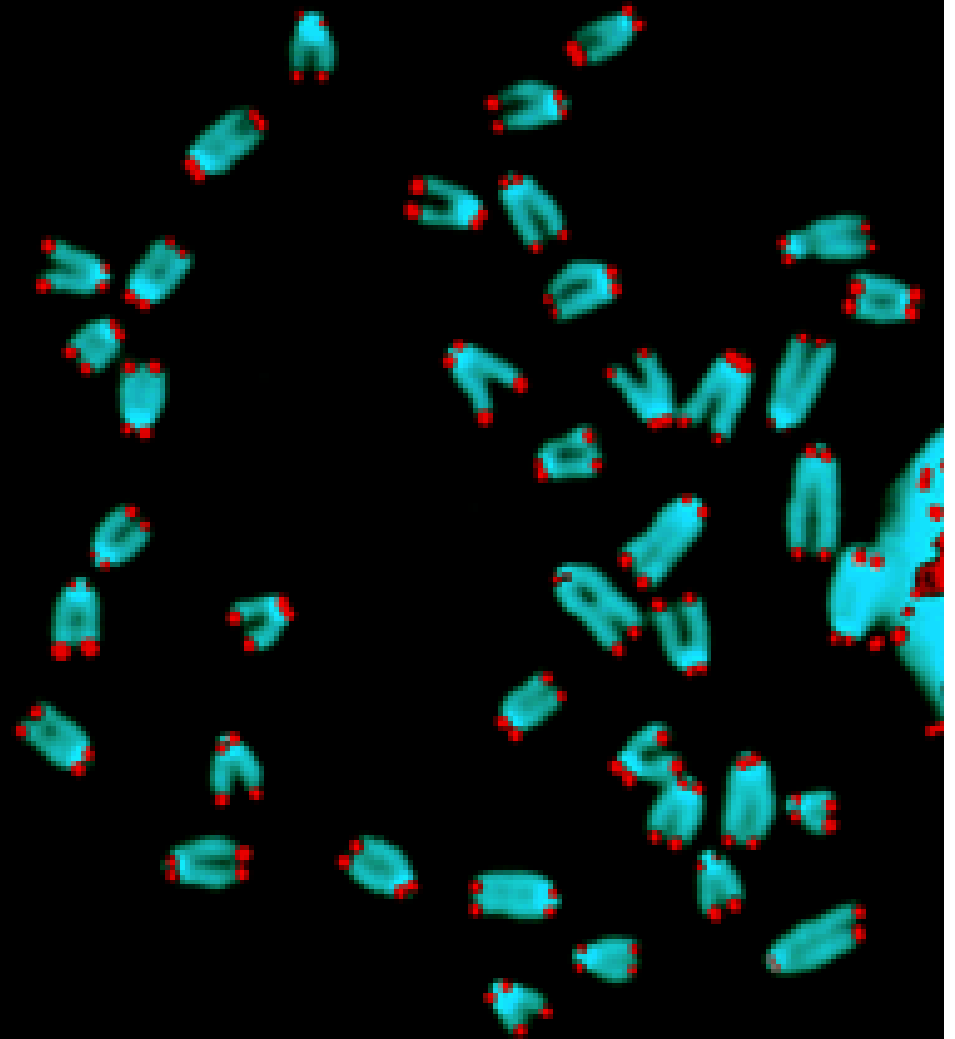


Telomerase and the consequences of telomere dysfunction

Carol W. Greider



Setting the stage: telomere function is conserved

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

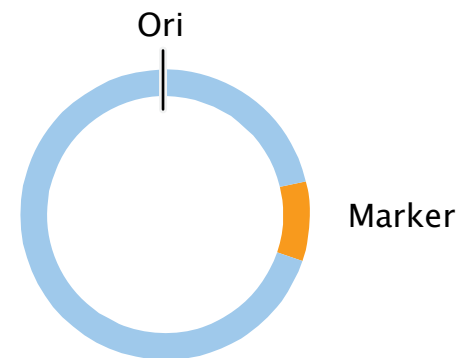
Cloning Yeast Telomeres on Linear Plasmid Vectors

Jack W. Szostak

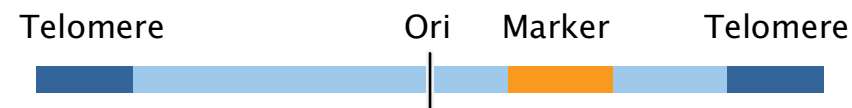
Sidney Farber Cancer Institute
and Department of Biological Chemistry
Harvard Medical School
Boston, Massachusetts 02115

Elizabeth H. Blackburn

Department of Molecular Biology
University of California
Berkeley, California 94720



Linear plasmid
Add Tetrahymena telomeres
Transform yeast



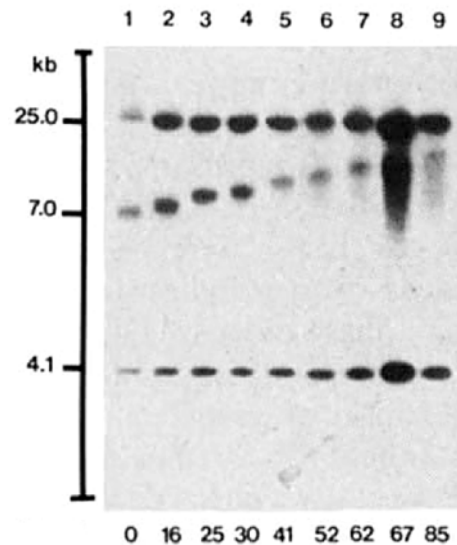
Cell May 1982

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

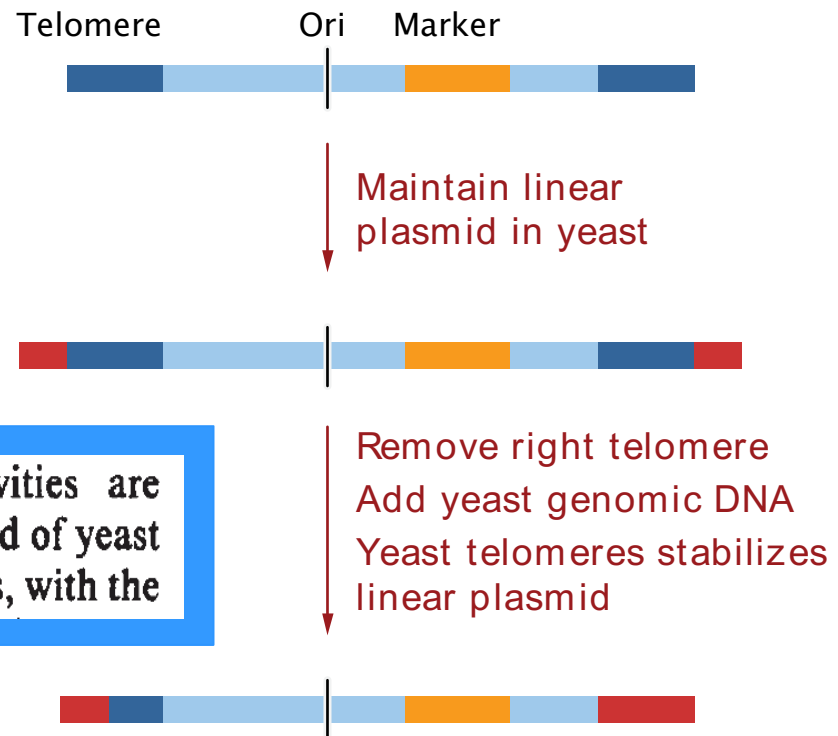
DNA sequences of telomeres maintained in yeast

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

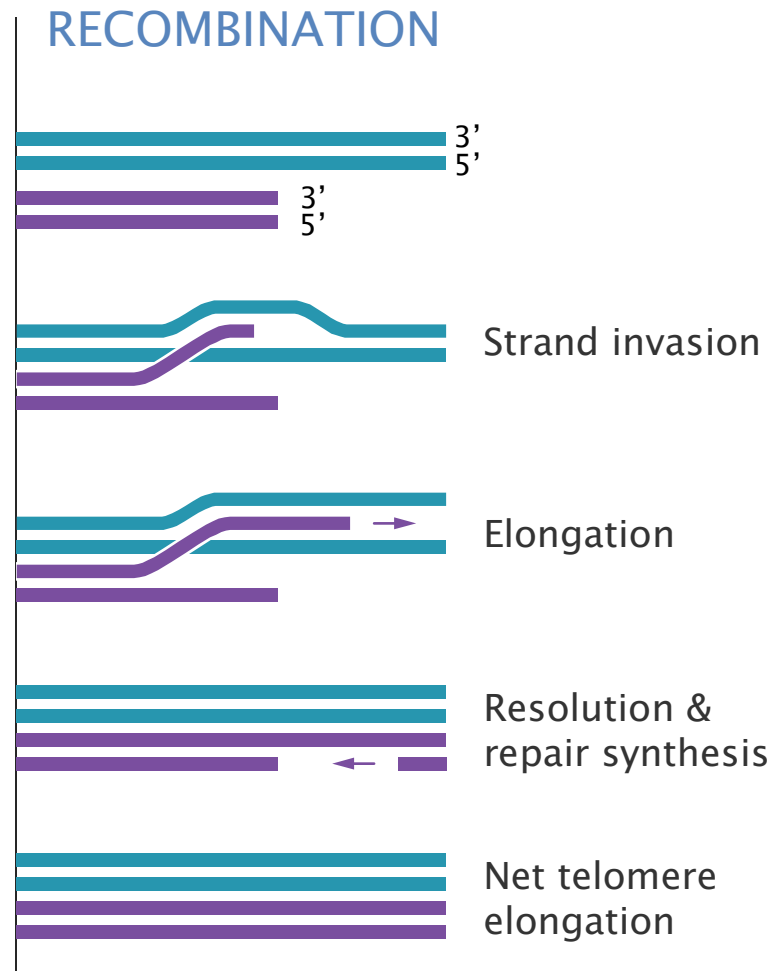
* Department of Molecular Biology, University of California, Berkeley, California 94720, USA

† 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*

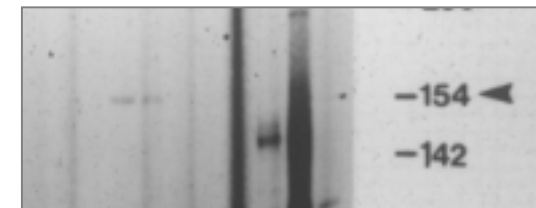
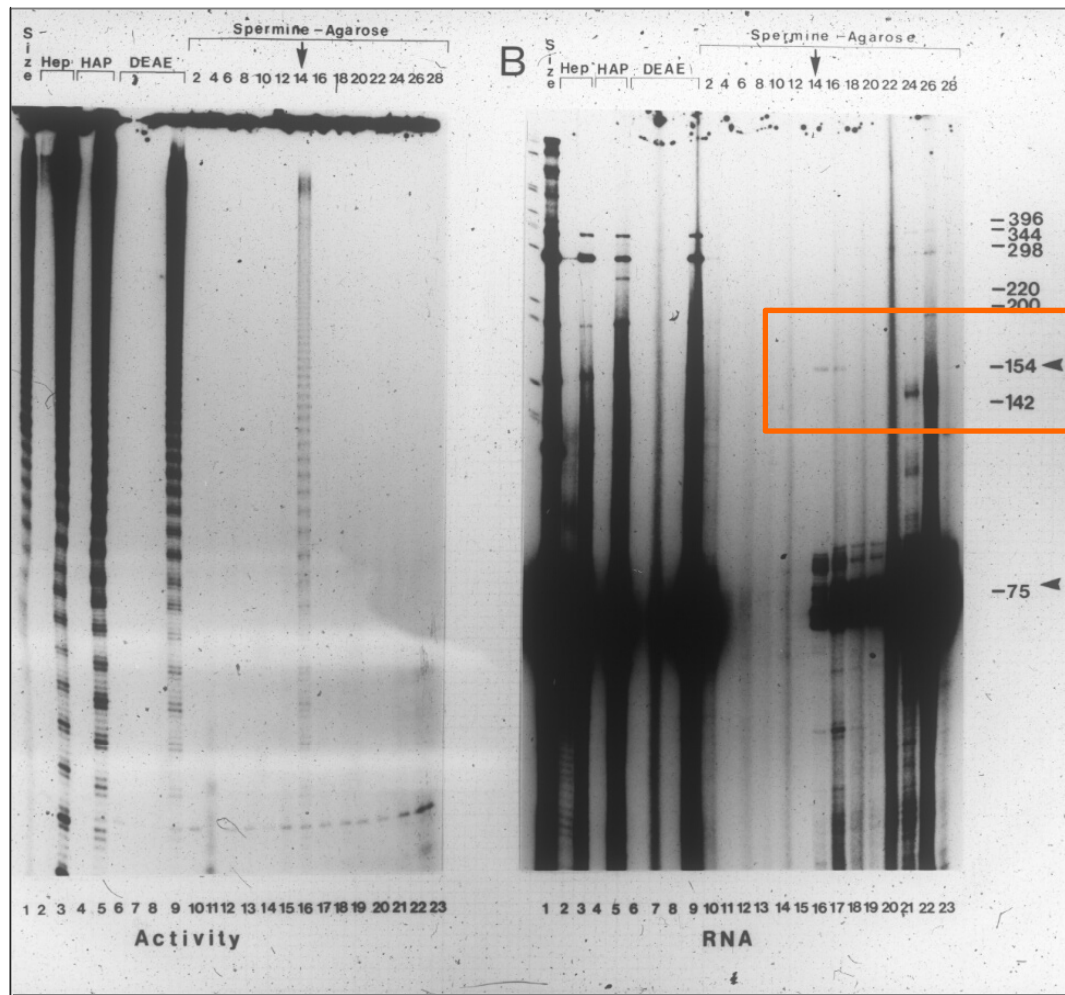
T dTTP

↓ Telomerase

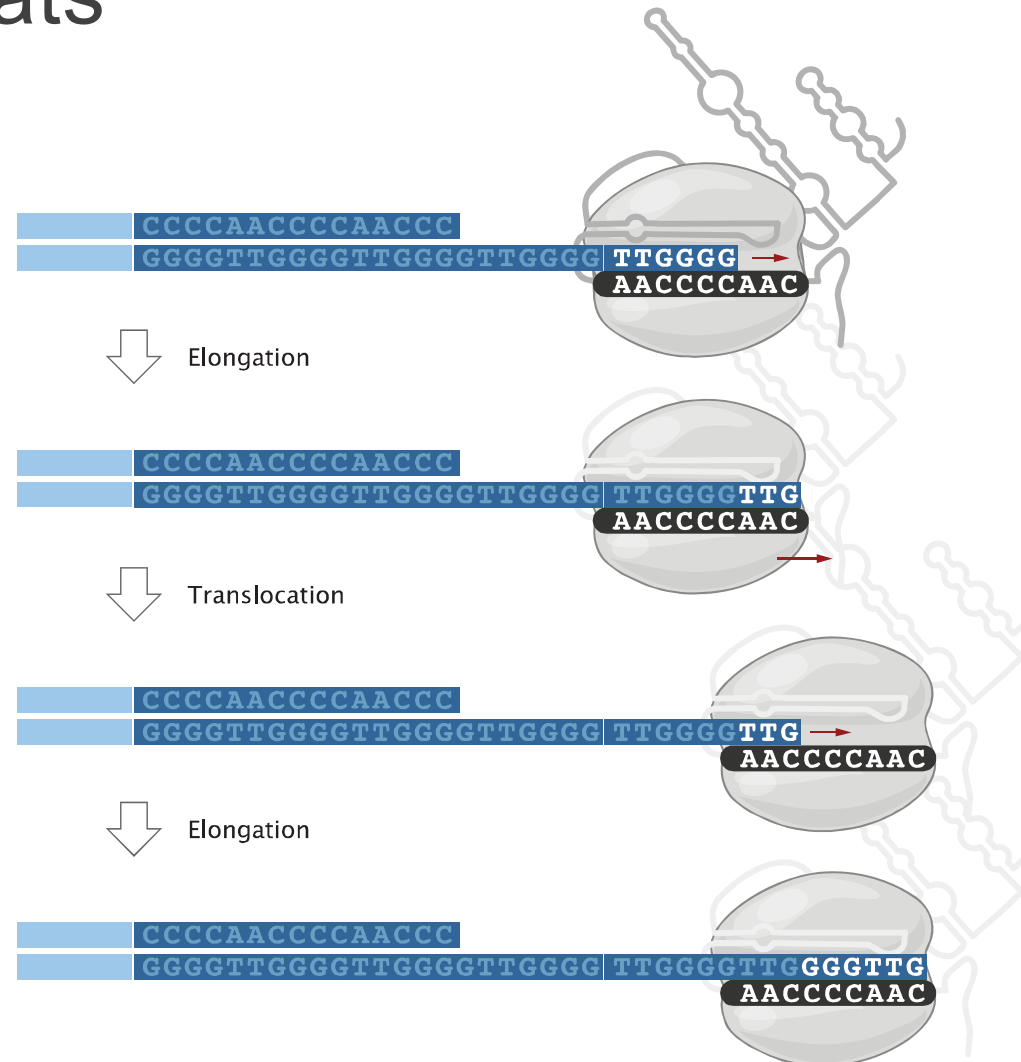
➔ **G**GGGTT**G**GGGTT**G**GGGTT**G**GGGTT**G**



Identification of the RNA component



Telomerase uses RNA template to add TTGGGG repeats



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



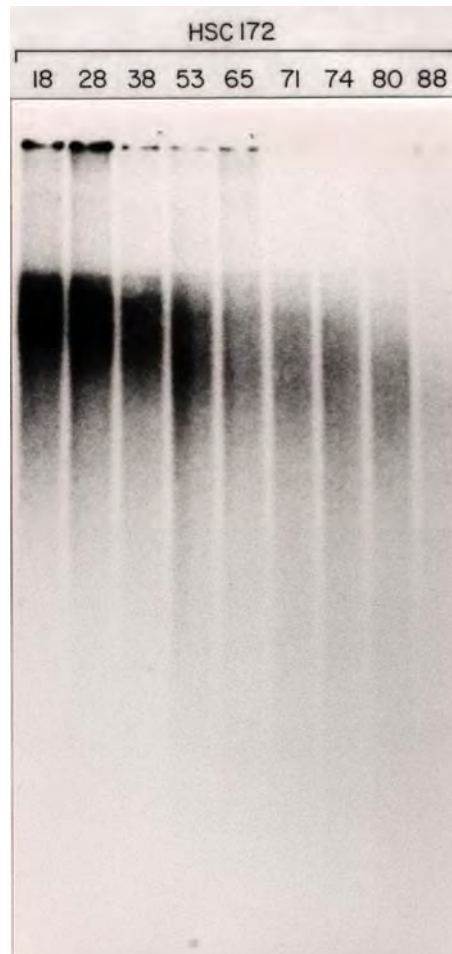
Telomere shortening in cellular senescence

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

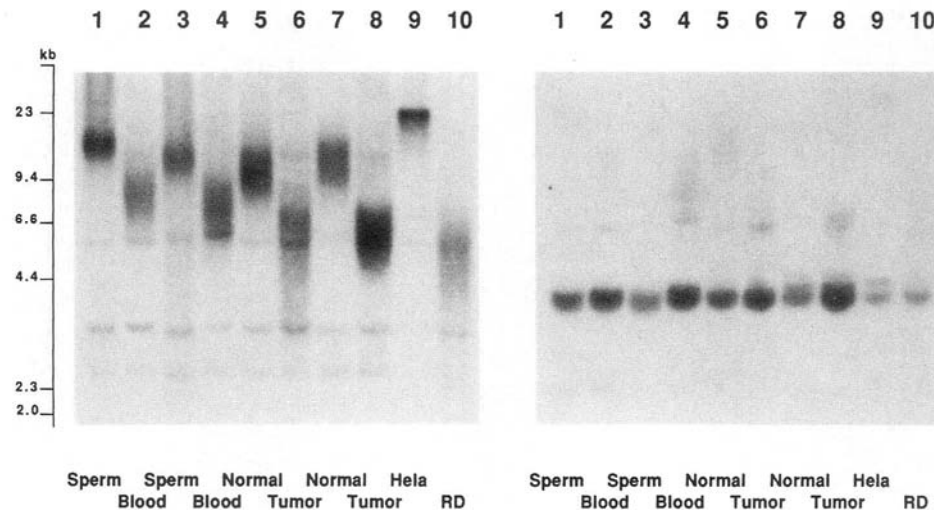
Telomeres are shorter in cancer cells

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}

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University of California, San Francisco, California 94143; Department of Cellular and Structural Biology,
University of Texas, San Antonio, Texas 78285⁵; and Department of Laboratory Medicine,
M. D. Anderson Tumor Institute, Houston, Texas 77030⁶*

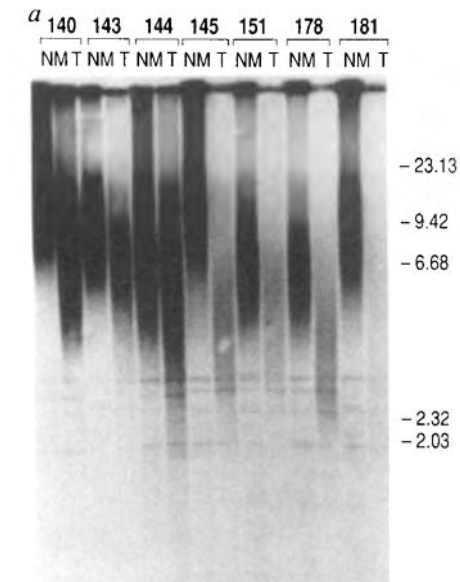
Received 21 August 1989/Accepted 17 October 1989



Telomere reduction in human colorectal carcinoma and with ageing

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

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



Telomerase protein component identified

RESEARCH ARTICLES

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 RNP 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 8×10^5 telomeres (13) and about 3×10^5 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).

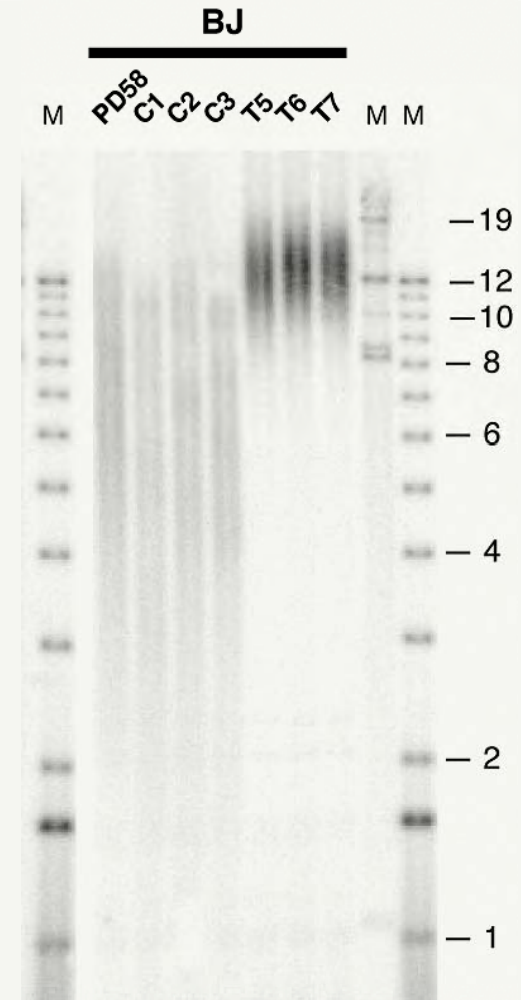
Here we characterize the p123 component of *Euplotes* telomerase and show that it contains sequence hallmarks of reverse transcriptases. Furthermore, it is the homolog of a yeast protein, Est2p, shown previously to function in telomere maintenance. Our genetic and biochemical analyses show that the reverse 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-



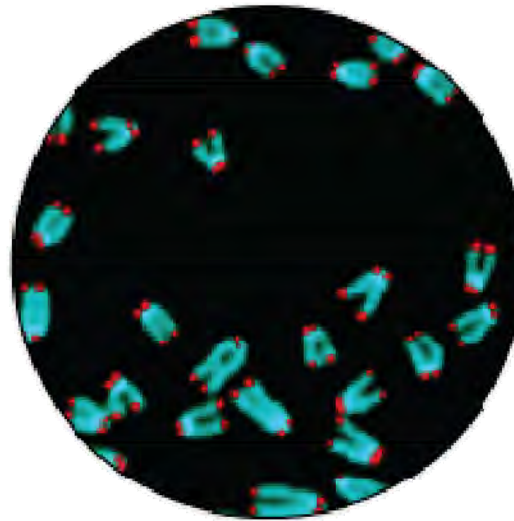
TERT transfection extends the lifespan of human cells

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†



Using genetics to examine the role of telomerase in mammals

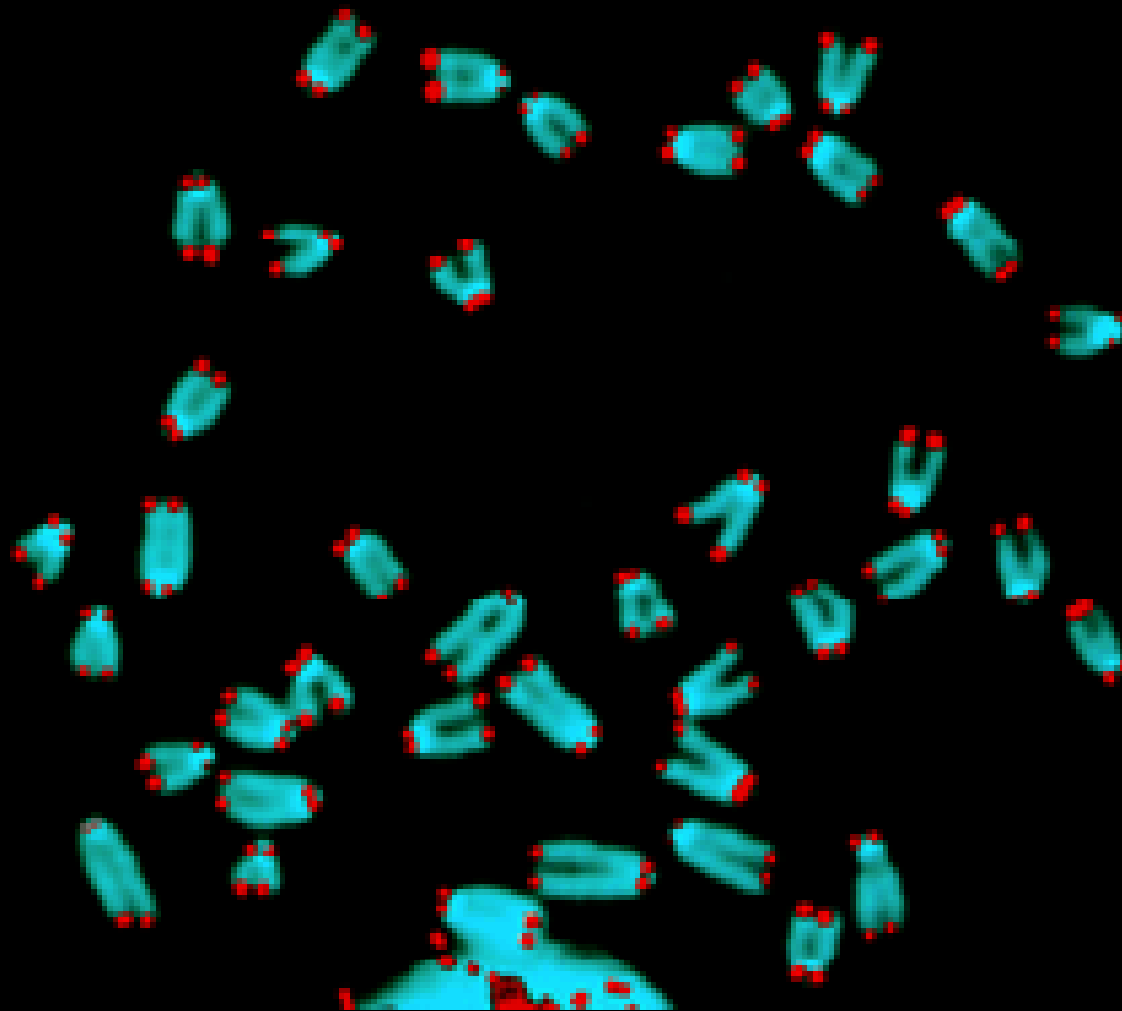


Maria Blasco

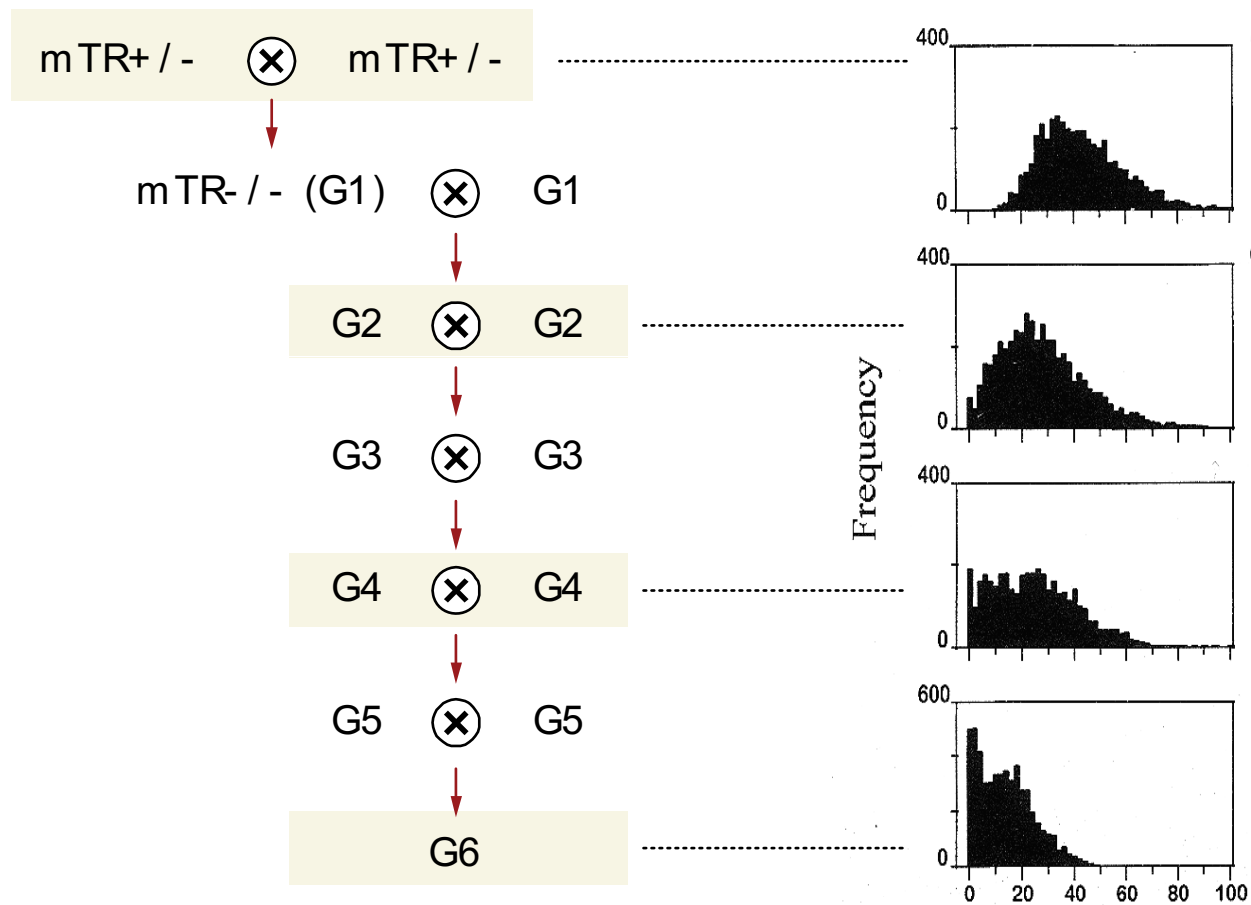


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Quantitative analysis of telomere length

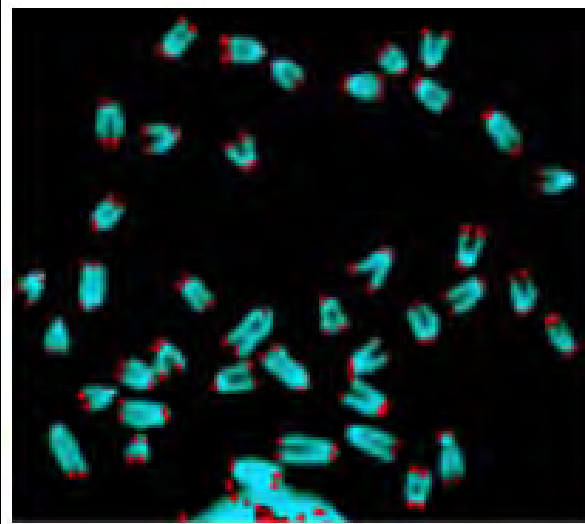


Telomeres shorten progressively in telomerase-null mice

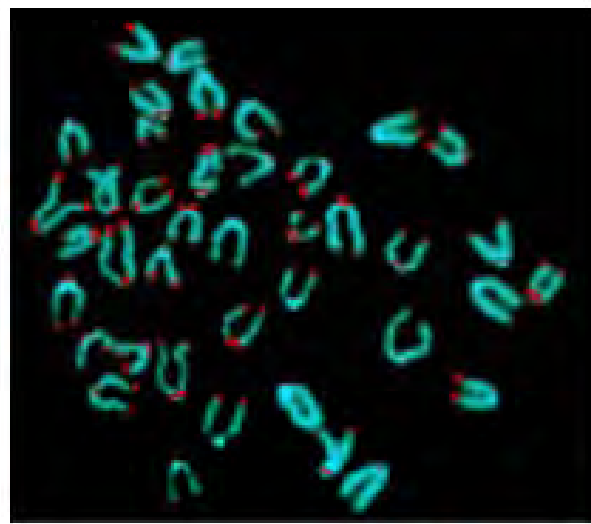


Loss of telomere sequence leads to loss of telomere function

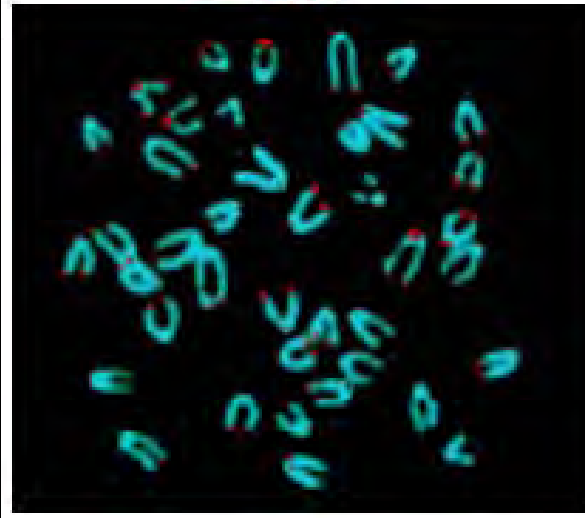
WT



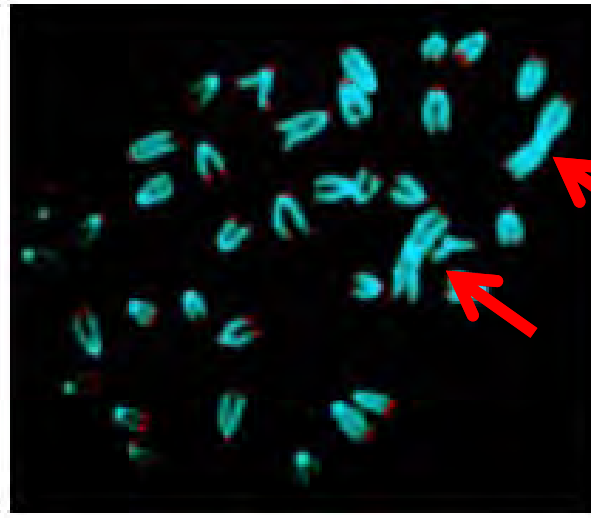
KO-G2



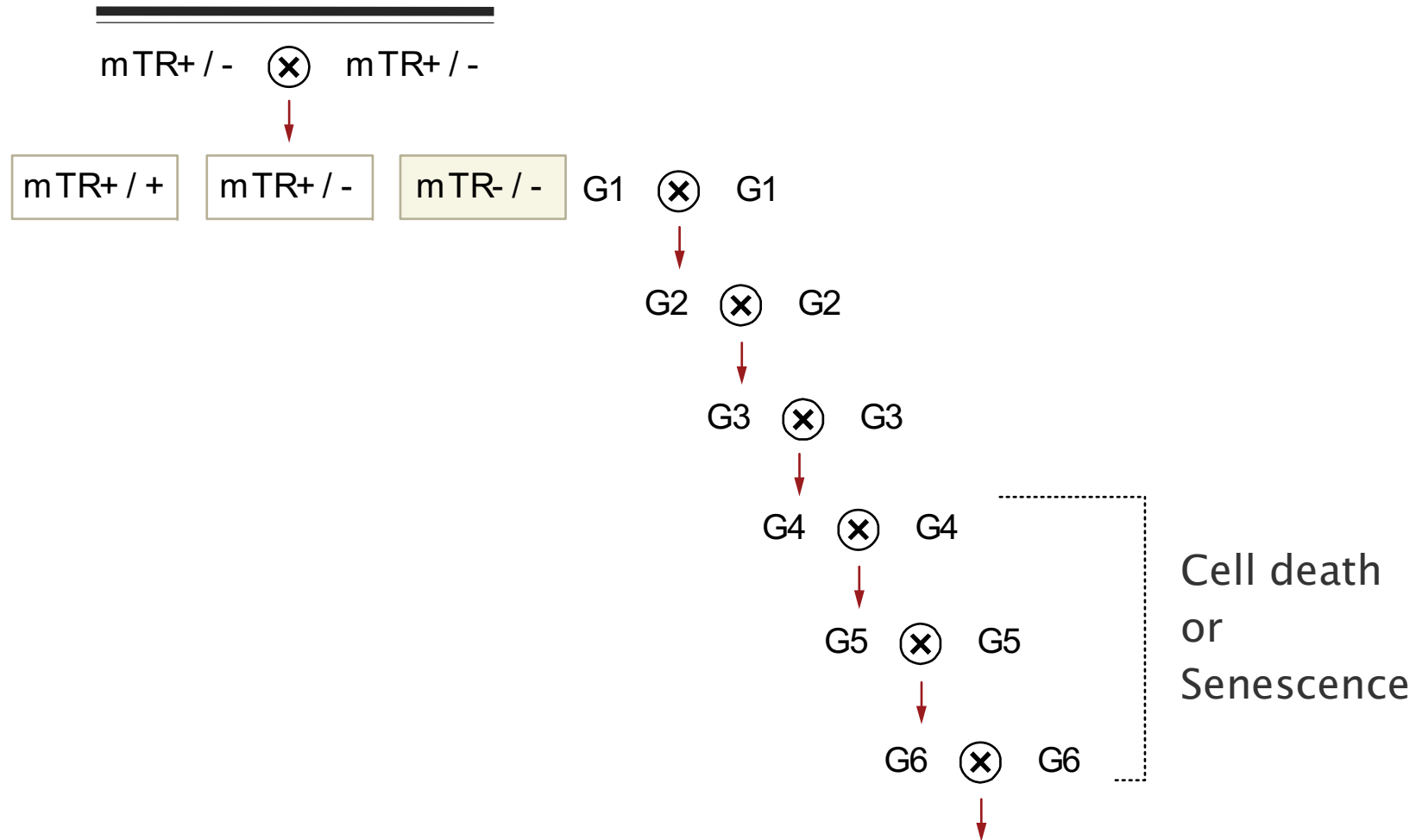
KO-G4



KO-G6



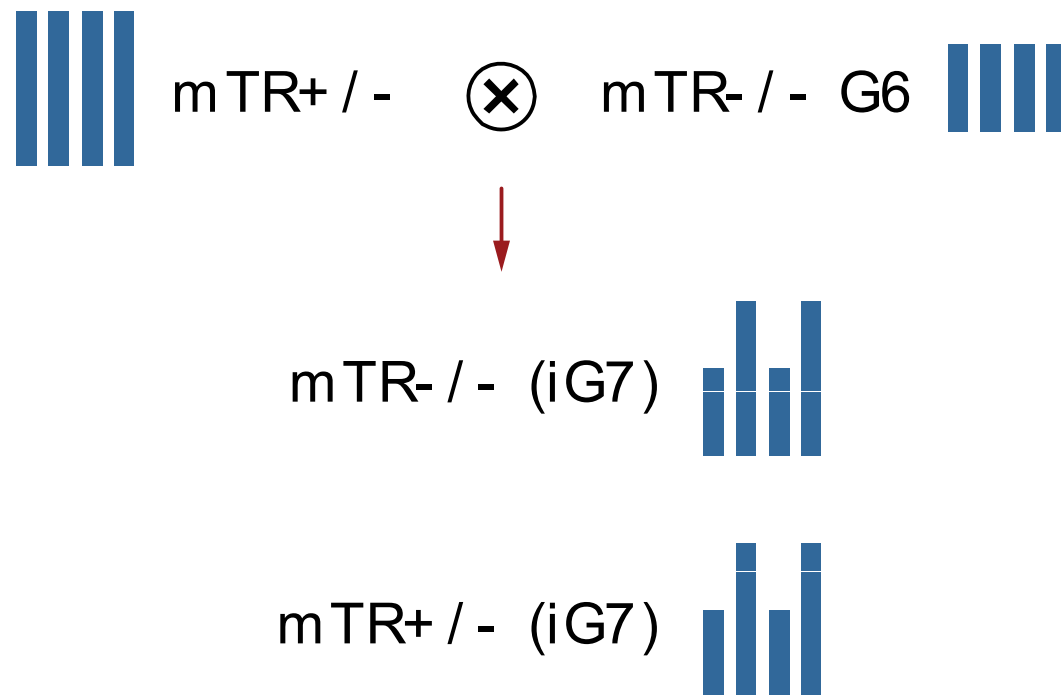
The consequences of short telomeres



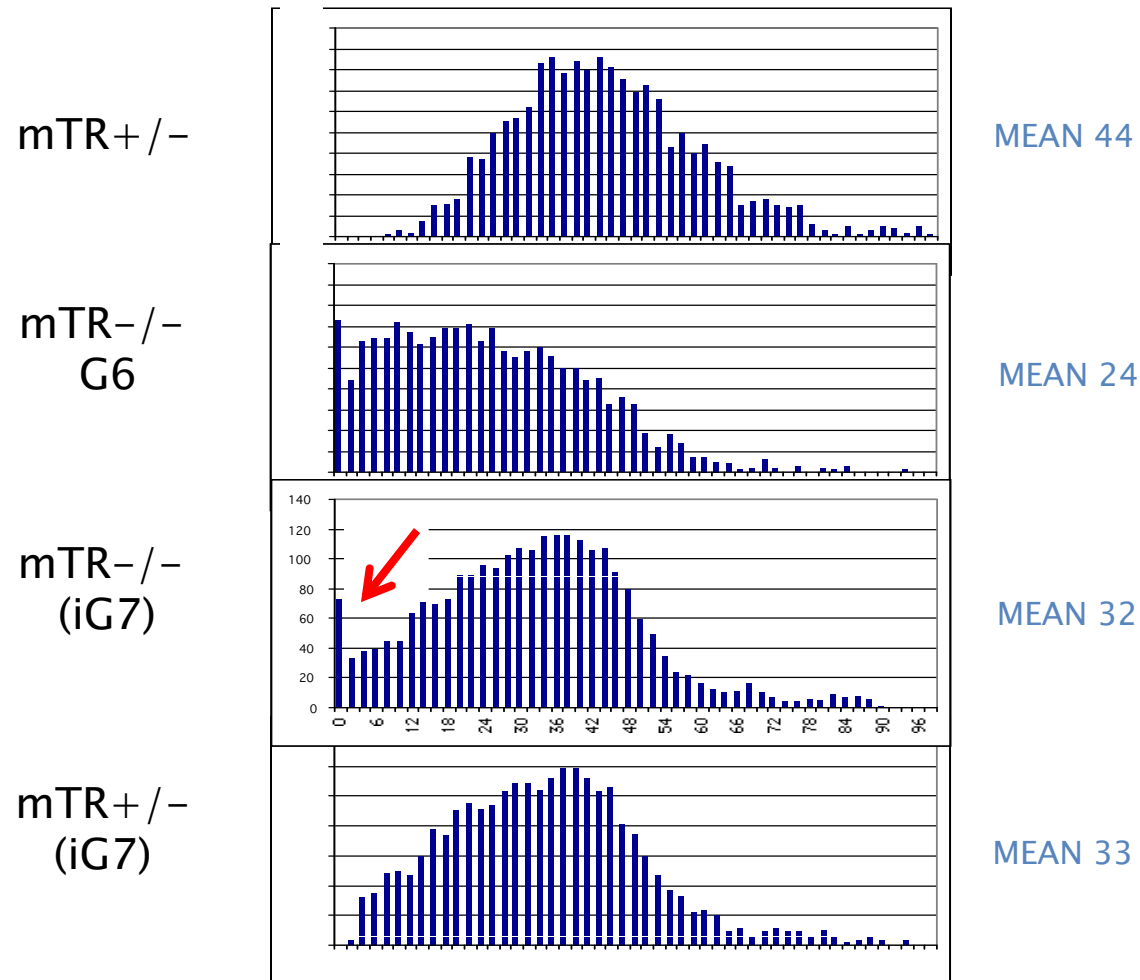
Mike Hemann



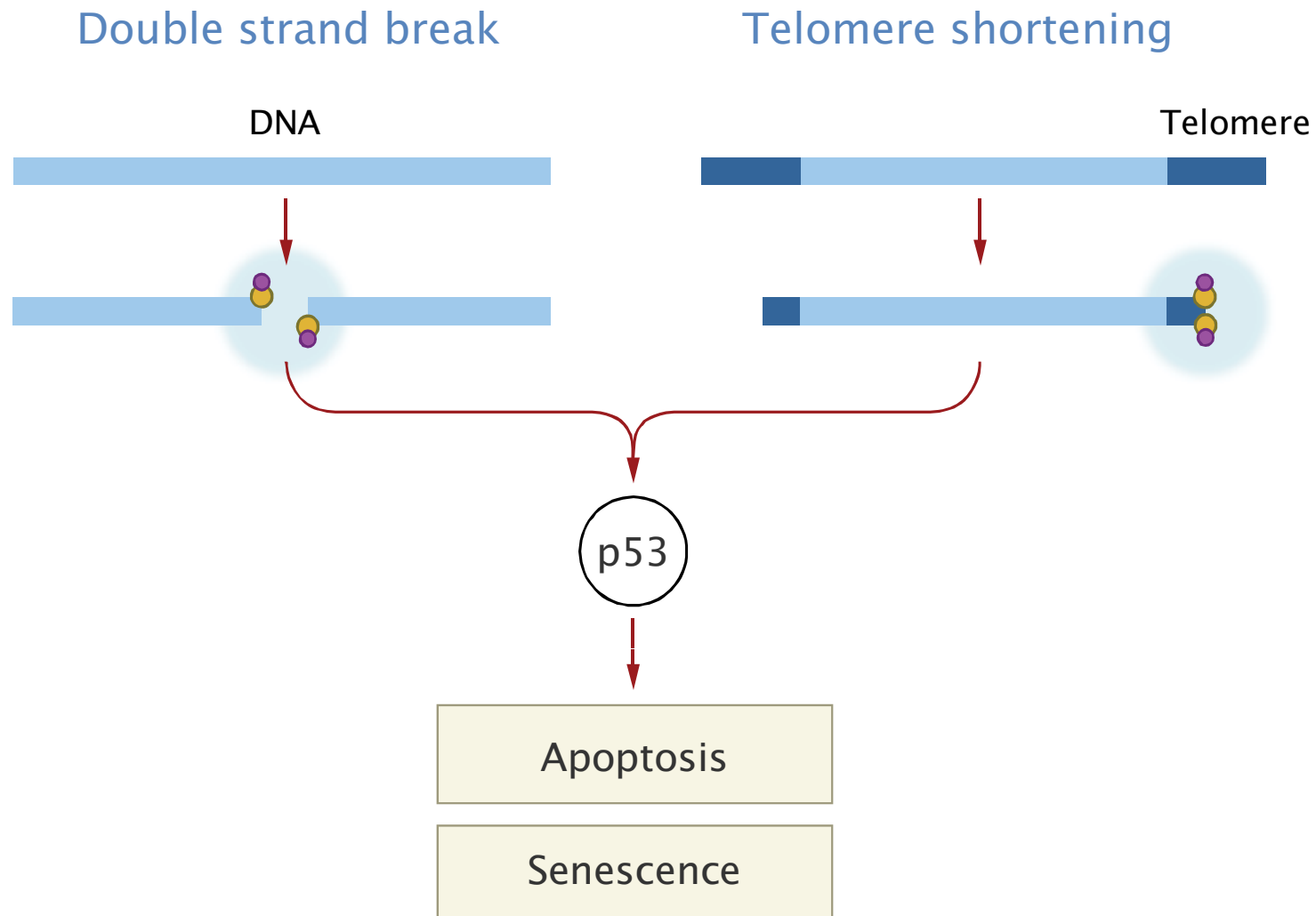
Mice with 1/2 short and 1/2 long telomeres show telomere dysfunction



The shortest telomere triggers cell death

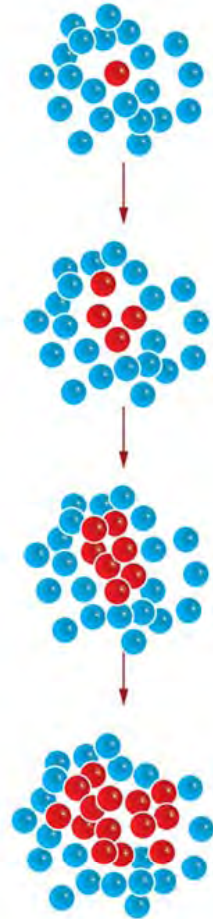


Short telomeres induce a DNA damage response



Telomerase is required for cells that must divide many times

CANCER CELLS



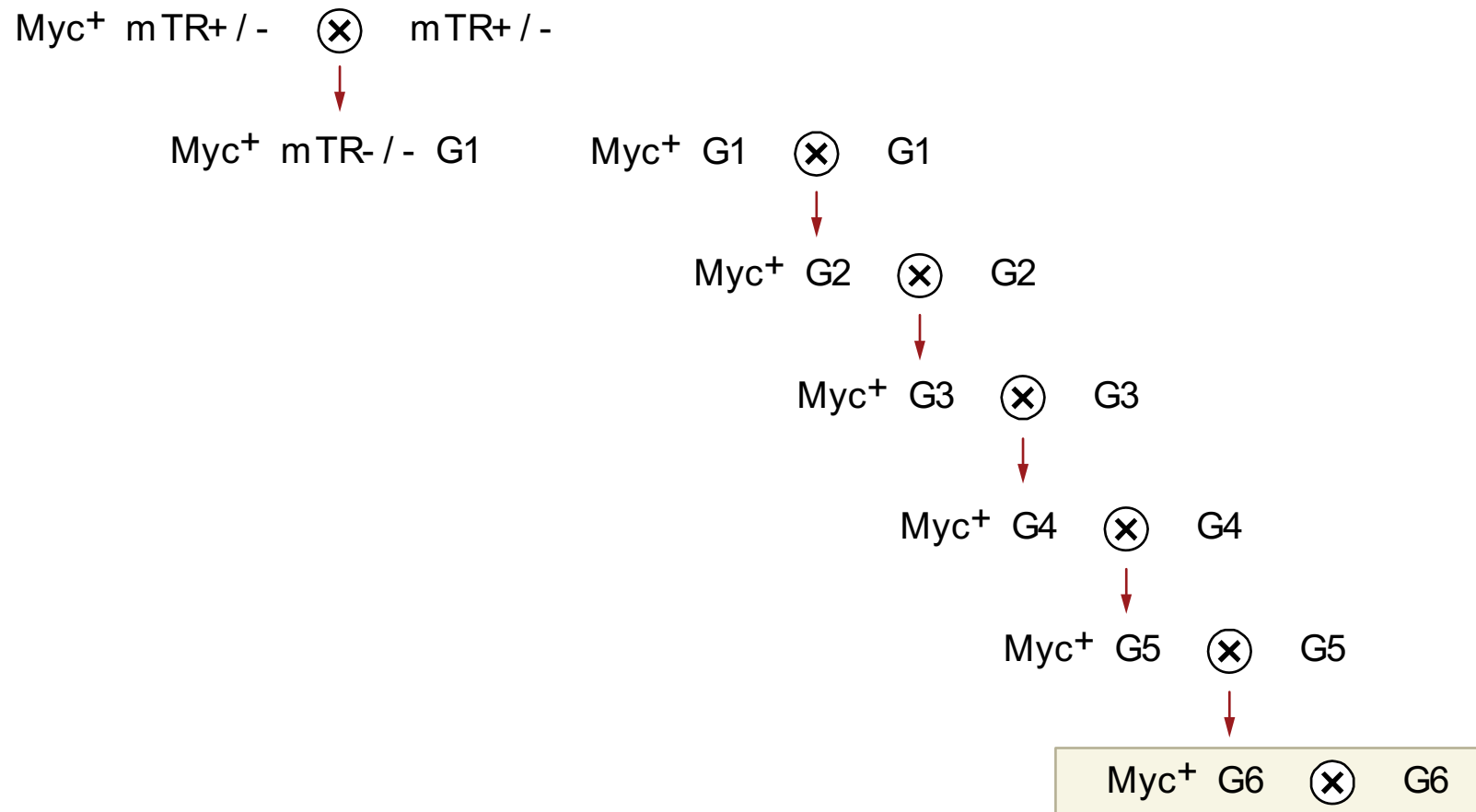
Short telomeres suppress tumorigenesis

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

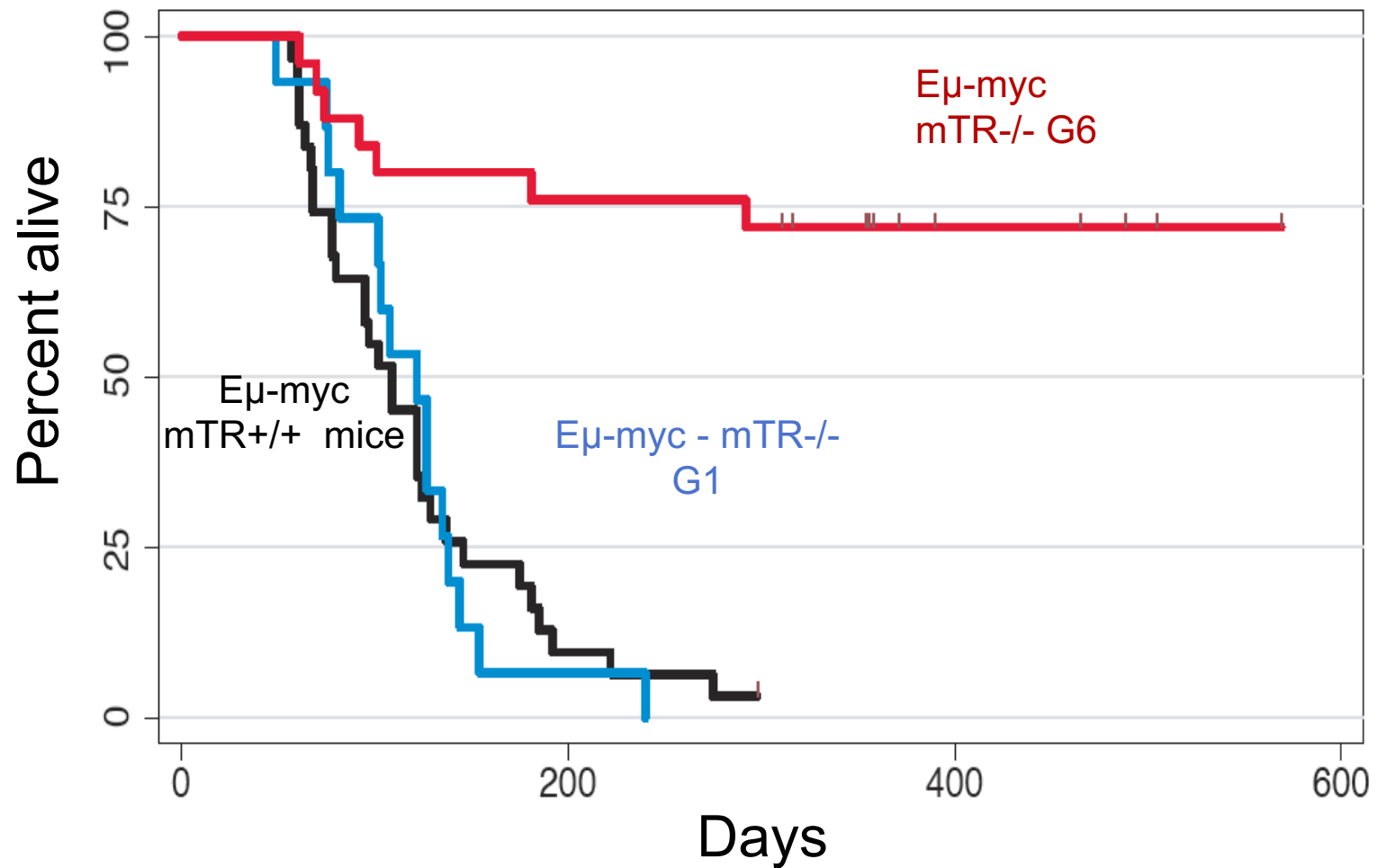
David Feldser



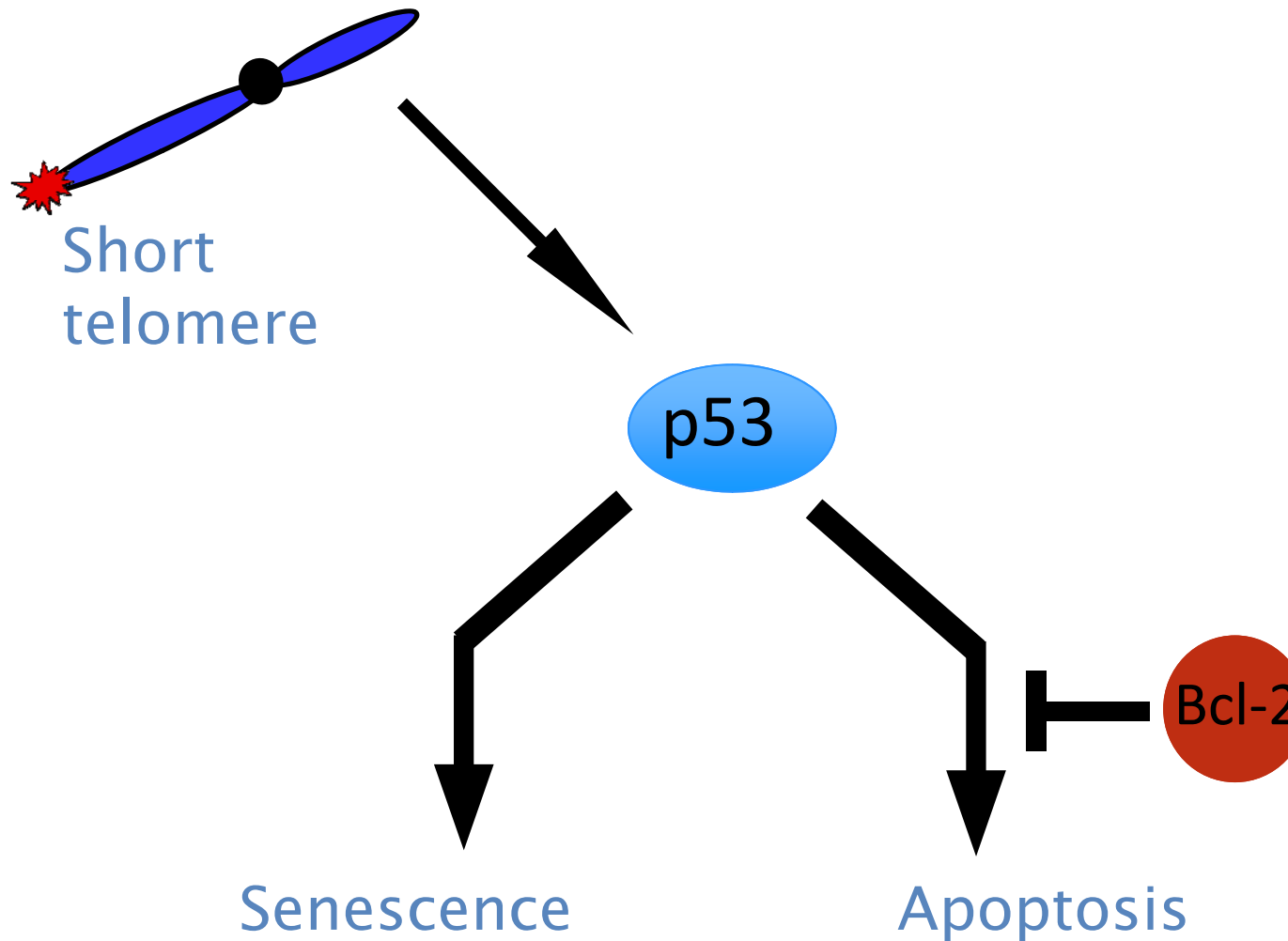
E μ -Myc induced B-cell lymphoma growth can be blocked by short telomeres



Short telomeres protect against B-cell lymphoma



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^{*}**

Clinical features of dyskeratosis congenita

- Dermatologic criteria
 - Skin hyperpigmentation
 - Oral leukoplakia
 - Nail dystrophy
 - Mortality
 - **Aplastic anemia**
 - Pulmonary fibrosis
 - Cancer
-

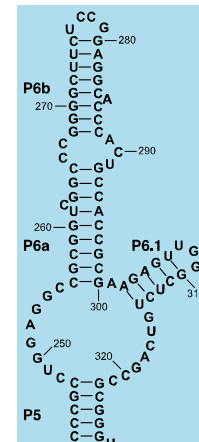
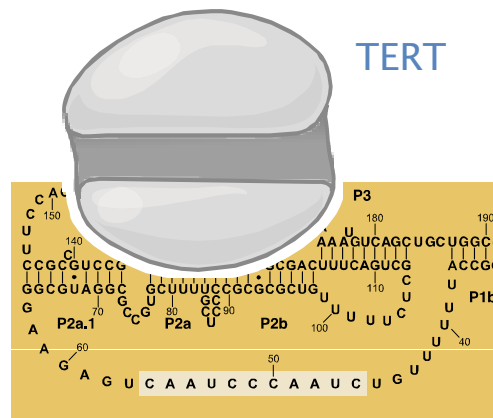
Julian Chen



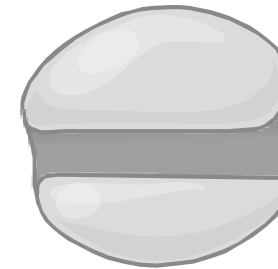
Telomerase RNA secondary structure

Human Telomerase RNA

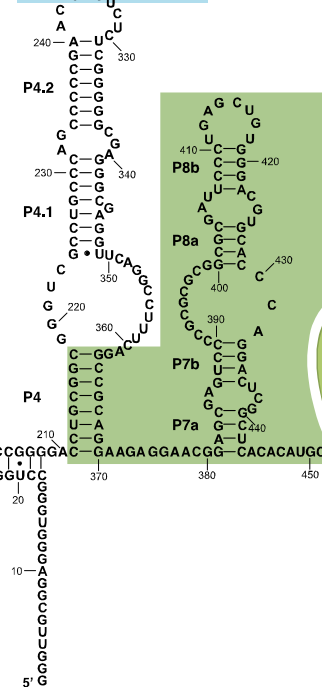
Pseudoknot/Template



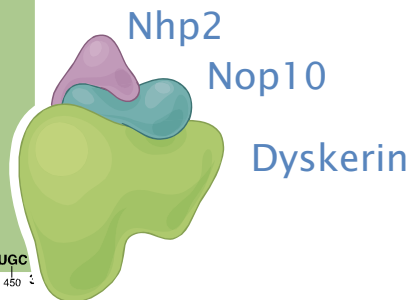
CR4/CR5



TERT



SnoRNA



Nhp2

Nop10

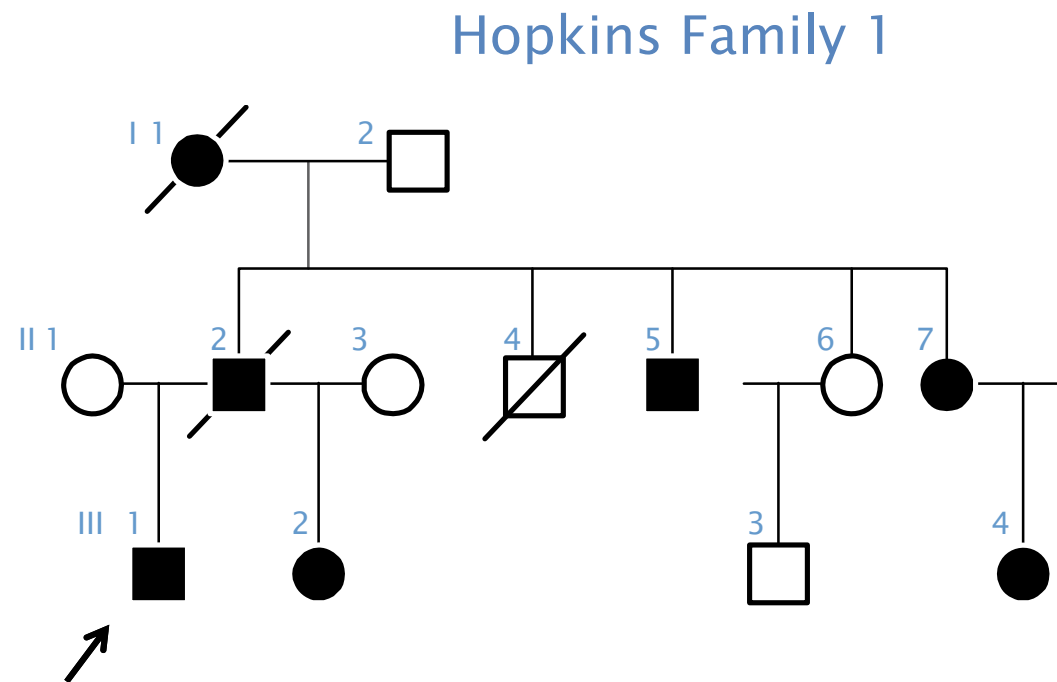
Dyskerin

Mitchell et al. *MCB*
1999

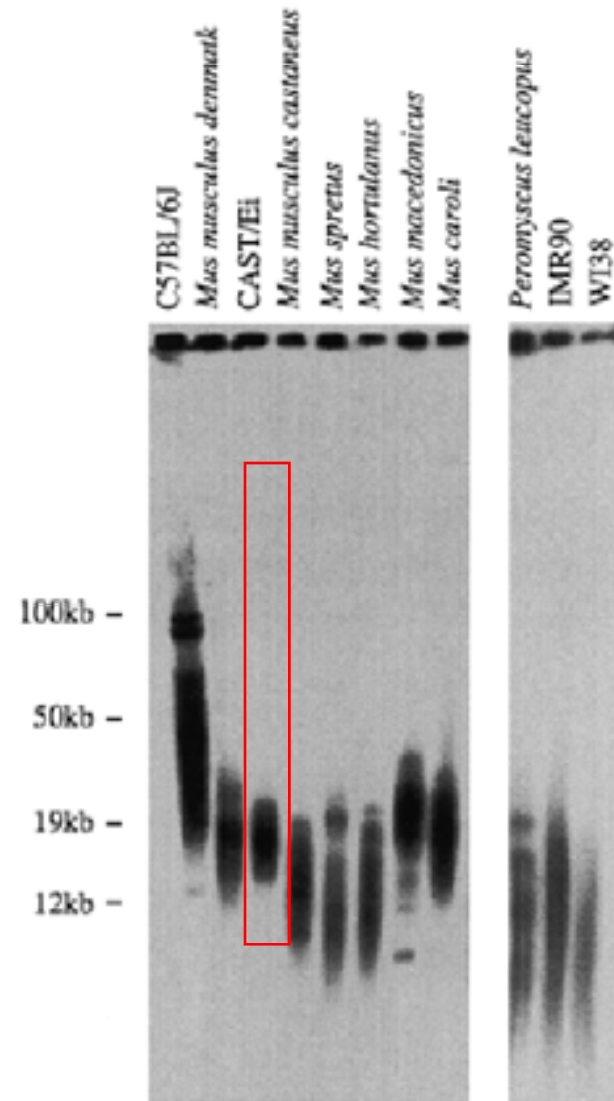
Chen et al. *Cell* 2000

TERT mutations cause autosomal dominant inheritance of dyskeratosis congenita

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



Cast/Ei mice have short telomeres similar to humans

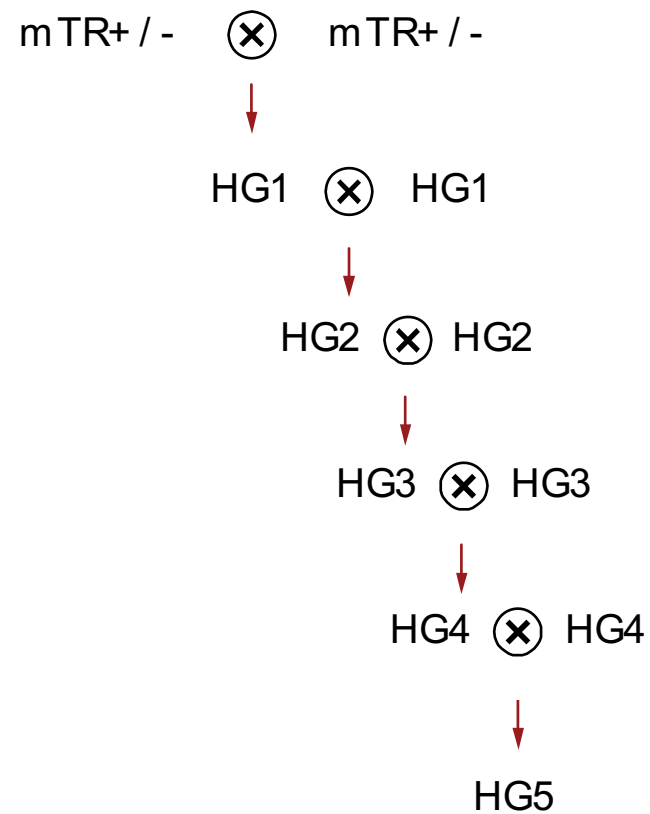


- Bred mTR null allele onto Cast/Ei background

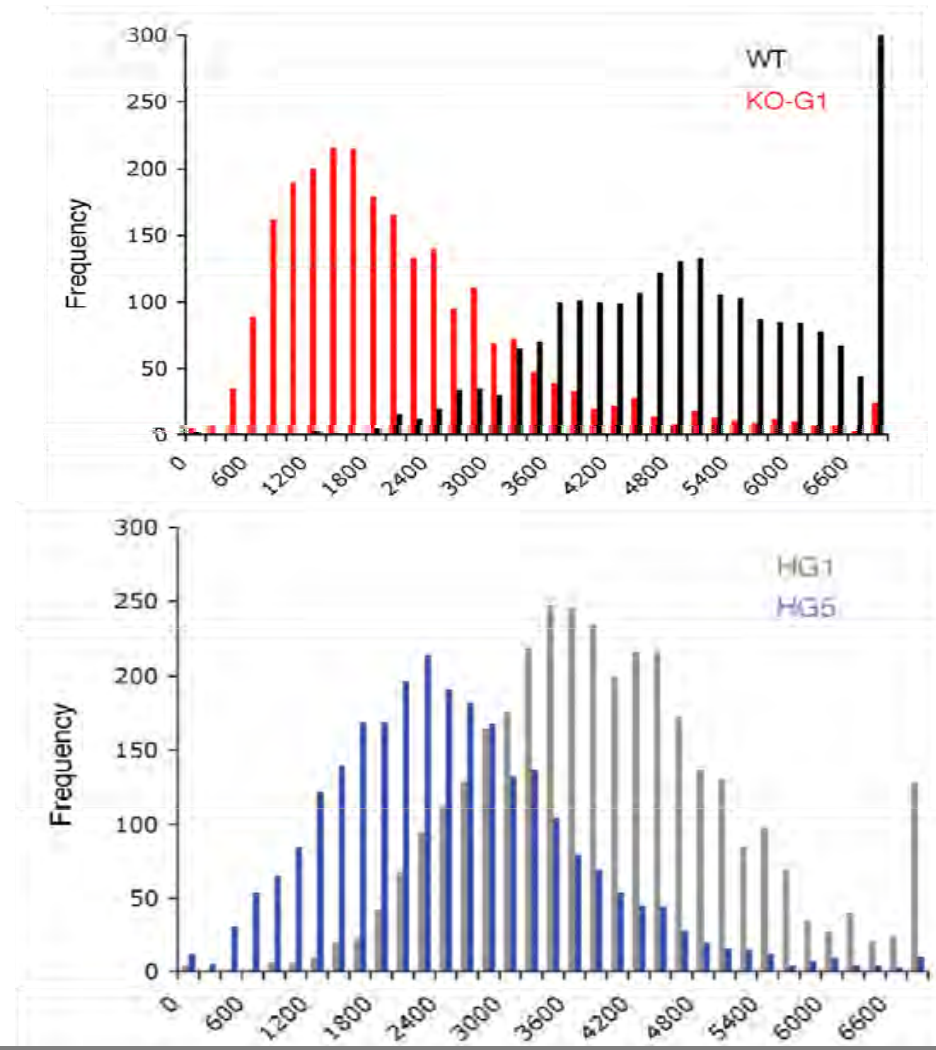
Ling-Yang Hao



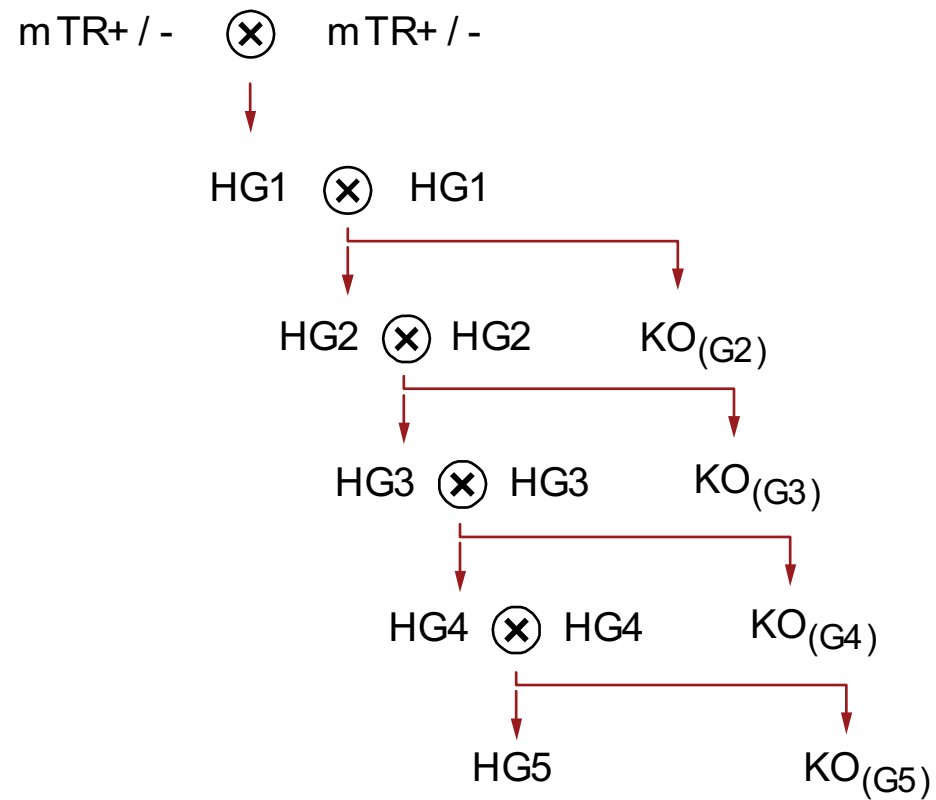
Progressive telomere shortening during mTR+/- breeding



Short telomeres in CAST/EiJ mTR+/- mice



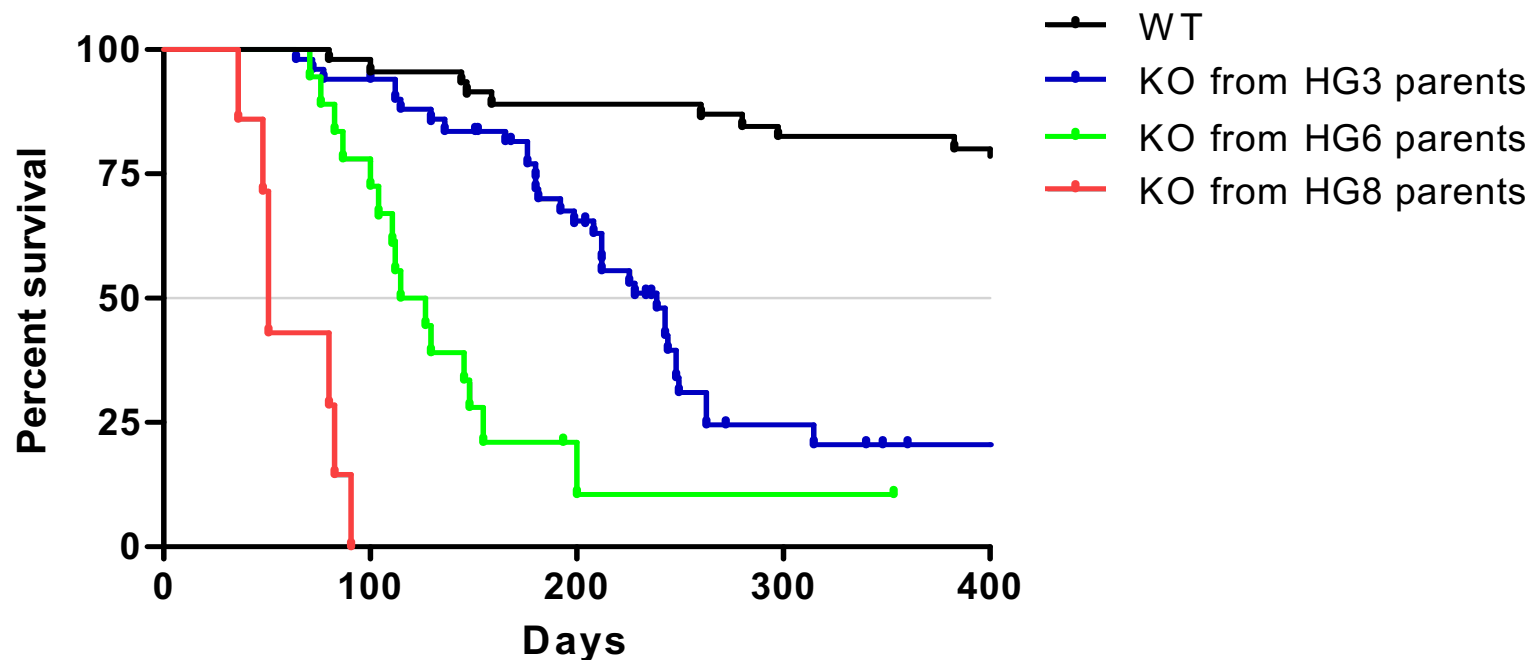
Inheritance of short telomeres decreases survival



Margaret Strong

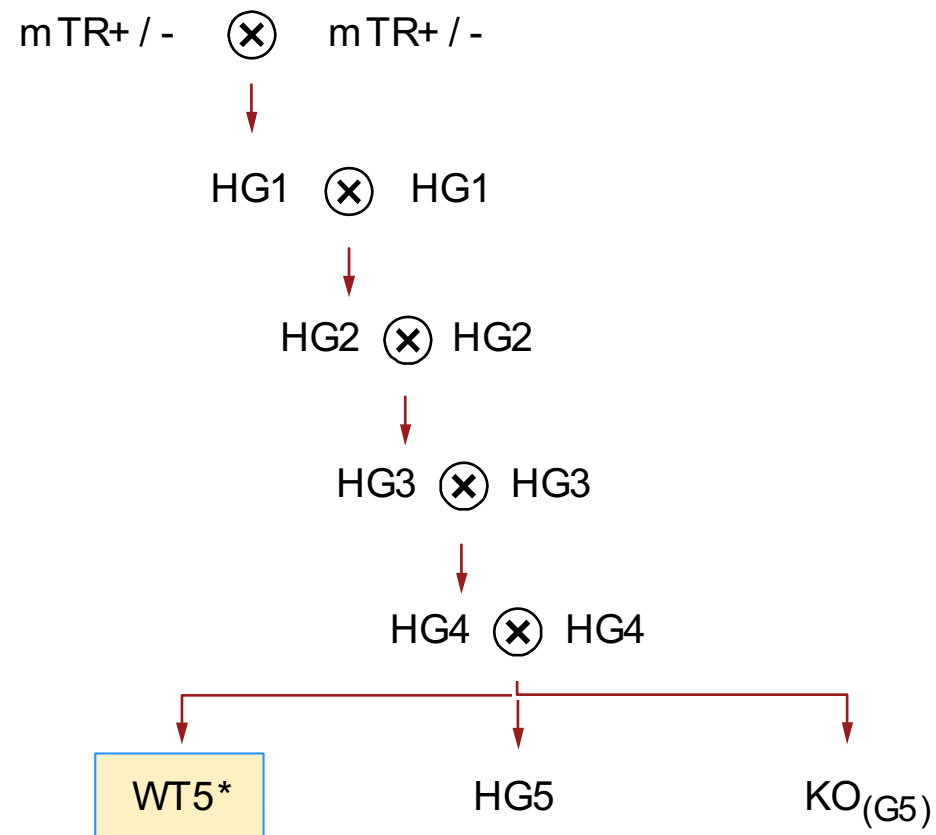


Decreased survival of mTR-/- from later generation heterozygous crosses

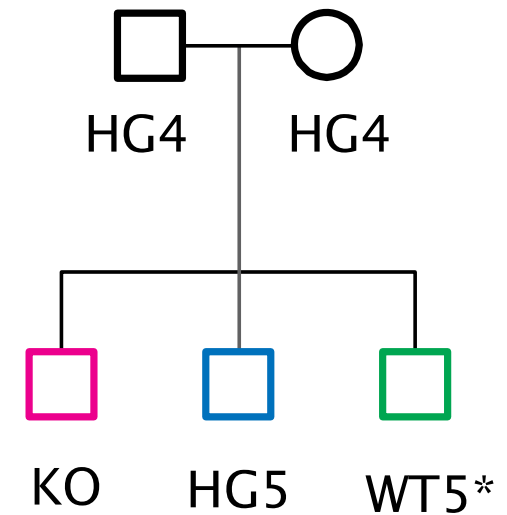
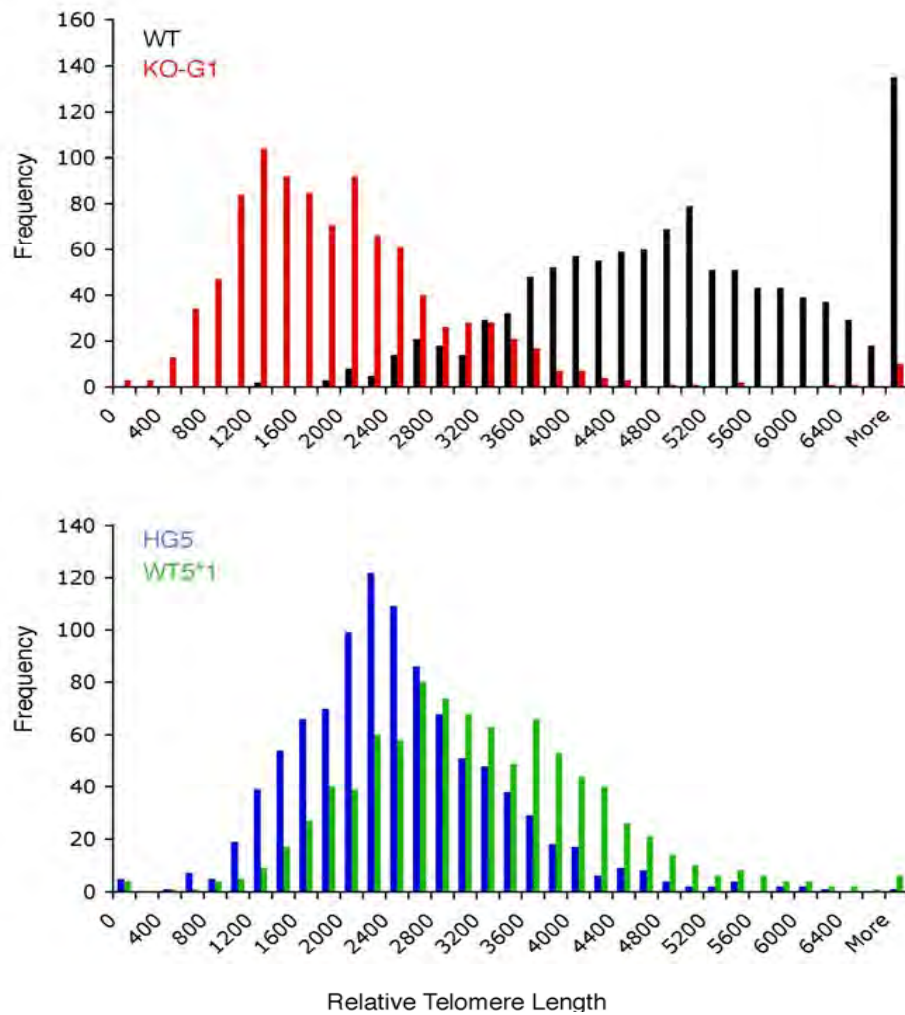


Inheritance of short telomeres decreases survival.
Resembles genetic anticipation in dyskeratosis congenita.

Telomere shortening during mTR+/- breeding affects wildtype offspring

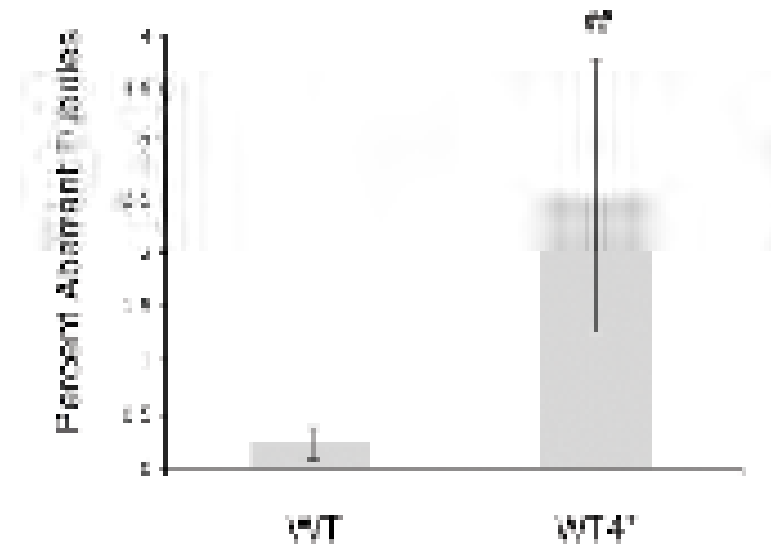


Short telomeres in Wt5* mice



Short telomeres cause phenotypes in Wt^* mice

Testis Apoptosis



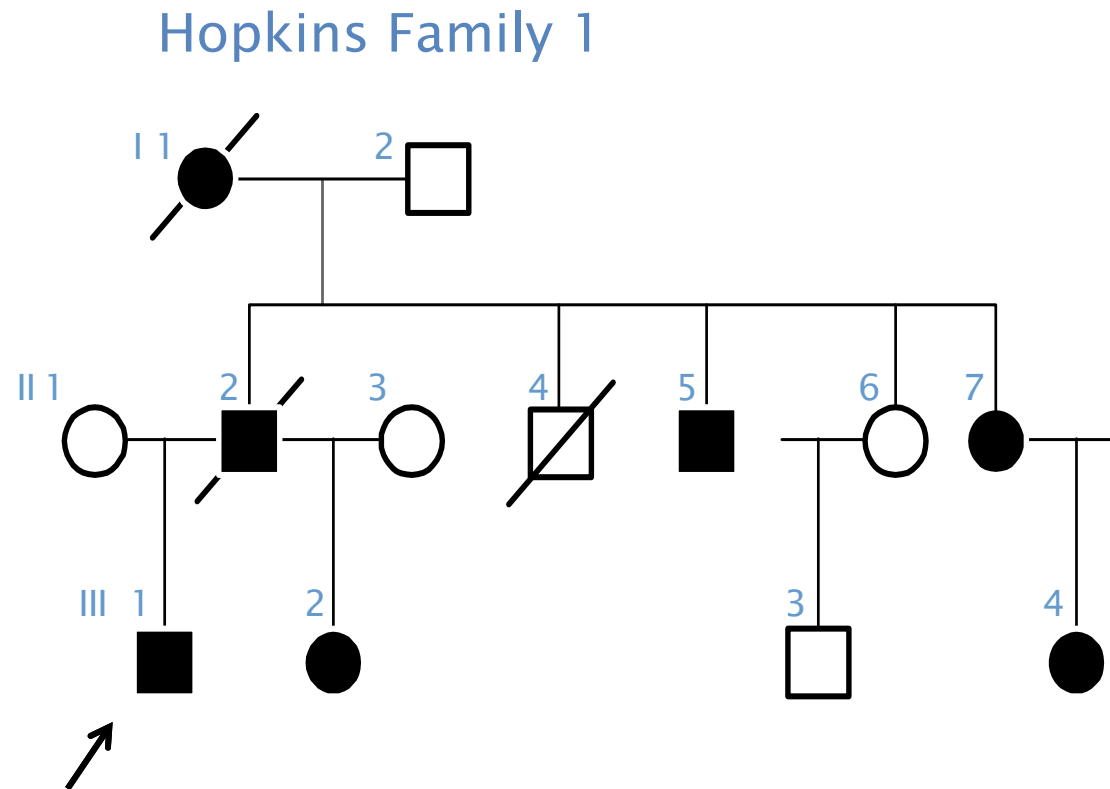
Genetic disease in the absence of telomerase mutation (Occult genetic disease)

Mary Armanios



Telomere shortening leads to genetic anticipation

- Genetic anticipation of pulmonary fibrosis.



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

From the Department of Oncology (M.Y.A., C.W.G.), the Graduate Program in Cellular and Molecular Medicine (J.K.A.), the Institute of Genetic Medicine (B.G.), and the Department of Molecular Biology and Genetics (C.W.G.), Johns Hopkins University School of Medicine, Baltimore; the Department of Chemistry and Biochemistry (J.J.-L.C., M.X.) and the School of Life Sciences (J.J.-L.C.), Arizona State University, Tempe; the Departments of Pediatrics (J.D.C., J.A.P.) and Medicine (C.M., W.E.L., J.E.L.), Vanderbilt University School of Medicine, Nashville; the Veterans Affairs Medical Center, Nashville (W.E.L.); and the Terry Fox Laboratory (I.V., P.M.L.) and the British Columbia Cancer Agency and the Department of Medicine (P.M.L.), University of British Columbia, Vancouver, BC, Canada. Address reprint requests to Dr. Armanios at the Department of Oncology, Johns Hopkins University School of Medicine, 1650 Orleans St., CRB 1-21231, or at marman1@jhmi.edu.

NEJM 357 p1317
(2007)

Syndromes of Telomere Shortening

Mary Armanios

Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21285; email: marmani1@jhmi.edu

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

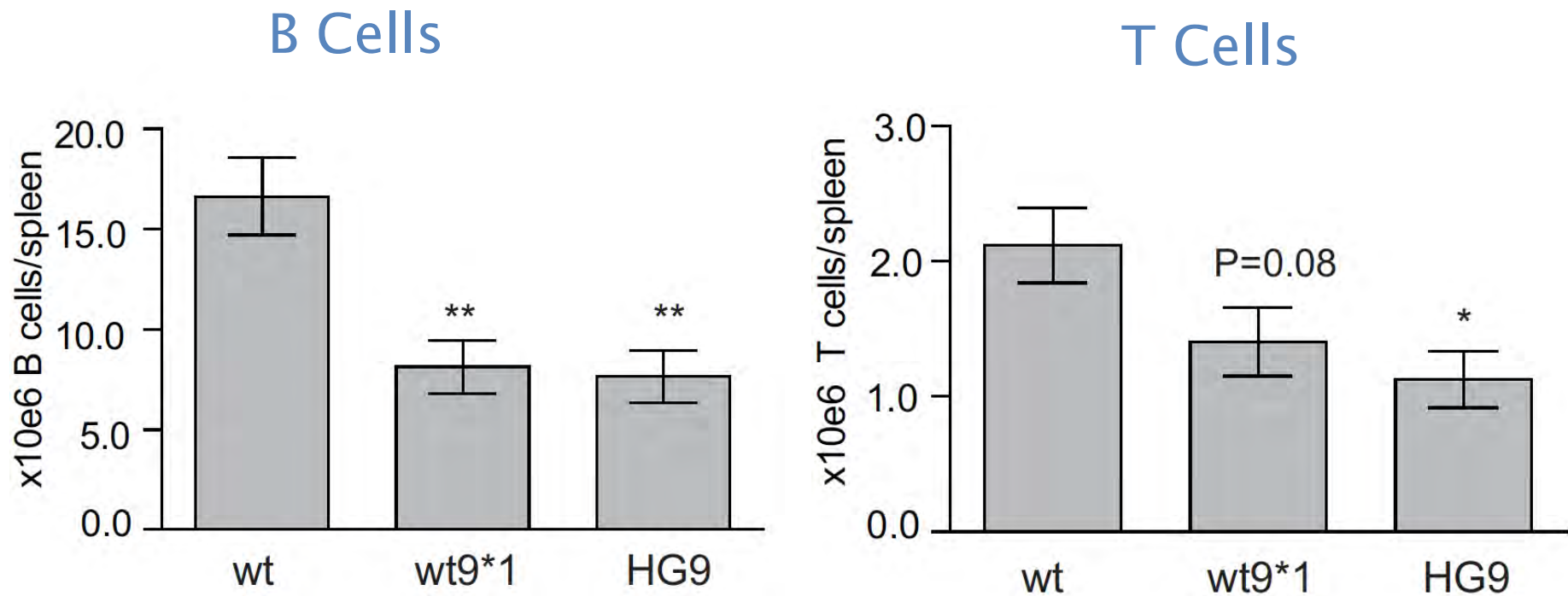
- Normal or mildly elevated transaminases
- Atrophic nodular liver on imaging studies
- Splenomegaly
- Cryptogenic liver fibrosis/cirrhosis

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

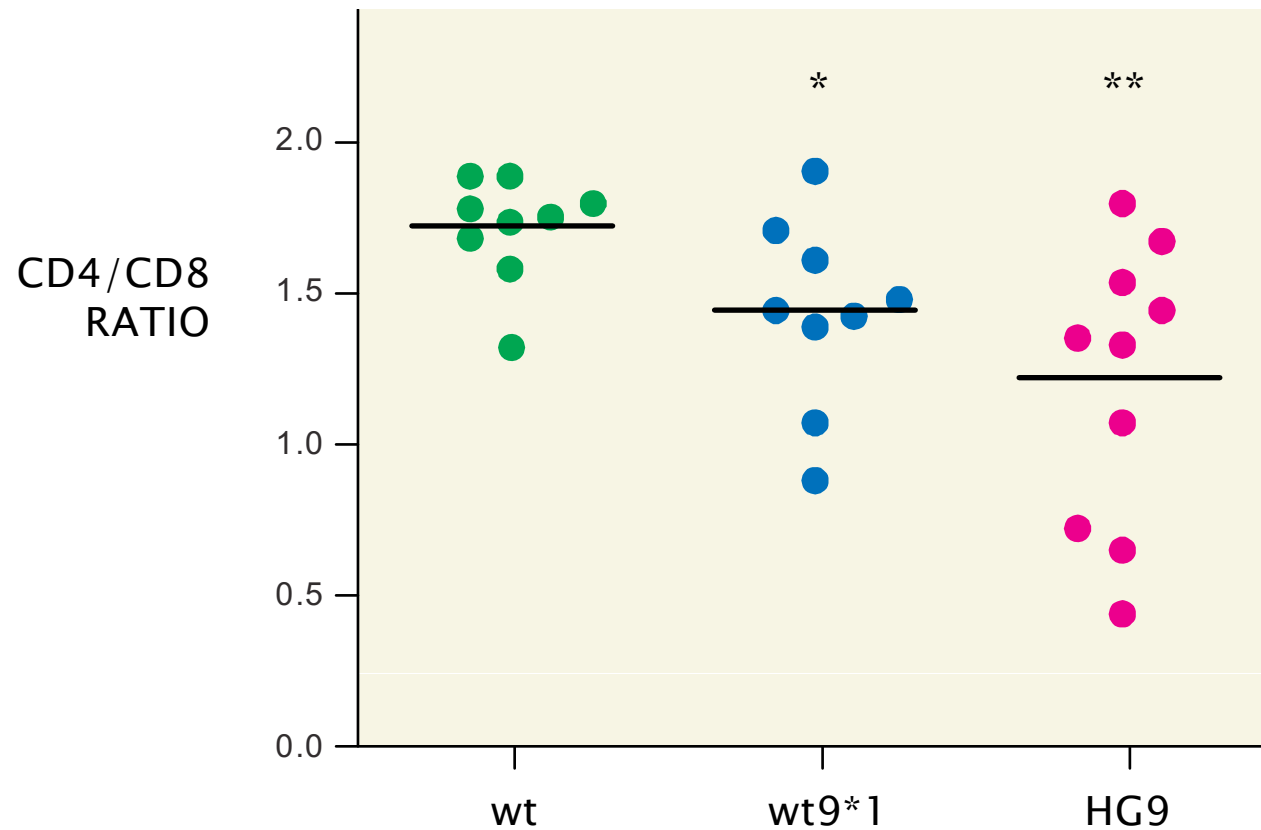
- Bone marrow failure
- Immune senescence
- Chemotherapy intolerance
- Pulmonary fibrosis
- Liver disease
- Increased cancer incidence

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

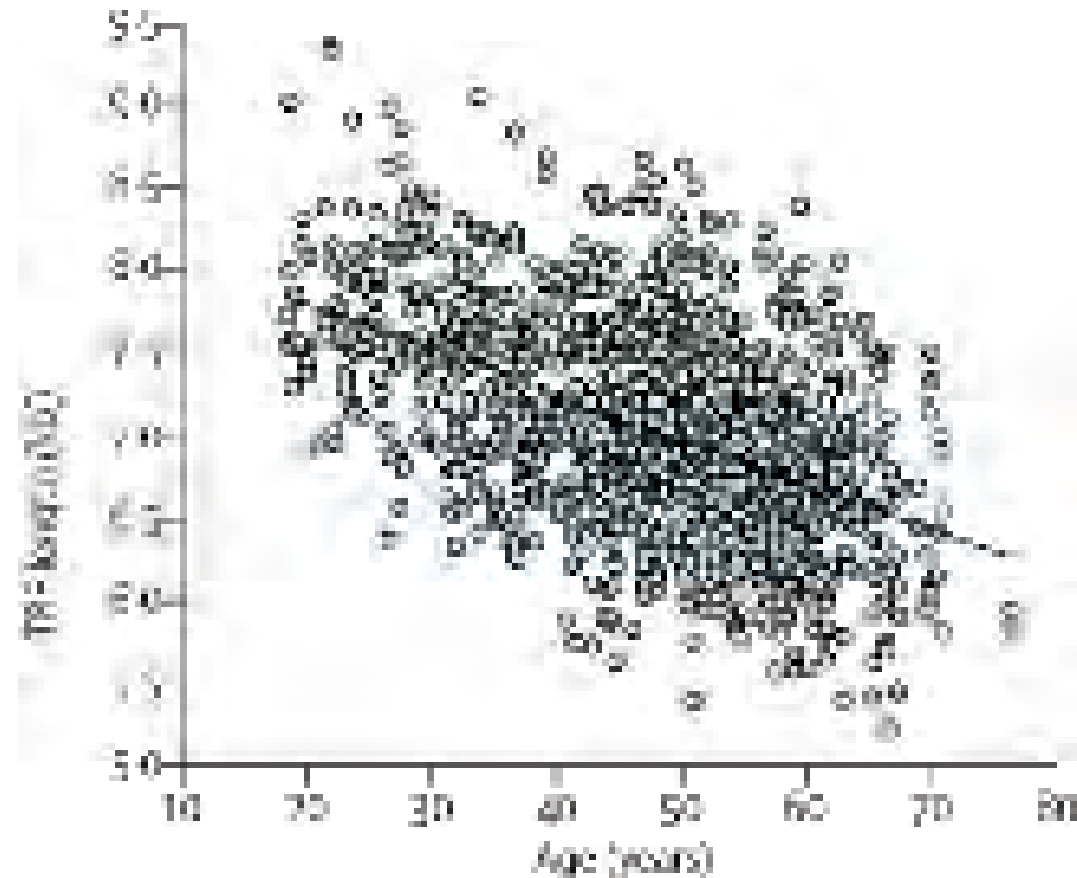
Wildtype mice with short telomeres show a decreased number of B and T lymphocytes



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



Extensive telomere length heterogeneity in the human population

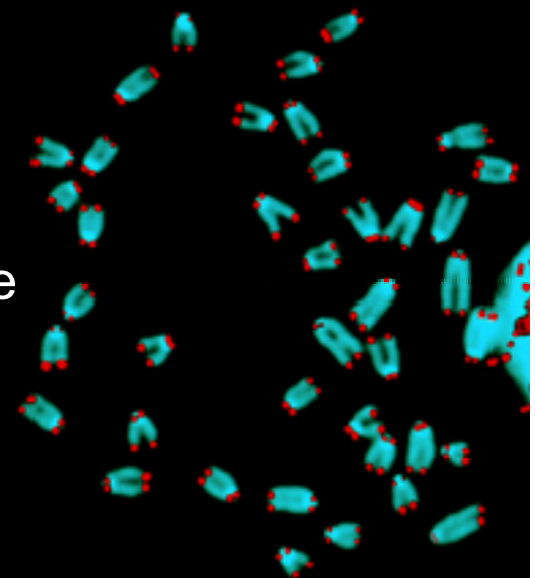


Valdes et al, 2005 *Lancet*
(2005)

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

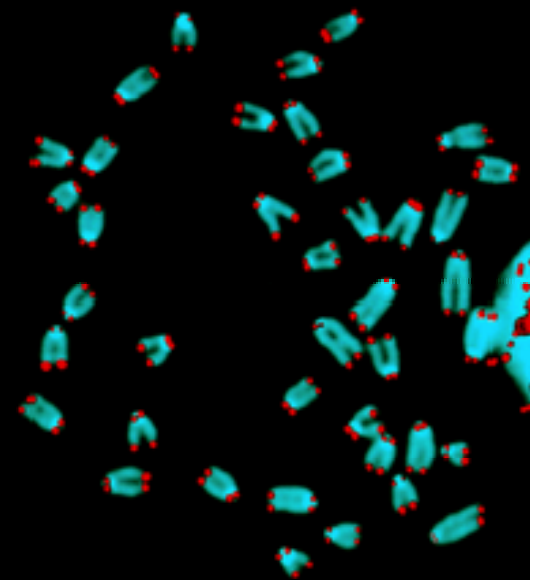
Summary and implications

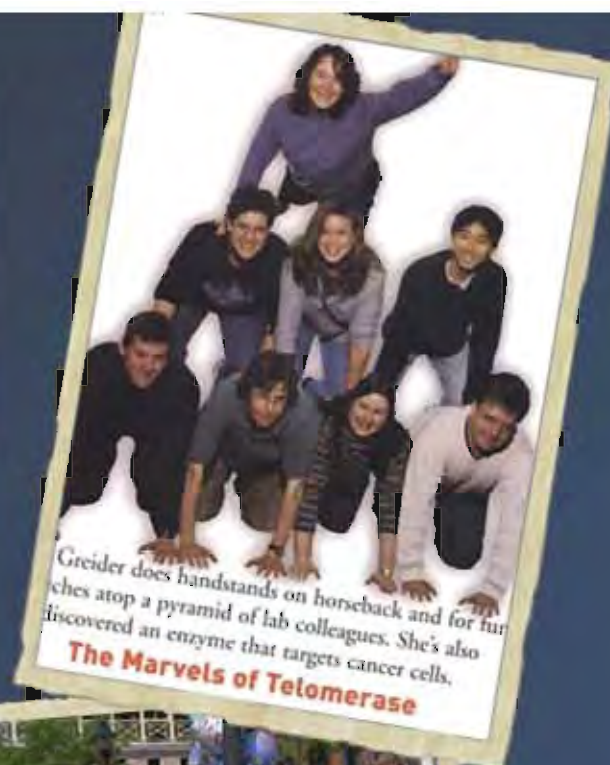
- Telomerase is essential for telomere maintenance.
- Telomere shortening leads to cell death or senescence after many cell divisions.
- Short telomeres inhibit tumor growth through apoptosis or senescence.
 - Telomerase inhibitors may be effective in cancer therapy.
- Haploinsufficiency for telomerase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



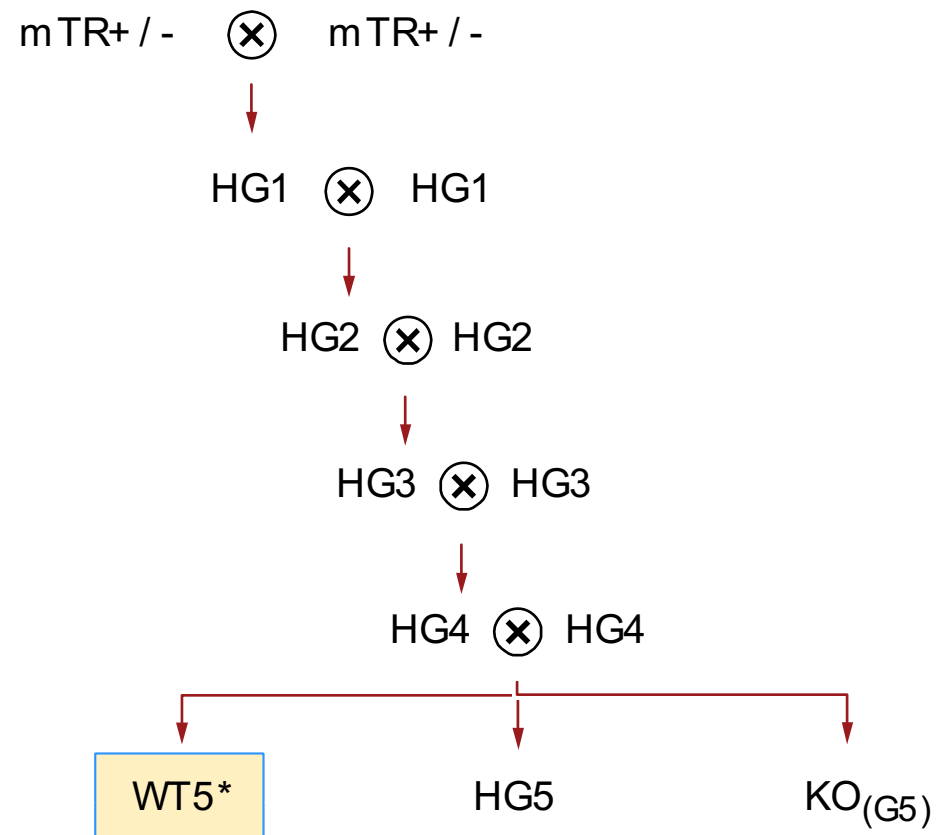
Summary and implications

- 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.

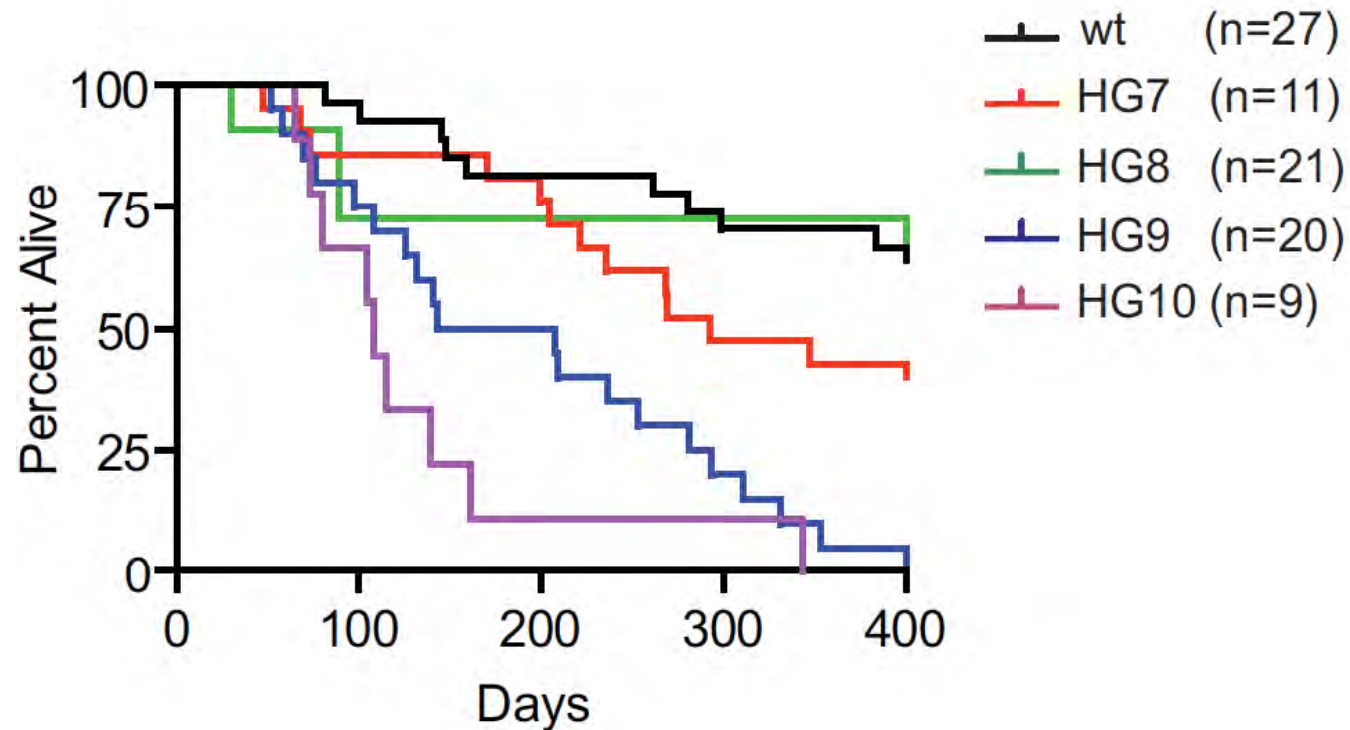




Telomere shortening during mTR+/- breeding affects wildtype offspring

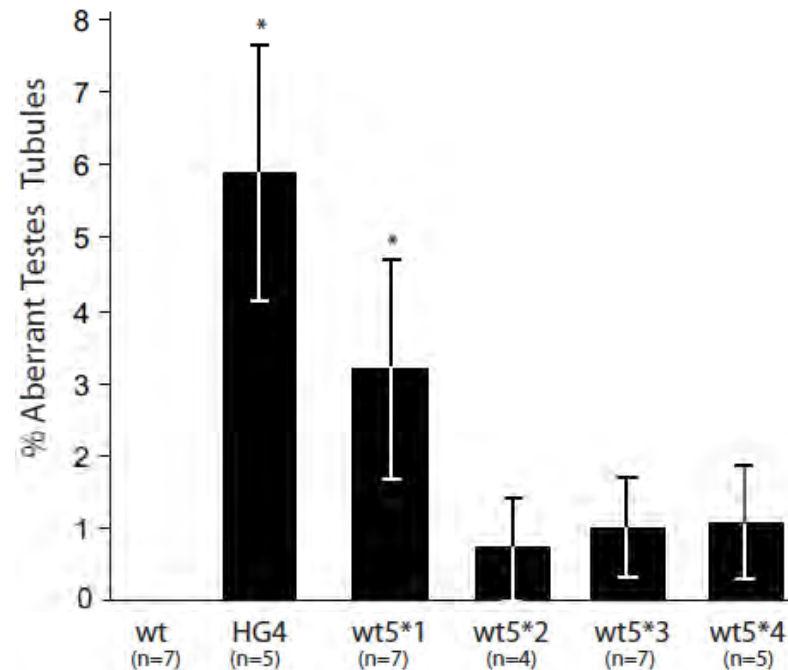
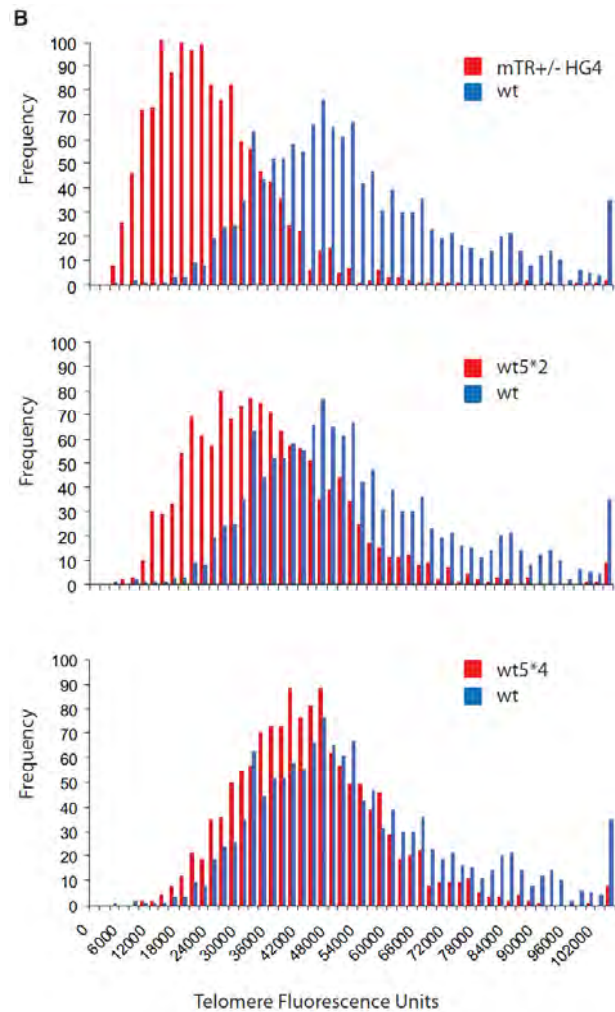


Decreased survival of mTR+/- from later generation heterozygous crosses



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

Telomere length equilibrium is reestablished after five generations



Wt* mice reestablish telomere equilibrium after several generations

