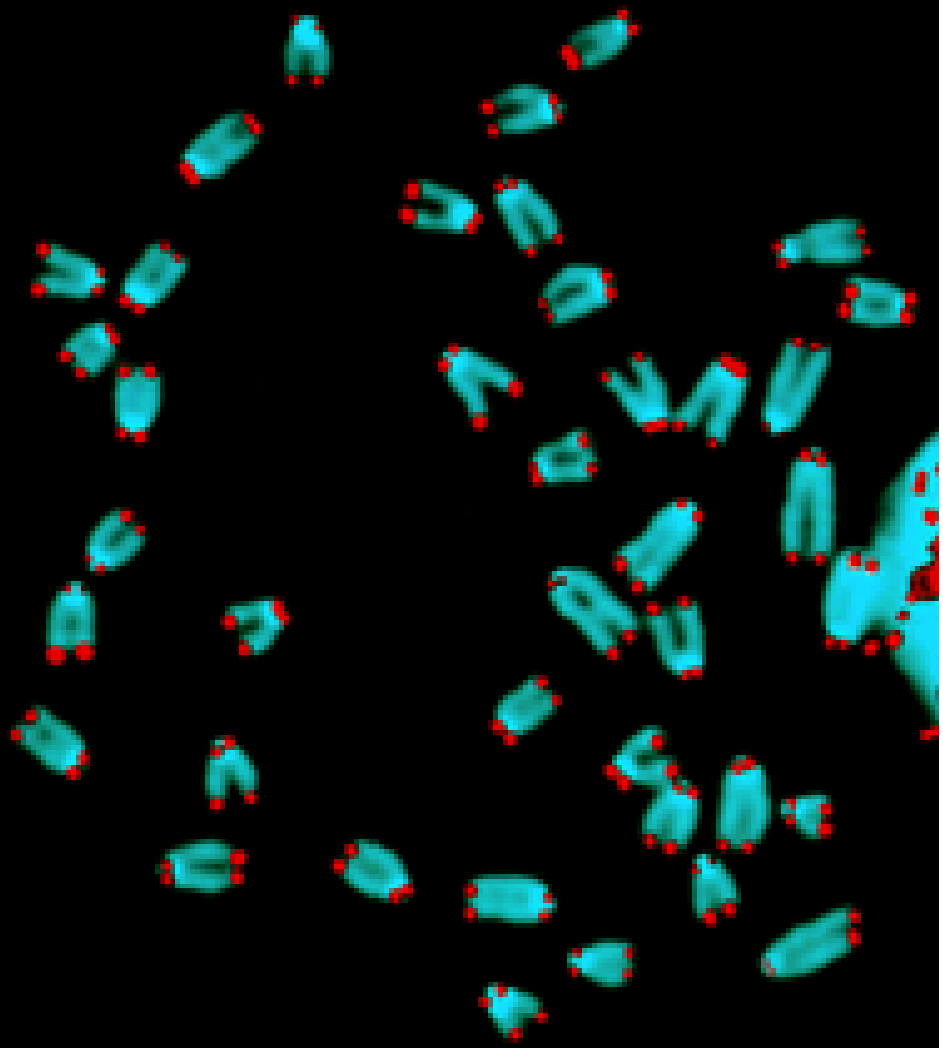
Telomerase and the consequences of telomere dysfunction



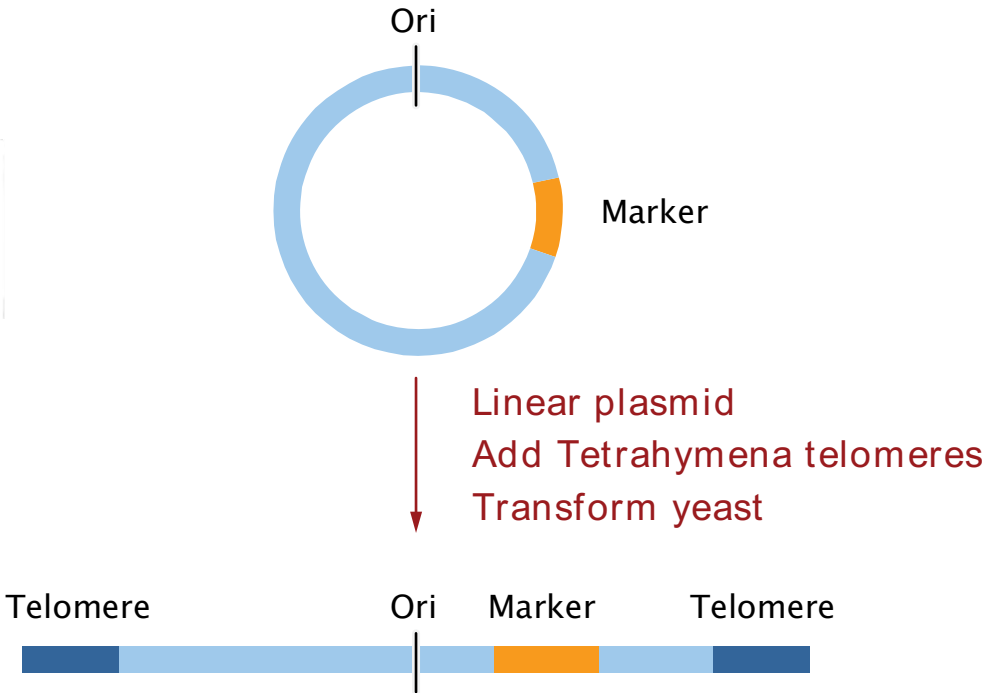
Carol W. Greider

Setting the stage: telomere function is conserved

Cell, Vol. 29, 245–255, May 1982, Copyright © 1982 by MIT

Cloning Yeast Telomeres on Linear Plasmid Vectors

Jack W. Szostak
Sidney Farber Cancer Institute
and Department of Biological Chemistry
Harvard Medical School
Boston, Massachusetts 02115
Elizabeth H. Blackburn
Department of Molecular Biology
University of California
Berkeley, California 94720



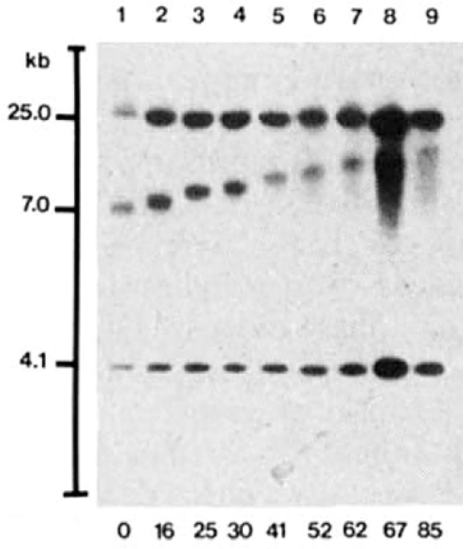
Cell May 1982

Setting the stage: telomere elongation in trypanosomes

Growth of chromosome ends in multiplying trypanosomes

André Bernards*, Paul A. M. Michels*, Carsten R. Lincke & Piet Borst*

Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, PO Box 60.000, 1005 GA Amsterdam, The Netherlands



Nature June 1983

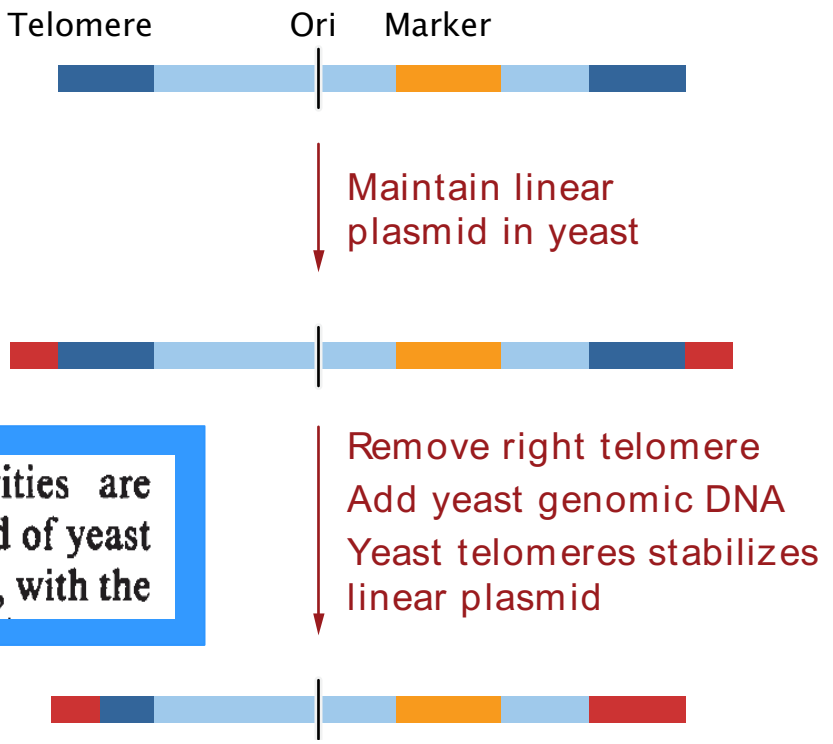
Setting the stage: Yeast sequences added onto Tetrahymena telomeres

DNA sequences of telomeres maintained in yeast

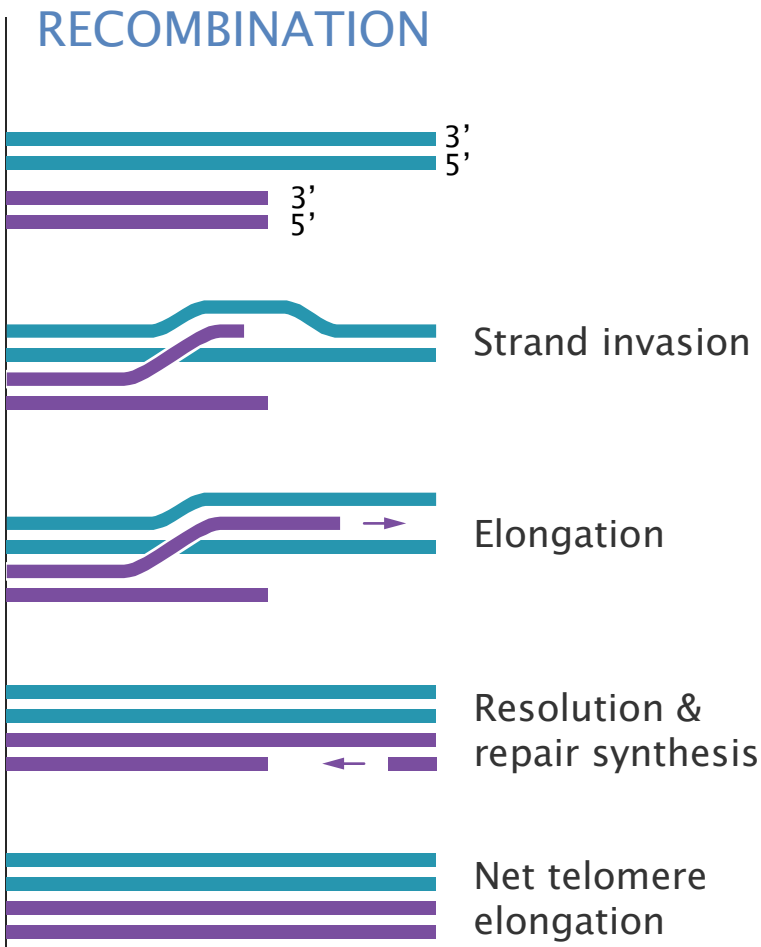
Janis Shampay*, Jack W. Szostak† & Elizabeth H. Blackburn*‡

* Department of Molecular Biology, University of California, Berkeley, California 94720, USA
† Dana-Farber Cancer Institute, and Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115, USA

We propose that terminal transferase-like activities are responsible for extending the 3′ end of the G+T strand of yeast telomeres. Such activities could add single nucleotides, with the



Two different models for elongation



The hunt for telomerase

Telomerase activity identified

Single stranded telomeric oligo-nucleotide primer

+

G ^{32}P-dGTP*

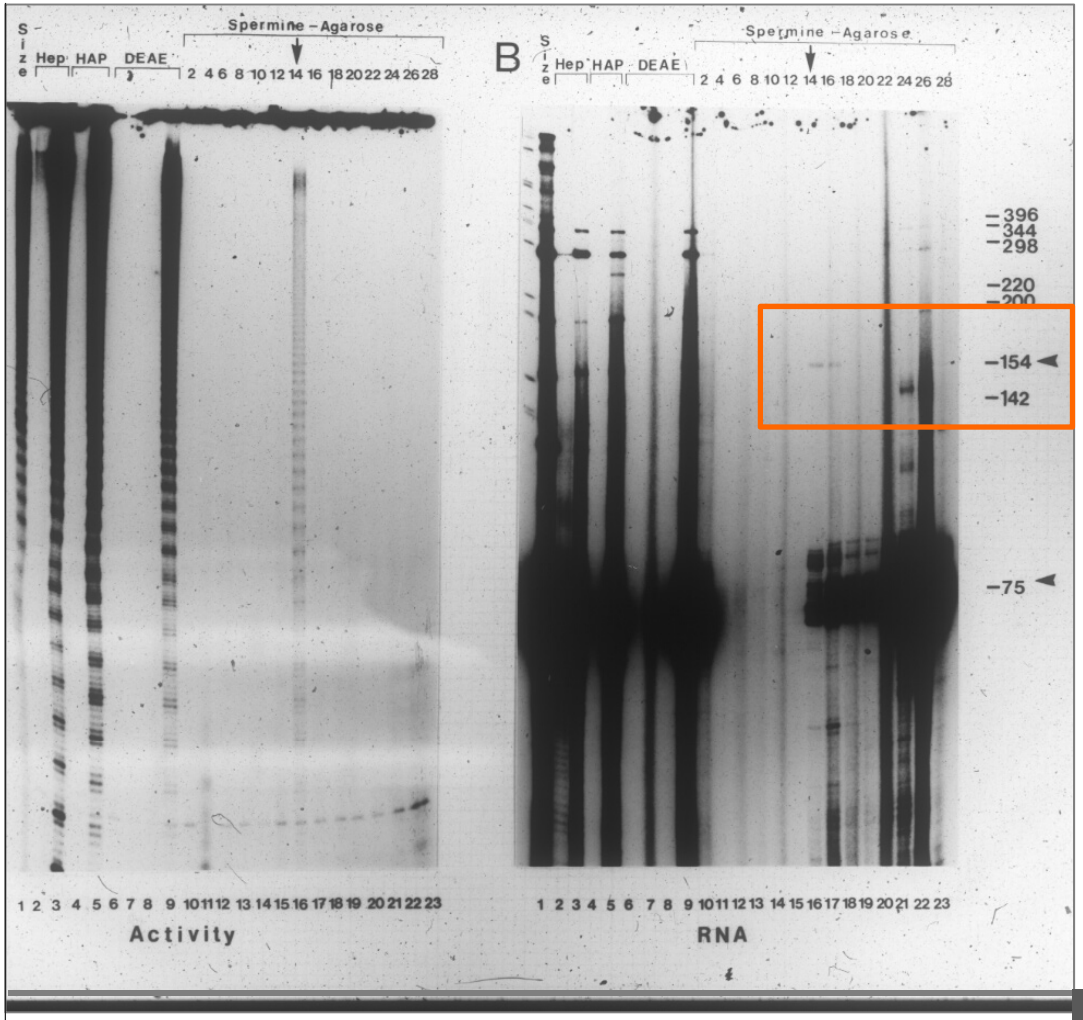
T dTTP

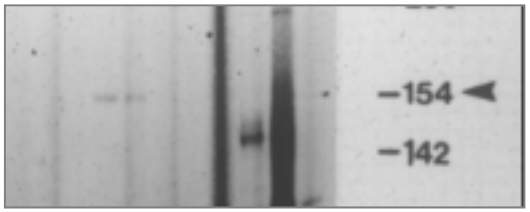
Telomerase

GGGGTTGGGGTTGGGGTTGGGGTTG

Greider & Blackburn *Cell* 1985

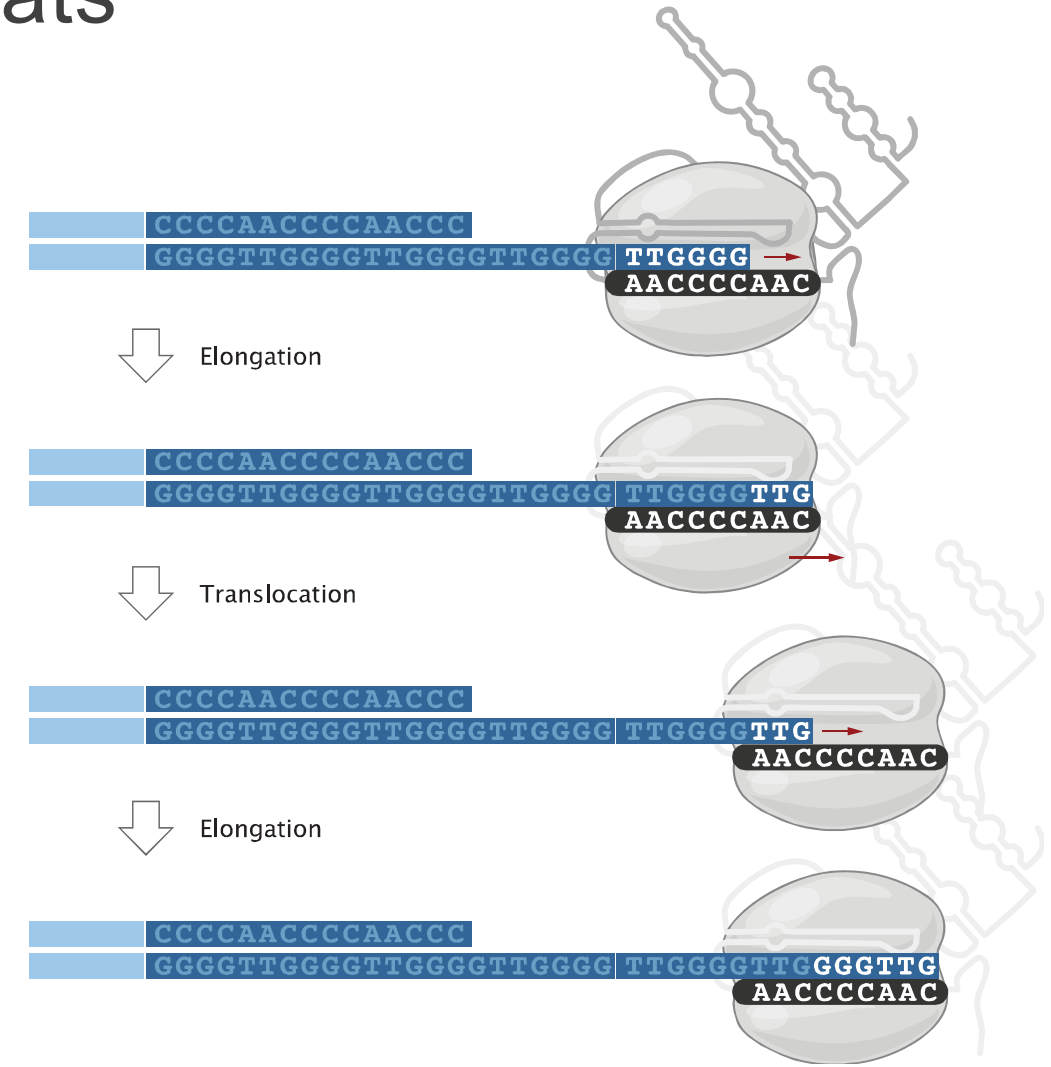
Identification of the RNA component





Greider & Blackburn *Cell* 1987

Telomerase uses RNA template to add TTGGGG repeats



Greider & Blackburn *Nature* 1989

What happens without telomerase?

Exp Cell Res. 1965 Mar;37:614-36.

THE LIMITED IN VITRO LIFETIME OF HUMAN DIPLOID CELL STRAINS.

HAYFLICK L.

A Theory of Marginotomy

The Incomplete Copying of Template Margin in Enzymic Synthesis of Polynucleotides and Biological Significance of the Phenomenon†

A. M. OLOVNIKOV

Laboratory of Chemistry and Synthesis of Antibodies, Gamaleya Institute for Epidemiology and Microbiology, Moscow, U.S.S.R.

J. theor. Biol. (1973) **41,** 181–190

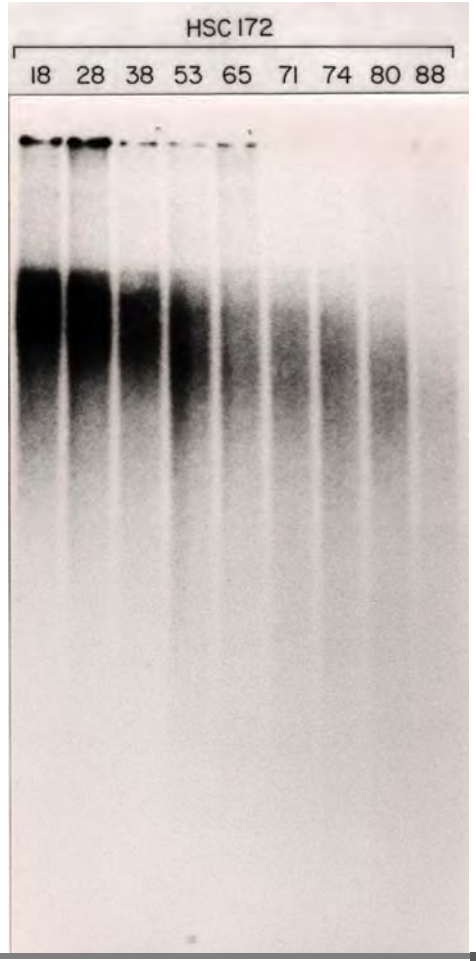
Greider & Harley 1988

Telomere shortening in cellular senescence

Telomeres shorten during ageing of human fibroblasts

Calvin B. Harley*, A. Bruce Futcher† & Carol W. Greider†

* Department of Biochemistry, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada
† Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA



Nature May 1990

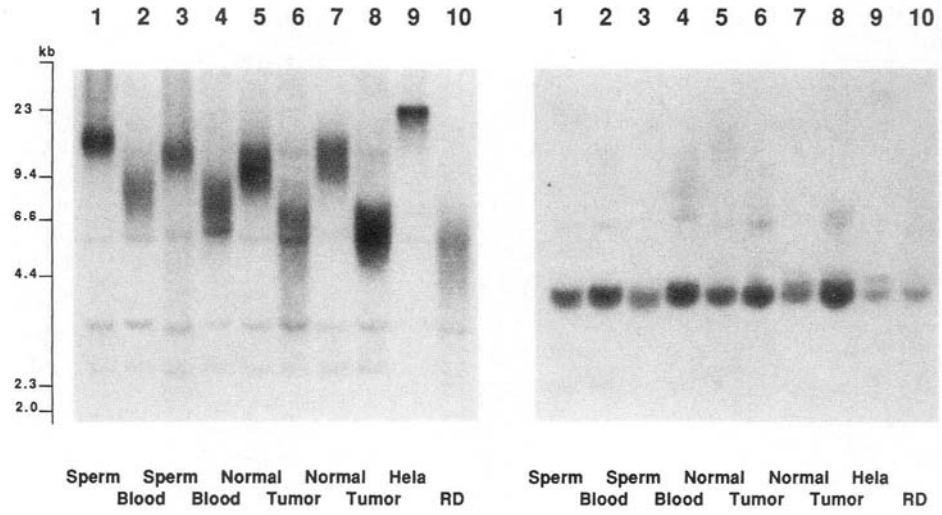
Telomeres are shorter in cancer cells

Structure and Variability of Human Chromosome Ends

TITIA DE LANGE,[1†*] LILY SHIUE,[1] RICHARD M. MYERS[2,3] DAVID R. COX,[2,4] SUSAN L. NAYLOR,[5] ANN M. KILLERY,[6] AND HAROLD E. VARMUS[1,2]

Departments of Microbiology and Immunology,[1] Biochemistry and Biophysics,[2] Physiology,[3] and Psychiatry,[4] University of California, San Francisco, California 94143; Department of Cellular and Structural Biology, University of Texas, San Antonio, Texas 78285[5]; and Department of Laboratory Medicine, M. D. Anderson Tumor Institute, Houston, Texas 77030[6]

Received 21 August 1989/Accepted 17 October 1989

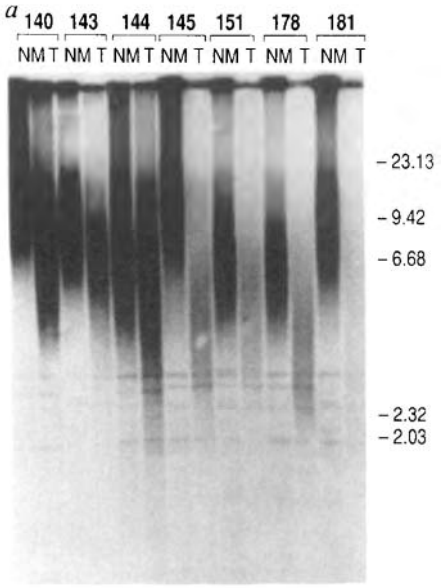


Mol Cell Biol. 1990

Telomere reduction in human colorectal carcinoma and with ageing

Nicholas D. Hastie, Maureen Dempster, Malcolm G. Dunlop*, Alastair M. Thompson*, Daryll K. Green & Robin C. Allshire*

MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK



Nature 1990

Telomerase protein component identified

Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase

Joachim Lingner,* Timothy R. Hughes, Andrej Shevchenko, Matthias Mann,† Victoria Lundblad,† Thomas R. Cech†

Telomerase is a ribonucleoprotein enzyme essential for the replication of chromosome termini in most eukaryotes. Telomerase RNA components have been identified from many organisms, but no protein component has been demonstrated to catalyze telomeric DNA extension. Telomerase was purified from *Euplotes aediculatus*, a ciliated protozoan, and one of its proteins was partially sequenced by nanoelectrospray tandem mass spectrometry. Cloning and sequence analysis of the corresponding gene revealed that this 123-kilodalton protein (p123) contains reverse transcriptase motifs. A yeast (*Saccharomyces cerevisiae*) homolog was found and subsequently identified as *EST2* (ever shorter telomeres), deletion of which had independently been shown to produce telomere defects. Introduction of single amino acid substitutions within the reverse transcriptase motifs of Est2 protein led to telomere shortening and senescence in yeast, indicating that these motifs are important for catalysis of telomere elongation in vivo. In vitro telomeric DNA extension occurred with extracts from wild-type yeast but not from *est2* mutants or mutants deficient in telomerase RNA. Thus, the reverse transcriptase protein fold, previously known to be involved in retroviral replication and retrotransposition, is essential for normal chromosome telomere replication in diverse eukaryotes.

Replication of chromosome ends, or telomeres, requires specialized factors that are not essential for replication of internal chromosome sequences. Conventional DNA polymerases cannot fully replicate blunt-ended DNA molecules (*1*) or eukaryotic chromosomes (*2*), which contain 3′-terminal extensions. The key to end replication is telomerase, a ribonucleoprotein

in telomere replication, their specific functions remain unclear. Neither protein has been reported to be essential for telomere synthesis, and neither has significant simi-

larity to known polymerases or reverse transcriptases (*11*).

Telomerase TNP has also been purified from *Euplotes aediculatus*, a hypotrichous ciliate only distantly related to *Tetrahymena* (*12*). The hypotrichs present a special opportunity for telomere studies because their macronuclei contain millions of gene-sized DNA molecules. Each cell has about 8×10^6 telomeres (*13*) and about 3×10^7 molecules of telomerase (*12*). Measurements of the specific activity of telomerase throughout the purification indicated that the major activity present in macronuclear extracts was purified (*12*). The active telomerase complex had a molecular mass of ~230 kD, corresponding to a 66-kD RNA subunit and two proteins of 123 kD and ~43 kD (*12*). Photocross-linking experiments implicated the larger protein in specific binding of the telomeric DNA substrate (*14*).

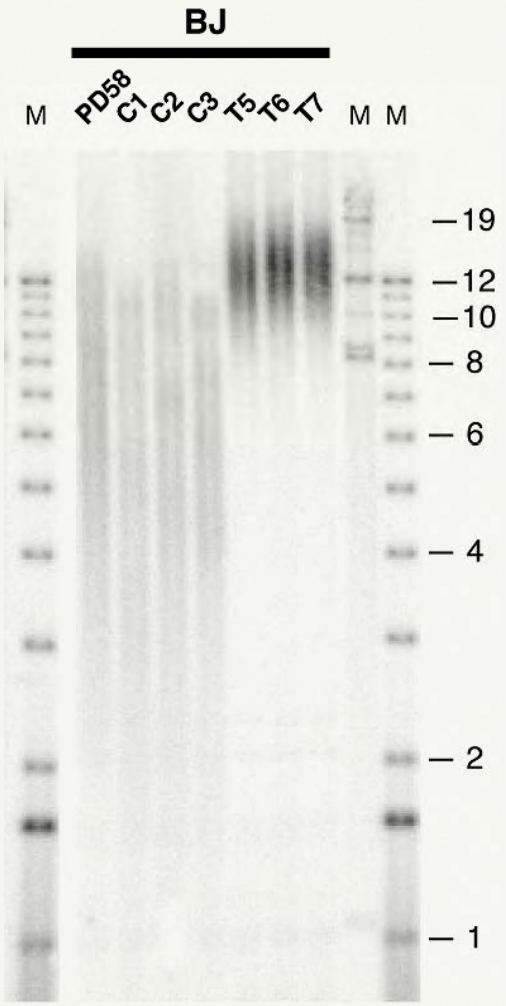
Here we characterize the p123 component of *Euplotes* telomerase and show that it contains sequence hallmarks of reverse transcriptases. Furthermore, it is the homolog of a yeast protein, Est2p, shown previously to function in telomere maintenance. Our genetic and biochemical analyses show that the reverse transcriptase motifs of Est2p are essential for telomeric DNA synthesis in vivo and in vitro. We propose that telomerase, frequently called "a specialized reverse transcriptase," is in fact a reverse tran-

Science 1997

TERT transfection extends the lifespan of human cells

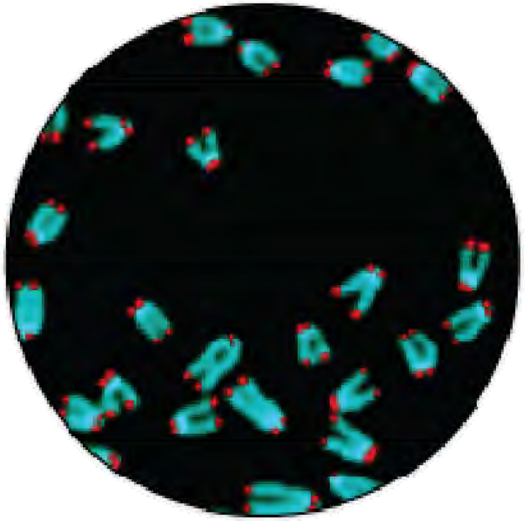
Extension of Life-Span by Introduction of Telomerase into Normal Human Cells

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†



Science 1998

Using genetics to examine the role of telomerase in mammals



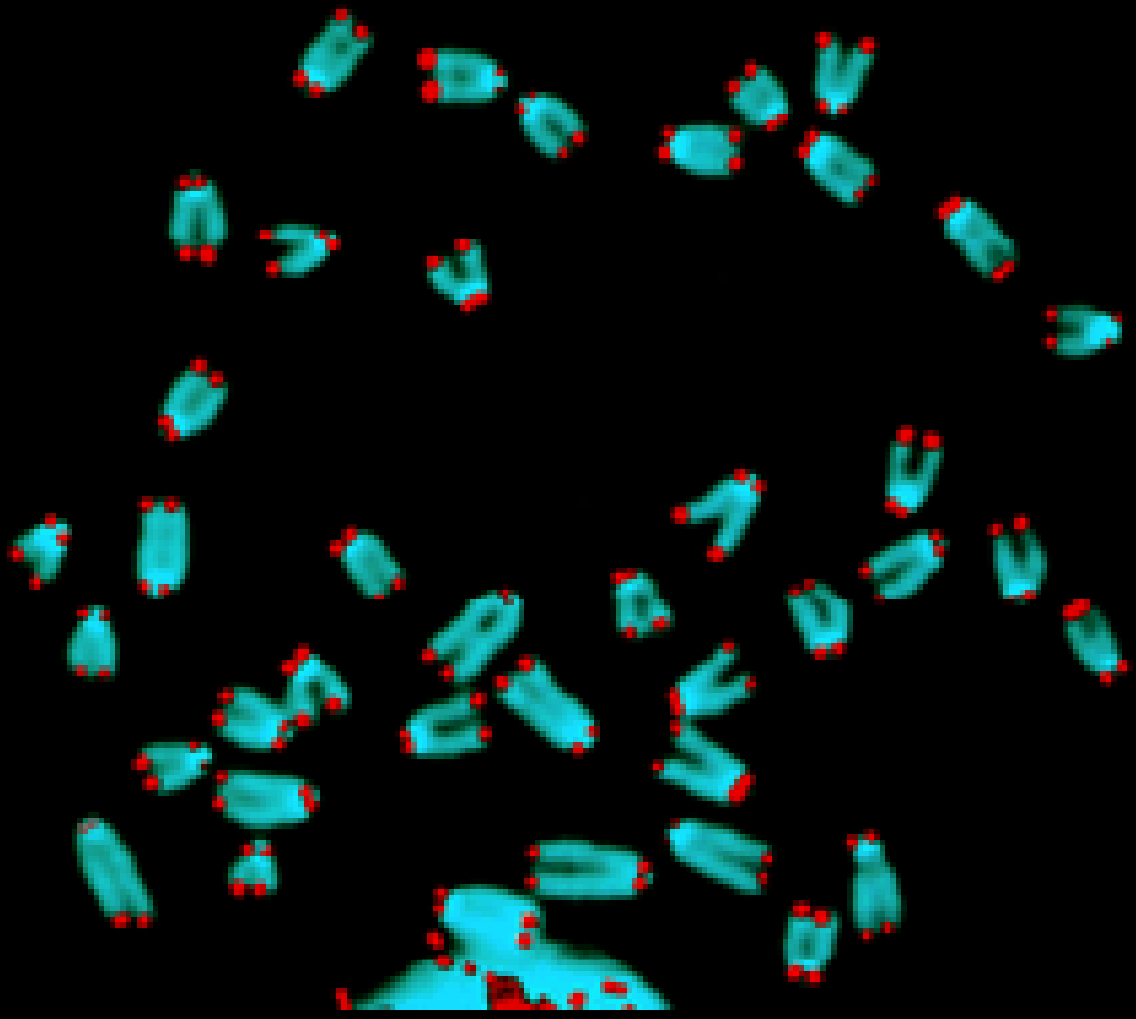
Maria Blasco

Telomerase knockout mouse

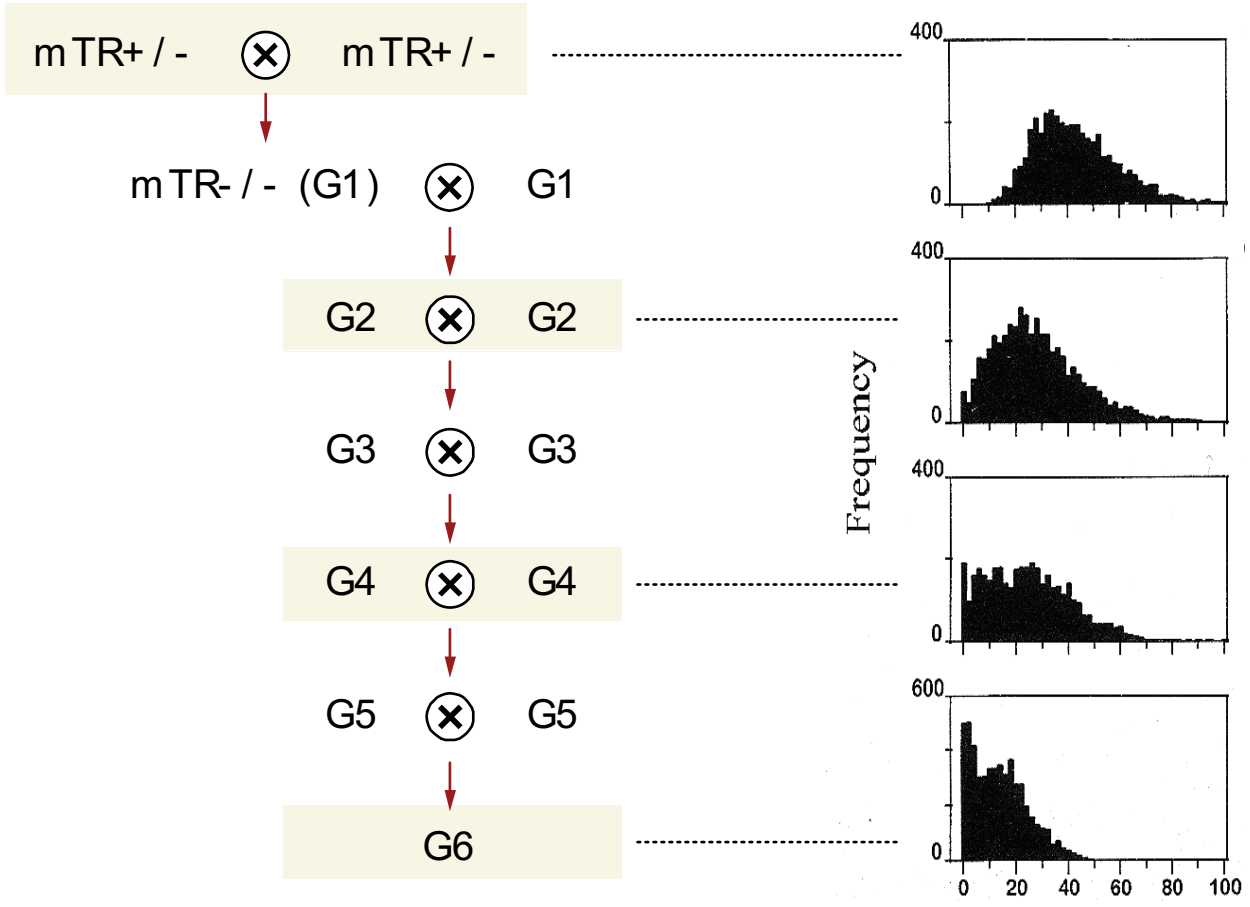
mTR+ / - ⊗ mTR+ / -

Blasco et al. *Cell* 1997

Quantiative analysis of telomere length

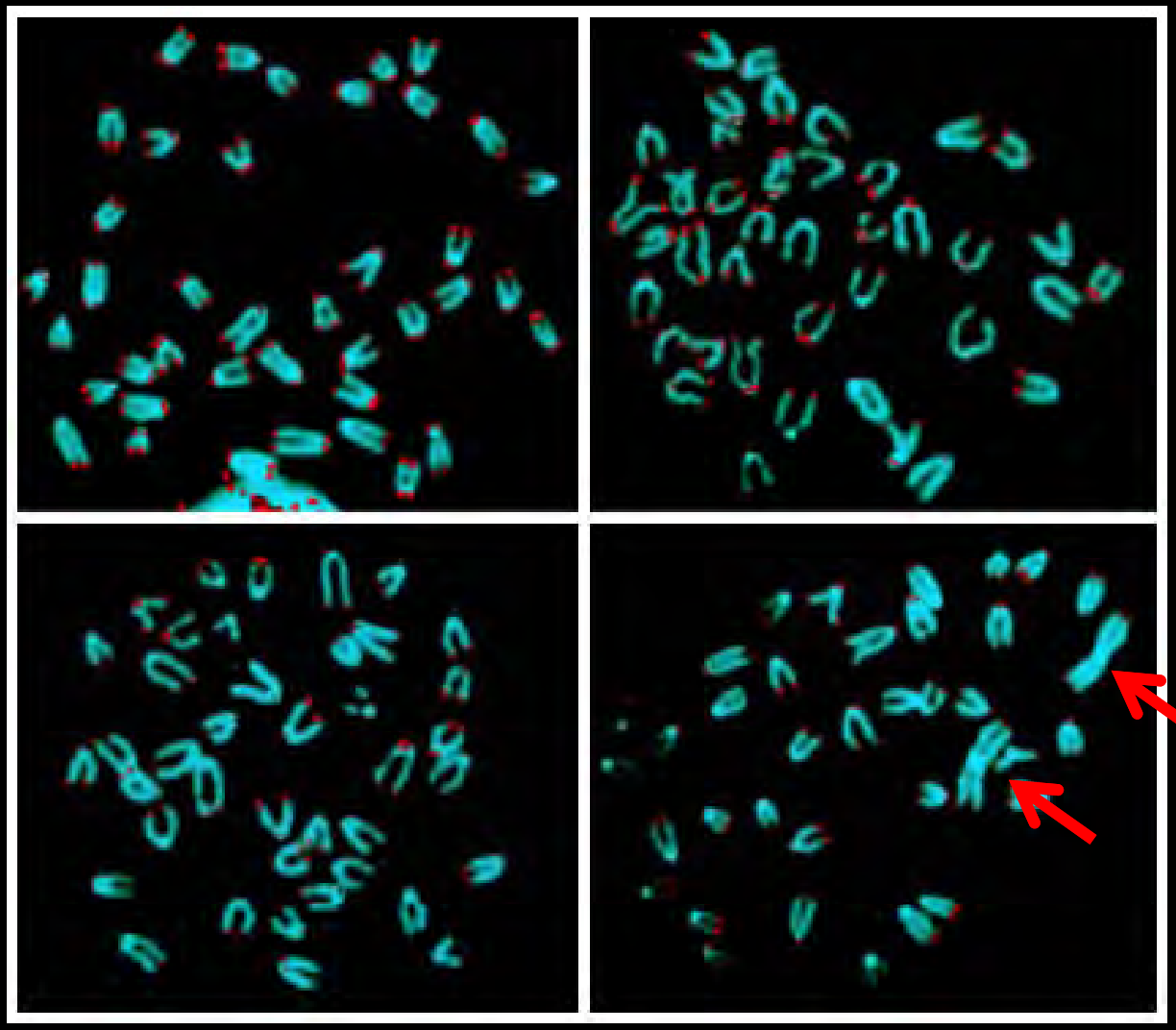


Telomeres shorten progressively in telomerase-null mice

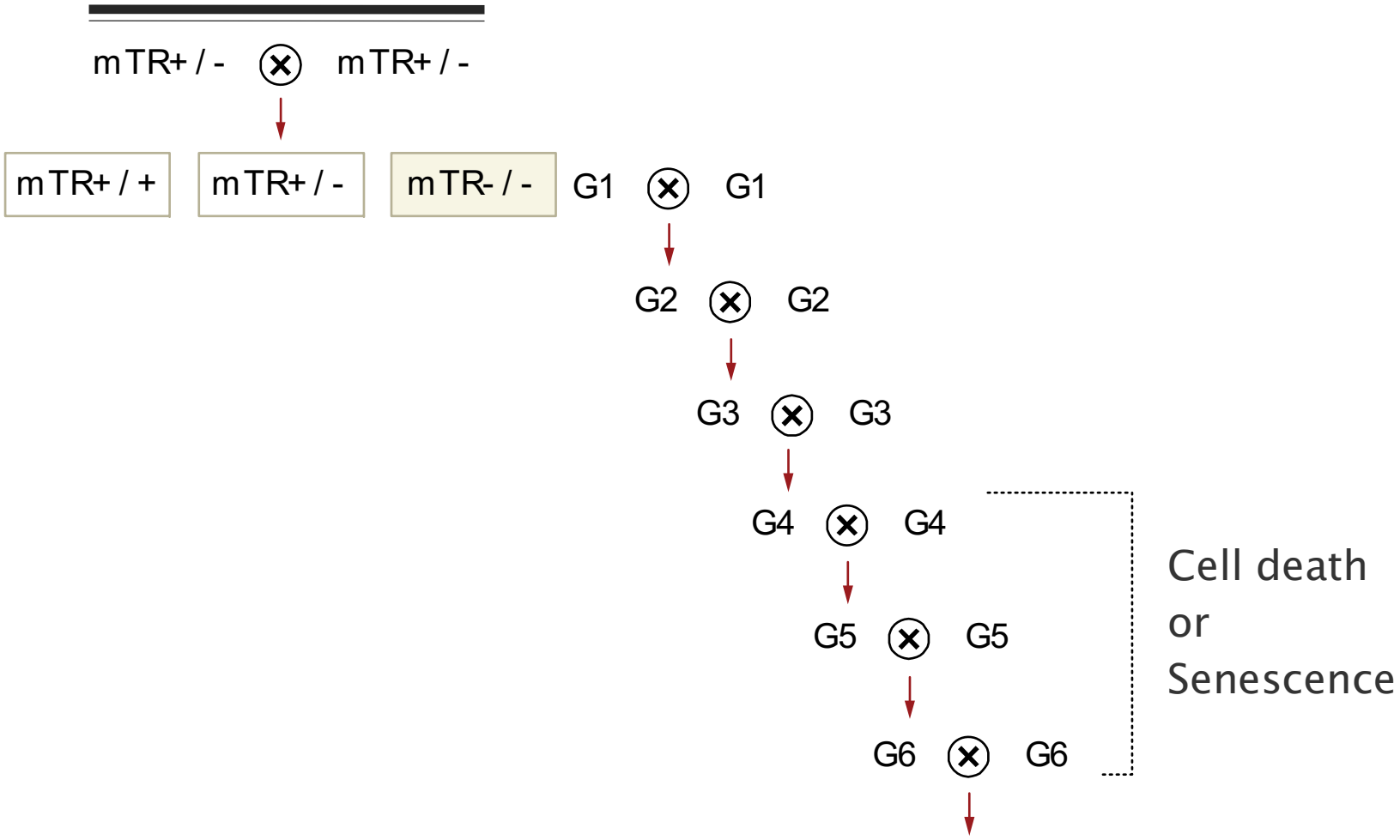


Blasco et al. *Cell* 1997

Loss of telomere sequence leads to loss of telomere function

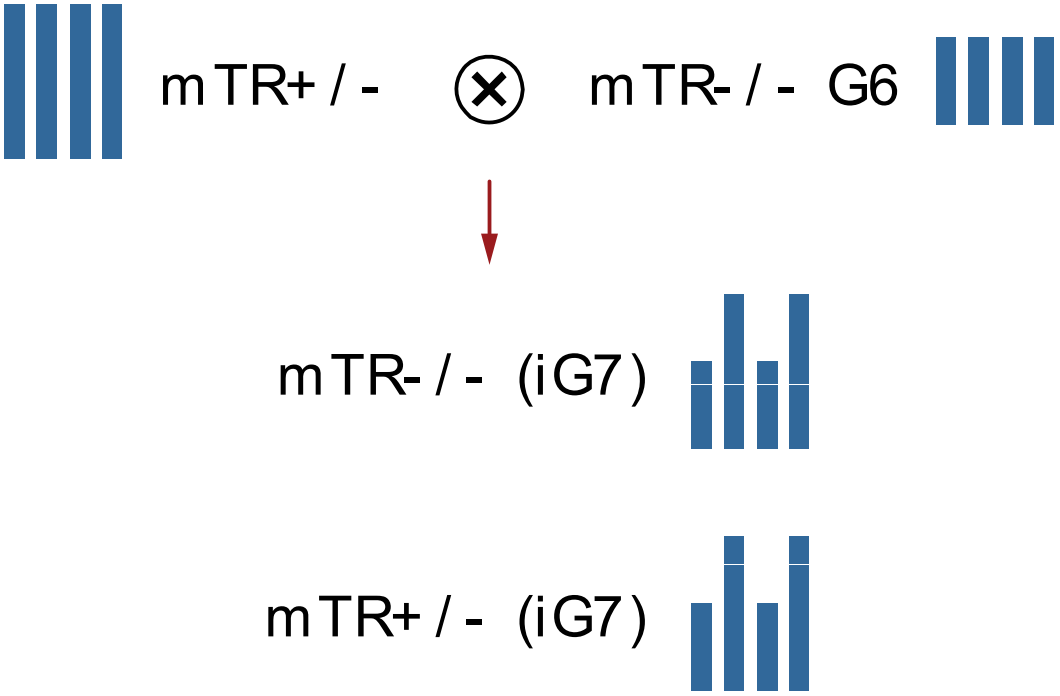


The consequences of short telomeres



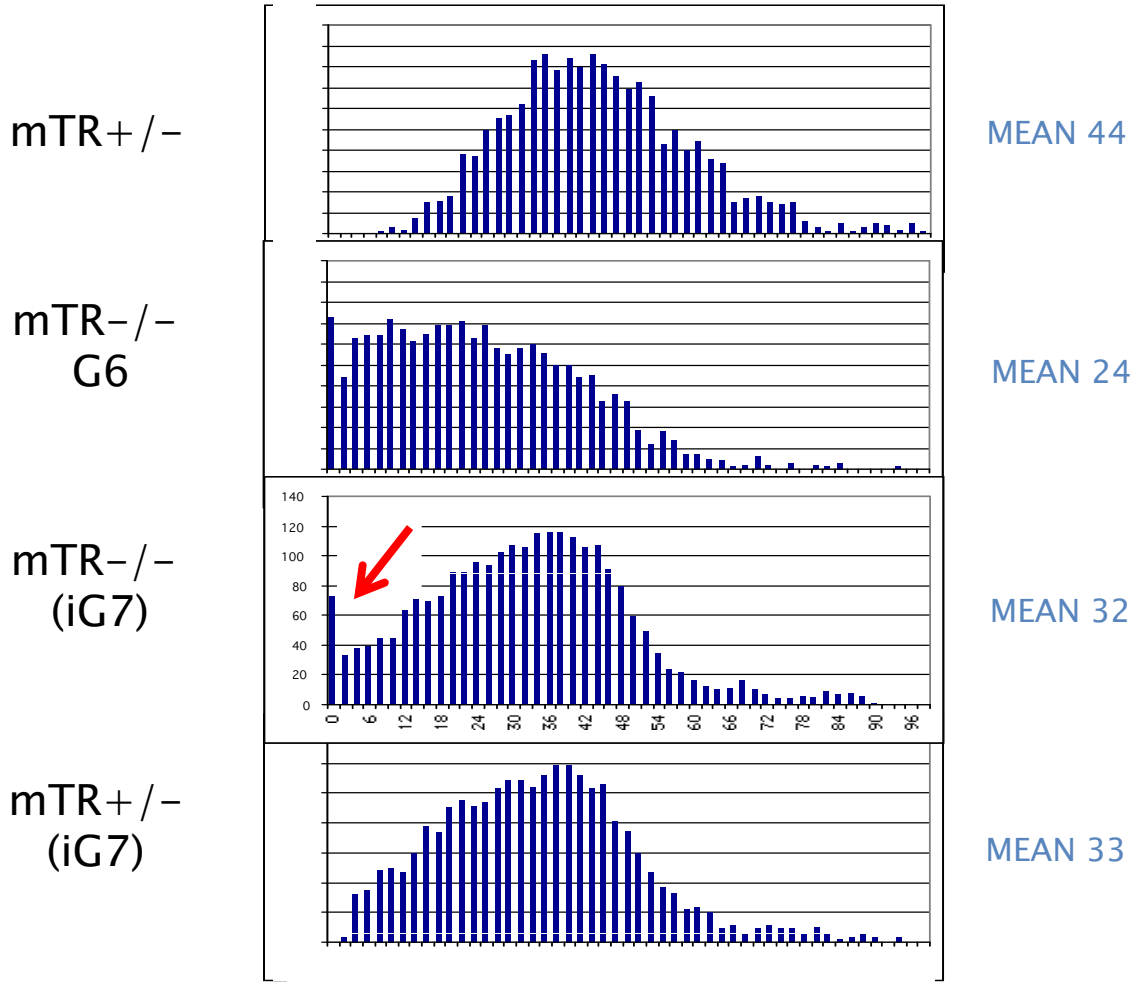
Mike Hemann

Mice with 1/2 short and 1/2 long telomeres show telomere dysfunction



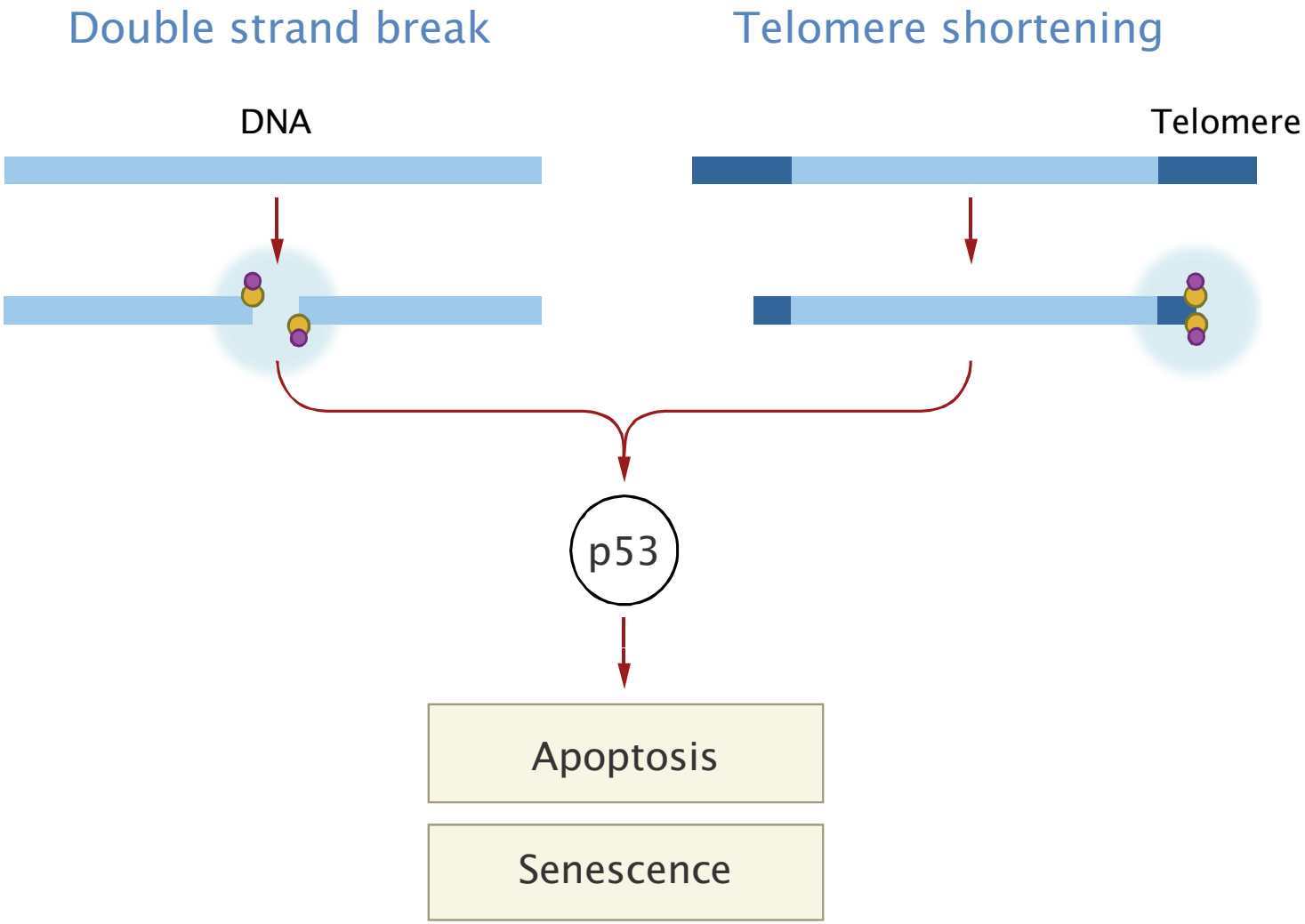
Hemann et al. *Cell* 2001

The shortest telomere triggers cell death



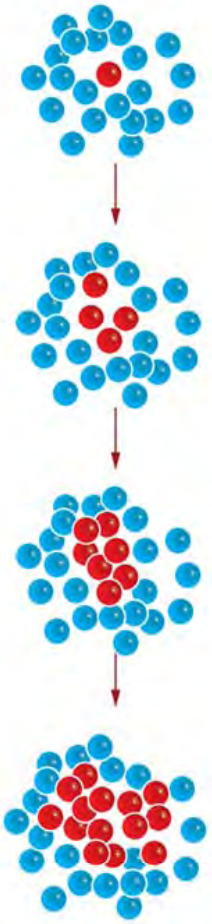
Hemann et al. *Cell* 2001

Short telomeres induce a DNA damage response



Telomerase is required for cells that must divide many times

CANCER CELLS

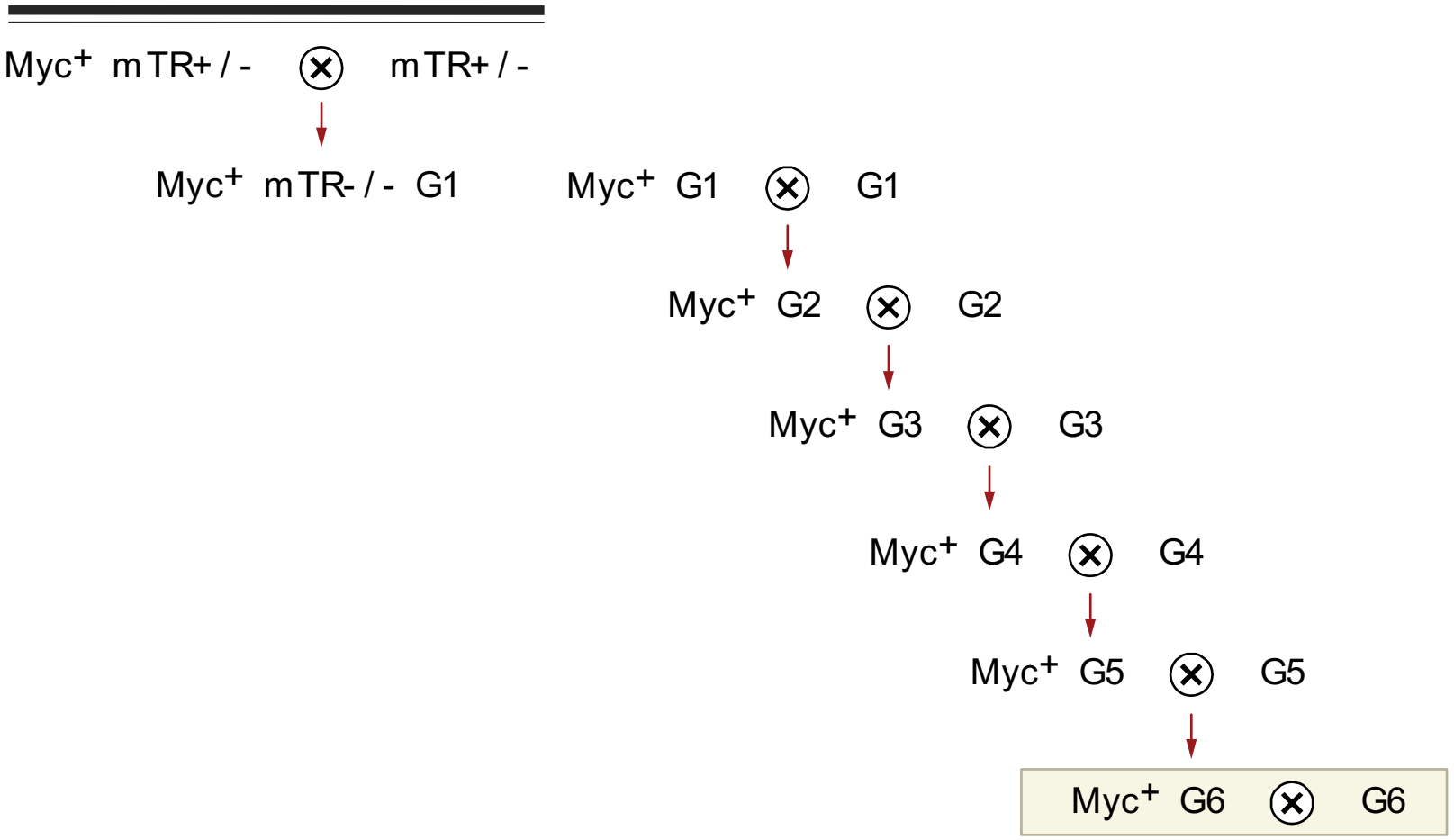


Short telomeres supress tumorigenesis

Cancer model	Effect of short telomeres	Reference
Squameous cell carcinoma (DMBA)	⇩ Decrease	Gonzalez-Suarez et al. *Nat Gen*. 2000
Colon cancer (APCmin)	⇩ Decrease (apoptosis)	Rudolph et al. *Nat Gen.* 2001
Lymphoma& fibrosarcoma (INK4a/Arf -/-)	⇩ Decrease	Greenberg et al. *Cell* 1999
T cell lymphoma (ATM-/-)	⇩ Decrease (apoptosis)	Qi et al. *Can. Res*. 2003 Wong et al. *Nature* 2003
Hepatocellular carcinoma (Alb-uPA-/-)	⇩ Decrease	Farazi et al. *Can. Res*. 2003
Soft tissue sarcoma (p53-/-)	⇧ Increase (loss of apoptosis)	Artandi et al. *Nature* 2000

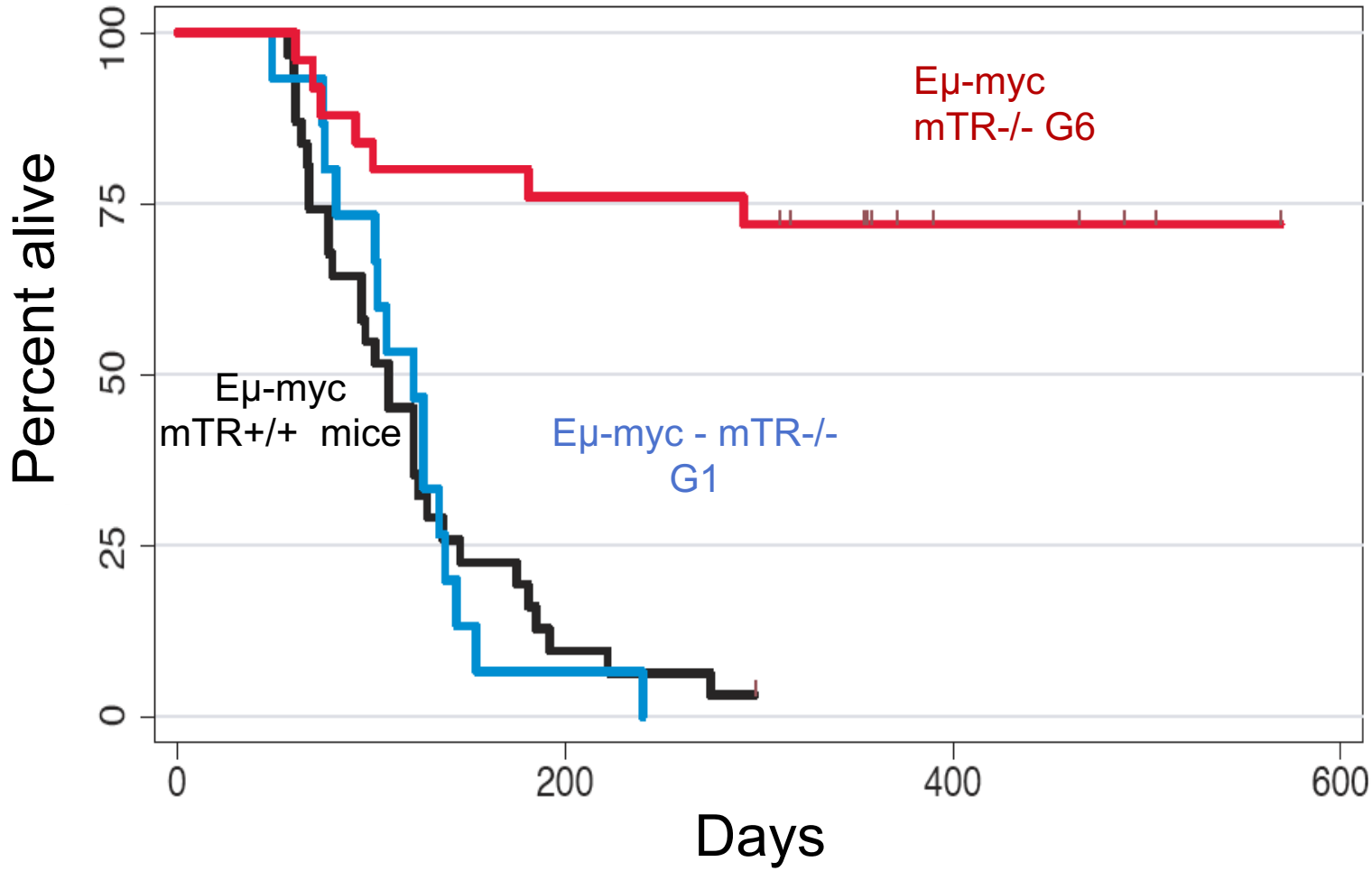
David Feldser

Eμ-Myc induced B-cell lymphoma growth can be blocked by short telomeres



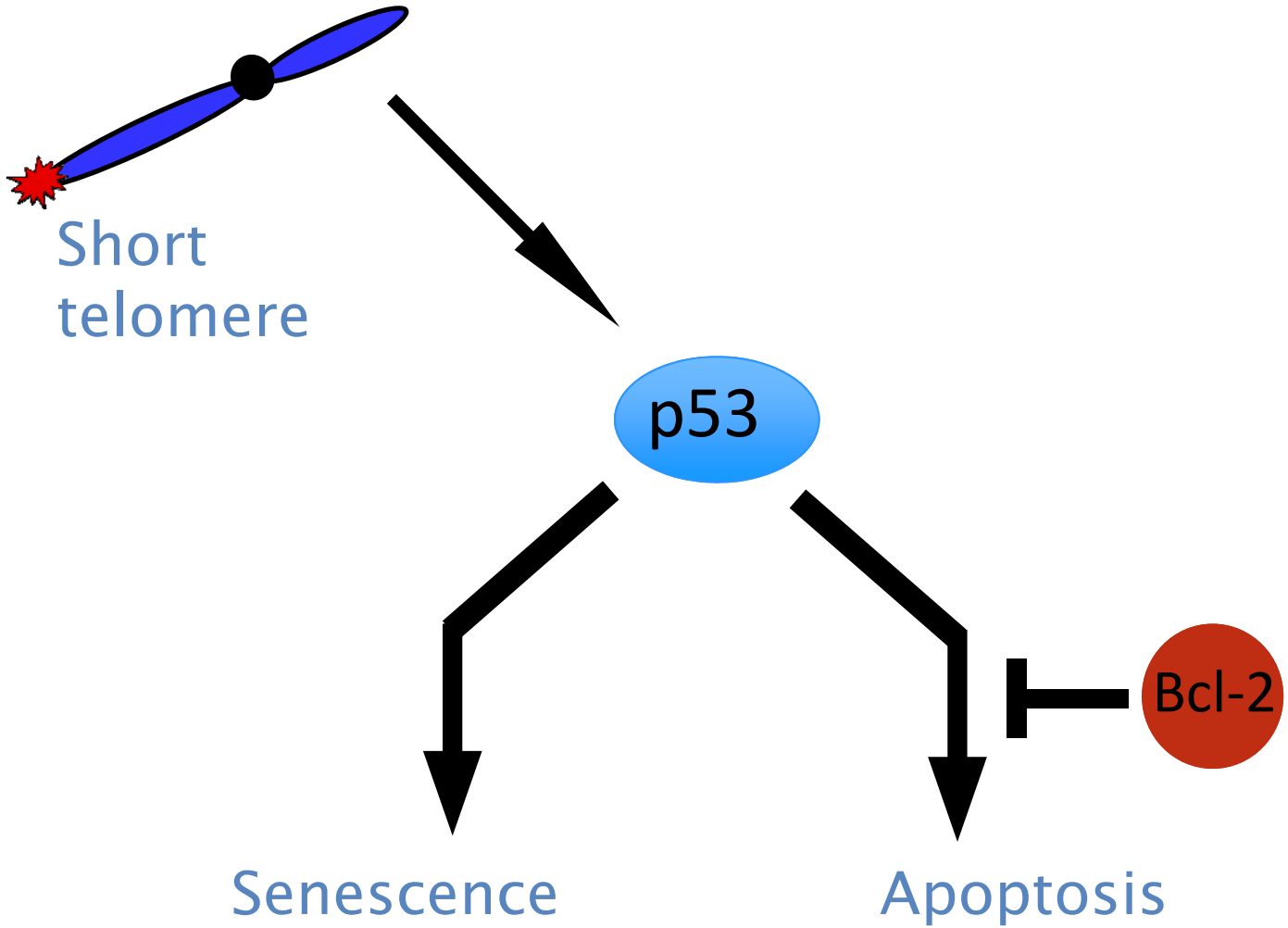
Feldser & Greider *Cancer Cell* 2007

Short telomeres protect against B-cell lymphoma



Feldser & Greider *Cancer Cell* 2007

Both apoptosis and senescence block tumor growth



The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita

Tom Vulliamy*, Anna Marrone*, Frederick Goldman†, Andrew Dearlove‡, Monica Bessler§, Philip J. Mason* & Inderjeet Dokal*

Nature 2001

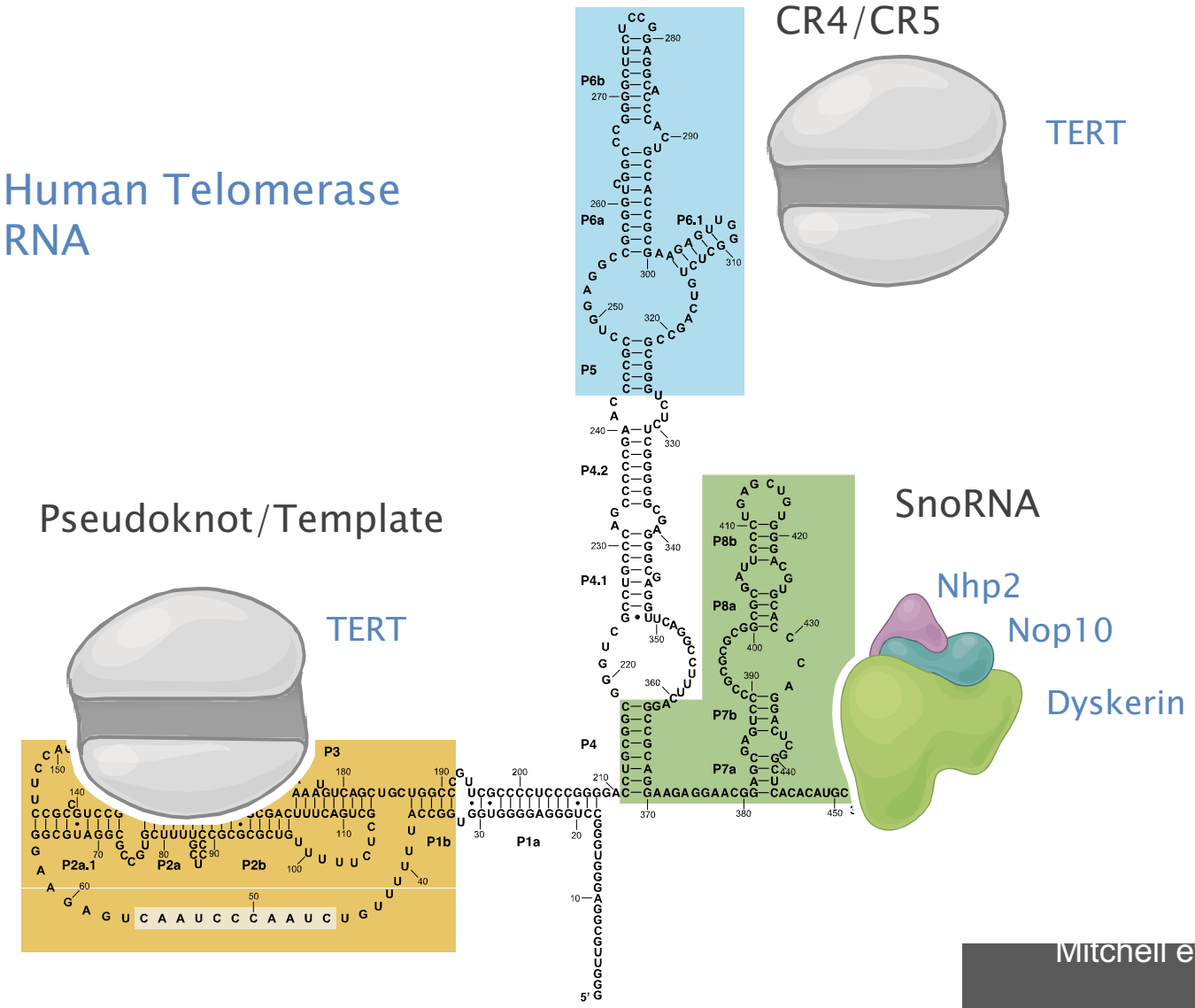
Clinical features of dyskeratosis congentia

- Dermatologic criteria
  - Skin hyperpigmentation
  - Oral leukoplakia
  - Nail dystrophy

- Mortality
  - **Aplastic anemia**
  - Pulmonary fibrosis
  - Cancer

Julian Chen

Telomerase RNA secondary structure

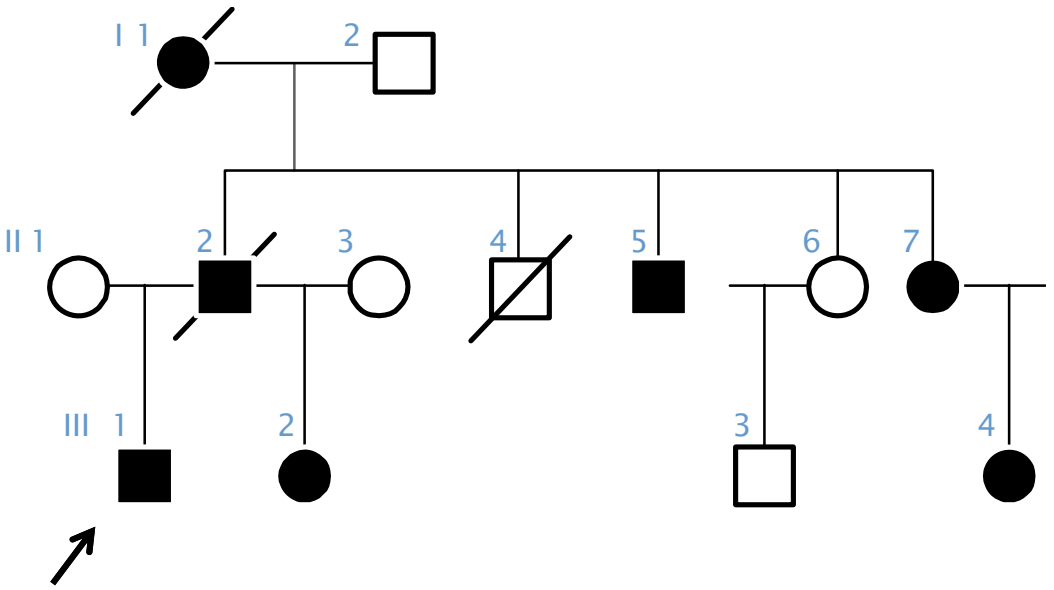


Mitchell et al. *MCB* 1999
Chen et al. *Cell* 2000

TERT mutations cause autosomal dominant inheritance of dyskeratosis congentia

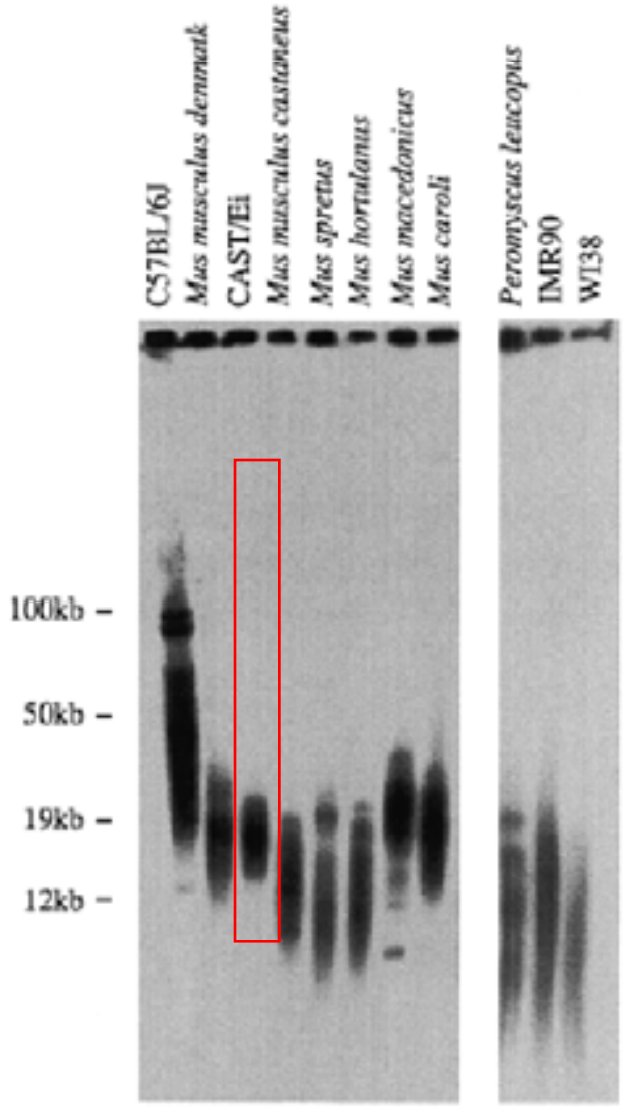
- Genetic anticipation.
- Affected people are heterozygous for hTR or hTERT mutations.
- Dominant negative or haploinsufficiency?

Hopkins Family 1



Armanios et al. *PNAS* (2005)

Cast/Ei mice have short telomeres similar to humans

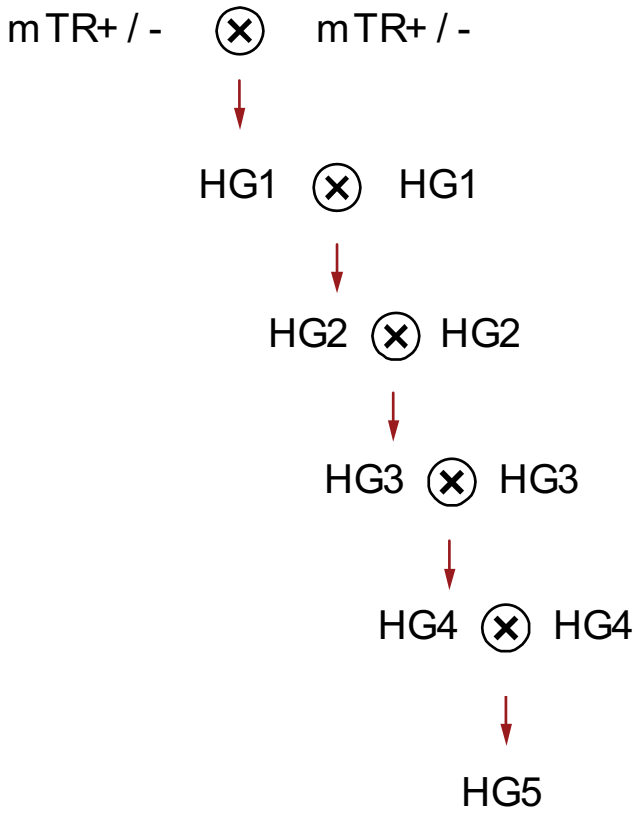


- Bred mTR null allele onto Cast/Ei background

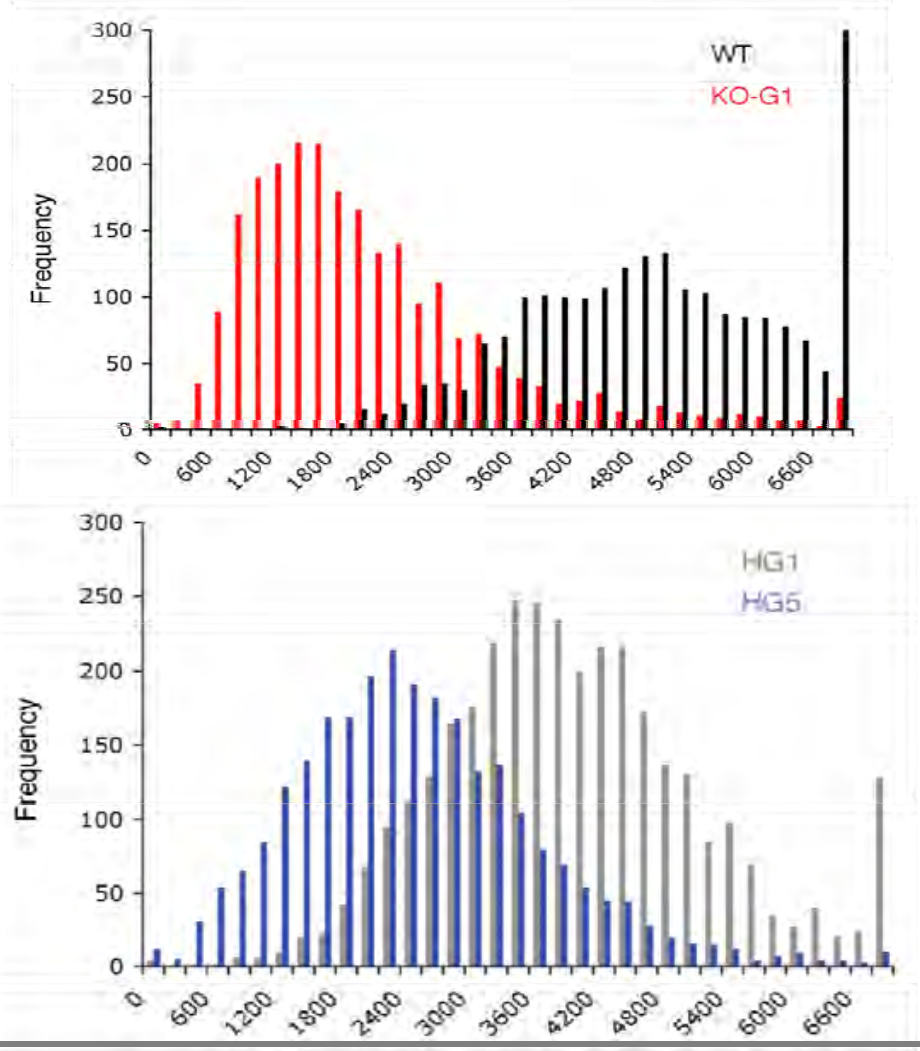
Hemann & Greider NAR 2000

Ling-Yang Hao

Progressive telomere shortening during mTR+/- breeding

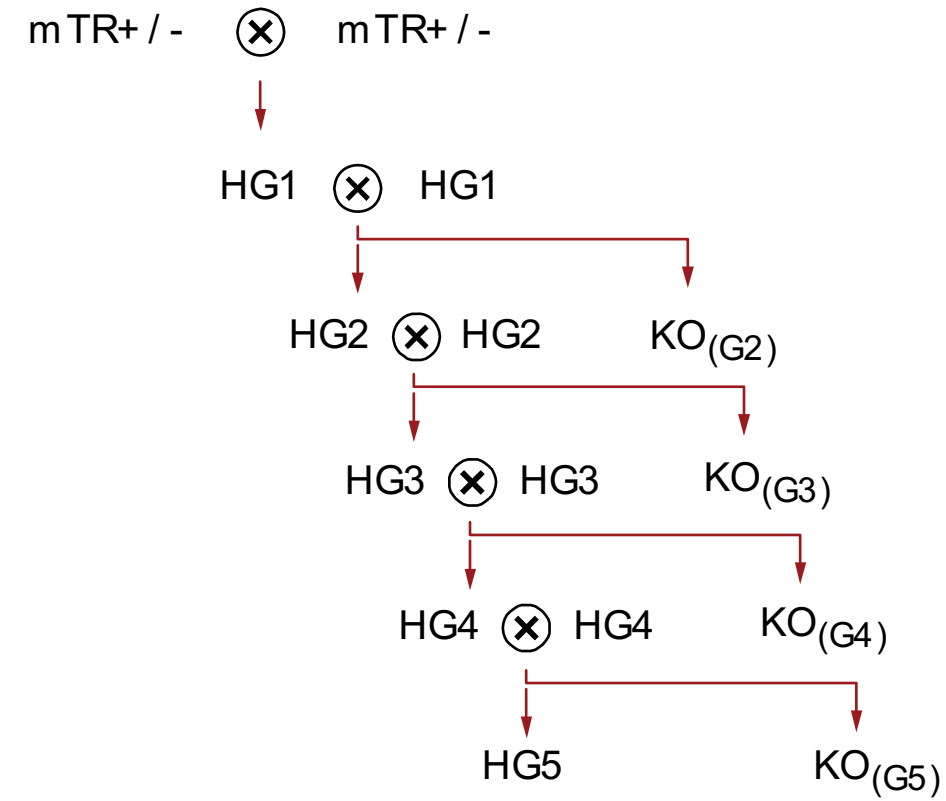


Short telomeres in CAST/EiJ mTR+/- mice



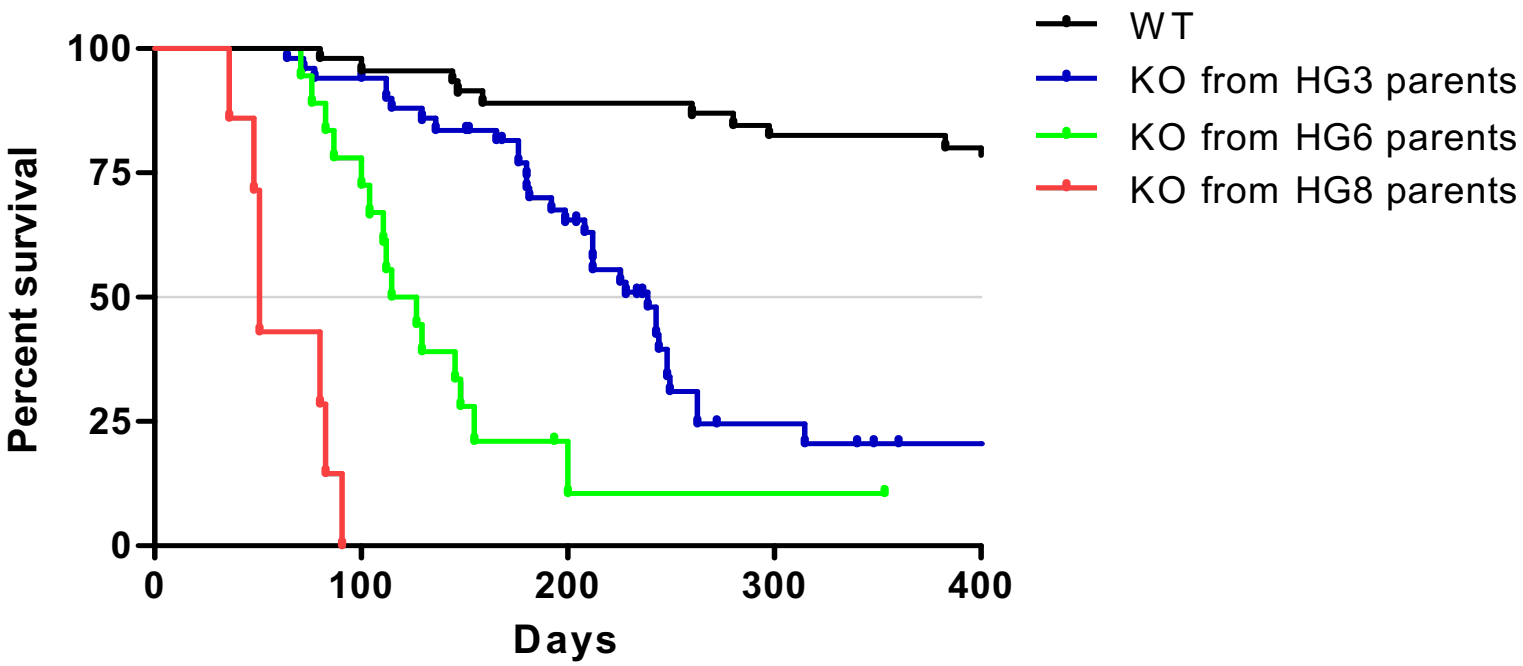
Hao et al. *Cell* 2005

Inheritance of short telomeres decreases survival



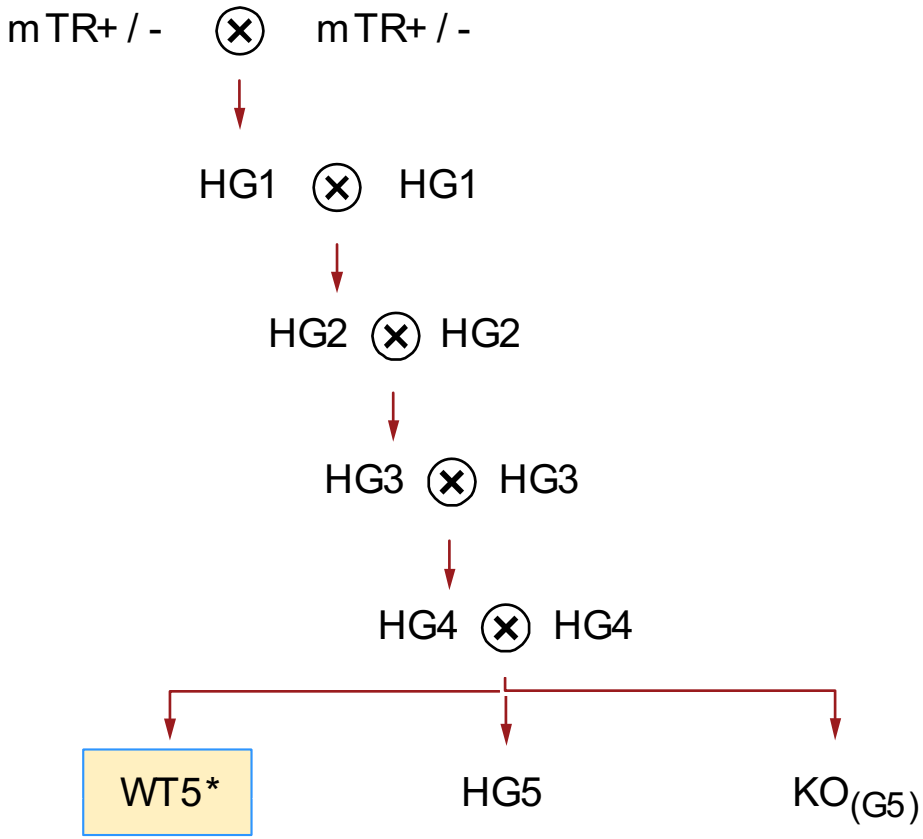
Margaret Strong

Decreased survival of mTR-/- from later generation heterozygous crosses

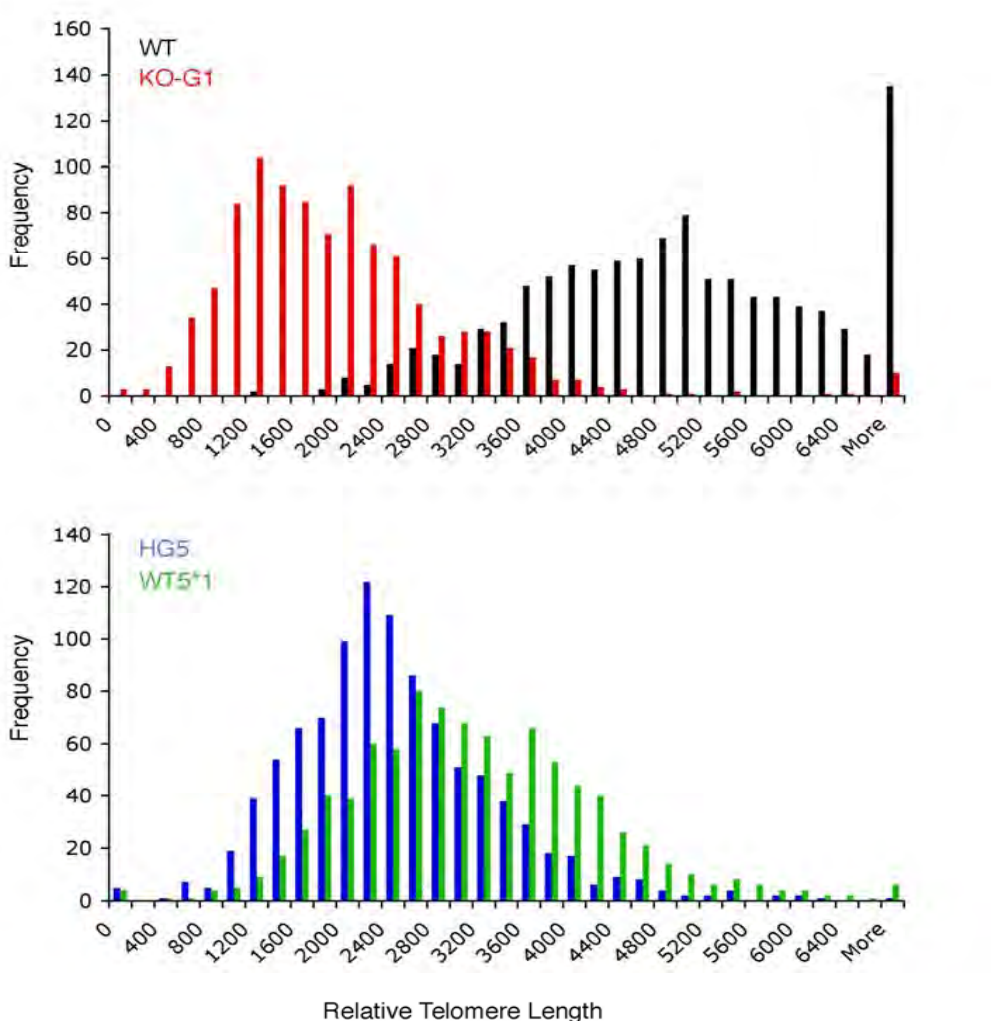


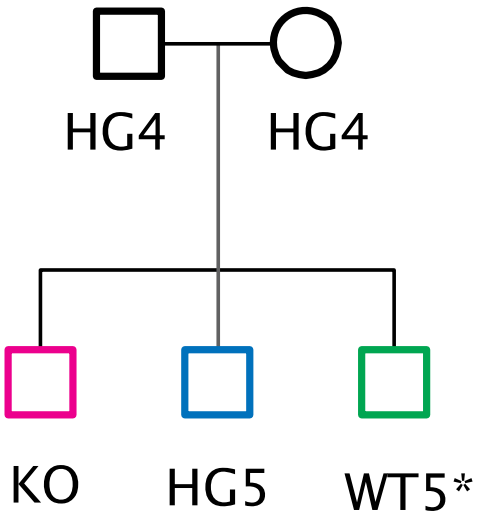
Inheritance of short telomeres decreases survival.
Resembles genetic anticipation in dyskeratosis congenita.

Telomere shortening during mTR+/- breeding affects wildtype offspring



Short telomeres in Wt5* mice

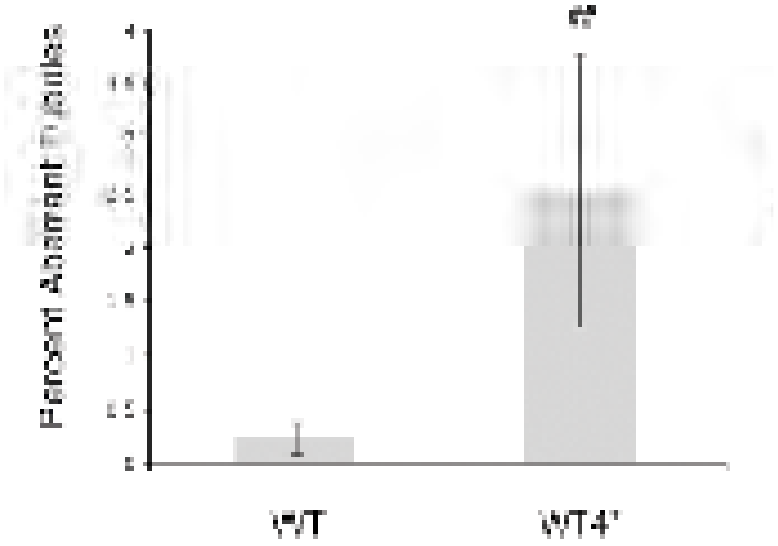




Hao et al. *Cell* 2005

Short telomeres cause phenotypes in Wt* mice

Testis Apoptosis

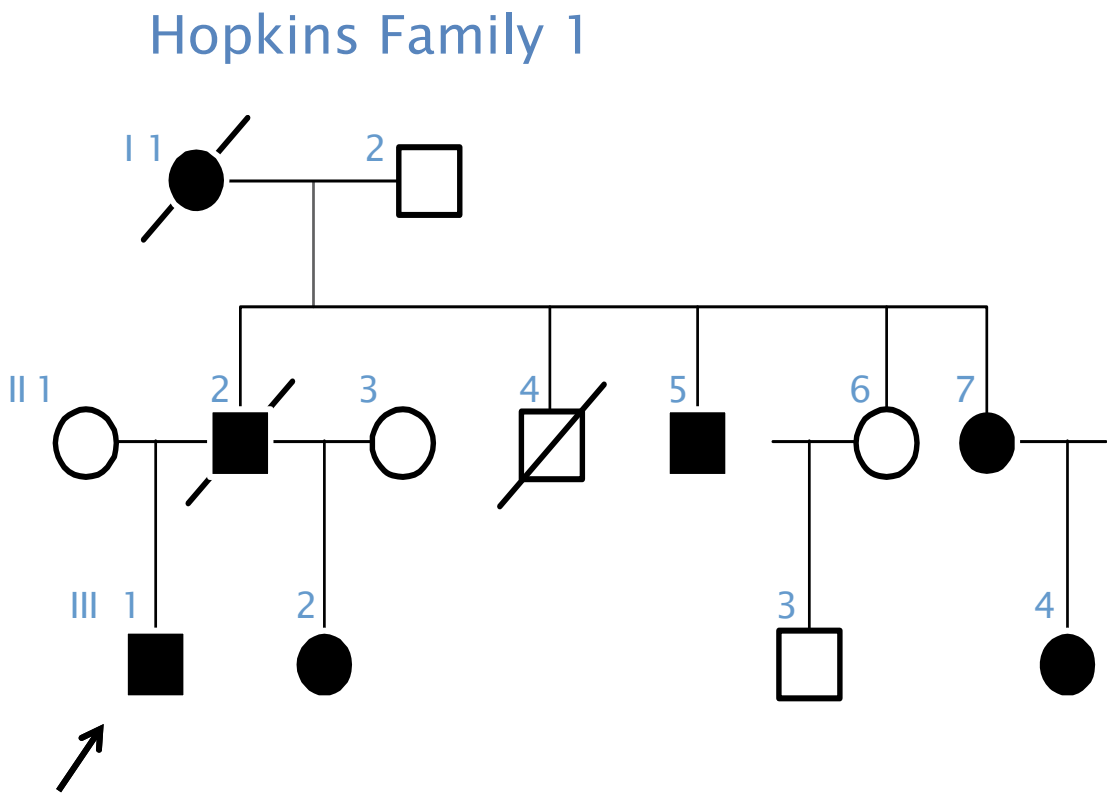


Genetic disease in the absence of telomerase mutation (Occult genetic disease)

Mary Armanios

Telomere shortening leads to genetic anticipation

- Genetic anticipation of pulmonary fibrosis.



Armanios et al. *PNAS* (2005)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Telomerase Mutations in Families with Idiopathic Pulmonary Fibrosis

Mary Y. Armanios, M.D., Julian J.-L. Chen, Ph.D., Joy D. Cogan, Ph.D., Jonathan K. Alder, B.A., Roxann G. Ingersoll, B.S., Cheryl Markin, B.S., William E. Lawson, M.D., Mingyi Xie, B.S., Irma Vulto, B.S., John A. Phillips III, M.D., Peter M. Lansdorp, M.D., Ph.D., Carol W. Greider, Ph.D., and James E. Loyd, M.D.

ABSTRACT

BACKGROUND

Idiopathic pulmonary fibrosis is progressive and often fatal; causes of familial clustering of the disease are unknown. Germ-line mutations in the genes *hTERT* and *hTR*, encoding telomerase reverse transcriptase and telomerase RNA, respectively, cause autosomal dominant dyskeratosis congenita, a rare hereditary disorder associated with premature death from aplastic anemia and pulmonary fibrosis.

METHODS

To test the hypothesis that familial idiopathic pulmonary fibrosis may be caused by short telomeres, we screened 73 probands from the Vanderbilt Familial Pulmonary Fibrosis Registry for mutations in *hTERT* and *hTR*.

RESULTS

Six probands (8%) had heterozygous mutations in *hTERT* or *hTR*; mutant telomerase resulted in short telomeres. Asymptomatic subjects with mutant telomerase also had

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Syndromes of Telomere Shortening

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Table 2 Spectrum of bone marrow, lung, and liver disease seen in individuals with syndromes of telomere shortening

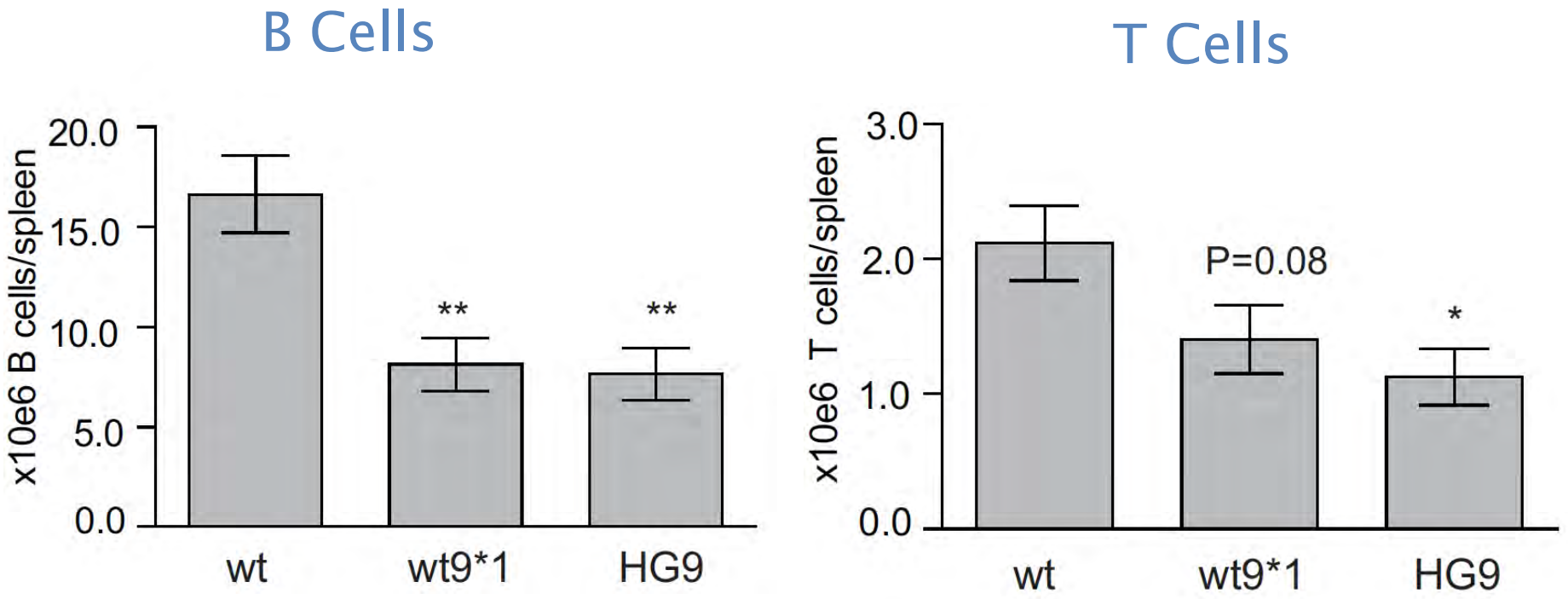
Hematologic features
Macrocytosis
Elevated hemoglobin F
Isolated cytopenias (most commonly thrombocytopenia)
Aplastic anemia
Myelodysplasia
Acute myeloid leukemia
Pulmonary fibrosis
Asymptomatic restrictive defects on pulmonary function studies
Idiopathic pulmonary fibrosis/usual interstitial pneumonia
Nonspecific interstitial pneumonia
Idiopathic interstitial pneumonia nonclassifiable on biopsy
Liver disease
Normal or mildly elevated transaminases
Atrophic nodular liver on imaging studies
Splenomegaly
Cryptogenic liver fibrosis/cirrhosis

Many phenotypes from telomere shortening syndromes share features of age related disease

- Bone marrow failure
- Immune senescence
- Chemotherapy intolerance
- Pulmonary fibrosis
- Liver disease
- Increased cancer incidence

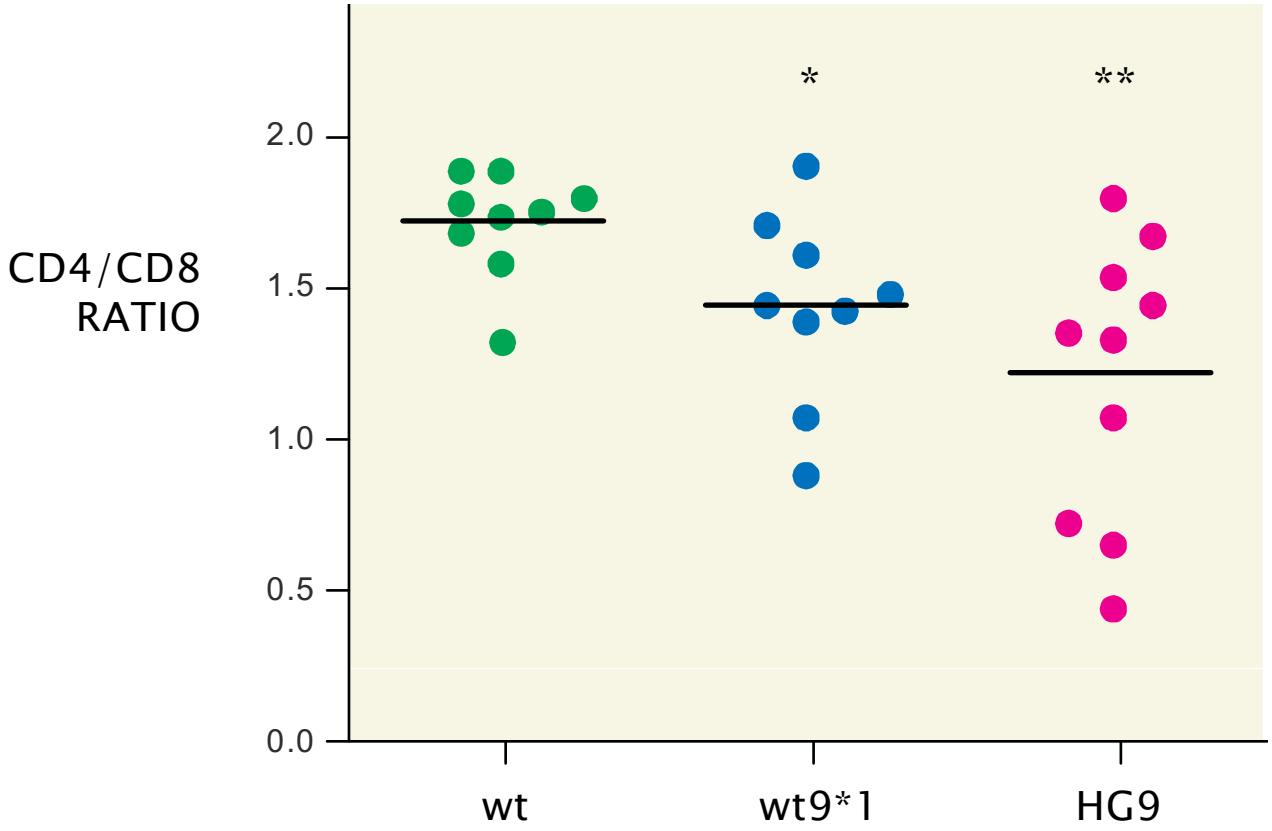
•Implies short telomeres may play a role in disease without mutation.

Wildtype mice with short telomeres show a decreased number of B and T lymphocytes



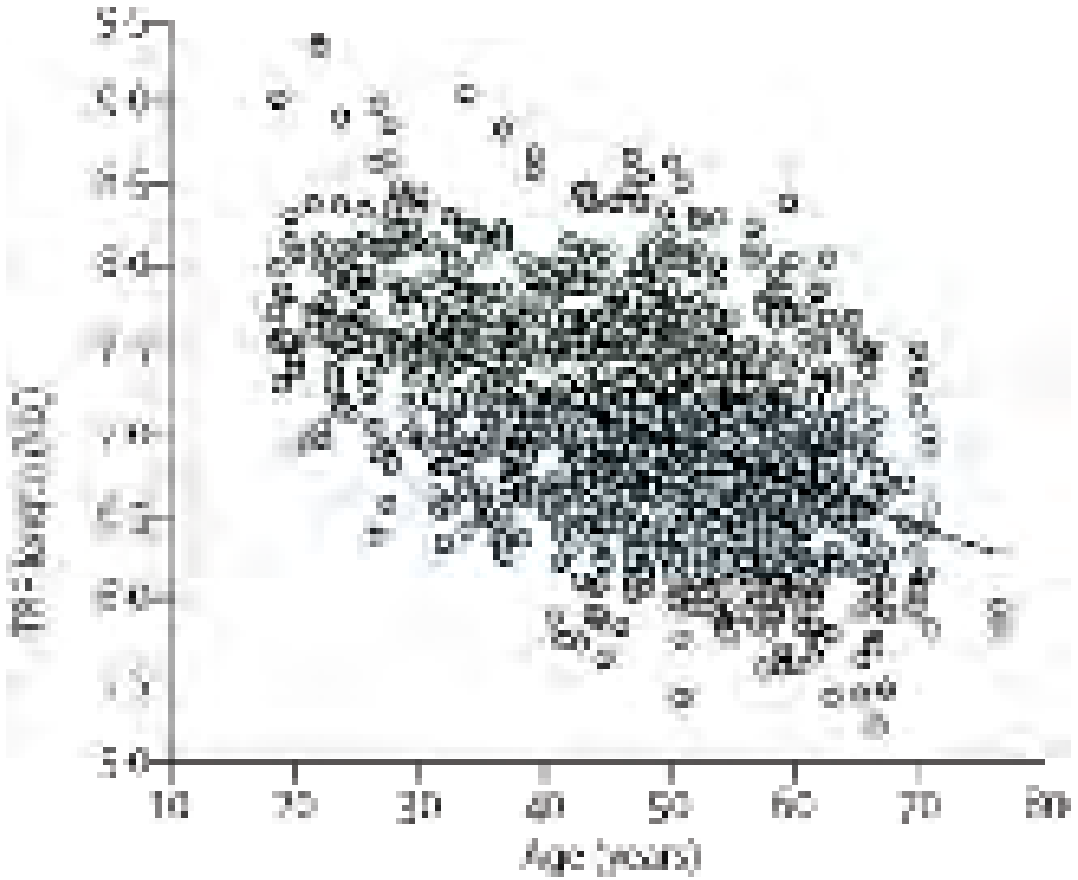
Armanios et al. *AJHG* 2009

Decreased CD4/CD8 ratio in mice with short telomeres is a hallmark of immune senescence



Armanios et al. *AJHG* 2009

Extensive telomere length heterogeneity in the human population

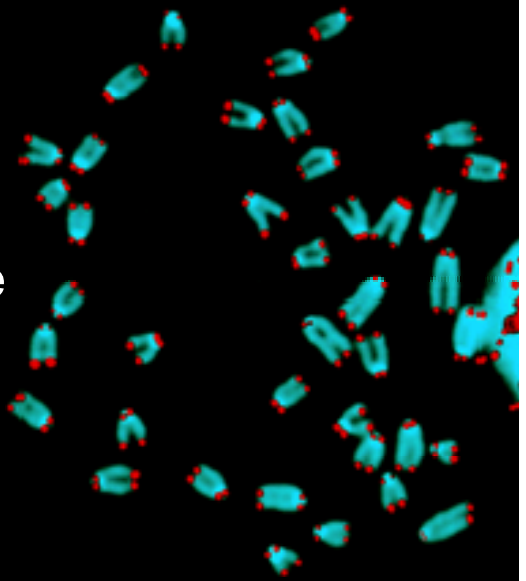


Valdes et al, 2005 *Lancet* (2005)

•Combined effect of initial telomere length and environmental history may contribute to age related disease.

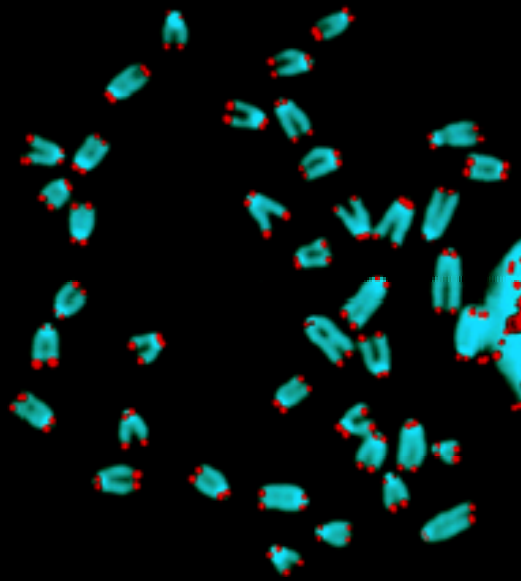
Summary and implications

- Telomerase is essential for telomere maintenance.
- Telomere shortening leads to cell death or senescence after many cell divisions.
- Short telomeres inhibit tumor growth through apoptosis or senescence.
  - Telomerase inhibitors may be effective in cancer therapy.
- Haploinsufficiency for telomerase causes telomere shortening.
- Short telomeres limit cell growth even in the presence of telomerase.
  - Short telomeres may limit long term stem cell division.



Summary and implications

- Inheritance of short telomeres causes phenotypes even in wildtype animals (Wt* or occult genetic disease).
  - Short telomeres may cause loss of tissue renewal in normal aging population.
  - Telomere length may predict onset of certain age related diseases.

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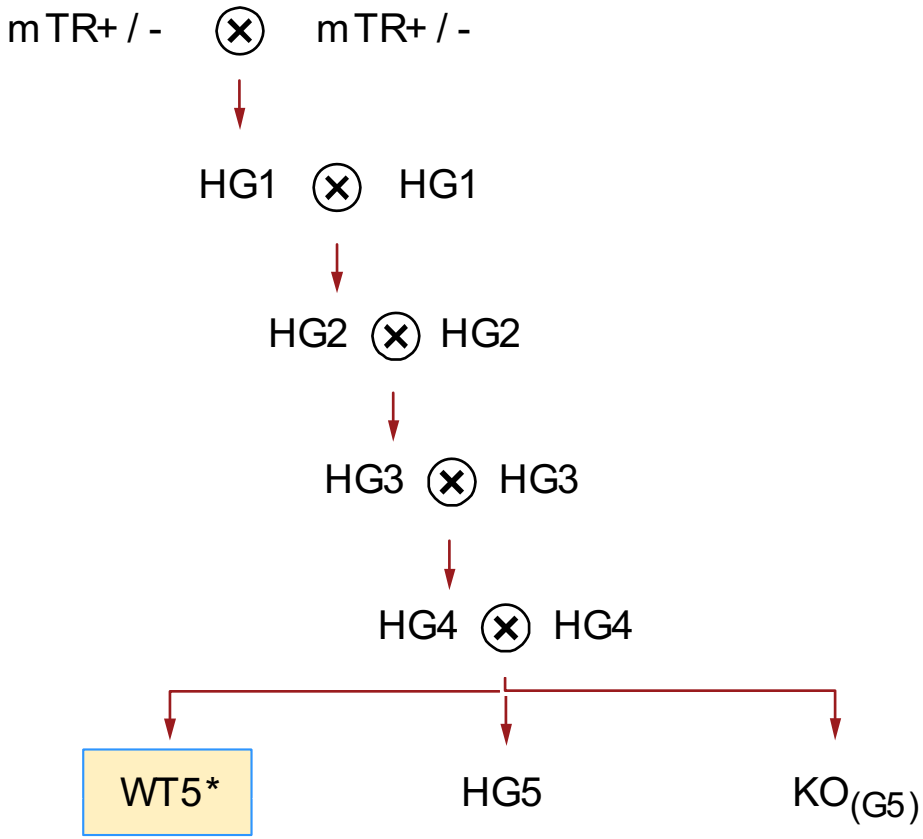
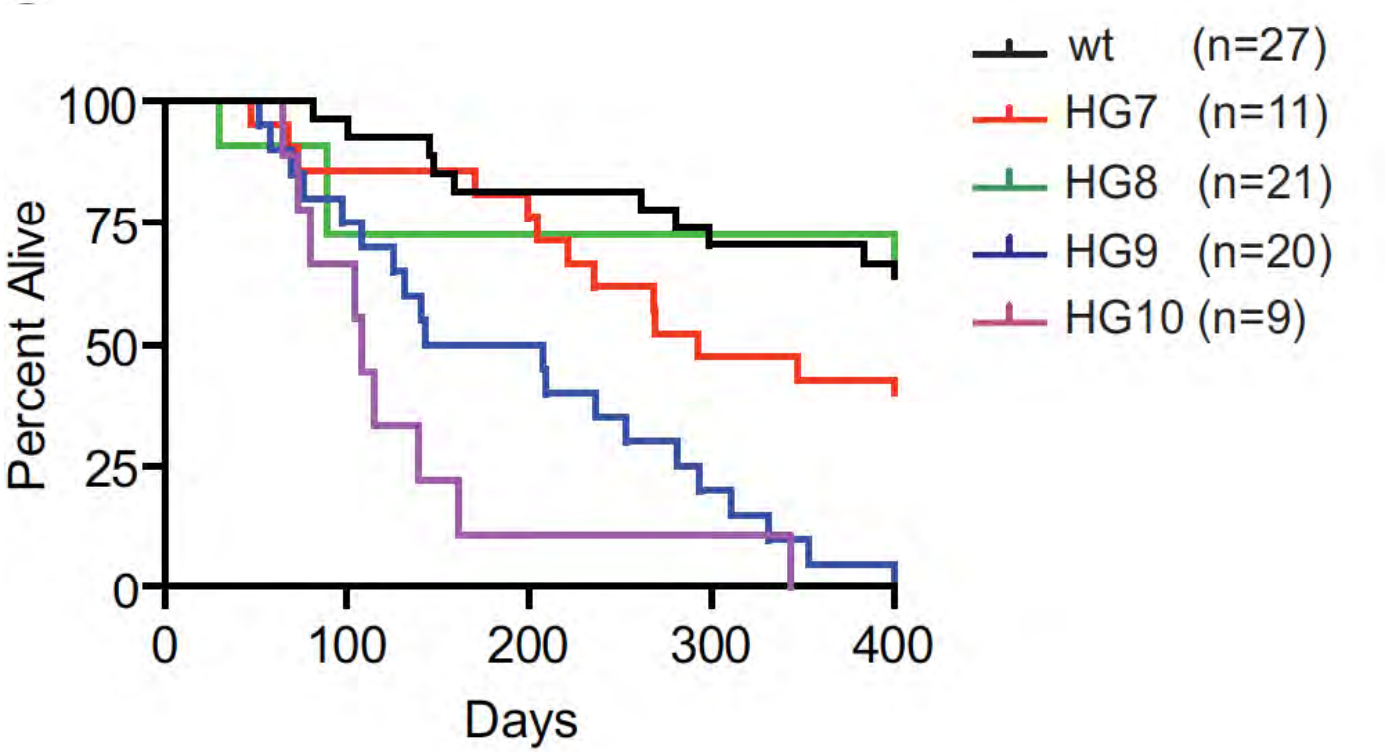
Greider does handstands on horseback and for fur
ches atop a pyramid of lab colleagues. She's also
discovered an enzyme that targets cancer cells.
The Marvels of Telomerase

Telomere shortening during mTR+/- breeding affects wildtype offspring



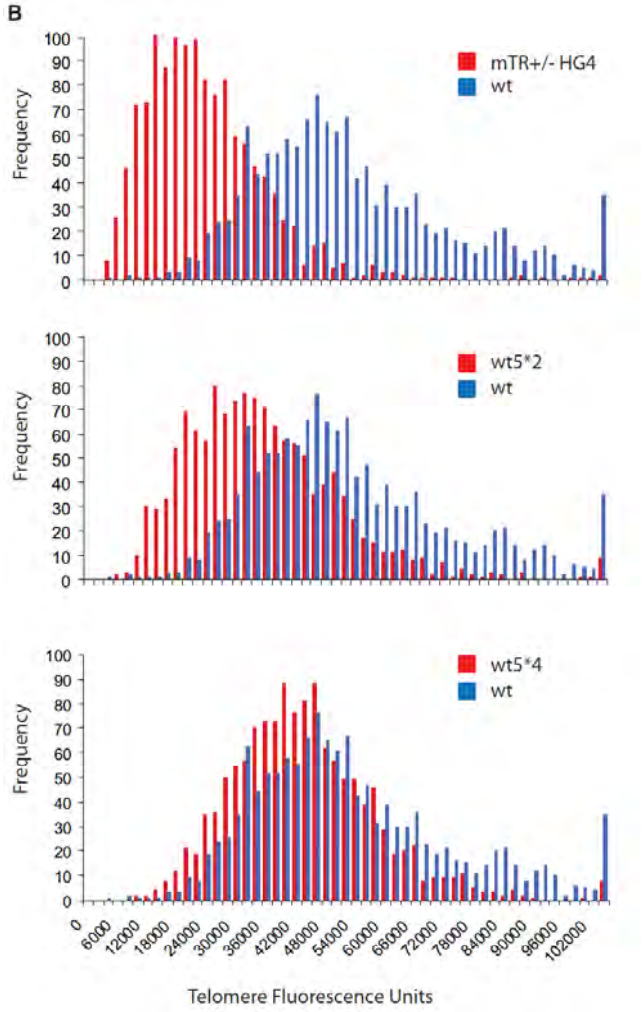
Decreased survival of mTR+/- from later generation heterozygous crosses

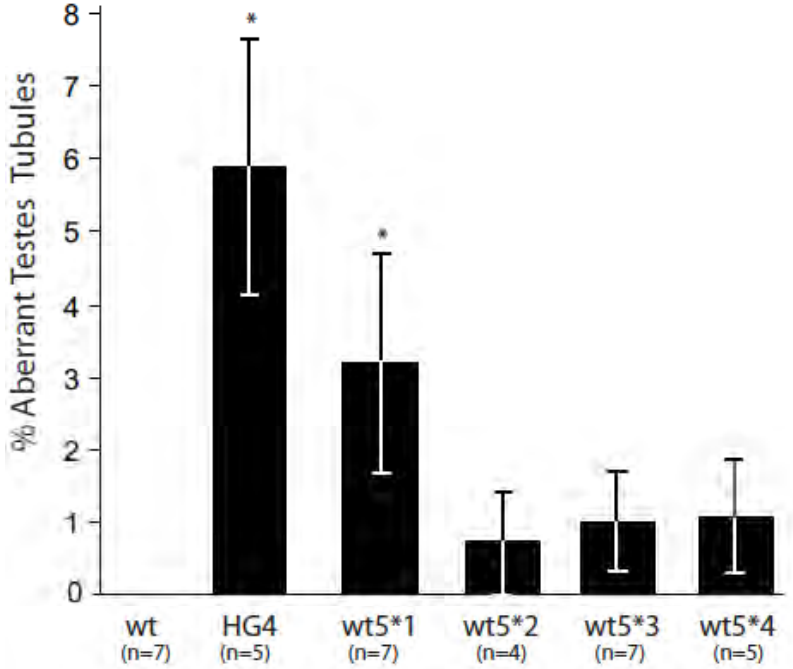


Inheritance of short telomeres decreases survival.
Resembles genetic anticipation in dyskeratosis congenita.

Armanios et al. *AJHG* 2009

Telomere length equlibrium is reestablished after five generations





Armanios et al. AJHG (2009)

Wt* mice reestablish telomere equilibrium after several generations

mTR+ / - HG4 ⊗ mTR+ / - HG4

↓

mTR+ / + WT5*1 ⊗ mTR+ / + WT5*1

↓

mTR+ / + WT5*2 ⊗ mTR+ / + WT5*2

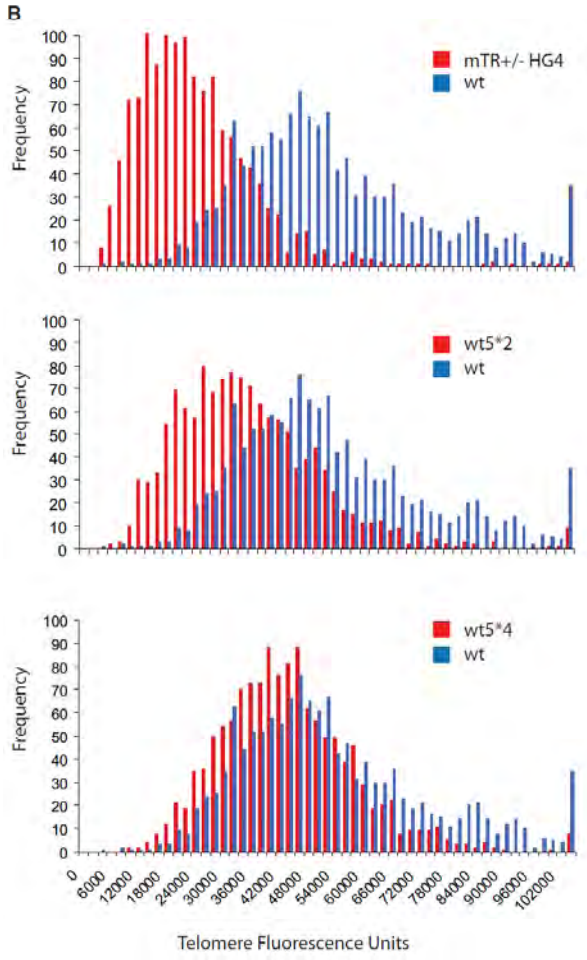
↓

mTR+ / + WT5*3 ⊗ mTR+ / + WT5*3

↓

mTR+ / + WT5*4 ⊗ mTR+ / + WT5*4

↓



Armanios et al. *AJHG* 2009