TELOMERI

La cromatina telomerica

Telomeres also bind to nucleosomes, which are rich in modified histones.

Major histone modifications *found in telomeres are*
*-H3K9 and H4K20 trimethylatio*n
-low abundance of acetylated H3 and H4

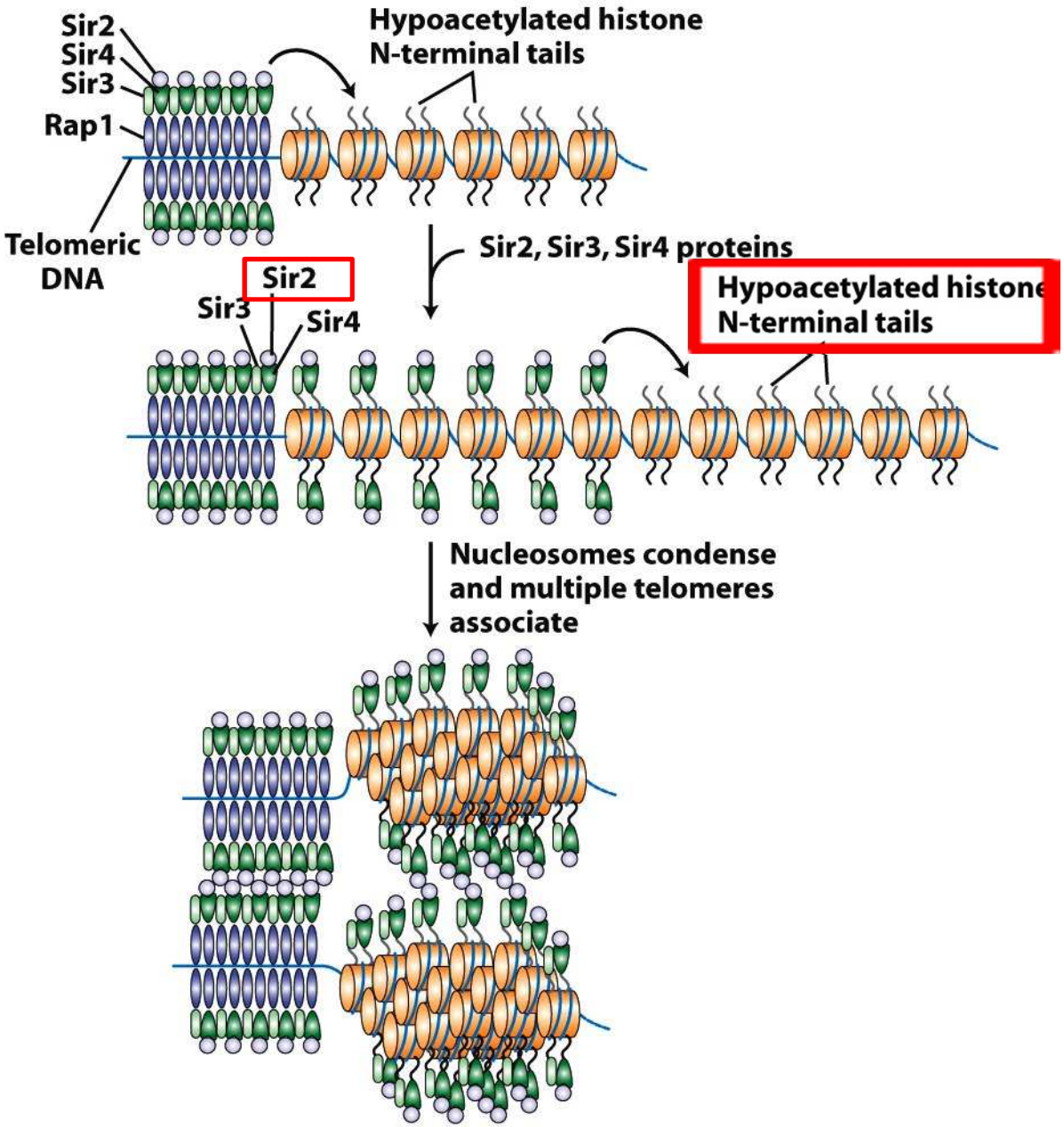


Figure 7-35
Molecular Cell Biology, Sixth Edition

SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

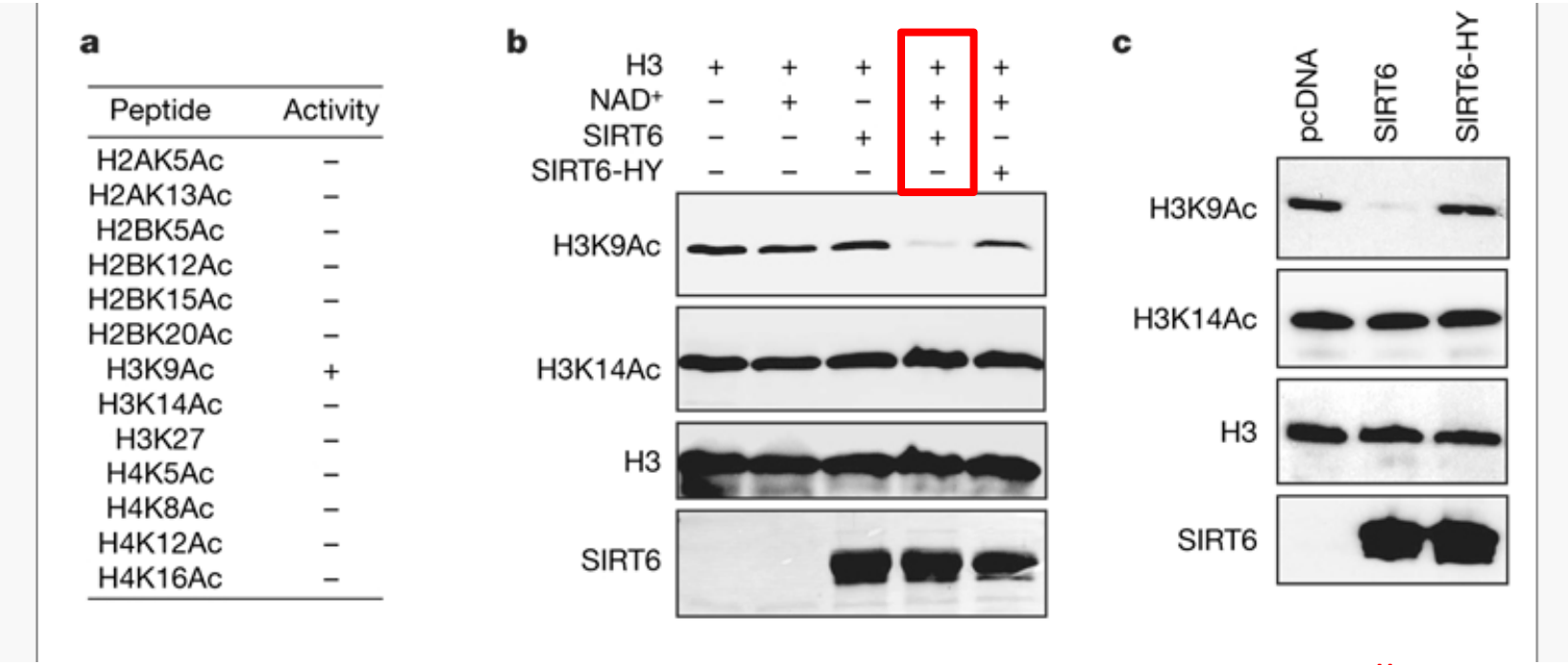
The Sir2 deacetylase regulates chromatin silencing and lifespan in Saccharomyces cerevisiae1,2.
Inmice, deficiency for the Sir2 family member SIRT6 leads to a shortened lifespan and a premature ageing-like phenotype.

SIRT6 is a chromatin-associated NAD+-dependent, histone H3 lysine 9 (H3K9) deacetylase that modulates telomeric chromatin.

SIRT6 associates specifically with telomeres, and SIRT6 depletion leads totelomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence. Moreover, SIRT6-depleted cells exhibit abnormal telomere structures

We propose that SIRT6 contributes to the propagation of a specialized chromatin state at mammalian telomeres, which in turn is required for proper telomere metabolism and function. Our findings constitute the first identification of a physiological enzymatic activity of SIRT6, and link chromatin regulation by SIRT6 to telomere maintenance and a human premature ageing syndrome

SIRT6 (sir 2) deacetylates lysine 9 of histone H3 at telomeric chromatin



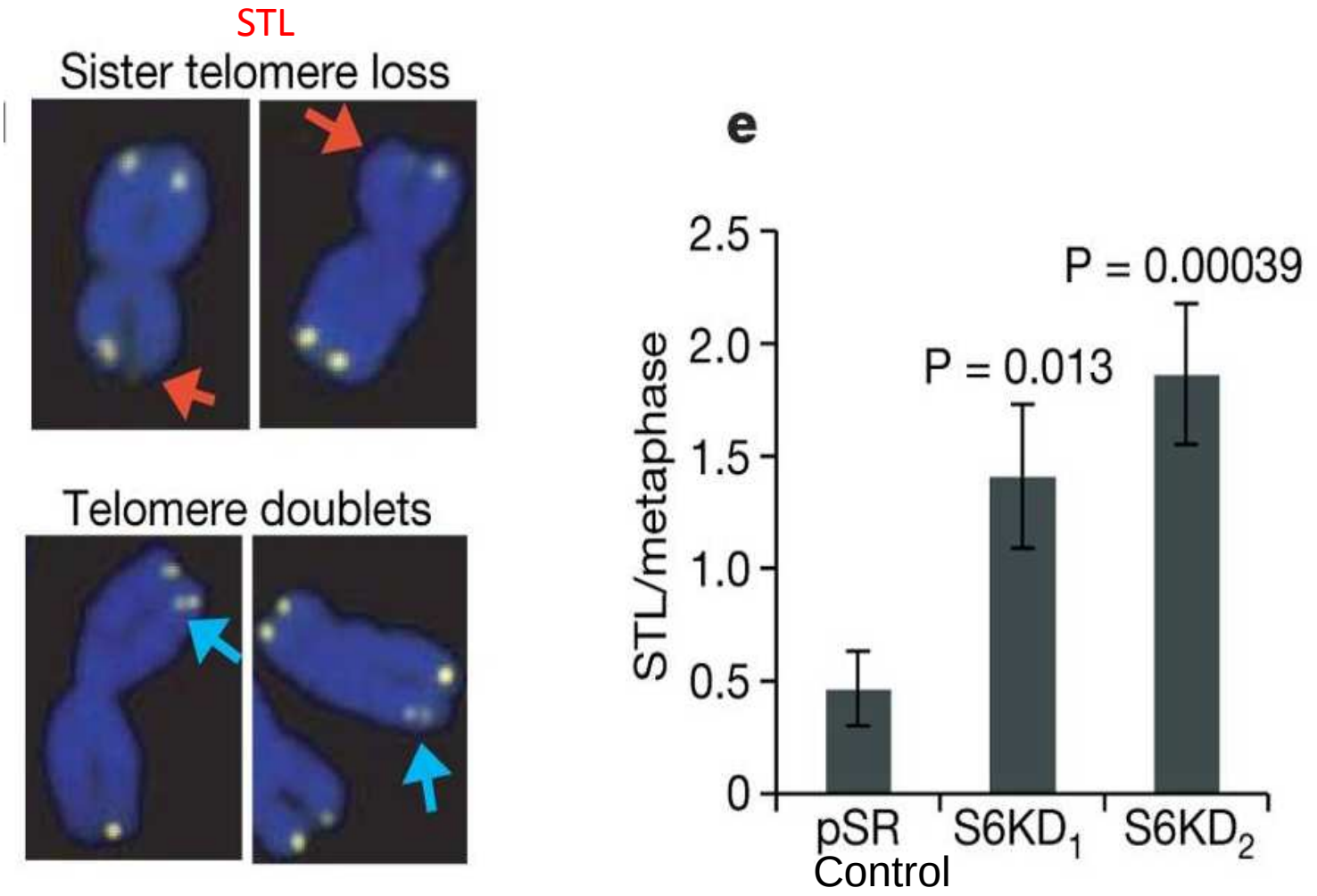
histone tail peptides

full-length histone H3

293Tcells overexpressing SIRT6

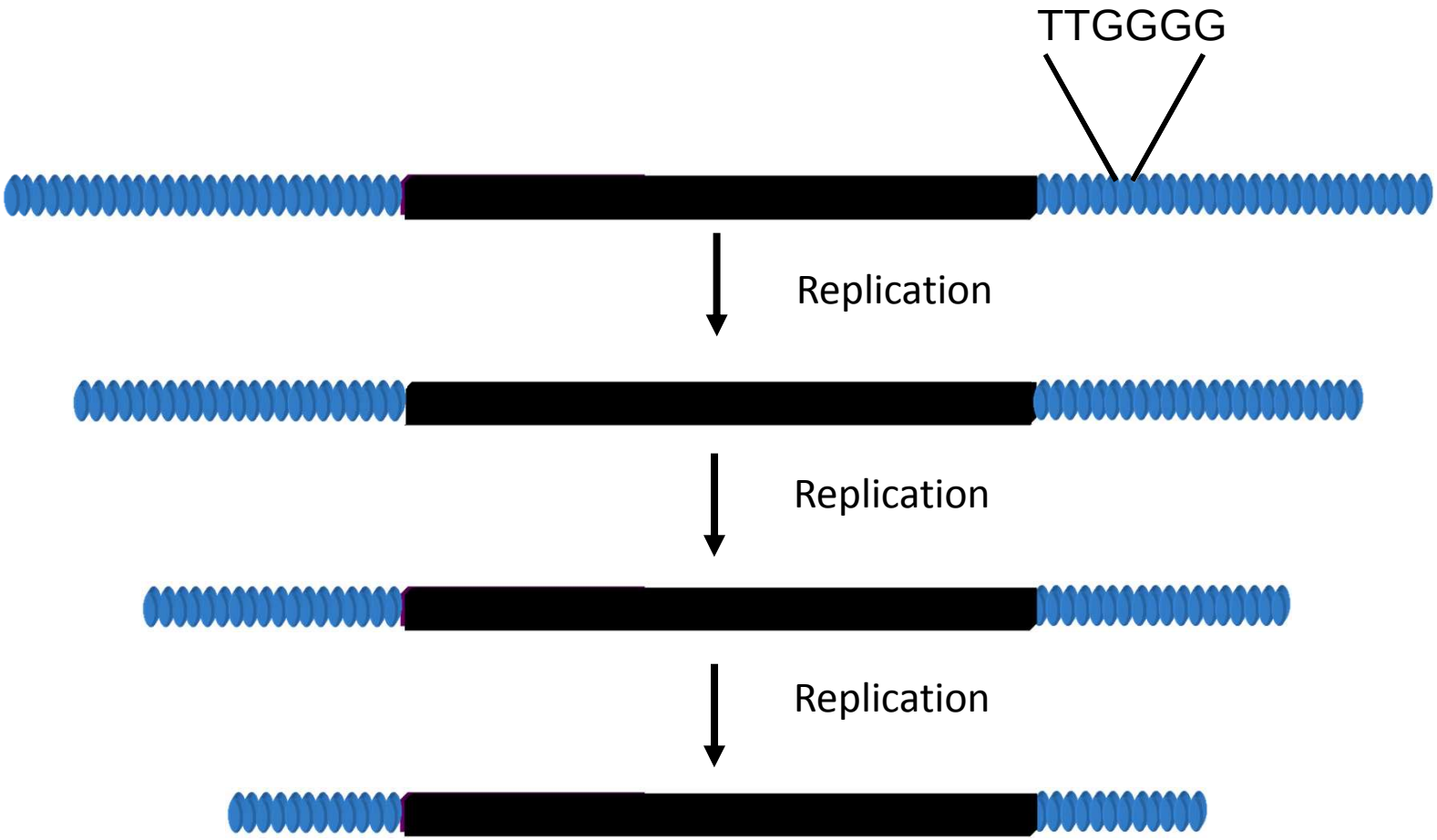
SIRT6-HY: *catalytic H133Y SIRT6 mutant protein*

SIRT6 knockdown (S6KD) cells

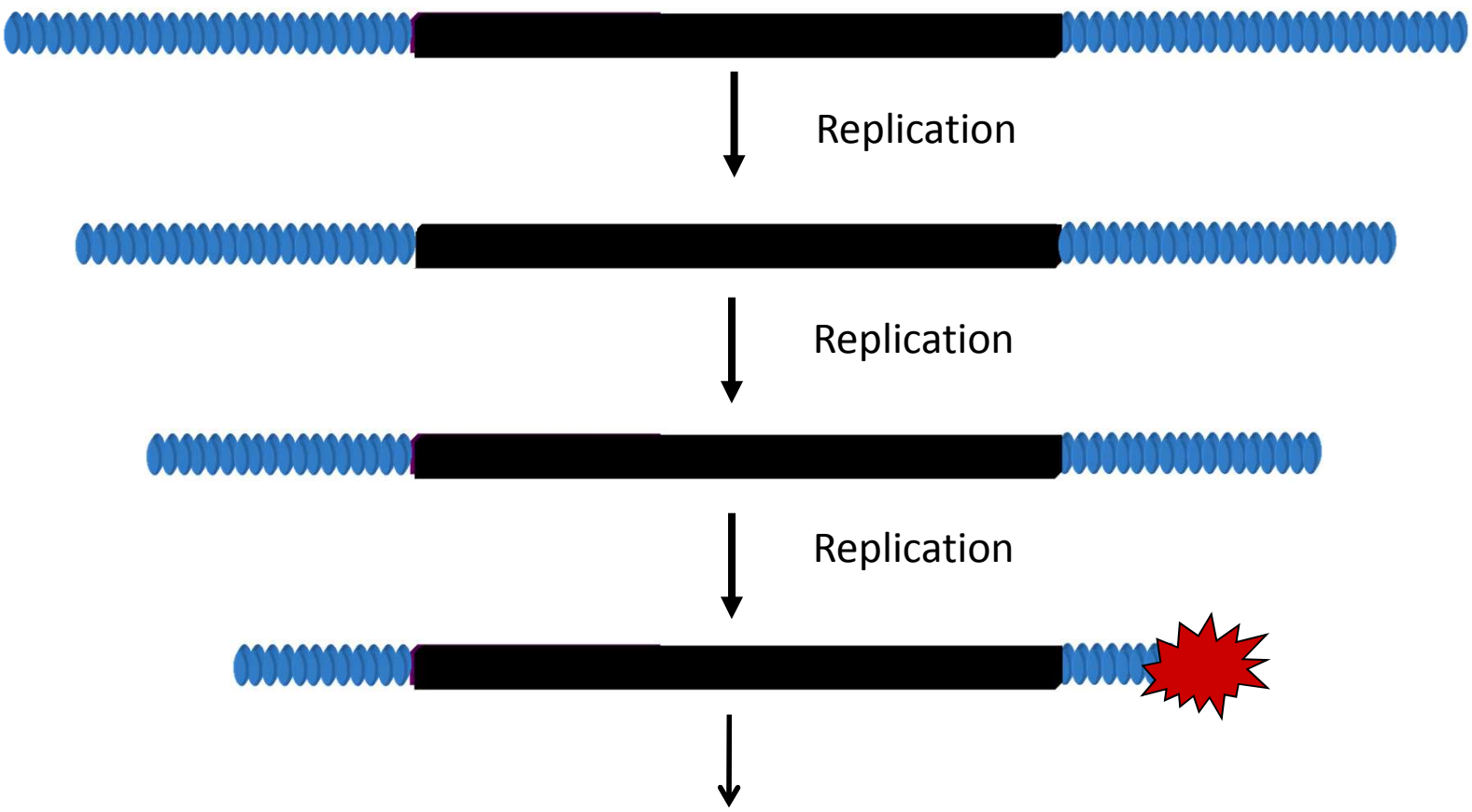


d, Representative S6KD metaphases showing aberrant telomere signals. Red arrows, sister telomere loss; blue arrows, telomere doublets. e, Quantification of sister telomere loss

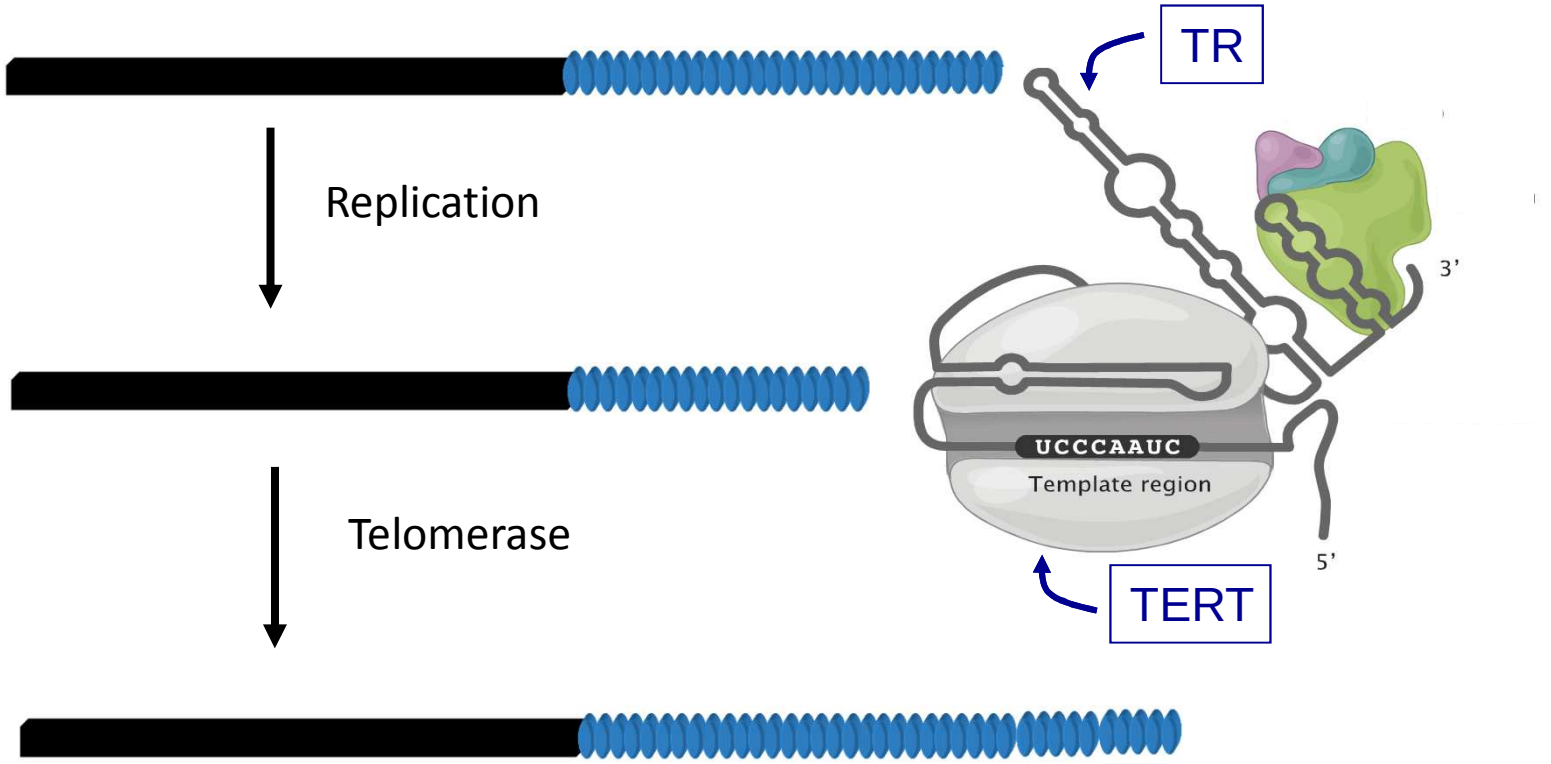
Telomeres shorten as cells divide



Telomere shortening leads to cell death



Telomerase allows telomere length equilibrium maintenance

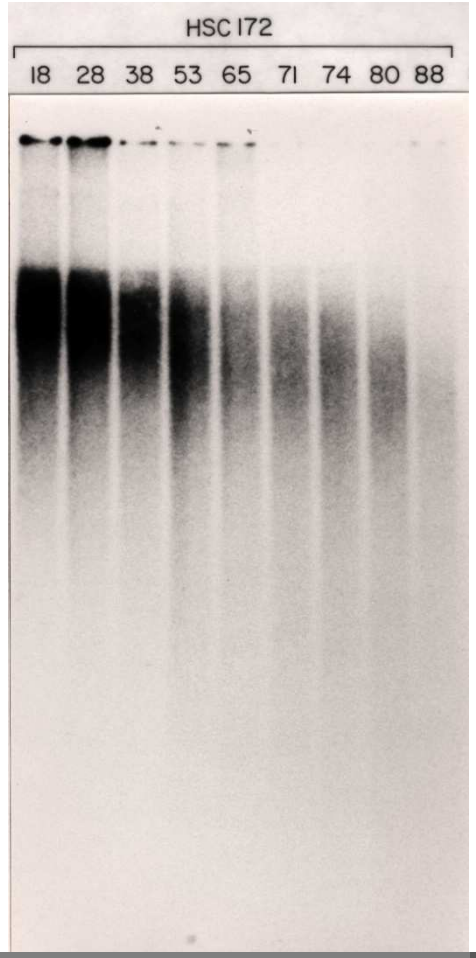


Telomere shortening in cellular senescence

Telomeres shorten during ageing of human fibroblasts

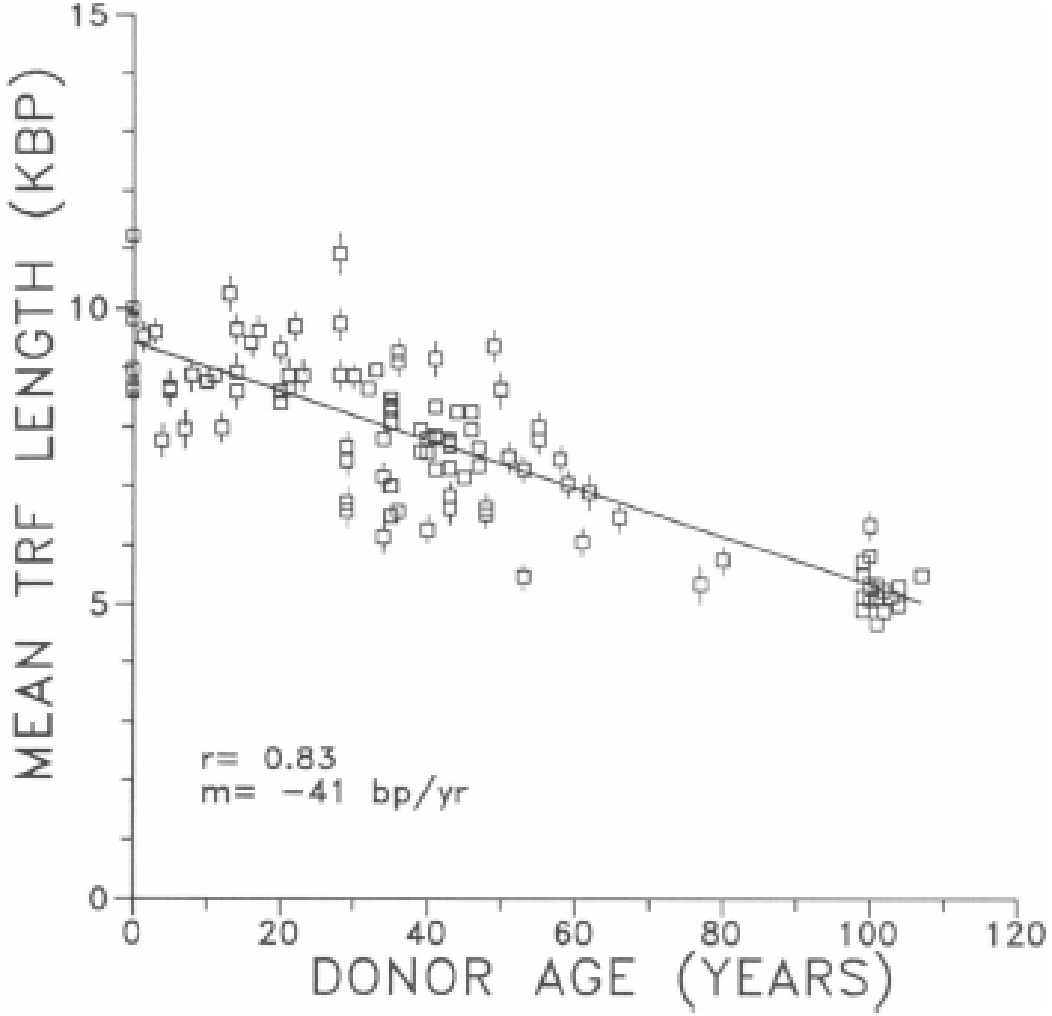
Calvin B. Harley*, A. Bruce Futcher† & Carol W. Greider†

* 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 shorten with age



Telomerase is limiting in cells

Vaziri et al. *AJHG* (1995)

- EMBO Mol Med. 2012 Aug;4(8):691-704. doi: 10.1002/emmm.201200245. Epub 2012 May 15.
- Telomerase **gene therapy in adult and old mice delays** aging **and increases longevity without increasing cancer.**
- Bernardes de Jesus B, Vera E, Schneeberger K, Tejera AM, Ayuso E, Bosch F, Blasco MA.
- Source
- Telomeres and Telomerase Group, Molecular Oncology Program, Spanish National Cancer Centre (CNIO), Madrid, Spain.
- Abstract
- A major goal in aging research is to improve health during aging. In the case of mice, genetic manipulations that shorten or lengthen telomeres result, respectively, in decreased or increased longevity. Based on this, we have tested the effects of a telomerase gene therapy in adult (1 year of age) and old (2 years of age) mice.

- EMBO Mol Med. 2012 Aug;4(8):691-704. doi: 10.1002/emmm.201200245. Epub 2012 May 15.
- Telomerase **gene therapy in adult and old mice delays** aging **and increases longevity without increasing cancer.**
- Abstract 2
- Treatment of 1- and 2-year old mice with an adeno associated virus (AAV) of wide tropism expressing mouse TERT had remarkable beneficial effects on health and fitness, including insulin sensitivity, osteoporosis, neuromuscular coordination and several molecular biomarkers of aging. Importantly, telomerase-treated mice did not develop more cancer than their control littermates, suggesting that the known tumorigenic activity of telomerase is severely decreased when expressed in adult or old organisms using AAV vectors.
- Finally, telomerase-treated mice, both at 1-year and at 2-year of age, had an increase in median lifespan of 24 and 13%, respectively. These beneficial effects were not observed with a catalytically inactive TERT, demonstrating that they require telomerase activity. Together, these results constitute a proof-of-principle of a role of TERT in delaying physiological aging and extending longevity in normal mice through a telomerase-based treatment, and demonstrate the feasibility of anti-aging gene therapy.

Technology Corporation for support. This work was also funded in part by the Ralph Hochstetter Medical Research Fund.

Correspondence and requests for materials should be addressed to F.S. (e-mail: sachs@buffalo.edu).

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*

** Department of Haematology, Division of Investigative Science, Faculty of Medicine, Imperial College School of Science, Technology and Medicine, Hammersmith Hospital, Ducane Road, London W12 ONN, UK*
† Department of Pediatrics, The University of Iowa Hospitals and Clinics, Iowa City, Iowa 52242-1083, USA
‡ MRC UK, HGMP Resource Centre, Hinxton Cambridge, CB10 1SB, UK
§ Division of Hematology, Washington University School of Medicine, St. Louis, Missouri 63110, USA

Dyskeratosis congenita is a progressive bone-marrow failure syndrome that is characterized by abnormal skin pigmentation, leukoplakia and nail dystrophy[1,2]. X-linked, autosomal recessive and autosomal dominant inheritance have been found in different pedigrees. The X-linked form of the disease is due to mutations in the gene *DKC1* in band 2, sub-band 8 of the long arm of the X chromosome (ref. 3). The affected protein, dyskerin, is a nucleolar protein that is found associated with the H/ACA class of small nucleolar RNAs and is involved in pseudo-uridylation of specific residues of ribosomal RNA[4]. Dyskerin is also associated with telomerase RNA (hTR)[5], which contains a H/ACA consensus sequence[6,7]. Here we map the gene responsible for dyskeratosis congenita in a large pedigree with autosomal dominant inheritance. Affected members of this family have an 821-base-pair deletion on chromosome 3q that removes the 3′ 74 bases of hTR. Mutations in hTR were found in two other families with autosomal dominant dyskeratosis congenita.

Three other proteins, GAR1, NHP2 and NOP10, are known to be present along with dyskerin in the nucleolar ribonucleoprotein complex and in the telomerase complex[5,8,9]. Telomerase is an RNA–protein complex that is essential for maintaining the nucleoprotein caps at the ends (telomeres) of eukaryotic chromosomes[10,11]. The principal components of telomerase are hTR[6] and a specialized reverse transcriptase (hTERT)[12]. Dyskeratosis congenita is a multisystem disease that affects tissues such as skin, gut and bone marrow, all of which require constant renewal that is dependent on stem-cell activity, and thus may be due to a defect in stem-cell turn over or proliferative capacity[2,13]. Defects in rRNA synthesis and/or in telomere maintenance might affect stem-cell function[14]. Dyskeratosis congenita patients have markedly shorter telomeres than normal individuals and this is apparent from an early age[15]. The relative importance of rRNA processing and telomere maintenance in the pathophysiology of dyskeratosis congenita may be clarified by the nature of the genetic loci causing the autosomal form(s) of the disease. Our finding of mutations in the telomerase RNA component (hTR) in three separate autosomal dominant pedigrees suggests that dyskeratosis congenita is due to defective telomerase activity.

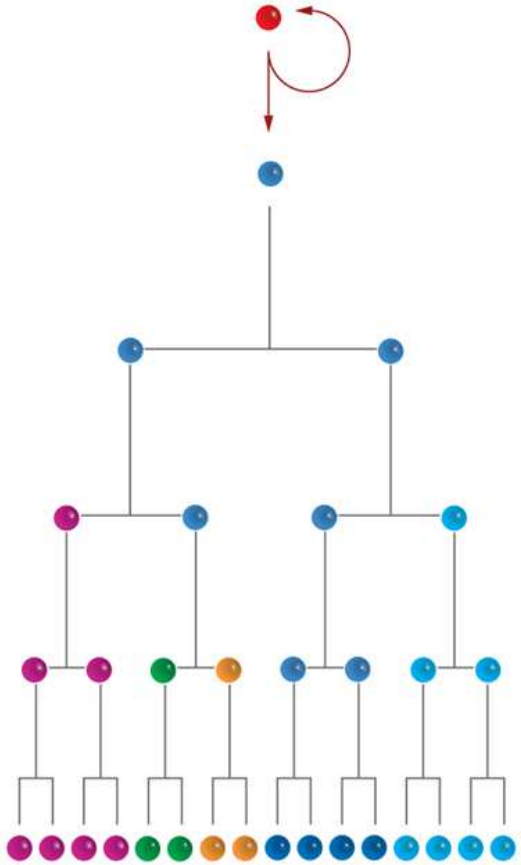
Among the families on the dyskeratosis congenita registry at the Hammersmith Hospital is a large family from Iowa, USA, with a mild form of dyskeratosis congenita and a[illegible] inheritance (DCR101; see Supplementary Info[illegible]

Nature 2001

Dyskeratosis congentia causes bone marrow failure

- Skin and nail problems
  - Skin hyperpigmentation
  - Rashes
  - Abnormal nail growth

- Mortality
  - Bone marrow failure
  - Cancer
  - other?



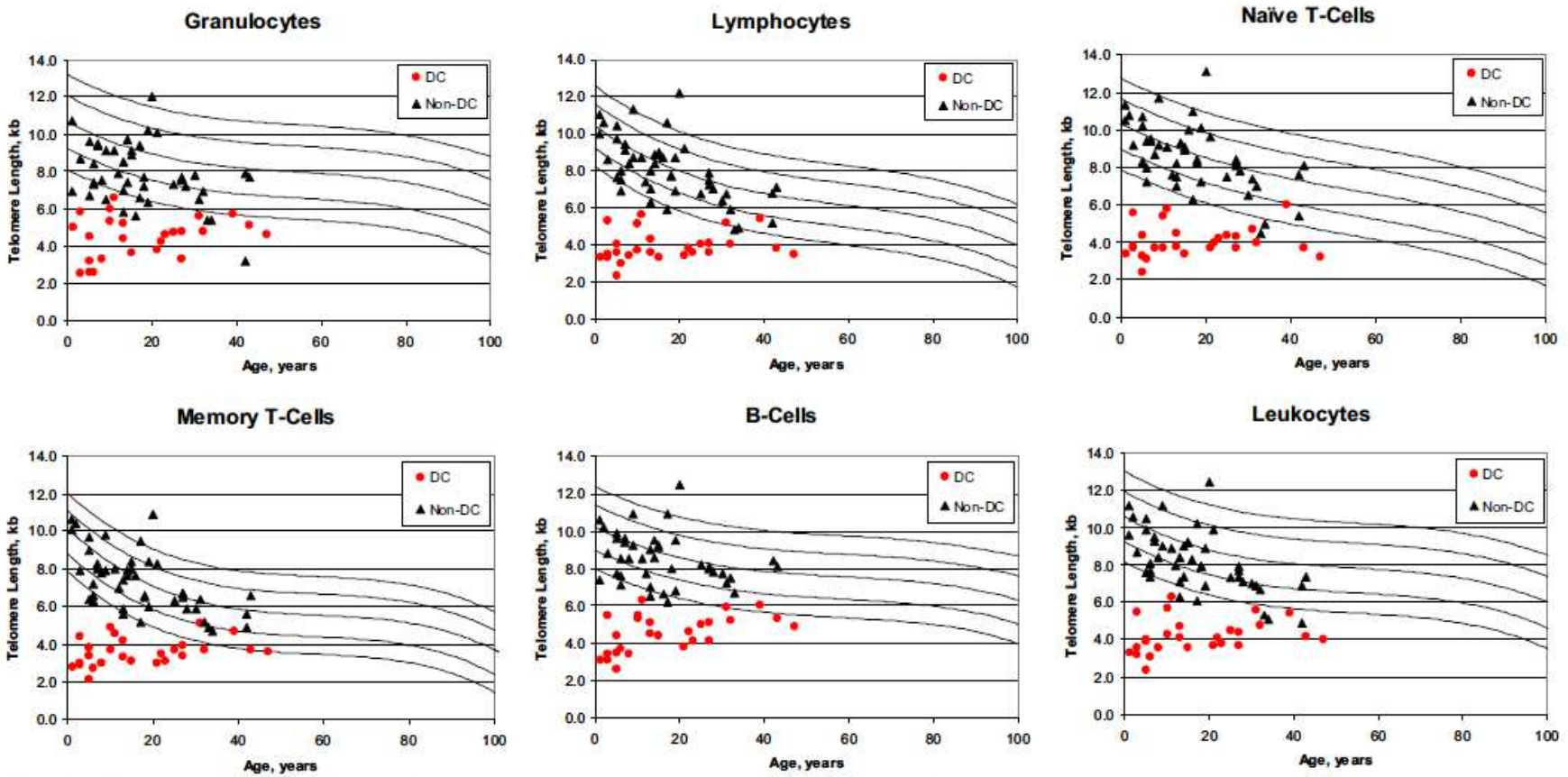


Figure 3. Telomere length according to age in dyskeratosis congenita and non–dyskeratosis congenita patients. The vertical axis represents telomere length in kilobytes. Lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal control subjects. Symbols represent subjects: 26 patients with dyskeratosis congenita (red solid circle), 46 non–dyskeratosis congenita patients (black solid triangle).

Short telomeres in lung disease

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

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 (R.G.I.), 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-[illegible], Baltimore, MD 21231, or at marmani1@jhmi.edu.

NEJM 357 p1317 (2007)

Table 1 Mutations in telomerase and telomere genes lead to a broad clinical spectrum of syndromes of telomere shortening

Gene Name	Diagnosis	Typical age of onset in years
hTR and *hTERT*	Sporadic IPF 1–3% Familial IPF[a] 8–15% Sporadic and familial aplastic anemia ~3–5% Autosomal dominant DC[b]	Broad range 5–77
DKC1	X-linked DC Hoyeraal-Hreiderasson	Less than 30 Less than 5
TINF2	Sporadic DC Autosomal dominant DC Hoyeraal-Hreiderasson	Less than 10 - Less than 5
NOP10	Autosomal recessive DC	-
NHP2	Autosomal recessive DC	-

[a]IPF refers to idiopathic pulmonary fibrosis.
[b]DC refers to dyskeratosis congenita.

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

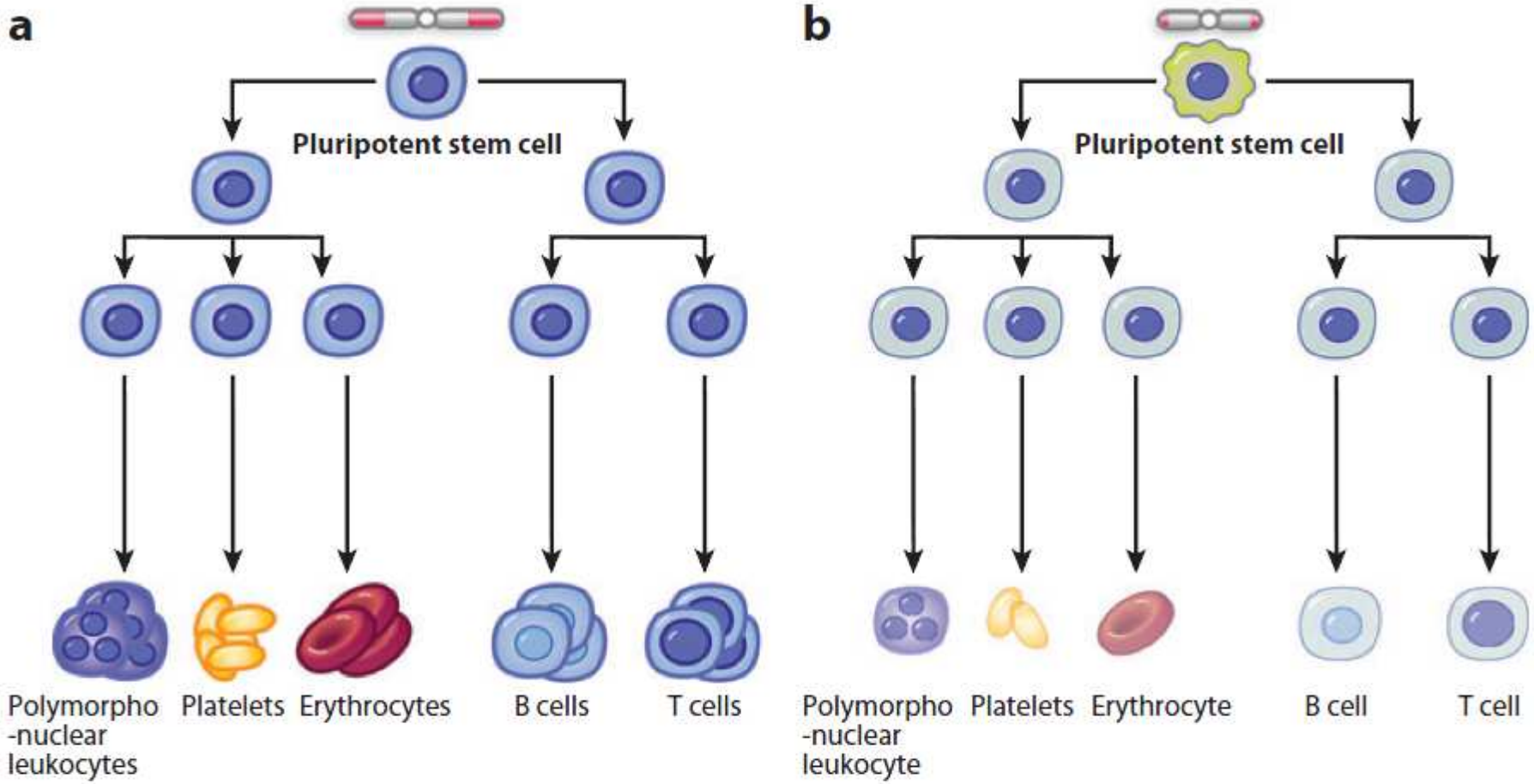


Figure 5

Short telomeres lead to stem cell failure in the bone marrow. (*a*) Normal hematopoiesis is hierarchical and relies on the intact capacity of a pleuripotent stem cell to self-renew and differentiate. When telomeres are short (*b*), stem cell function is impaired and the impairment leads to a progressive decline in the production of mature blood lineages in aplastic anemia.

SUMMARY POINTS

1. Mutations in telomerase and telomere components lead to a broad spectrum of disease that has clinical presentations in children and adults. The extent of telomere shortening determines the onset and severity of these disorders.
2. The study of families with mutations in telomerase components allows the identification of a distinct disease entity marked by organ failure in the bone marrow and a clustering of pulmonary and liver fibrosis. This syndrome frequently appears in adulthood and is distinct from DC, though it falls on the same spectrum.
3. IPF is the most common manifestation of a syndrome of telomere shortening. The causal role implicating short telomeres in IPF provides evidence that short telomeres are sufficient to cause common, age-related disease with its most common manifestation in the lung.
4. Syndromes of telomere shortening are unique among progeroid disorders in that they phenocopy a process that occurs in humans as they age.