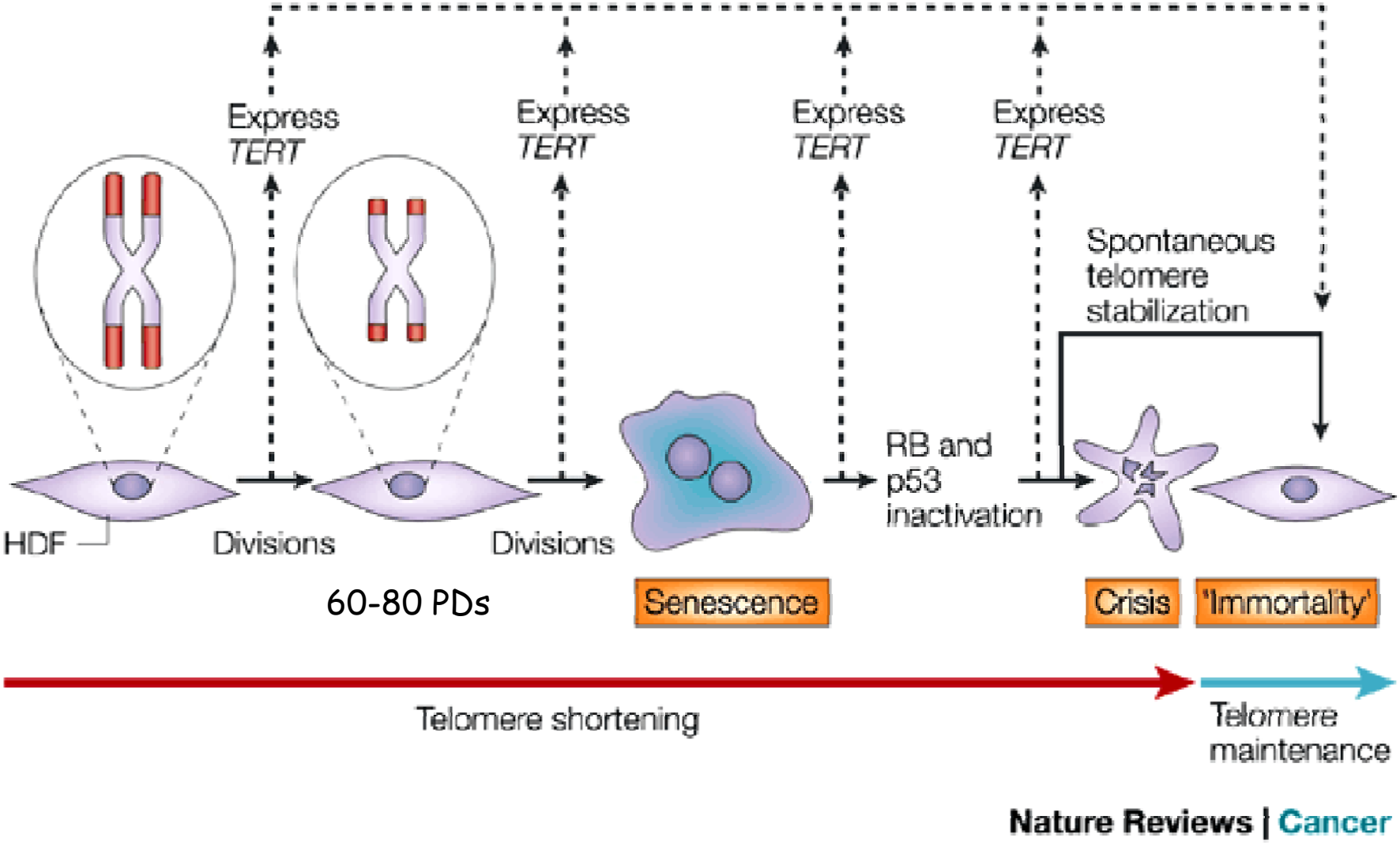
What is Aging?

- Aging is associated with the gradual decline in performance and reserve capacity in organ systems.

- Aging of organ systems is often associated with a decrease in the number and/or function of cells.

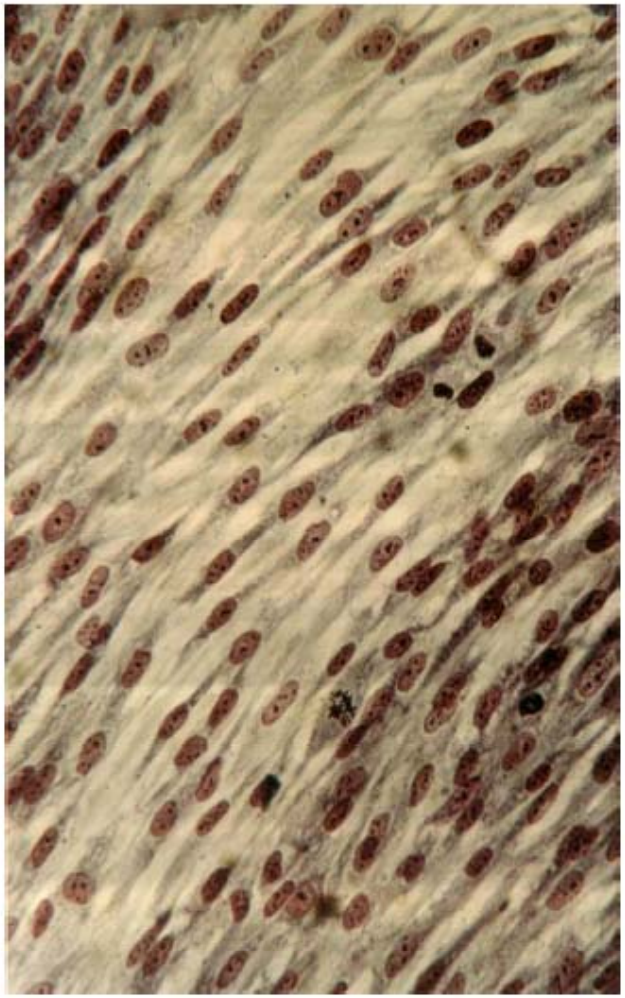
- Old cells do not maintain and repair tissues as well as young cells, leading to decreased overall vitality.

Normal human fibroblasts have a set, finite replicative lifespan



Understanding cellular aging

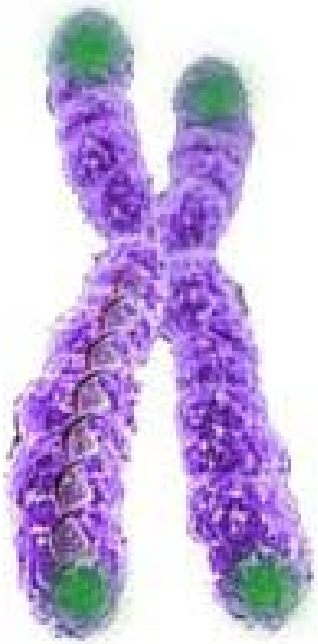
Early passage

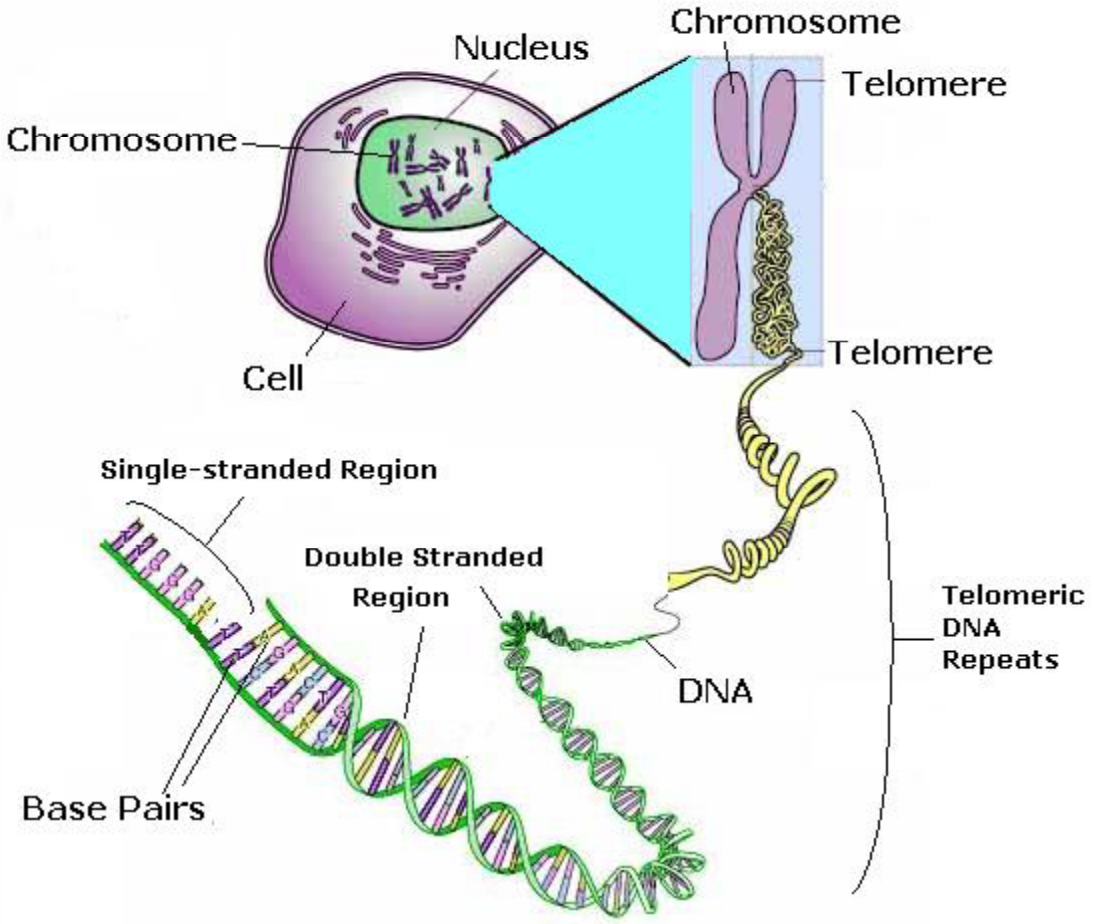


Late passage

Figures from original Hayflick paper

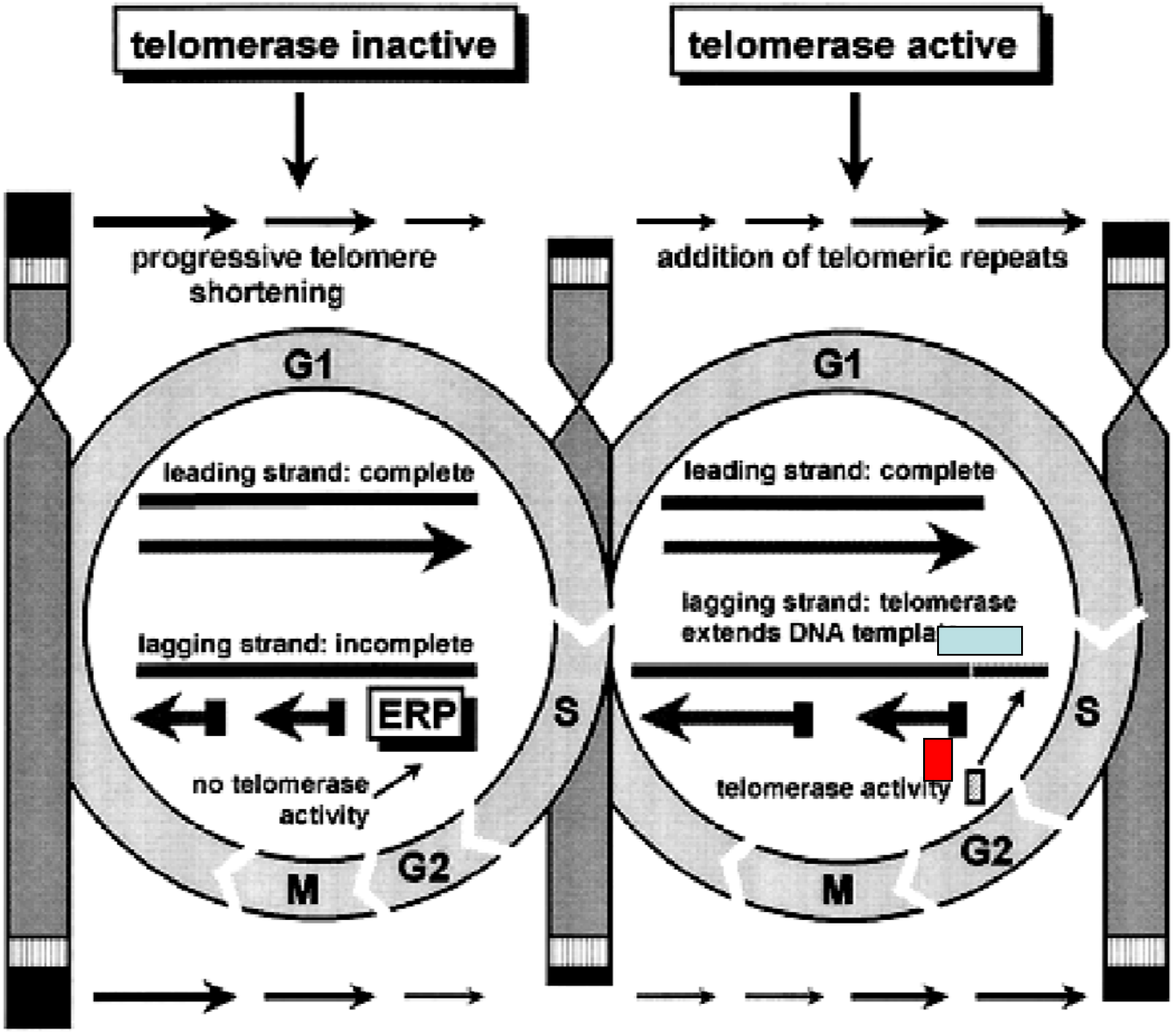
Telomeres cap the ends of all mammalian chromosomes



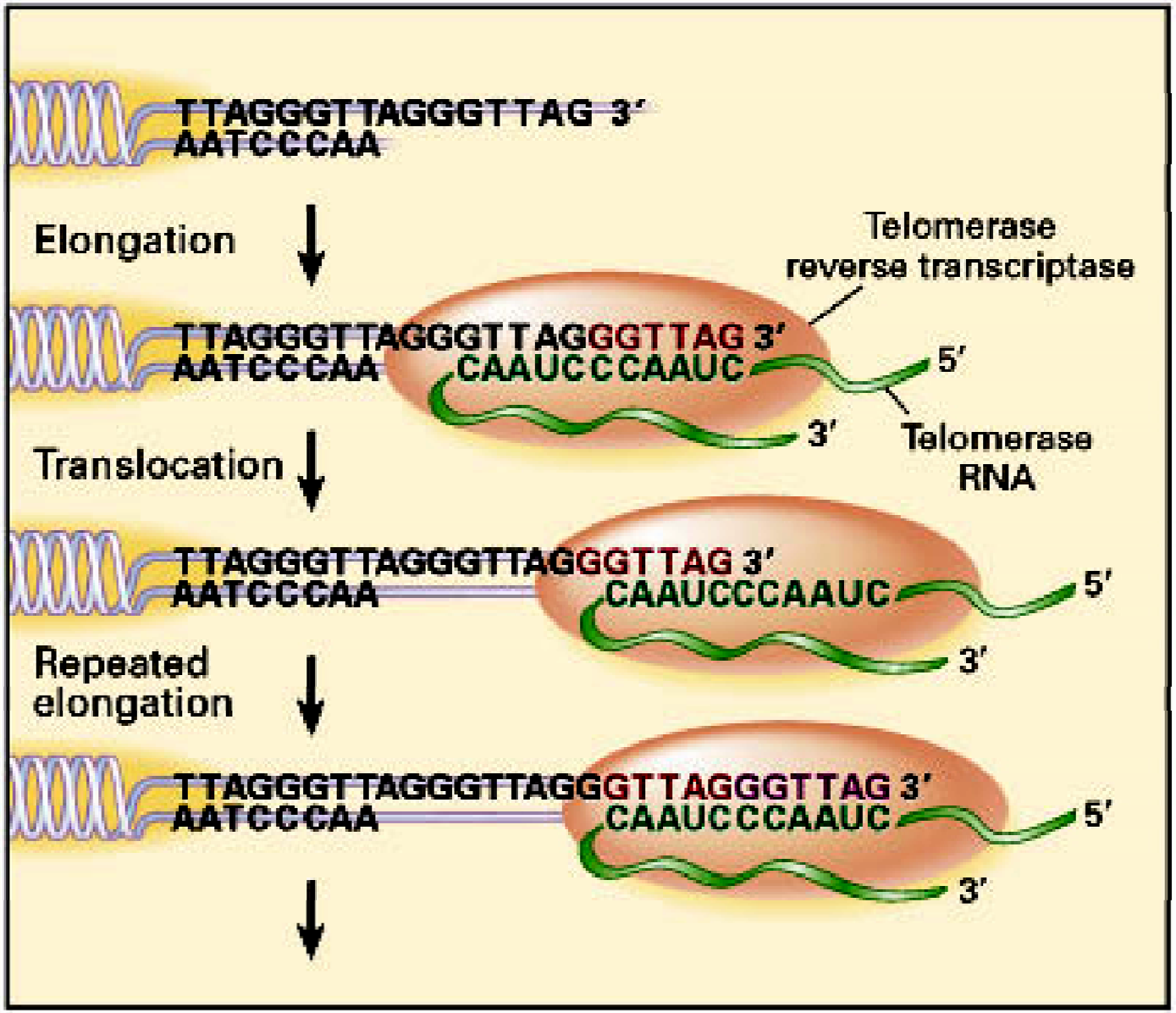


Most normal human somatic cells

Human germ and stem cells



Telomeres are maintained by the enzyme telomerase



Lasker Award for Basic Science 2006
Nobel Prize in Medicine 2009

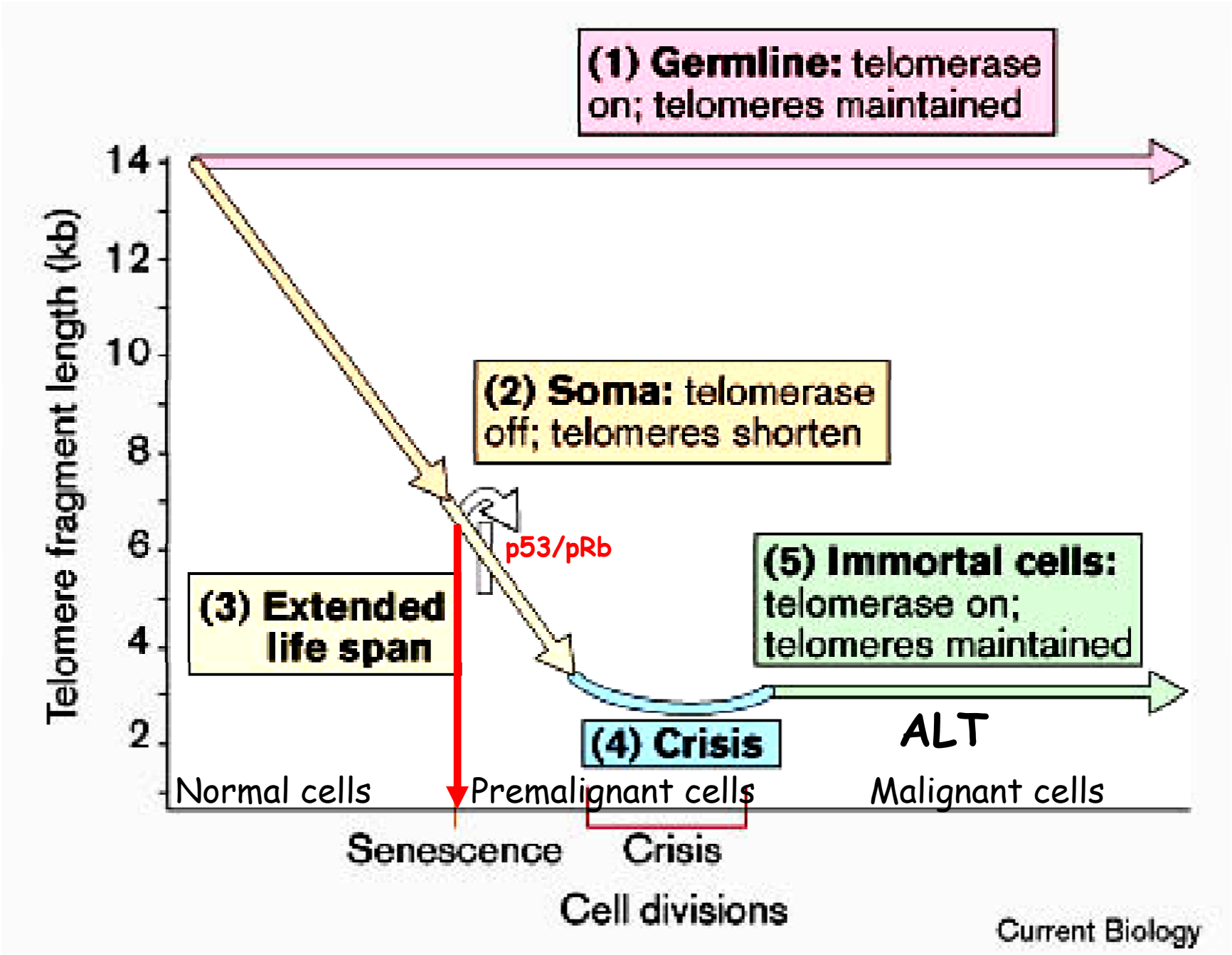
Elizabeth Blackburn

Jack Szostak

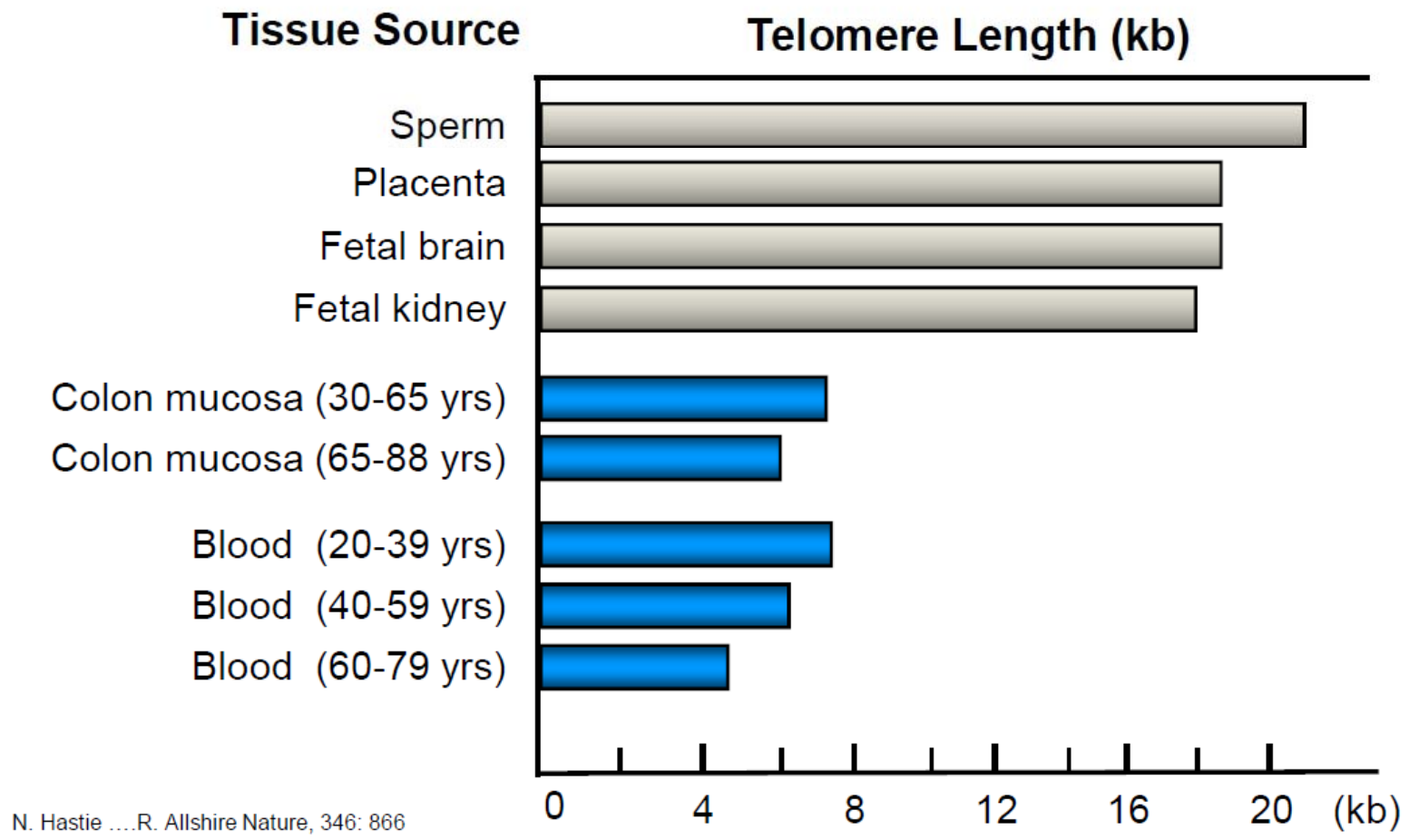
Carol Greider

"For the prediction and discovery of telomerase, a remarkable RNA-containing enzyme that synthesizes the ends of chromosomes, protecting them and maintaining the integrity of the genome."

Laskerfoundation.org September 17, 2006


(1) Germline: telomerase on; telomeres maintained
(2) Soma: telomerase off; telomeres shorten
p53/pRb
(3) Extended life span
(4) Crisis
(5) Immortal cells: telomerase on; telomeres maintained
ALT
Telomere fragment length (kb)
14
12
10
8
6
4
2
Normal cells
Premalignant cells
Malignant cells
Senescence
Crisis
Cell divisions
Current Biology

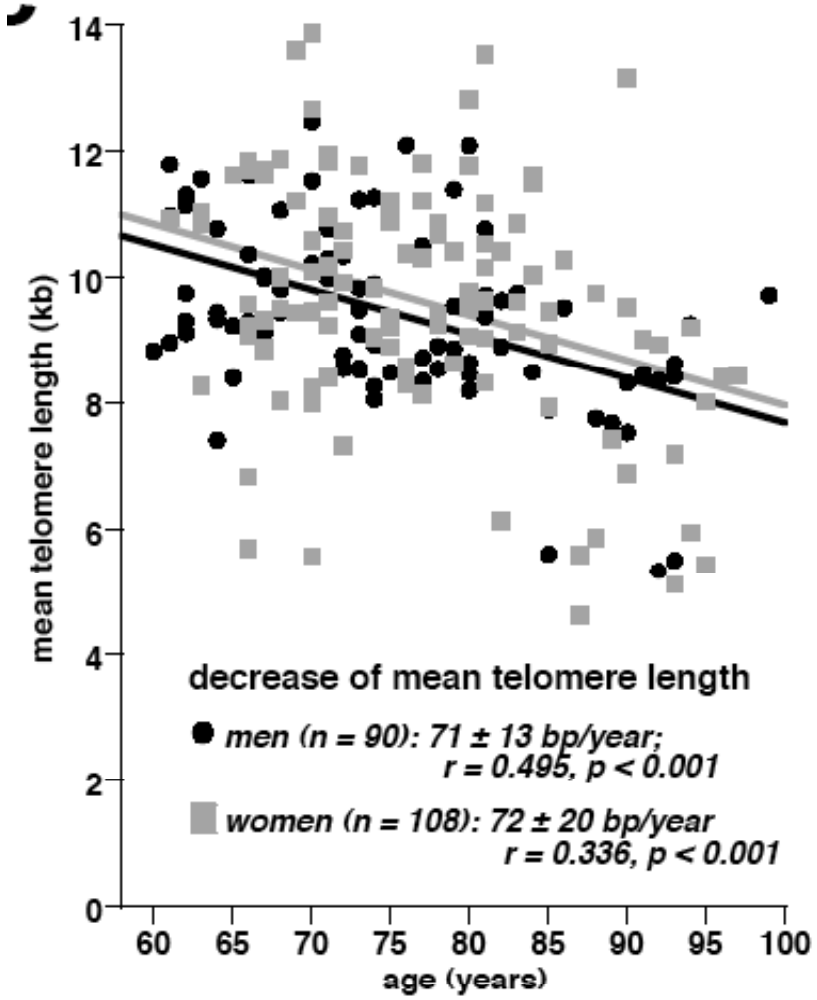
Mean human telomere length decreases with increasing age



N. Hastie ….R. Allshire Nature, 346: 866

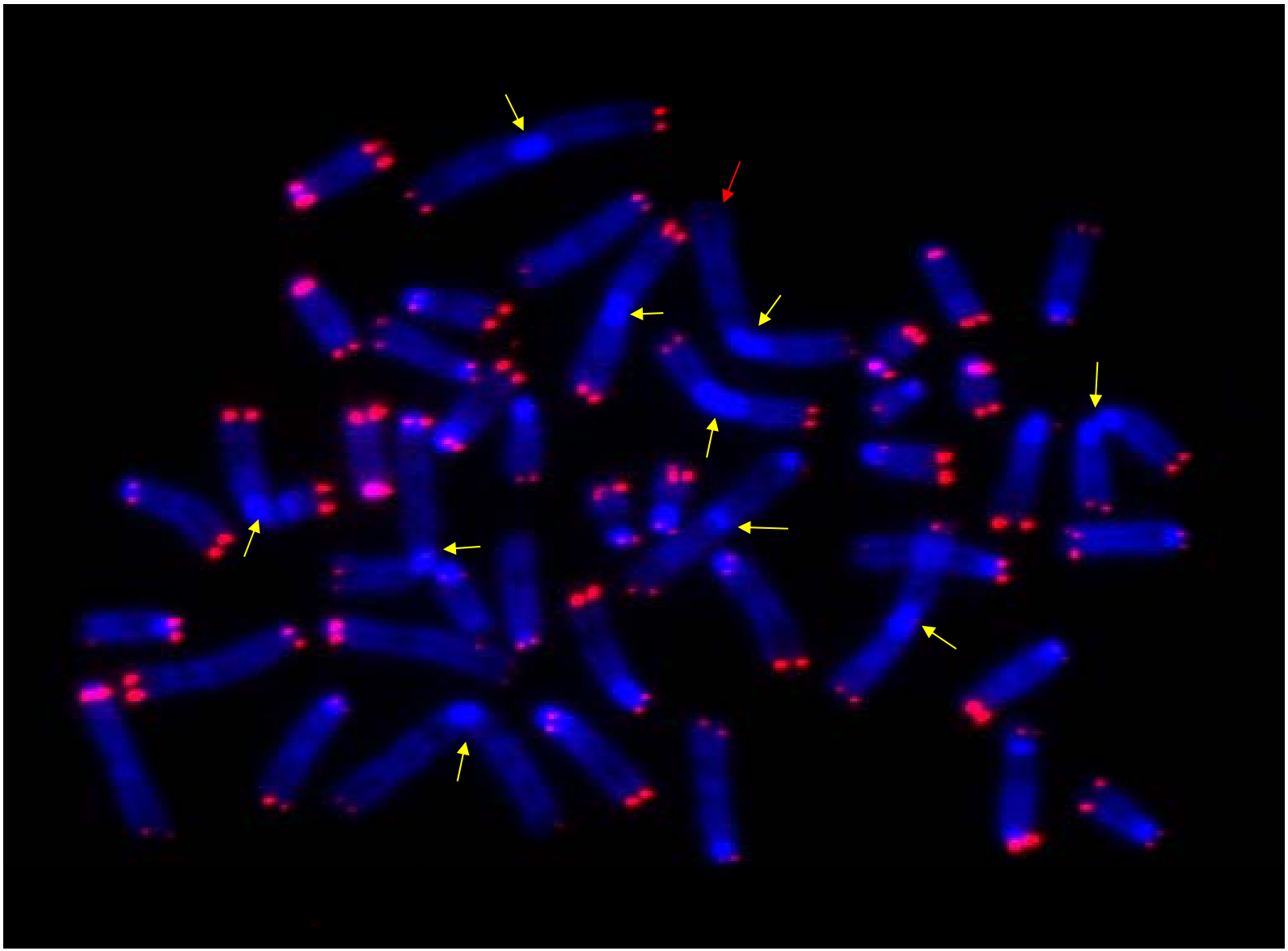
Short telomeres correlate with increased age

Mean human telomere length decreases with increasing age



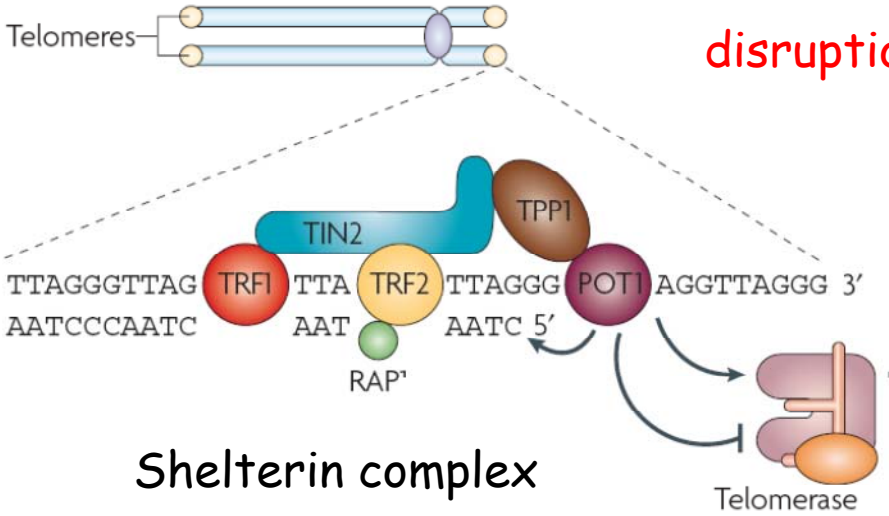
Canela et al., PNAS 2007

Telomere FISH reveals multiple chromosomal fusions in mouse tumors without telomerase



Chang et al., Genes and Dev 2002

Telomere binding proteins are also essential for telomere maintenance



Shelterin complex

disruption of telomere binding proteins

telomere dysfunction

absence of telomerase

DNA damage response

p53

no p53

stem cell defects

apoptosis

cancer

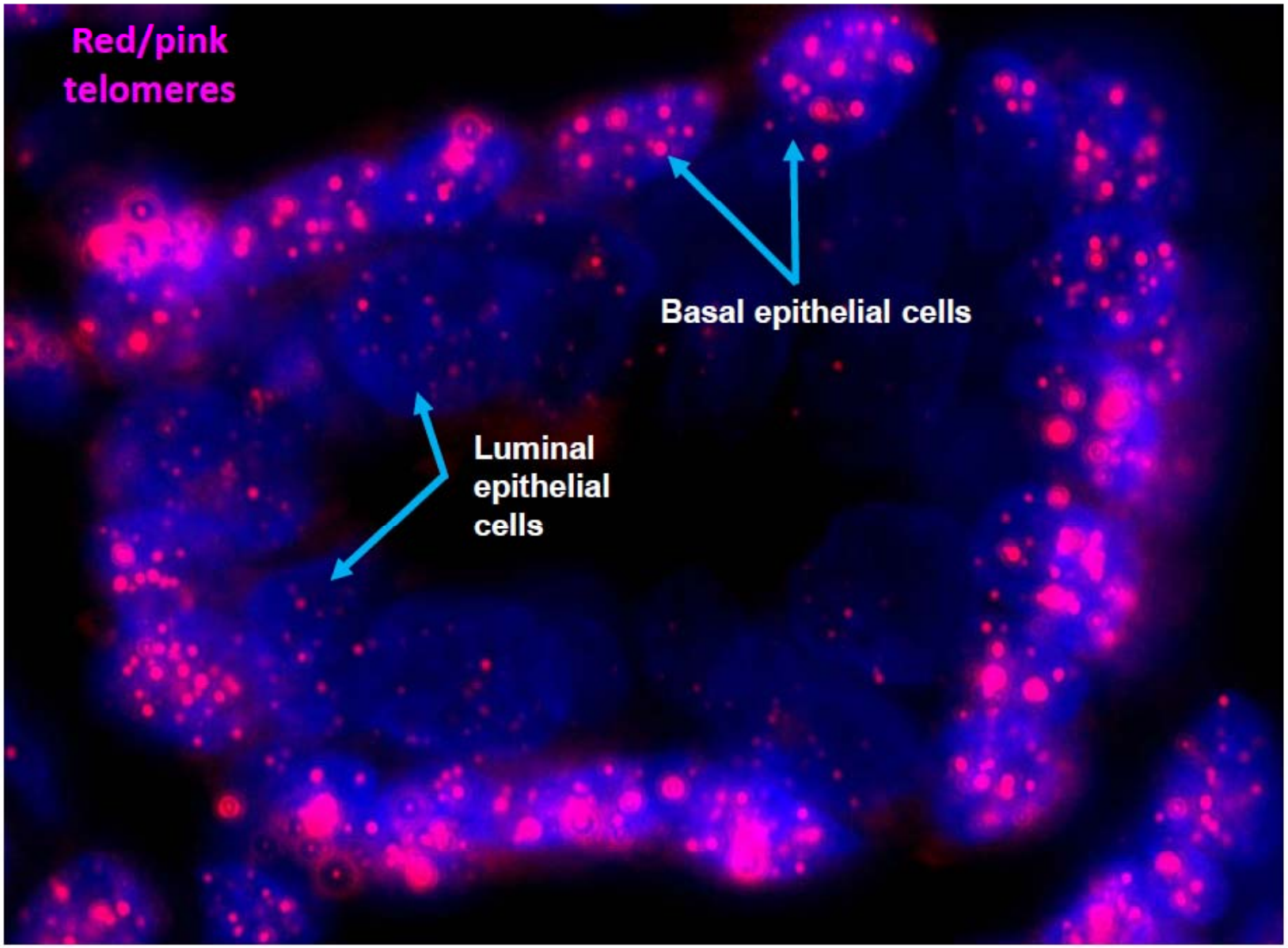
premature aging

cellular senescence

Short telomeres are found in almost all human preneoplastic lesions

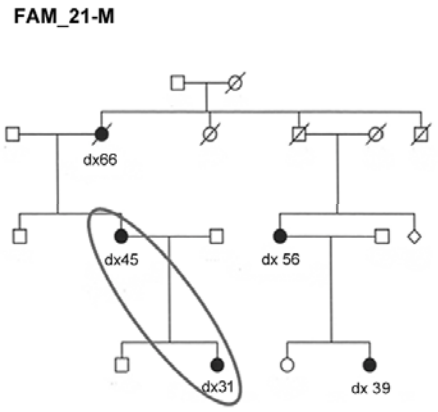
- Ductal Carcinoma In Situ (DCIS)
  - breast cancer
- Prostatic Intraepithelial Neoplasia (PIN)
  - prostate cancer
- Cervical Intraepithelial Neoplasia (CIN)
  - cervical cancer
- Barrett's esophagus
  - esophageal cancer
- Ulcerative colitis
  - colorectal cancer
- Liver cirrhosis
  - hepatocellular carcinoma
- Myeloproliferative disorders
  - decreased general immunity, leukemia

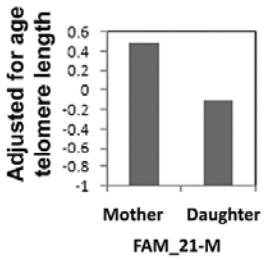
Critical telomere shortening in breast DCIS

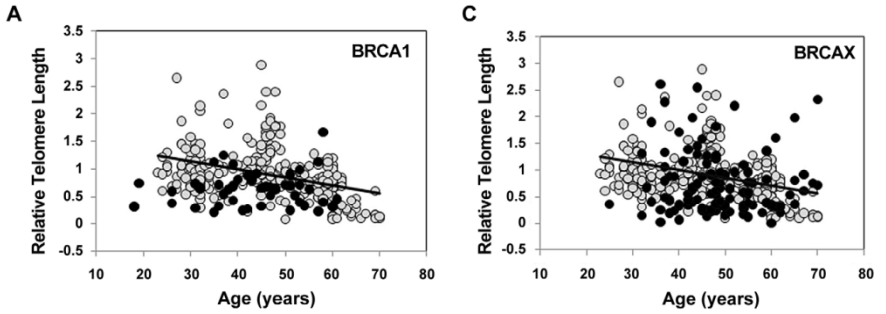


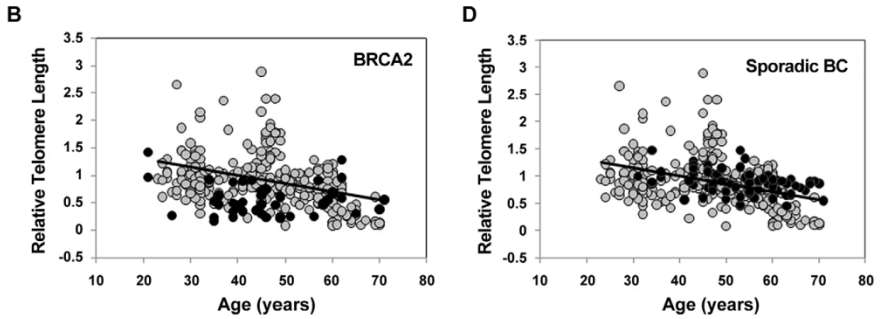
Alan Meeker

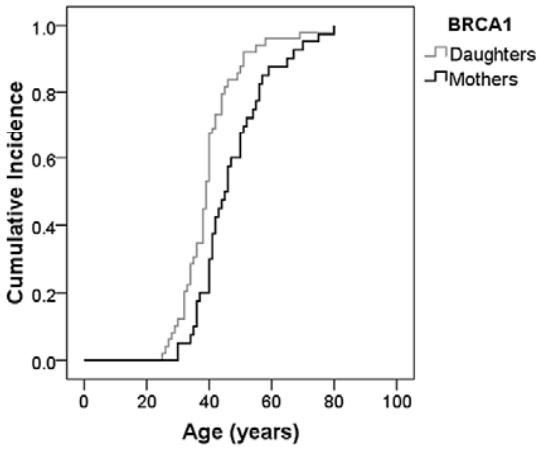
Short telomeres correlate with early onset of hereditary breast cancers

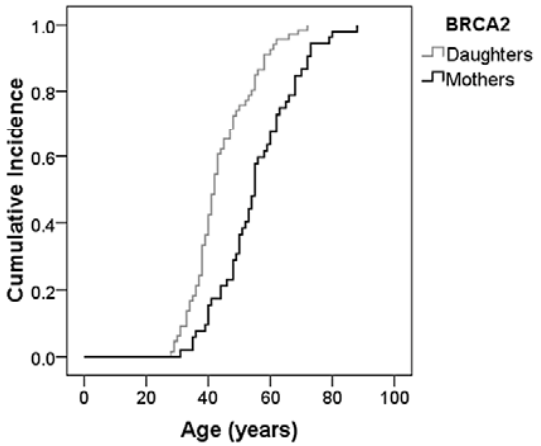






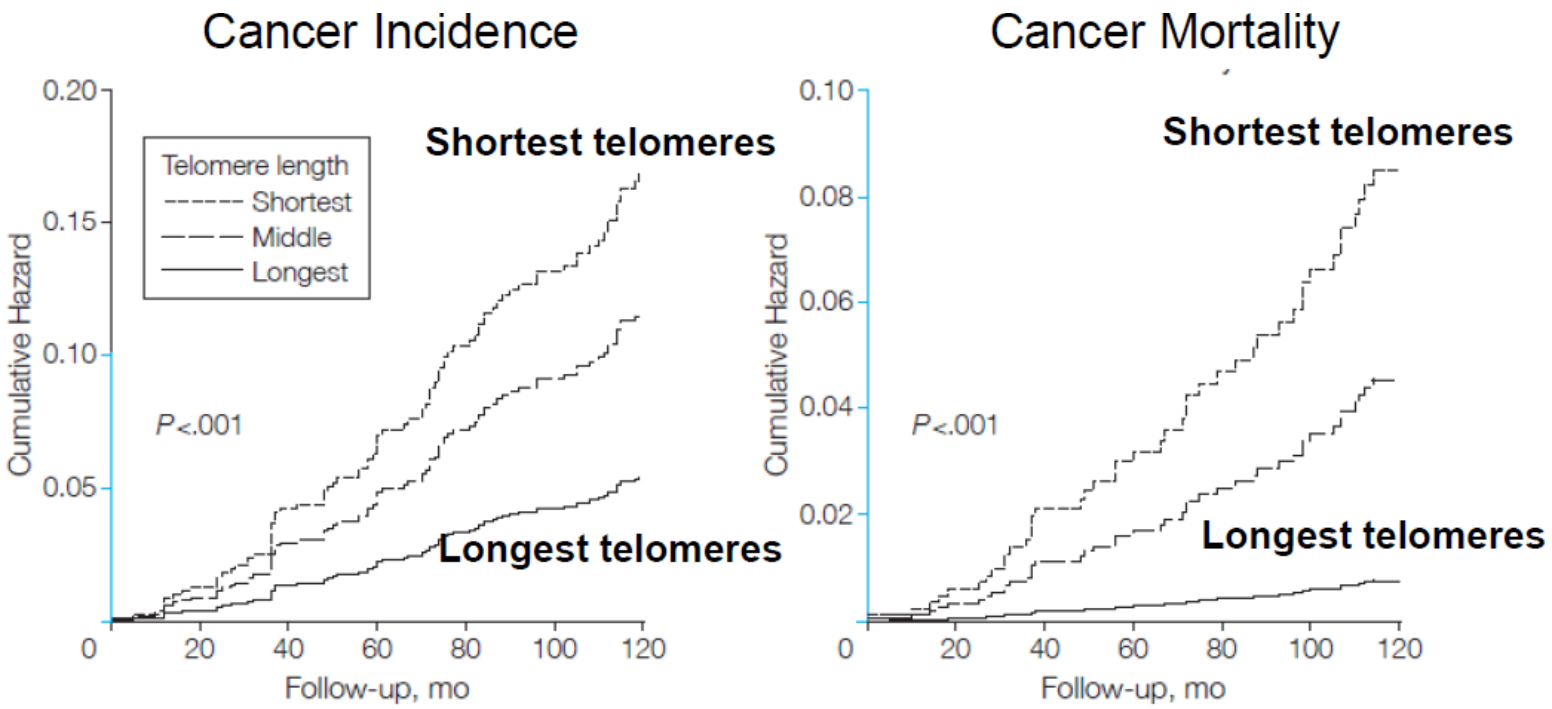






Martinez-Delgado et al., Plos Gen 2011

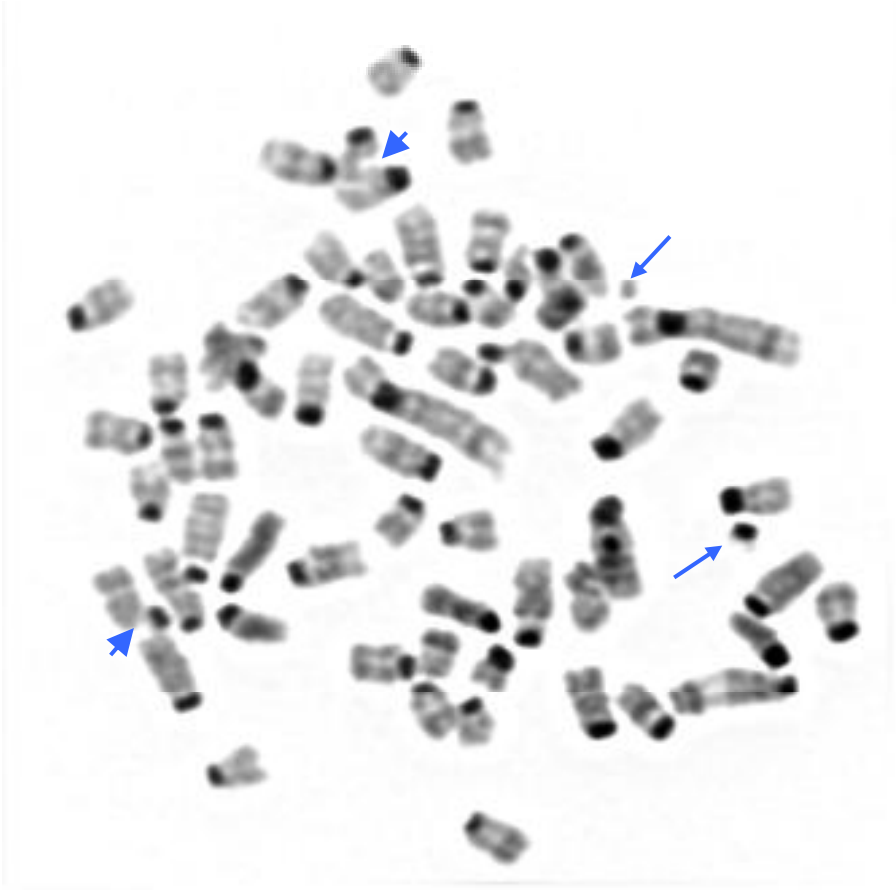
Short telomeres correlate with increased cancer incidence and mortality



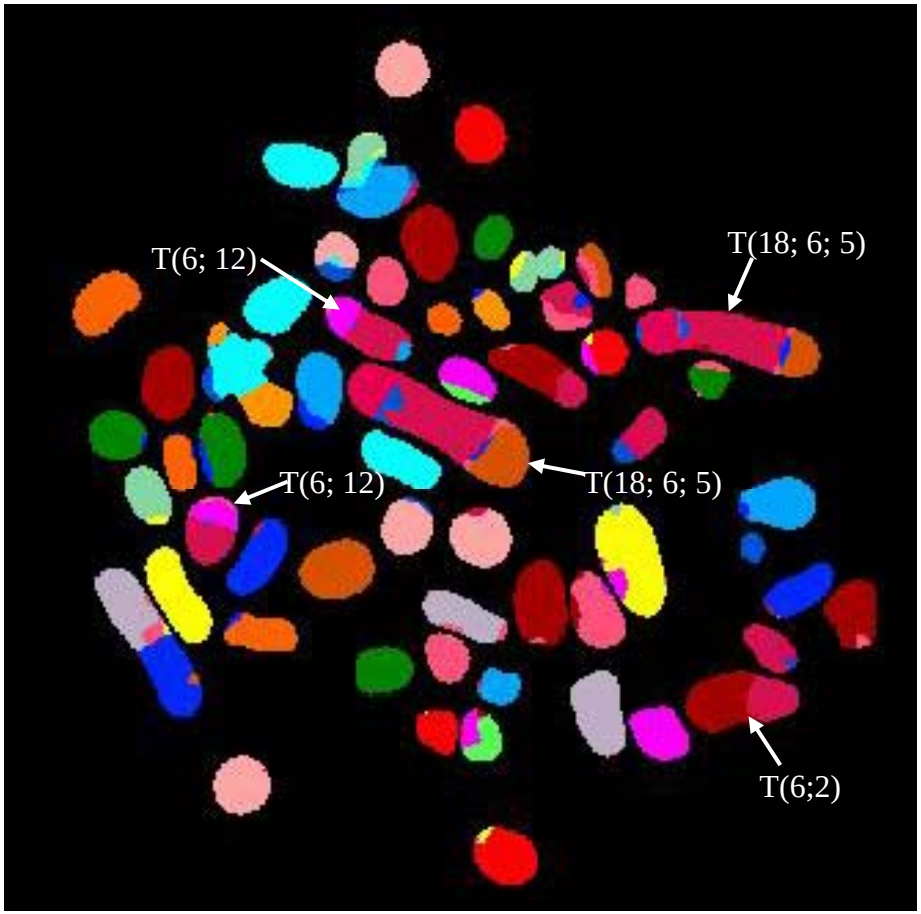
Willeit et al. JAMA 304:69-75, 2010 Short telomeres are associated with an enhanced risk of fatal cancers

Bruneck, Italy Study 1995-2005, 787 Participants (Ages 45-85), 92 Cases of Cancer

Multiple non-reciprocal chromosomal translocations are present in carcinomas from mice with short telomeres



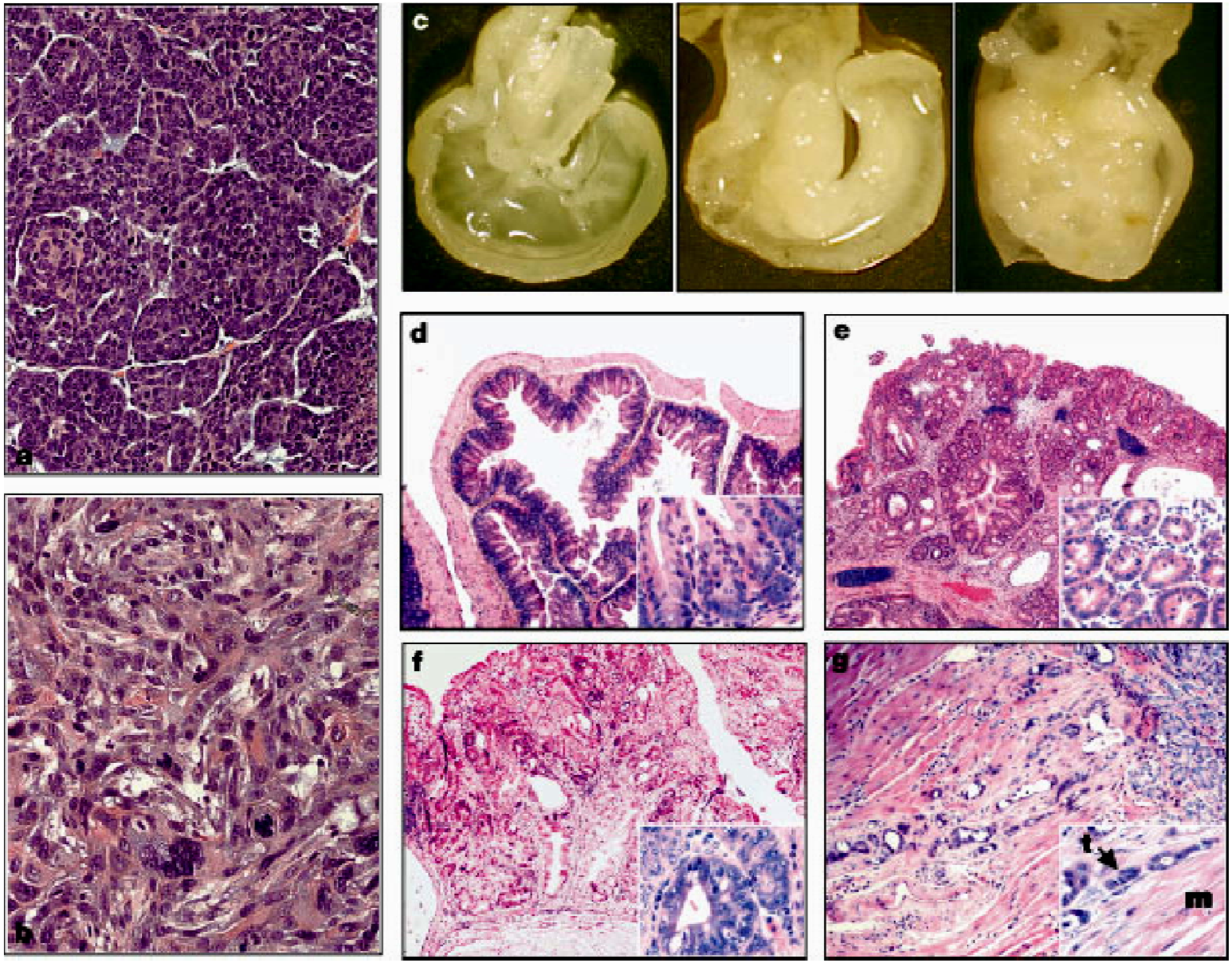
Inverse DAPI image



Classified spectral image

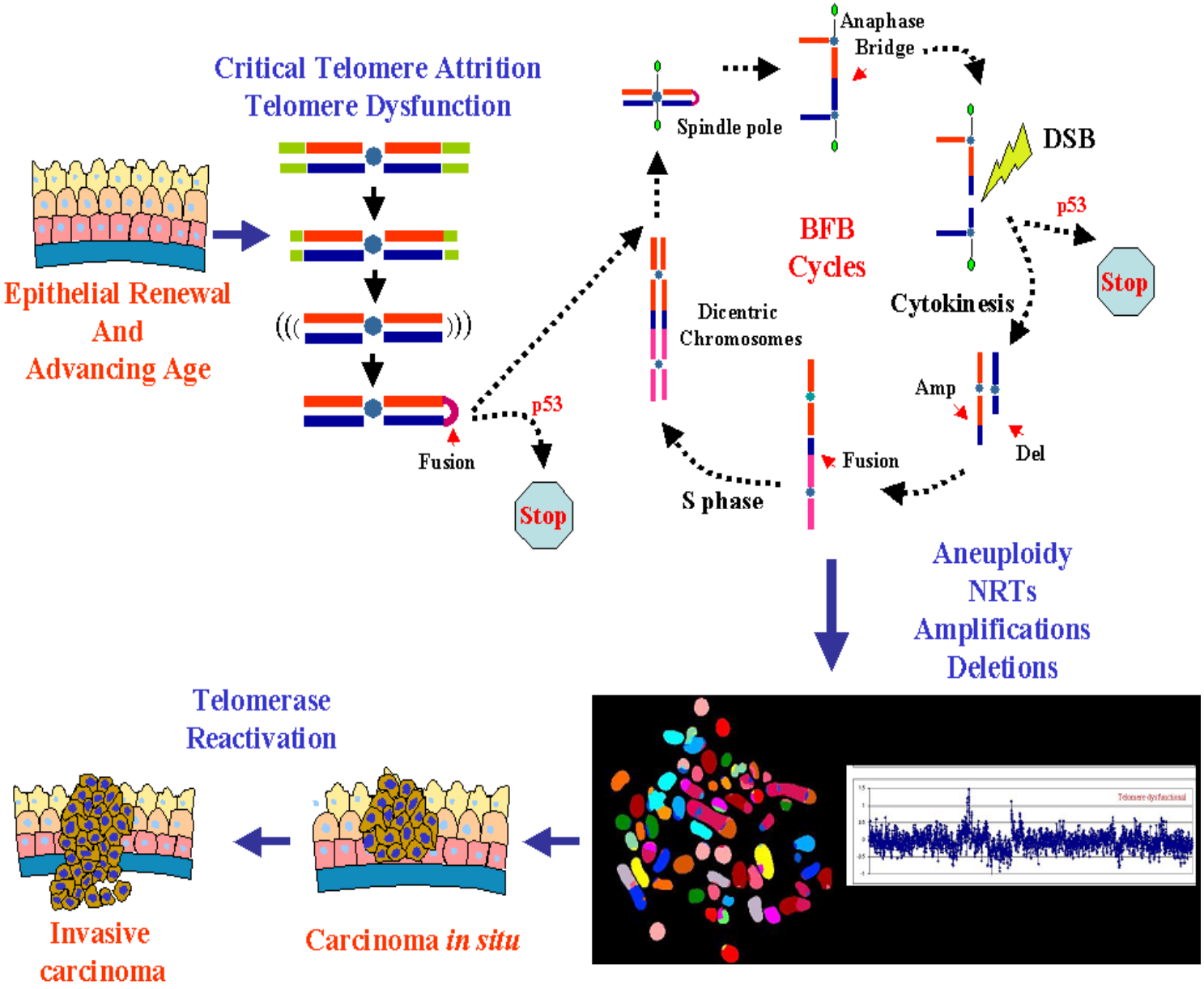
Artandi et al., Nature 2000

Mice engineered with short telomeres develop cancer

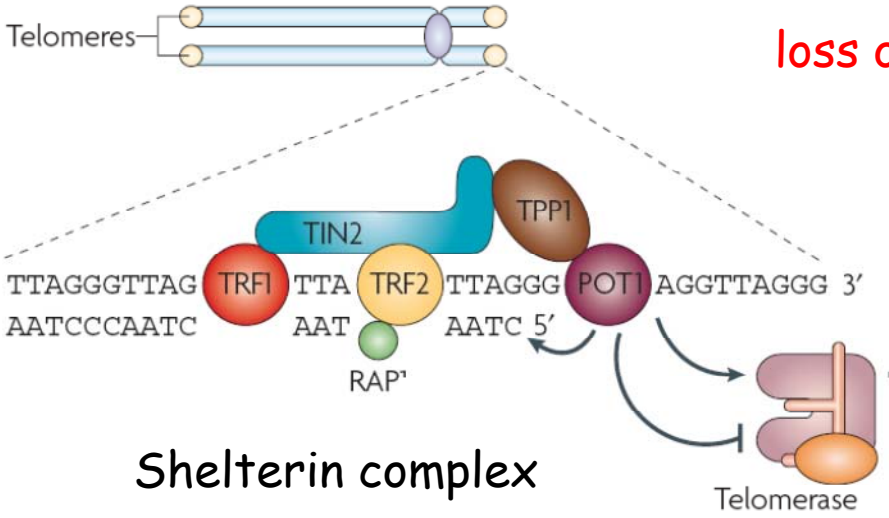


Artandi et al., Nature 2000

Dysfunctional telomeres promote genome instability and cancer



Telomere binding proteins are also essential for telomere maintenance



Shelterin complex

loss of telomere binding proteins

telomere dysfunction

absence of telomerase

DNA damage response

p53

no p53

stem cell defects

apoptosis

cancer

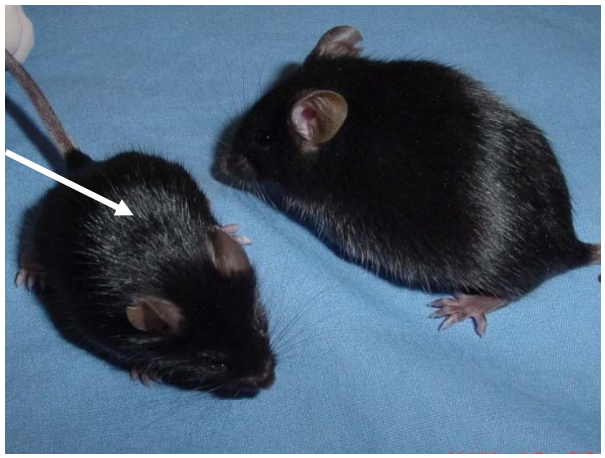
premature aging

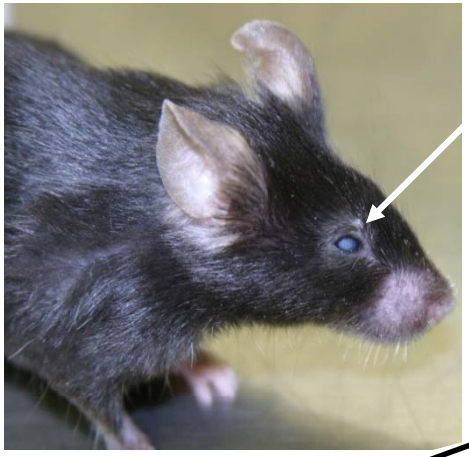
cellular senescence

Mice engineered with short telomeres display features of premature aging

stunted growth

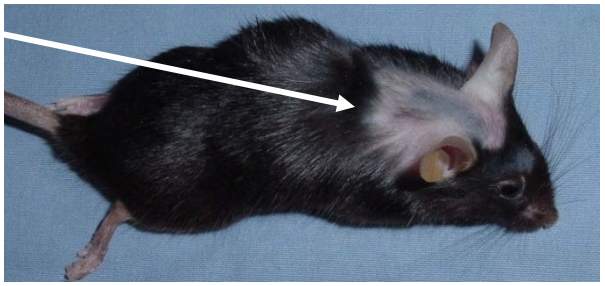
alopecia & hair greying

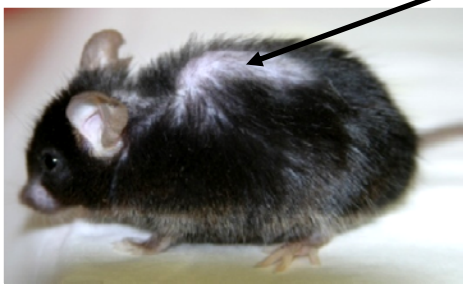




cataract

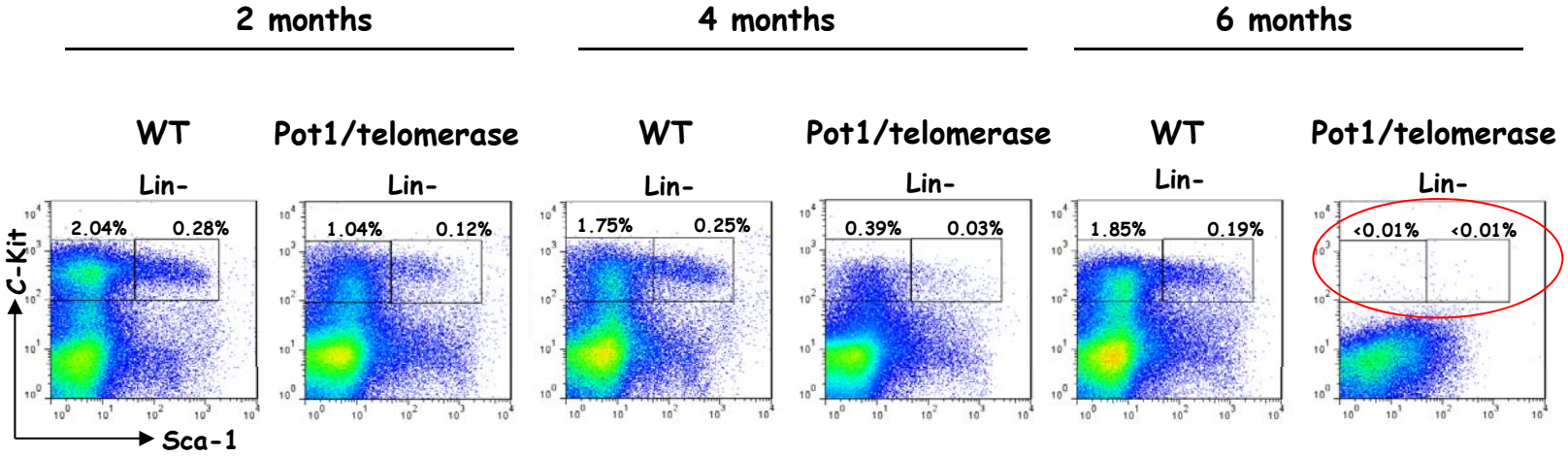
kyphosis



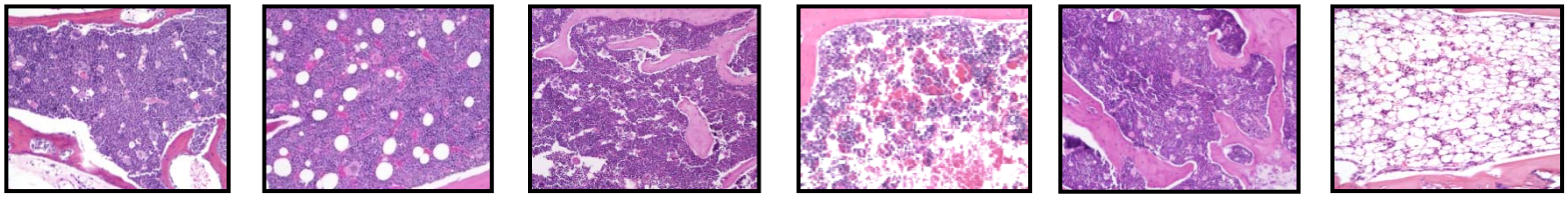


Progressive stem cell failure in mice with telomere dysfunction

A

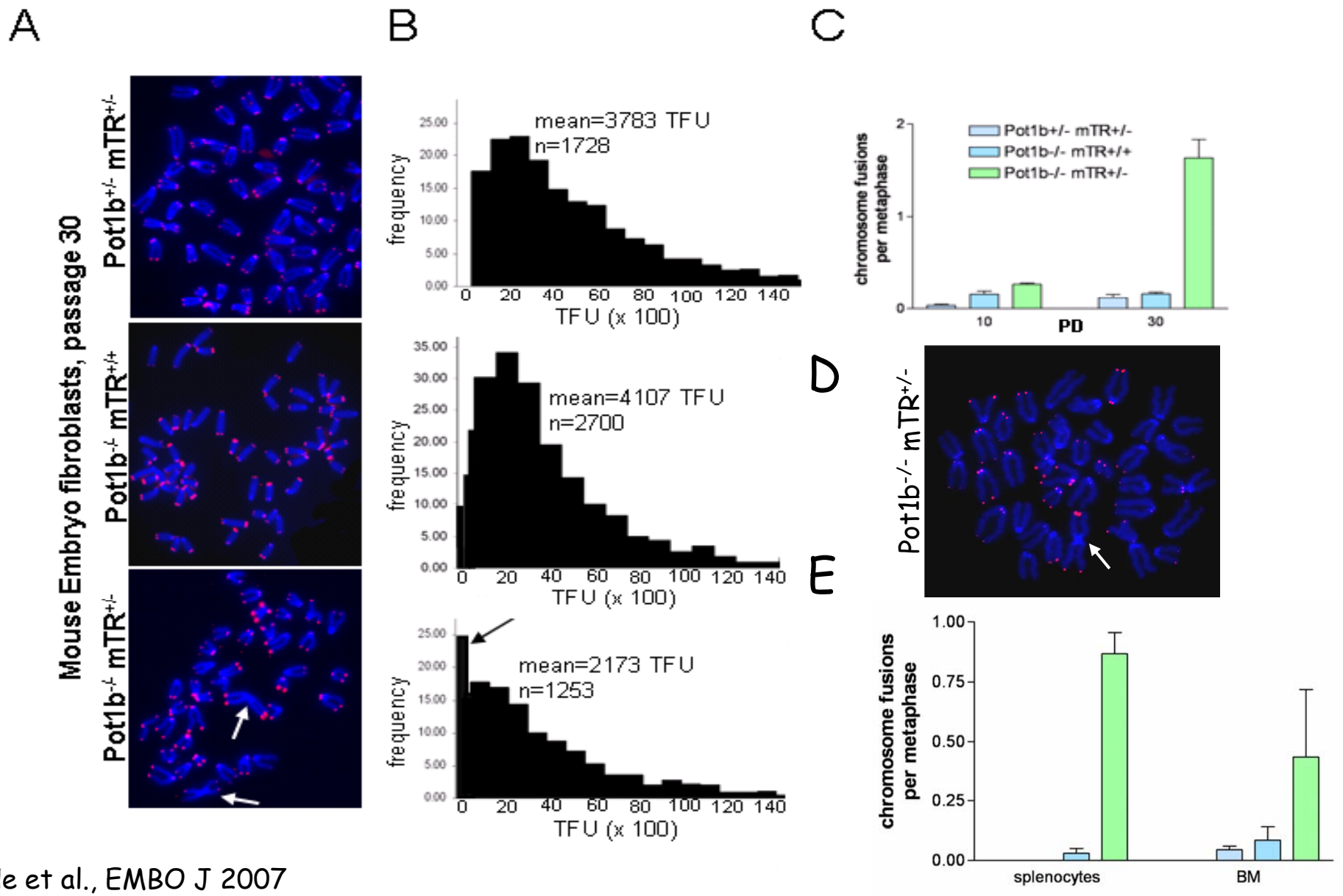


B



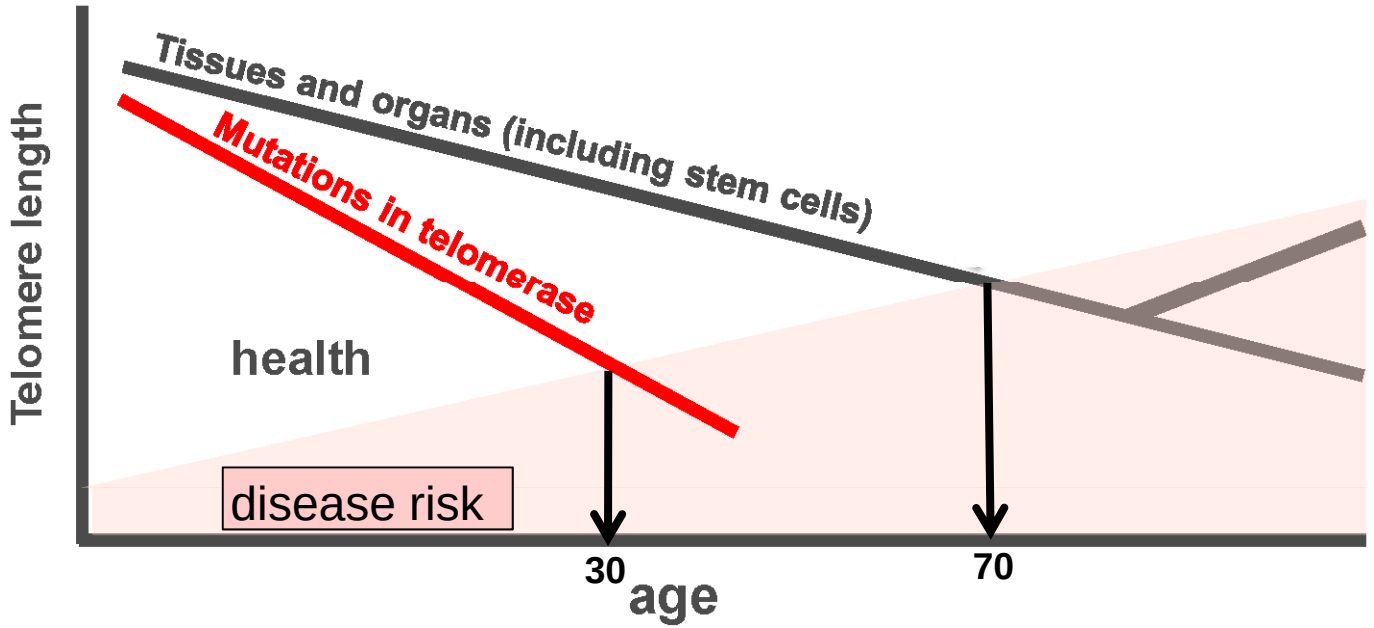
BM failure!

Increased telomere shortening and chromosome fusions in mice with short telomeres

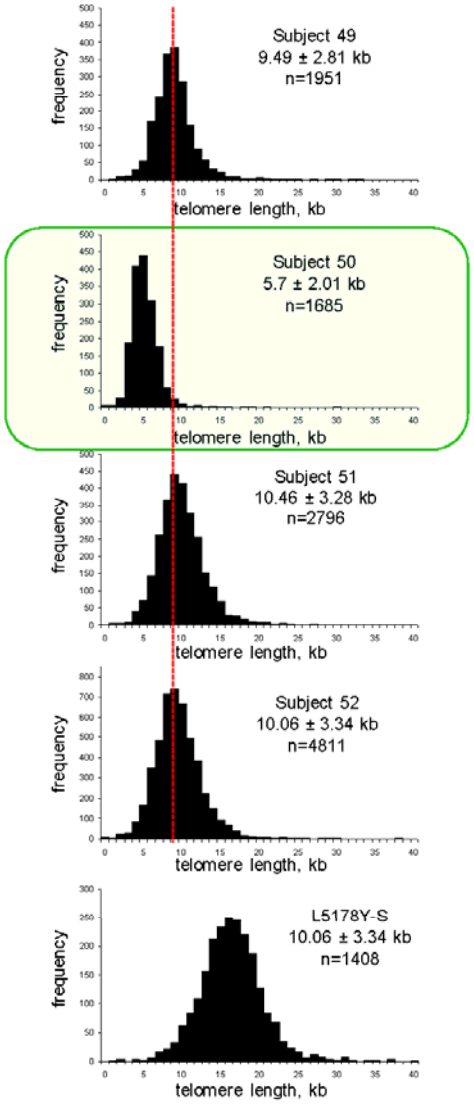


He et al., EMBO J 2007

Telomere length: biomarker of cellular aging



Mutations in genes involved in telomere maintenance results in very short telomeres that can be measured



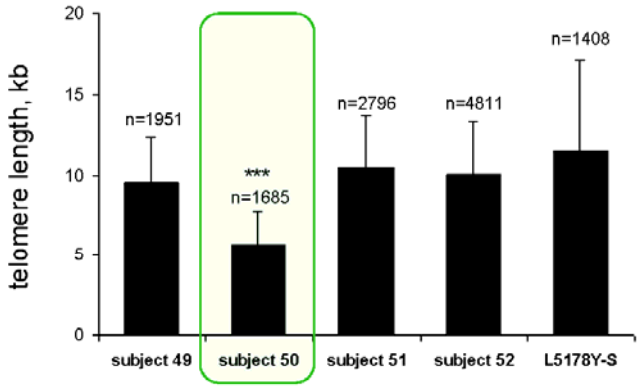
Subject 49- Male 02/02/78 healthy

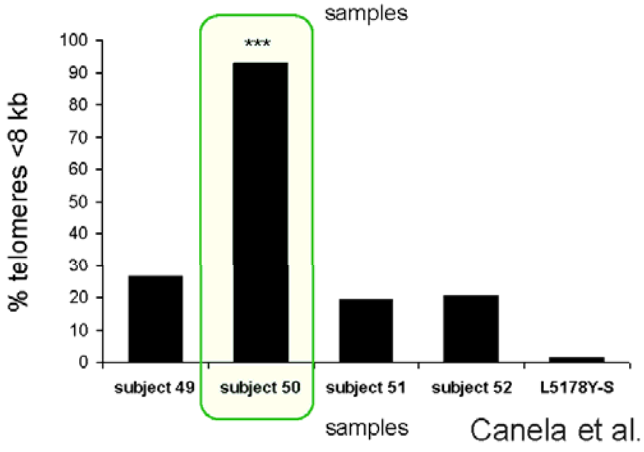
Subject 50- Male 22/09/77 medular aplasia, TERC mut

Subject 51- Male 17/05/78 healthy

Subject 52- Male 06/06/78 healthy

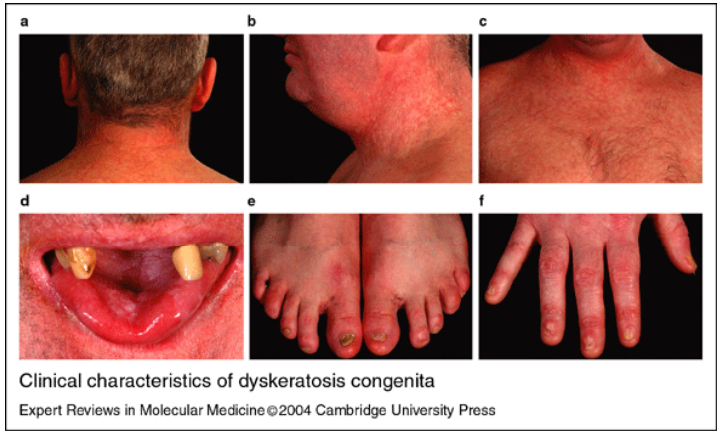
Hospital Vall d´Hebrón



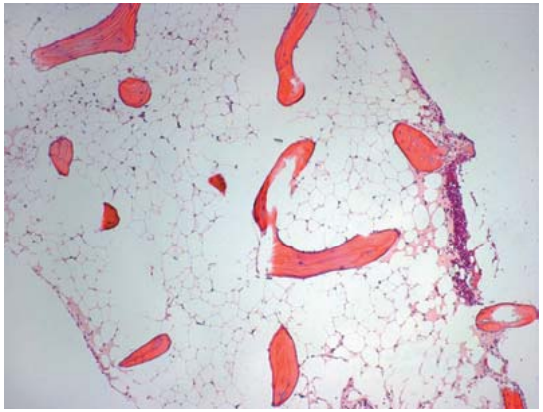


Canela et al., unpublished

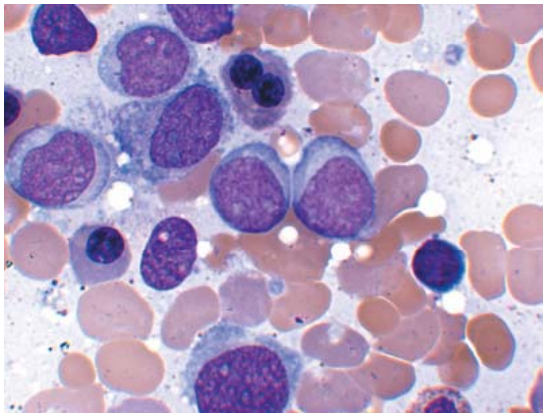
Dyskeratosis congenita-inherited telomere disease



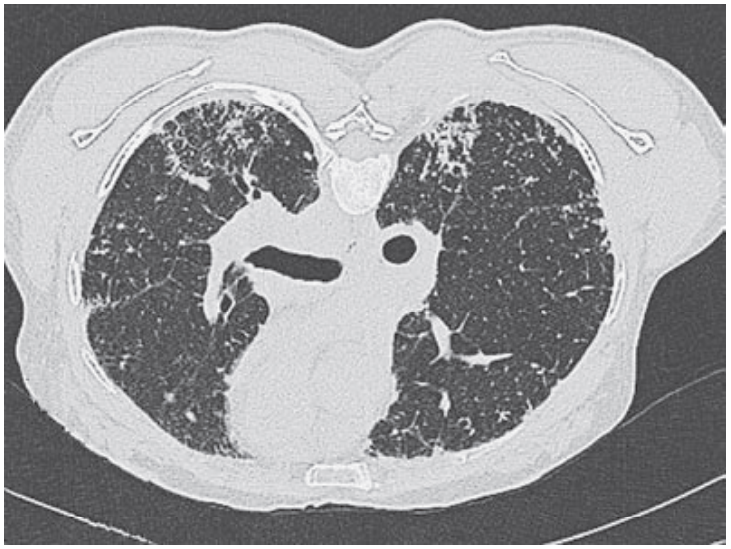
Clinical characteristics of dyskeratosis congenita



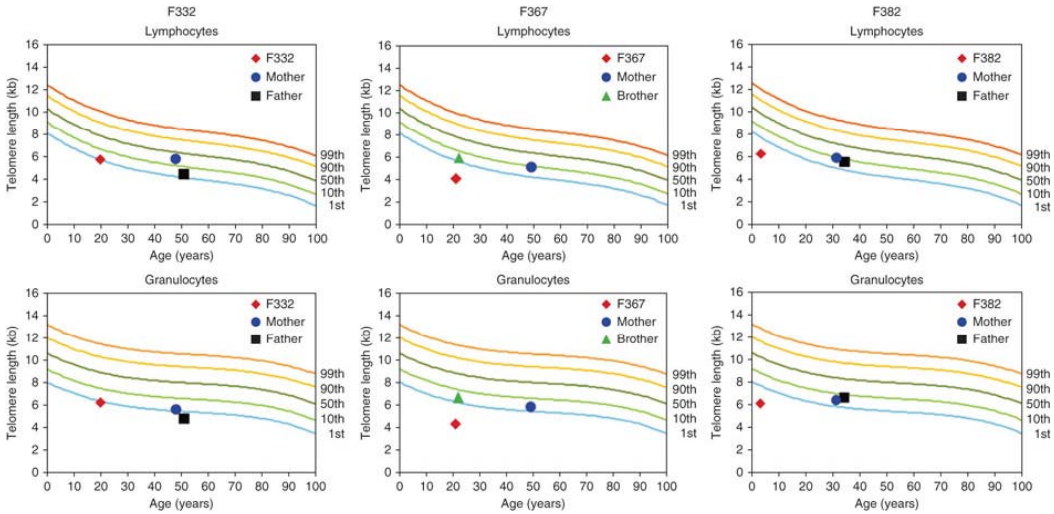
BM failure



AML



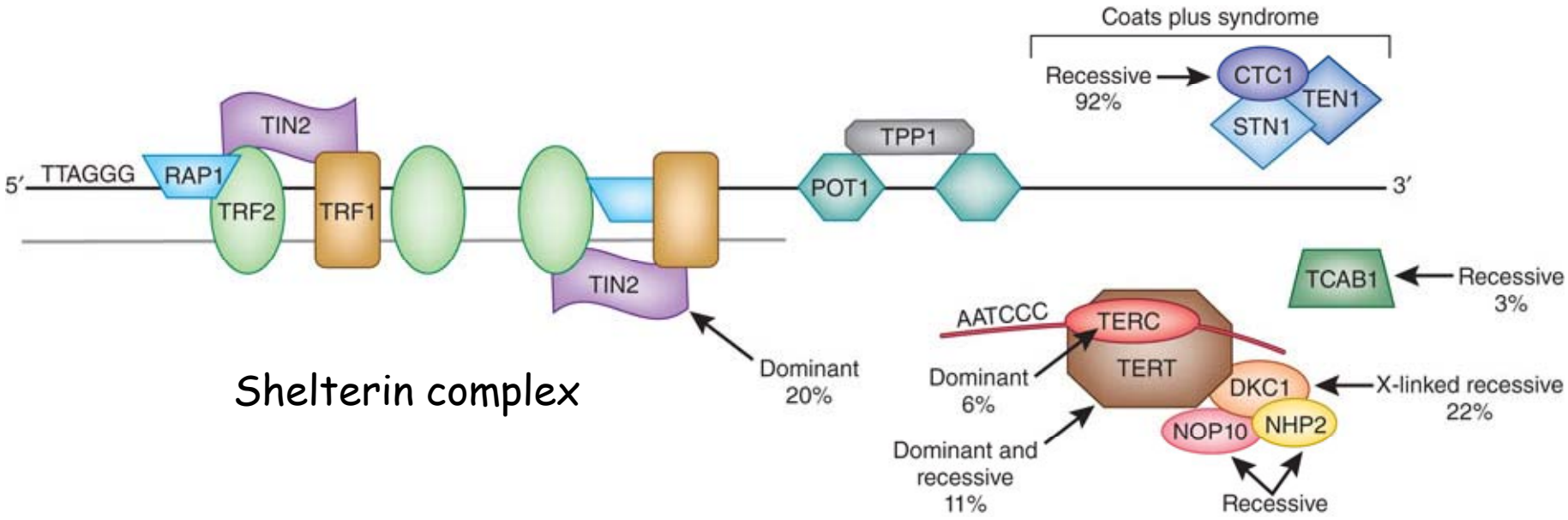
Pulmonary fibrosis



Critically short telomeres

Calado et al., NEJM 2009; Anderson et al., Nat Gen 2012

Inherited human telomere diseases disrupt telomere maintenance pathways

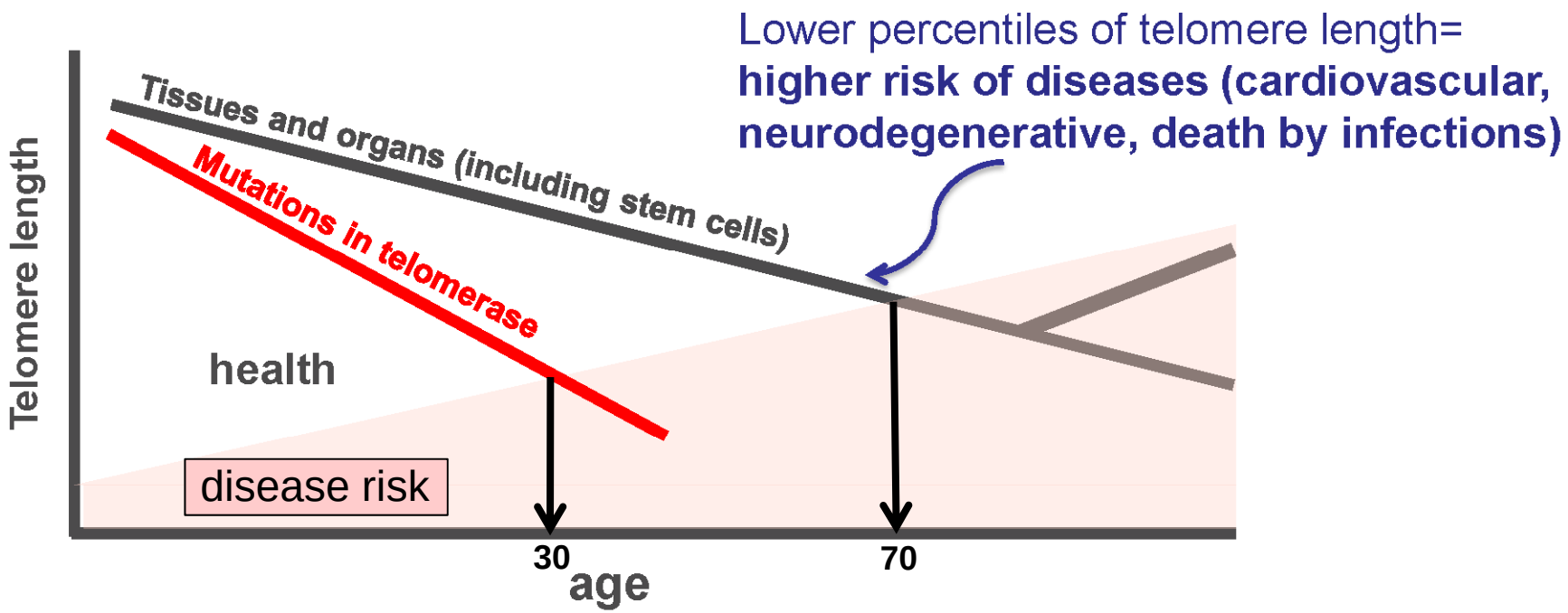


Shelterin complex

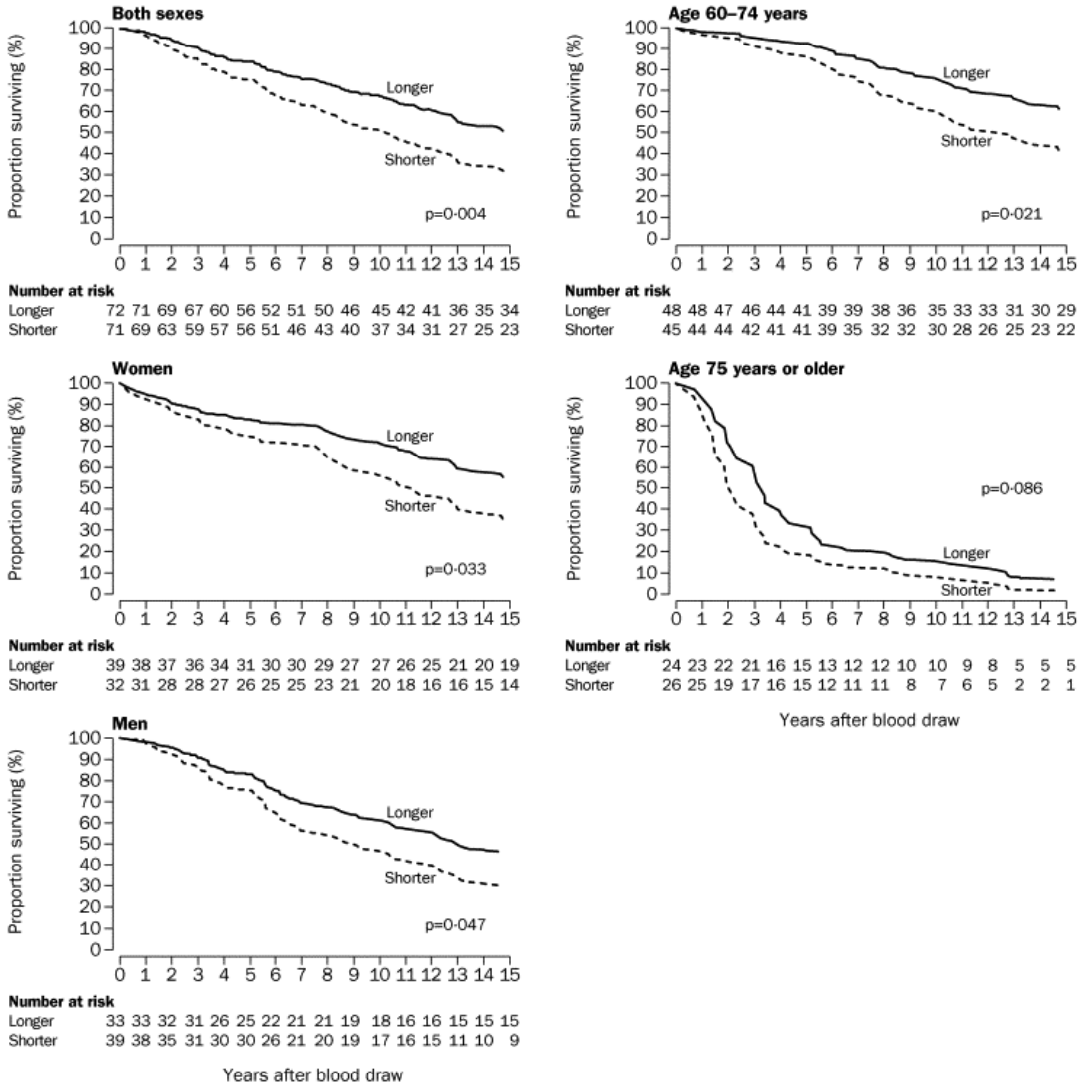
Telomerase complex

Savage, Nature Genetics 2012

What about "normal" individuals without mutations in telomere maintenance genes?



Telomere shortening correlates with decreased overall survival



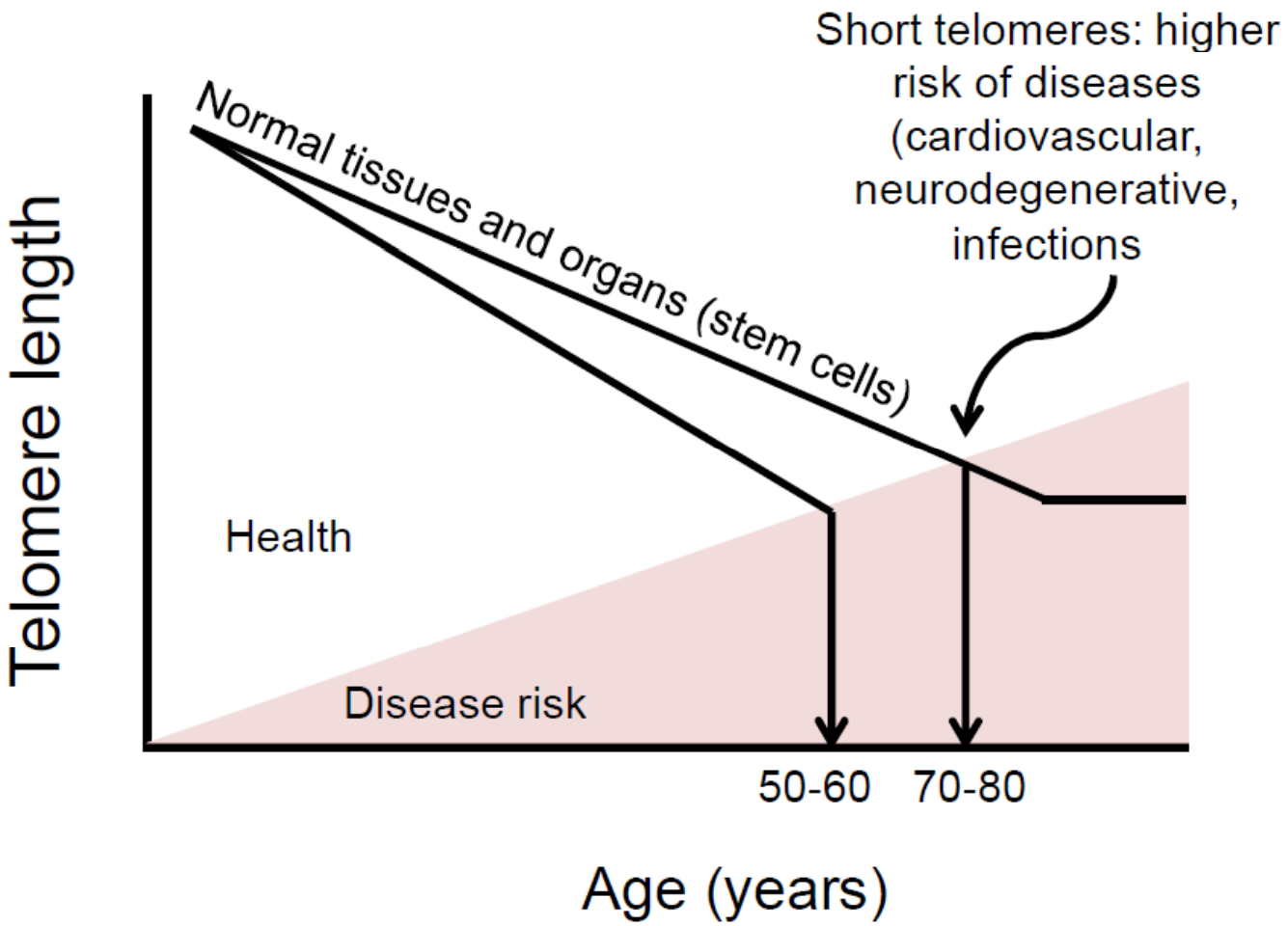
Patients with short telomeres have a mortality rate twice that of those with longer telomeres.

Heart disease mortality increased 3X.

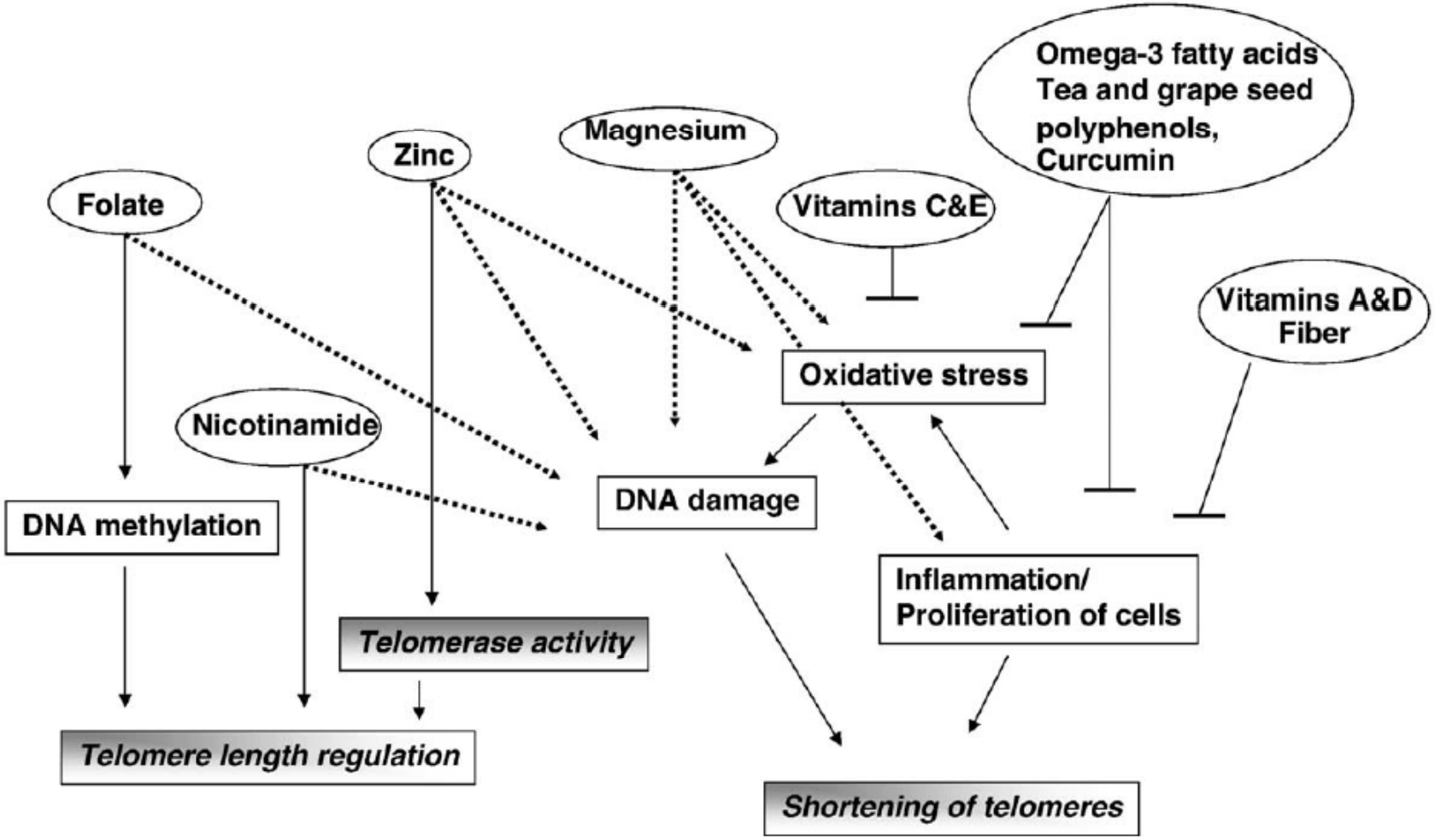
Infectious disease mortality increased 8X.

Cawthon et al., Lancet 2002

Stress, diet, and inflammation results in oxidative stress that can accelerate telomere shortening



Diet and nutrition can modify telomere length



L Paul, J. Nutritional Biochemistry, 22:895-901, 2011

In this era of personalized medicine, should we use telomere length as a diagnostic tool?

Whole genome sequencing

disease gene identification

potential for tailored therapeutic intervention

still expensive-bioinformatics

too much information!

Telomere length determination

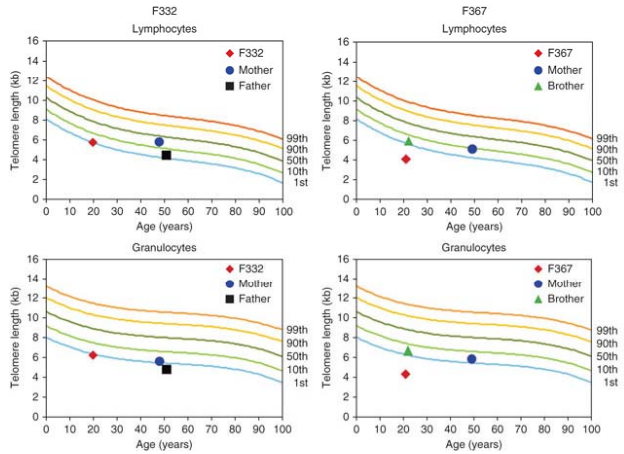
identify inherited telomere disorders

therapeutic intervention-BM transplantation

identify "healthy" individuals with accelerated biological aging

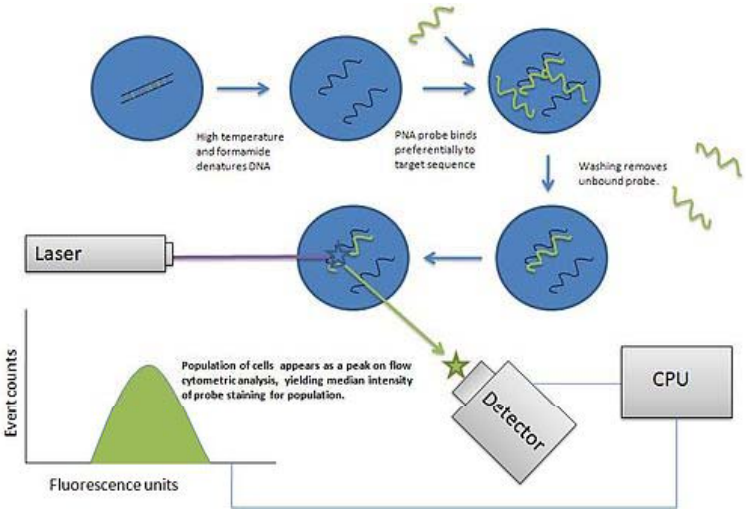
potential to enable early intervention-lifestyle modifications to reduce cellular stress

inexpensive



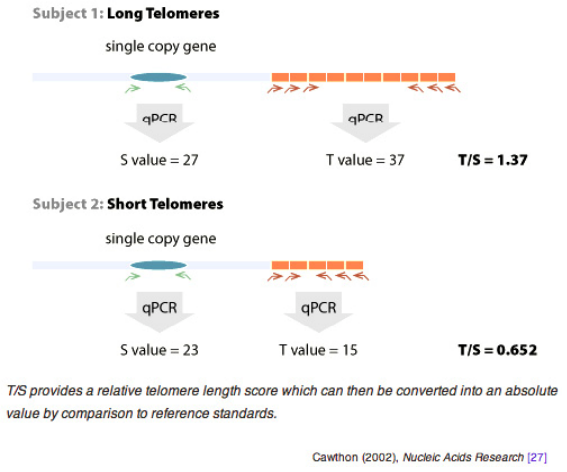
Common methods to determine telomere length

Telomere Flow-FISH



- Intensity of fluorescence signals correlate directly with telomere length
- Ability to process large sample volumes
- Only gives telomere length of a population of cells-cannot examine length of individual telomeres
- Cannot inform on spatial distribution of cells with the shortest telomeres in a tissue sample
- Does not work well on solid tissues

Quantitative telomere-PCR

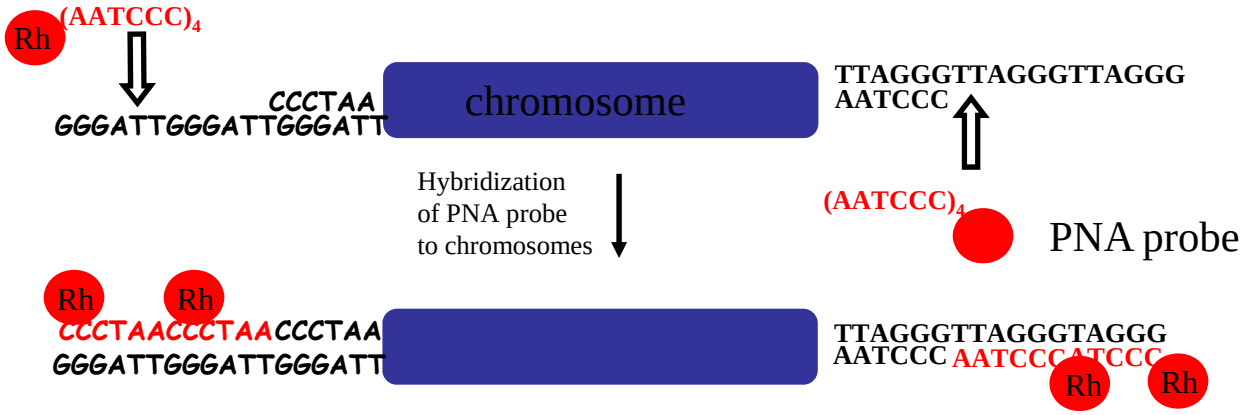


- Quantity of telomere PCR product correlates directly with telomere length
- Ability to process large sample volumes
- Only gives telomere length of a population of cells-cannot examine length of individual telomeres
- Cannot inform on spatial distribution of cells with the shortest telomeres in a tissue sample

Telome Health

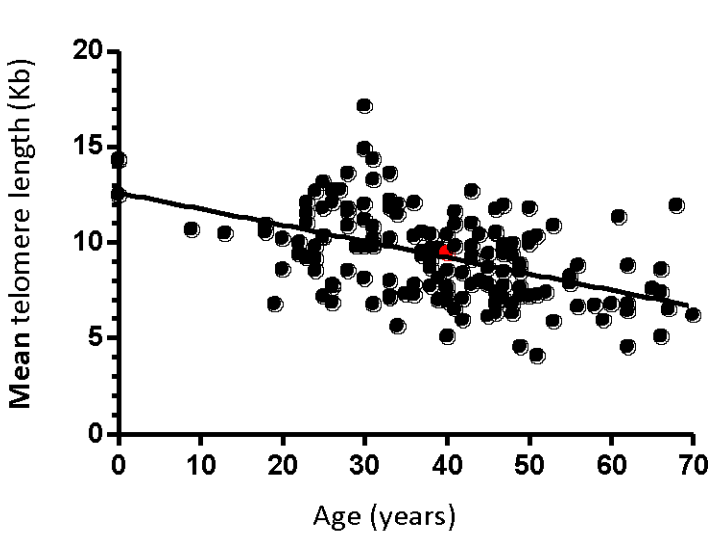
Quantitative telomere-FISH

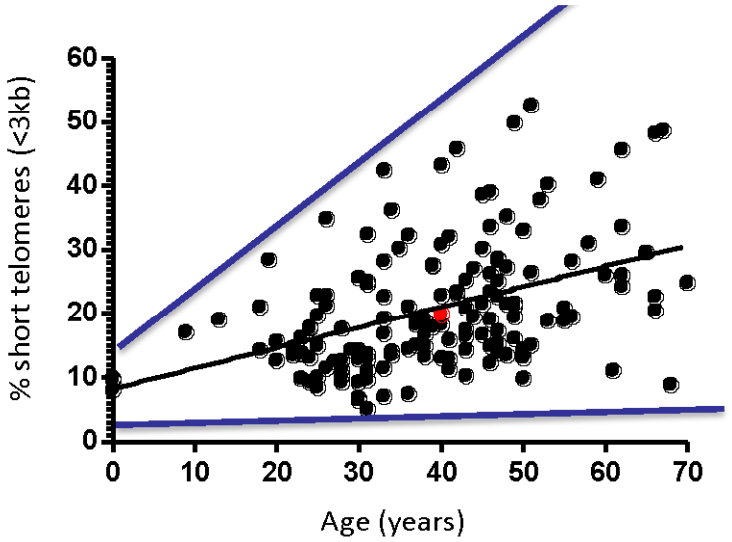
Telomeric peptide nucleic acid (PNA)-FISH



- Intensity of signals correlate directly with telomere length.
- Only method that could detect the critically shortest telomeres in a cell.
- Measures telomere length on metaphase chromosomes or nuclei.
- Measures telomere length on clinical tissue samples-valuable spatial information intact.
- Automated microscopic imaging techniques enable processing of large sample volumes.

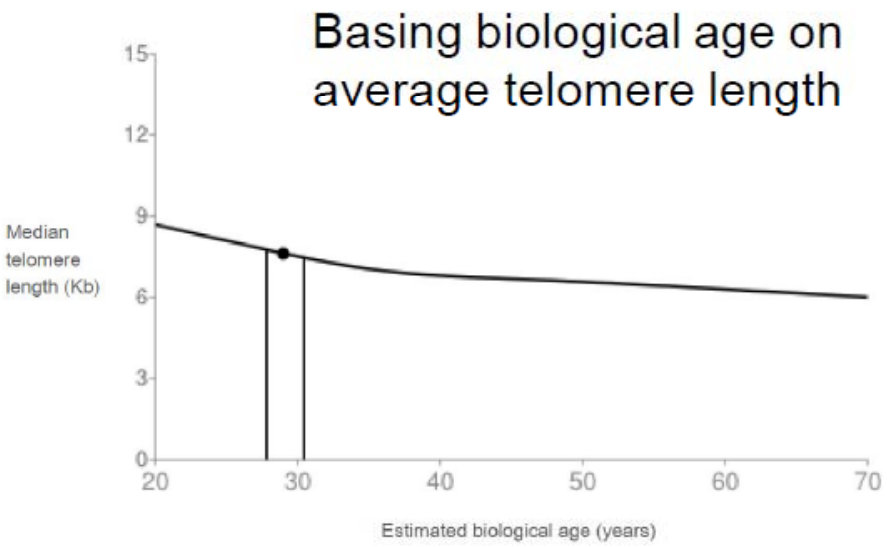
Telomere length measurements: need to determine length of shortest telomeres

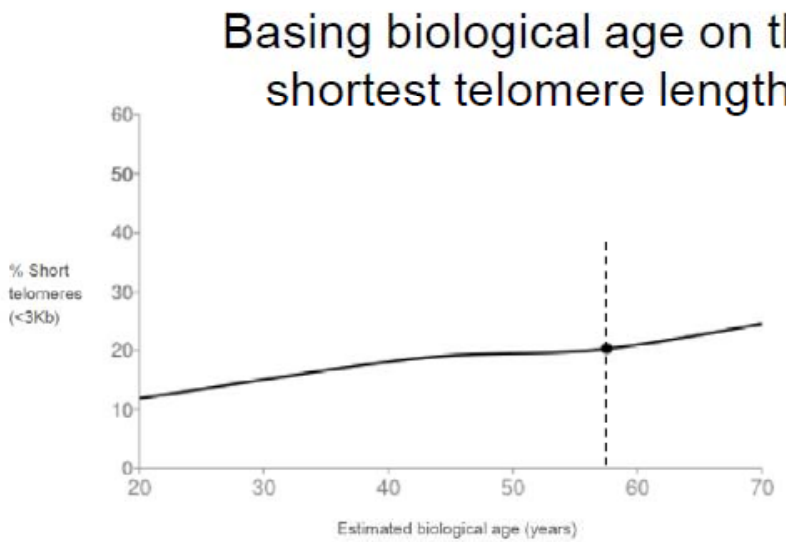




- Only a few critically short telomeres needed to push human cells into senescence.
- % shortest telomeres detect more differences between individuals than average length.
- % shortest telomeres show higher dispersion with increasing age.
- % shortest telomeres could be a marker for environmentally induced damage.

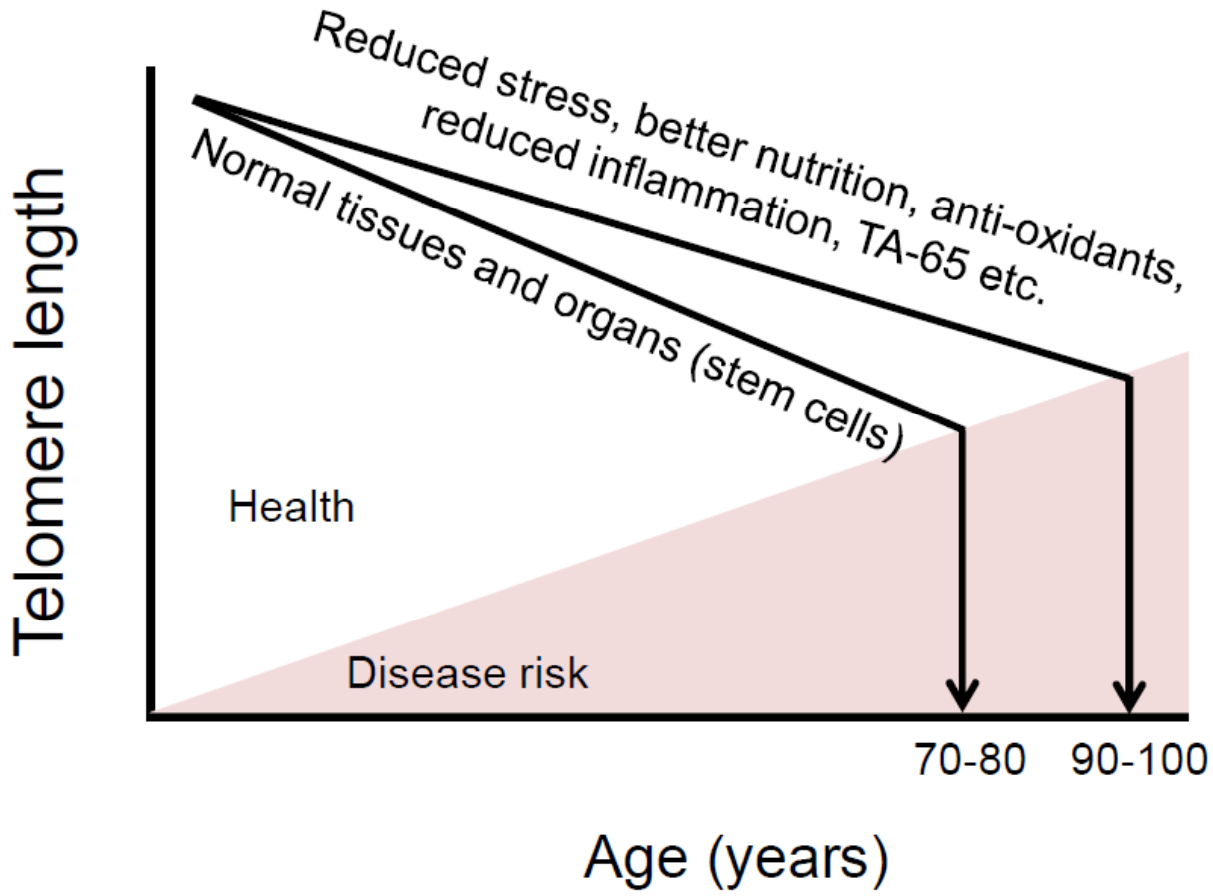
The percentage of short telomeres, not average telomere length, determines biological age





Chronological Age (years): 66.3
Estimated biological age (years): 57.5
Percentage of short telomeres (<3Kb): 20%
Median telomere length (Kb): 7.6
Percentile of medium telomere length: 81%

Will telomere length modification delay cellular aging in healthy individuals?



Possible effects of transient telomerase activation in patient cells

- Slow the rate of telomere loss
- Improved immune cell structure/function
- Prevention or slow down rate of genomic instability?
- Activation of renewal pathways
- Increase repair, resistance to stress-induced apoptosis

- Increased doublings for normal cells = increased chance of mutations occurring
- Increased doublings for premalignant cells = increased chance of mutation to next tumor stage

Why use telomere length determinations in your clinical practice?

- To identify inherited telomere disorders in patients
- To identify “healthy” individuals with accelerated biological aging
- To enable early intervention-lifestyle modifications to reduce stress, inflammation, oxidative damage and reduce accelerated telomere loss
- To provide therapeutic interventions to slow down or reverse telomere loss (stem cells, bone marrow transplantations, tissue engineering, supplements)

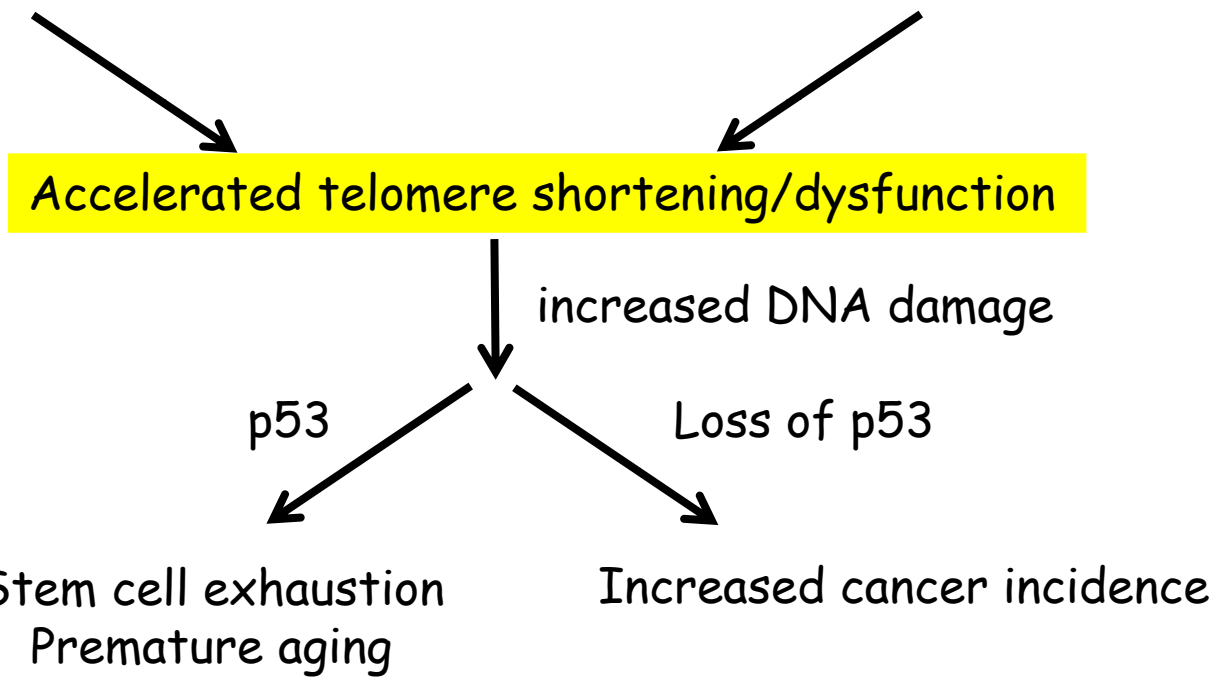
Conclusions

Inherited factors

mutations in telomere maintenance genes
certain inherited cancer syndromes
mutations in DNA repair pathways

Environmental factors

carcinogens
ROS
other stressors?



Telomere length measurements as a predictor of increased cellular risk arising from short telomeres.

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