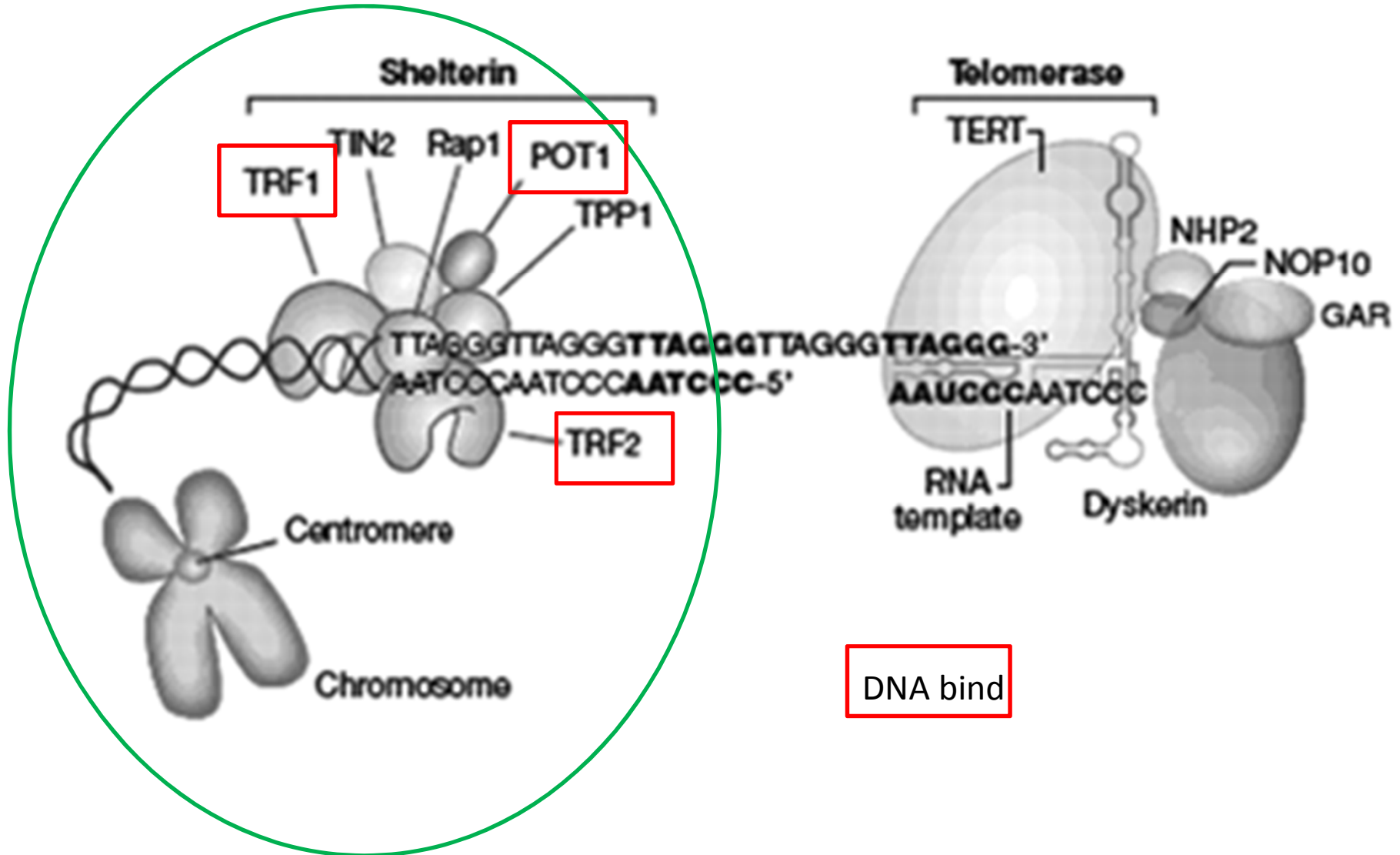


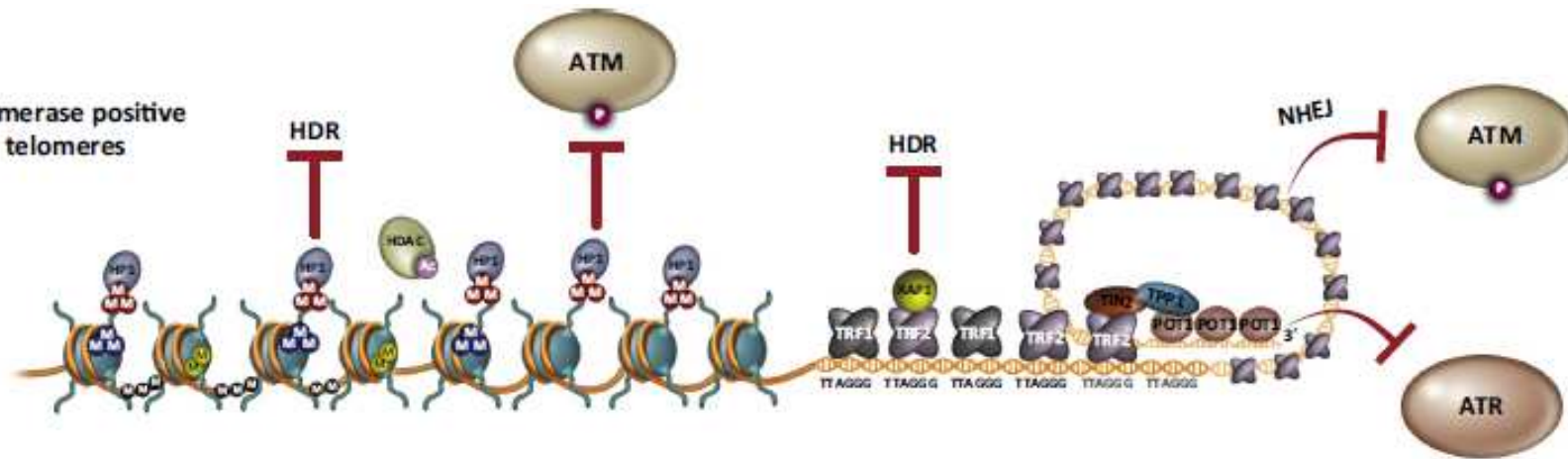
TELOMERI

Complessi macromolecolari associati al Telomero ed alla Telomerasi



(A)

Telomerase positive telomeres

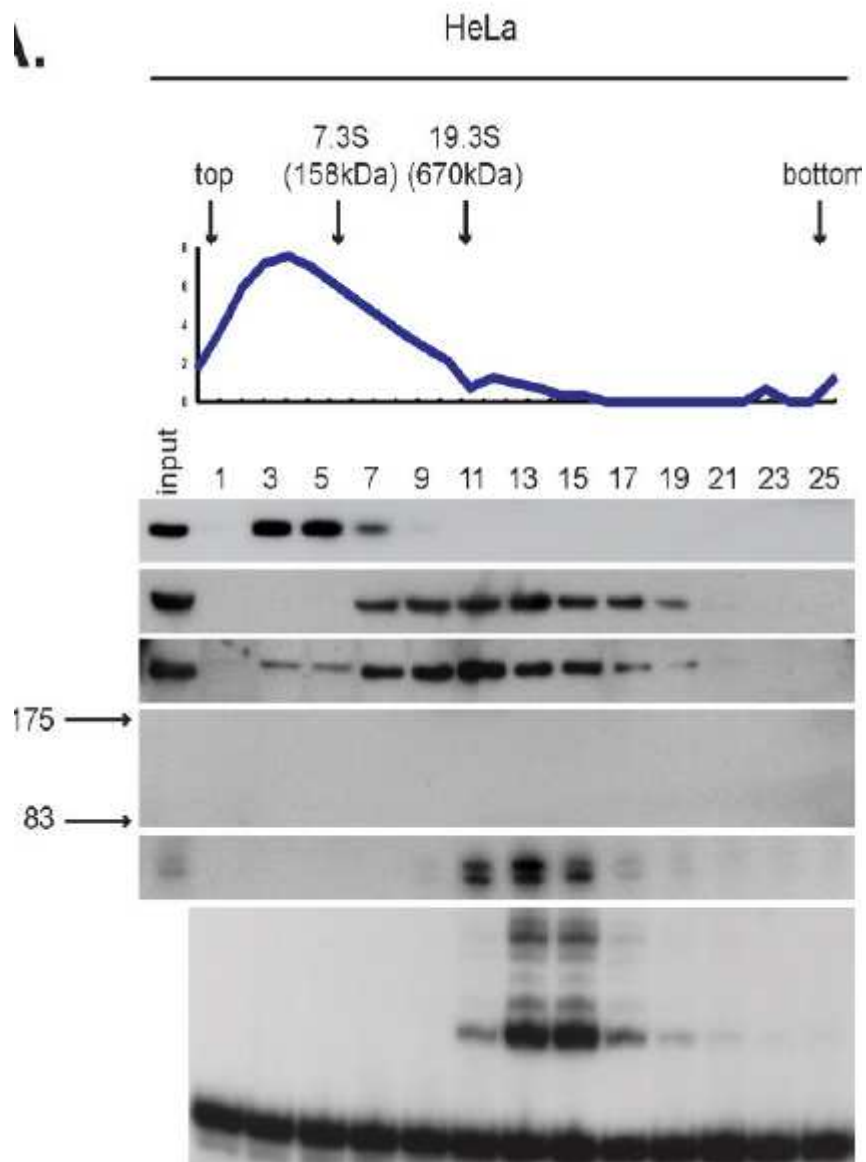


Key:	T-loop associated shelterin	Histone acetylation
T-loop associated NR2F2	H2A K15 mono-ubiquitination	Lysine poly-ubiquitination
DNA methylation	Protein phosphorylation	Protein sumoylation
H3K9me3	γH2AX (phosphorylation of serine 139 of histone H2AX)	
H4K20me3		
H4K20me2		
H3K79me2		

Few other proteins are known to be required for human telomerase function, significantly limiting our understanding of both telomerase regulation and mechanisms of telomerase action.

The ATPases **pontin and reptin** are telomerase components as indicated by Affinity purification of TERT from human cells.

Pontin interacts with both TERT and dyskerin,



1

Total protein

IB: α -tubulin

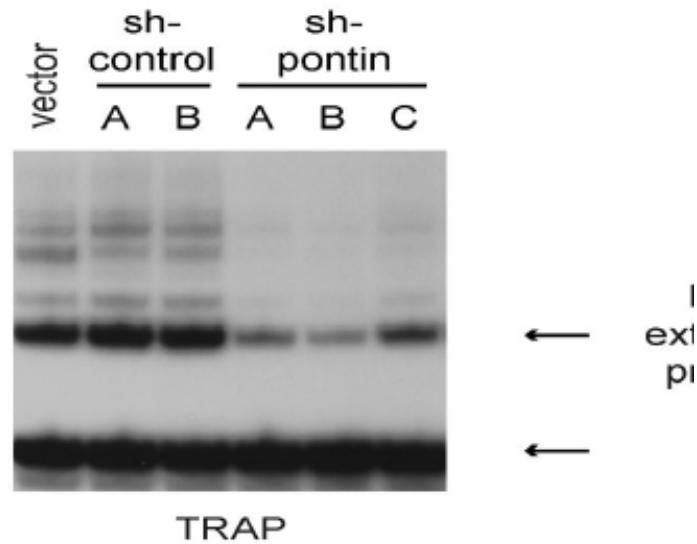
IB: pontin

IB: reptin

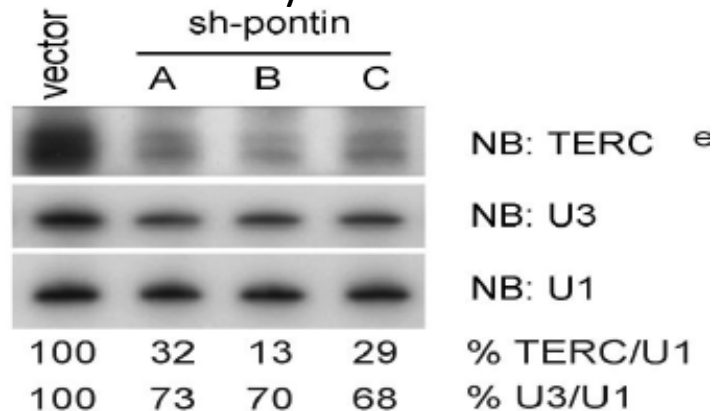
NB: TERC

TRAP

Depletion of pontin and reptin markedly impairs telomerase activity



Depletion of pontin and reptin markedly impairs accumulation of the telomerase RNP, indicating an essential role in telomerase assembly.

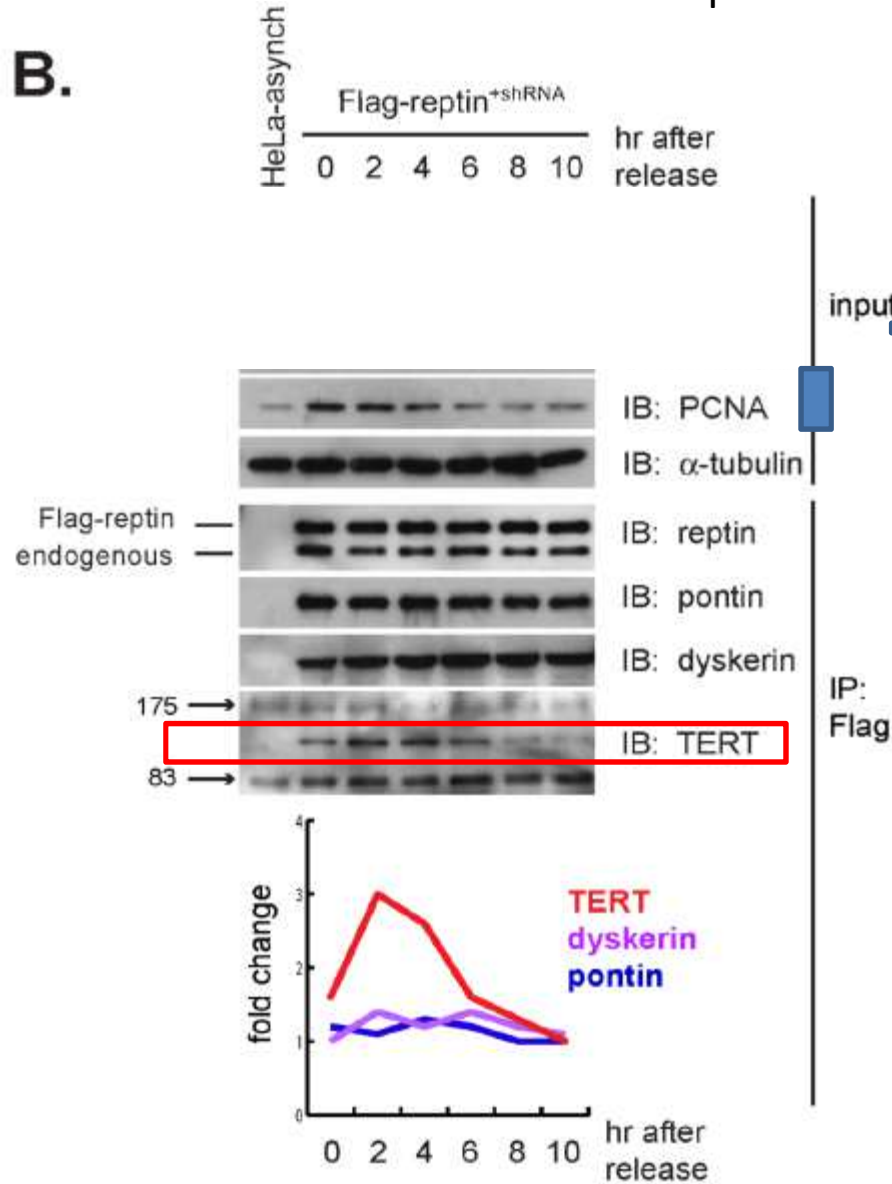


the amount of TERT bound to pontin and reptin
peaks in S phase,
evidence for dynamic cell cycle-dependent
regulation of TERT

These findings reveal an unanticipated
requirement for additional enzymes in
telomerase RNP biogenesis and suggest new
approaches for inhibiting telomerase in human
cancer.

the amount of TERT bound to pontin and reptin peaks in S phase,

B.



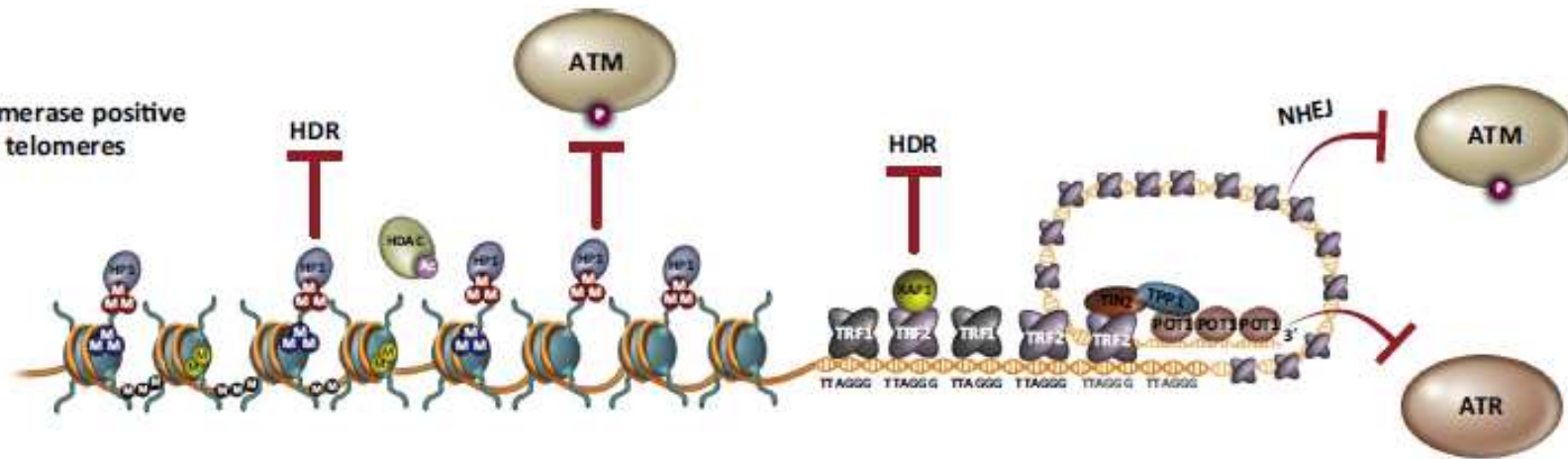
C.



evidence for dynamic cell cycle-dependent regulation of TERT.

(A)

Telomerase positive telomeres



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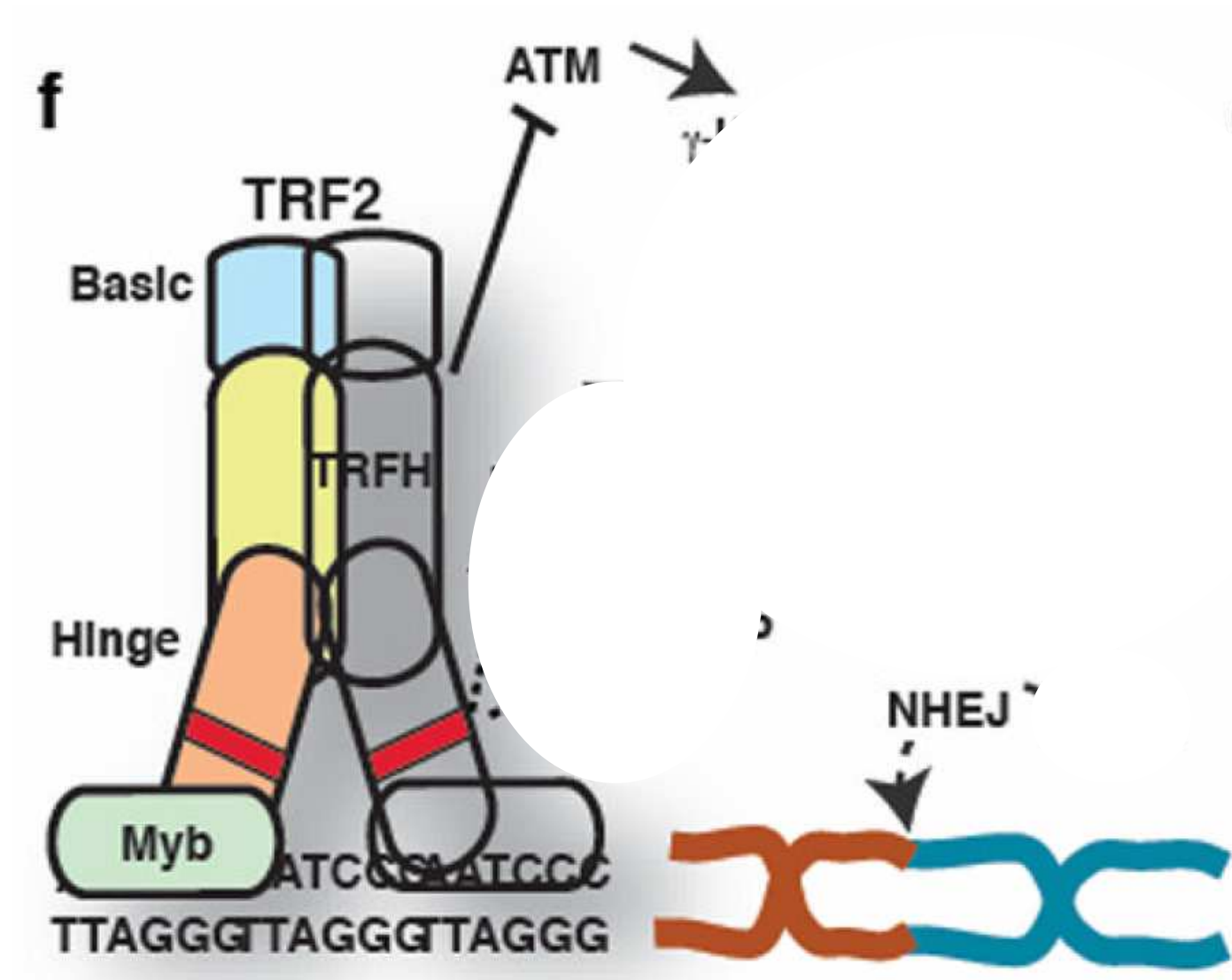
Within the protective complex shelterin, **TRF2** plays a crucial role in end-protection as it is required to suppress ATM activation and the formation of end-to-end chromosome fusions.

a two-step mechanism for TRF2-mediated end protection:

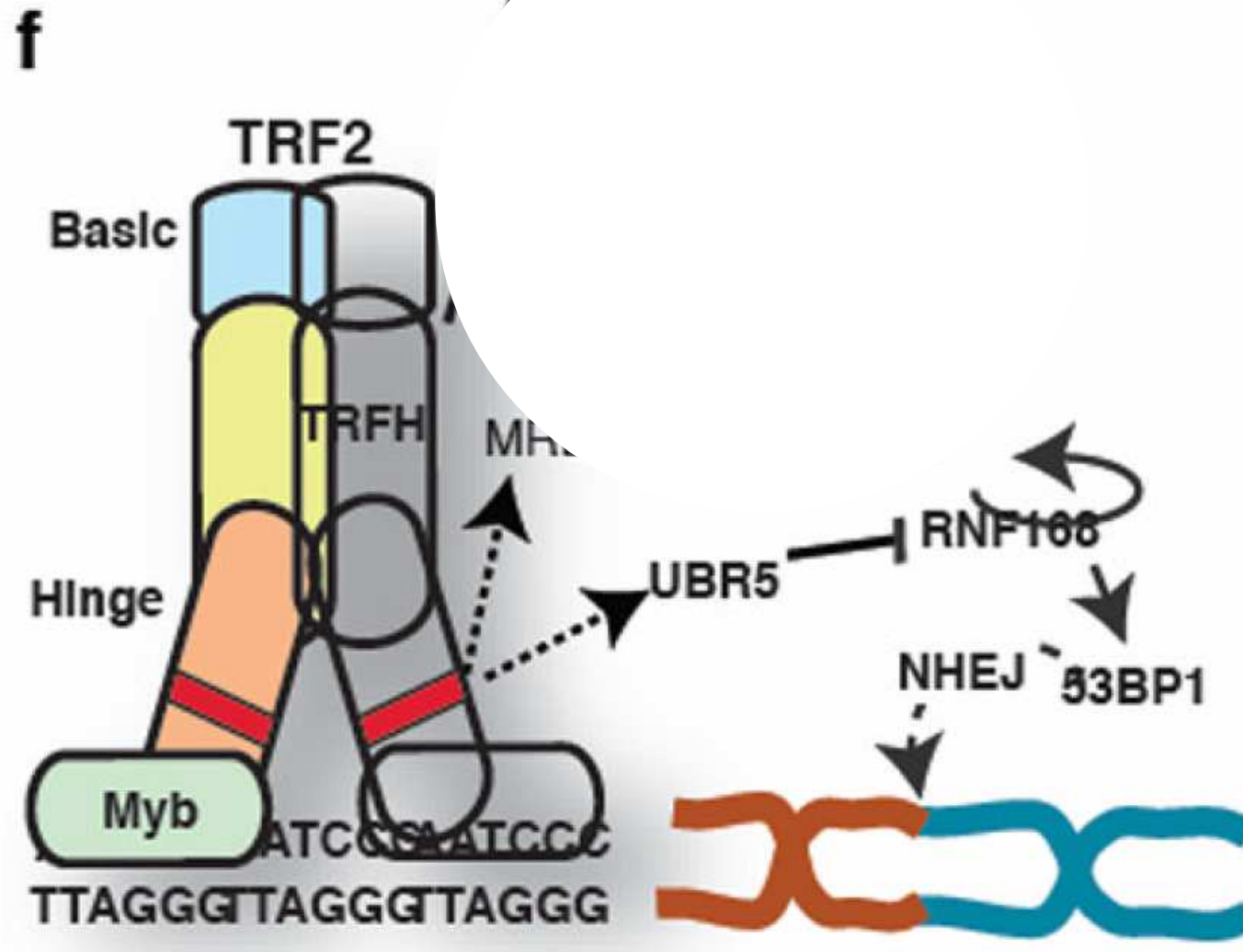
1) the dimerization domain of TRF2 is required to inhibit ATM activation, the key initial step involved in activation of a DNA damage response.

.

mechanism for TRF2-mediated chromosome end protection



mechanism for TRF2-mediated chromosome end protection



Within the protective complex shelterin, TRF2 plays a crucial role in end-protection as it is required to suppress ATM activation and the formation of end-to-end chromosome fusions.

2) TRF2 independently suppresses the propagation of DNA damage signaling downstream of ATM activation.

This novel modulation of the DNA damage response at telomeres occurs at the level of the E3 ubiquitin ligase RNF168

Inhibition of RNF168 at telomeres involves the ubiquitin ligase UBR5 and is sufficient to suppress chromosome end-to-end fusions.

Mitosis Inhibits DNA Double-Strand Break Repair to Guard Against Telomere Fusions

Orthwein Science 2014

Mitotic cells inactivate DNA double-strand break (DSB) repair, but the rationale behind this suppression remains unknown.

How mitosis blocks DSB repair?

Mitotic kinases phosphorylate the E3 ubiquitin ligase RNF8 and the nonhomologous end joining factor 53BP1 to inhibit their recruitment to DSB-flanking chromatin.

The capacity of mitotic DSB repair to destabilize the genome explains the necessity for its suppression during mitosis, principally due to the fusogenic potential of mitotic telomeres

Alternative lengthening of telomeres (ALT)

Alternative lengthening of telomeres (ALT) telomeres are considerably longer (4–50 kb) and contain an increased presence of **variant repeats** such as C-type TCAGGG repeats.

These provide a platform for the binding of proteins such as the **nucleosome remodeling** complex, NuRD-ZNF827.

ALT telomeres display reduced levels of H3K9me3.

As a result, they may contain elevated levels **of histone acetylation** as suggested by the reduced compaction of nucleosomal arrays.

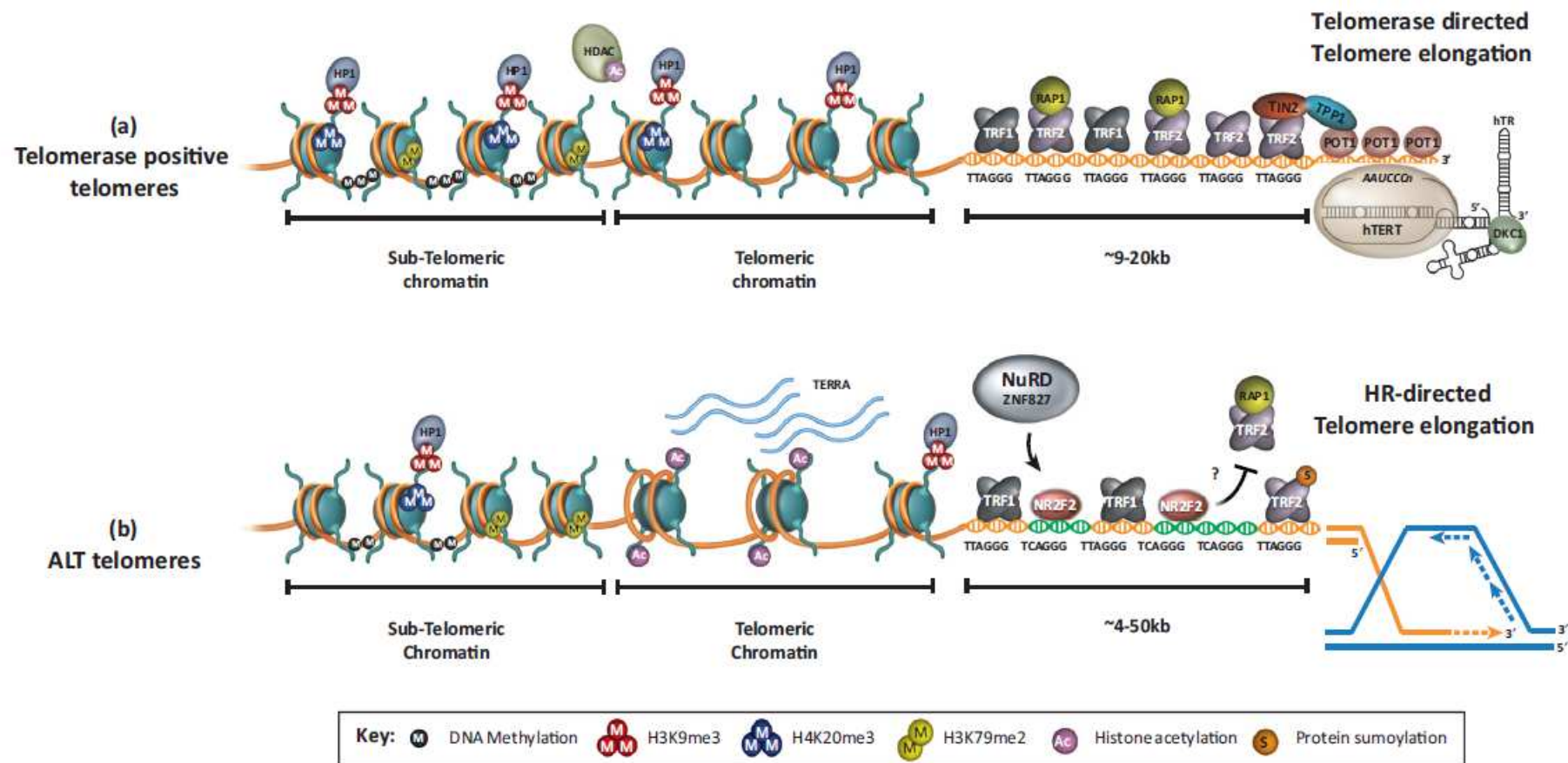
Sub-telomeric chromatin also displays reduced heterochromatic marks and altered DNA methylation patterns.

The more open chromatin configuration of ALT telomeres may promote homologous recombination directed telomere elongation and greater transcription of the telomeric non-coding RNA (TERRA).

ALT telomeres are distinct in that they contain variant C-type TCAGGG repeats and sumoylated TRF2.

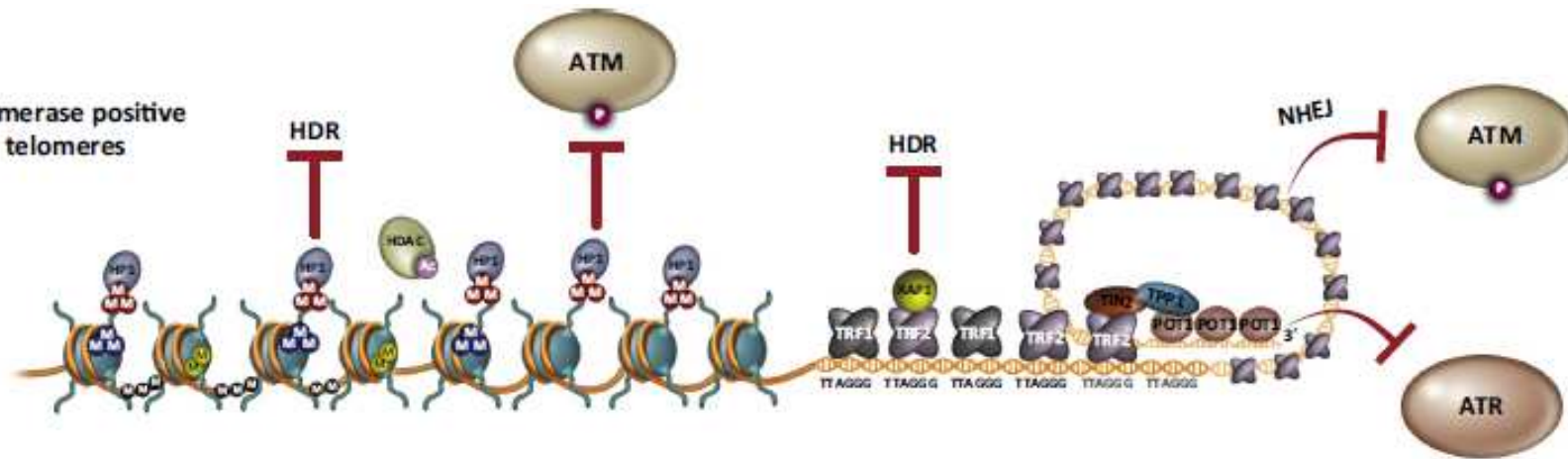
These features of ALT telomeres may lead to displacement of TRF2 and perhaps its binding partner, RAP1.

A small but significant number of cancers do so via the exchange of telomeric DNA between chromosomes by alternative lengthening of telomeres - ALT



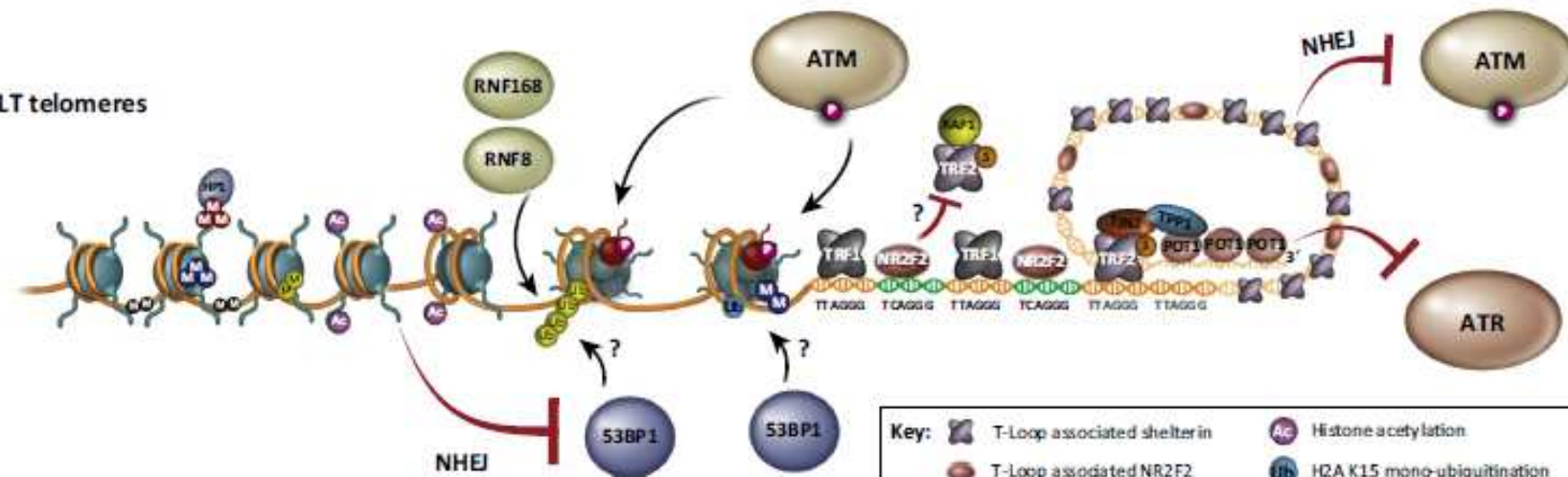
(A)

Telomerase positive telomeres



(B)

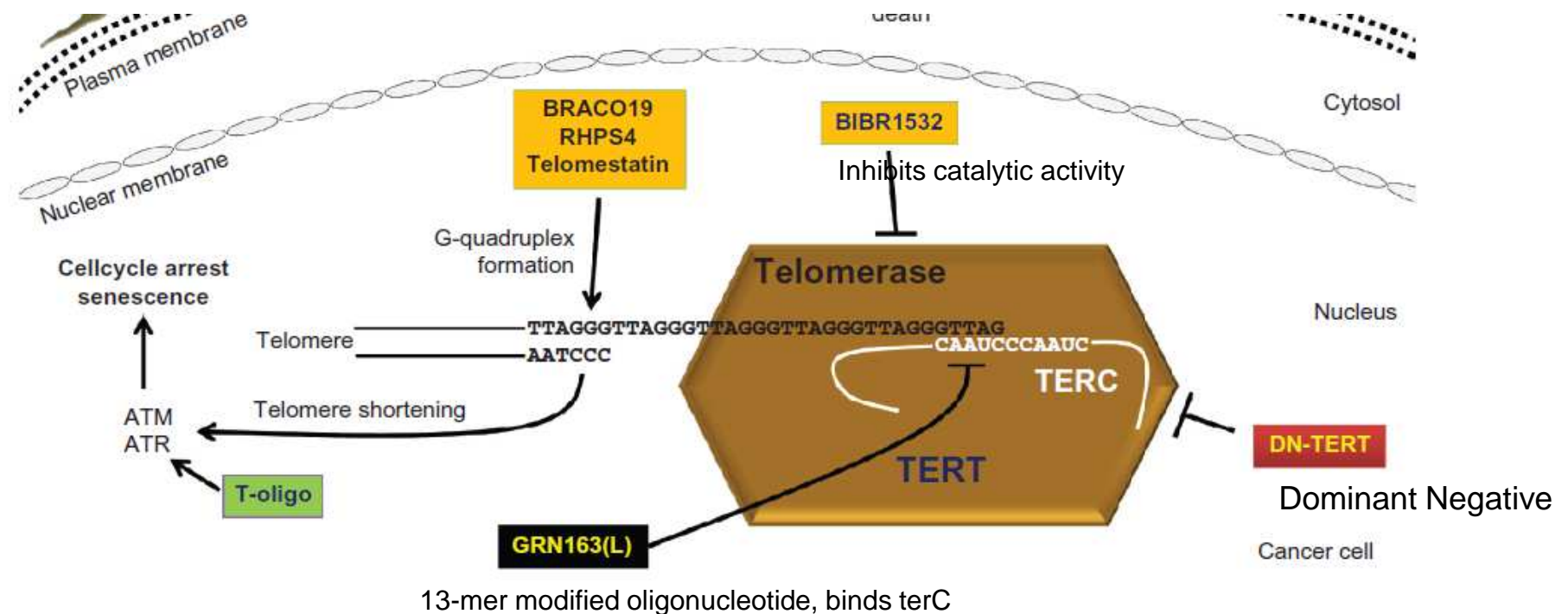
ALT telomeres



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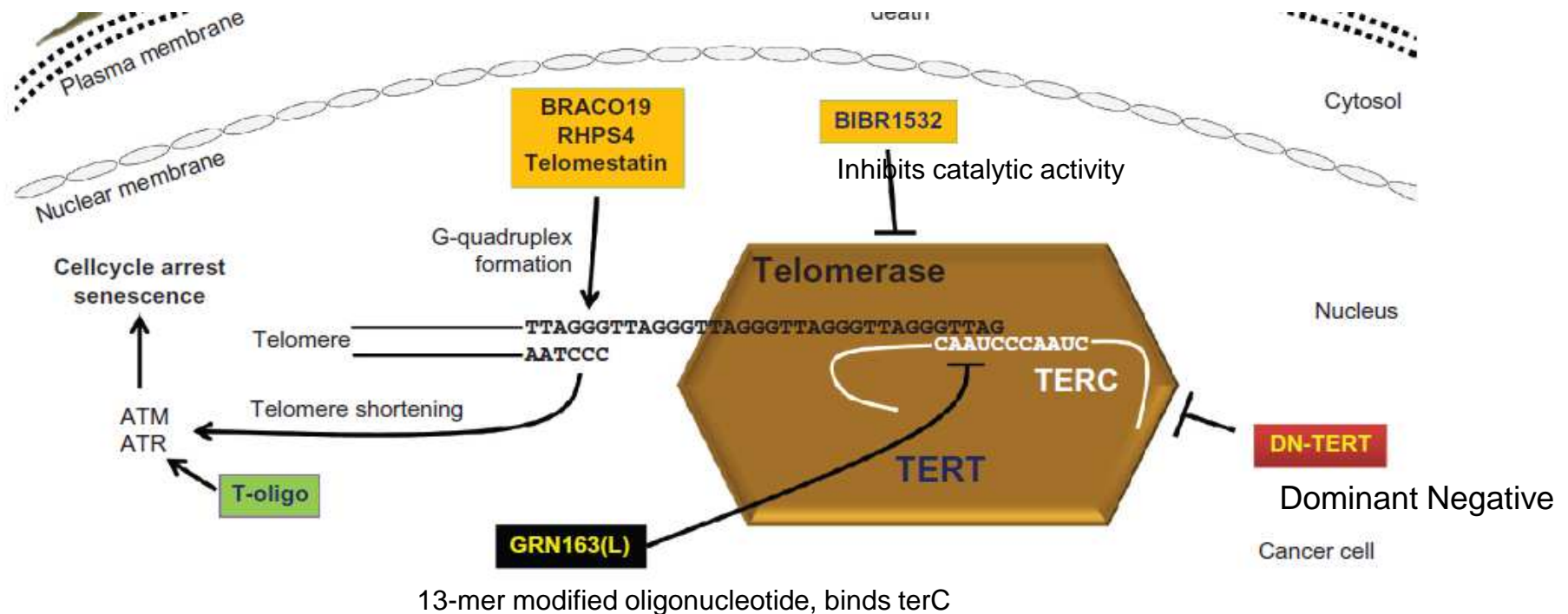
Strategie anti tumorali basate sulla inibizione della telomerasi

GRN163(L), DN-TERT, and BIBR1532 directly inhibit telomerase



BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

T-oligo mimics dysfunctional telomeres

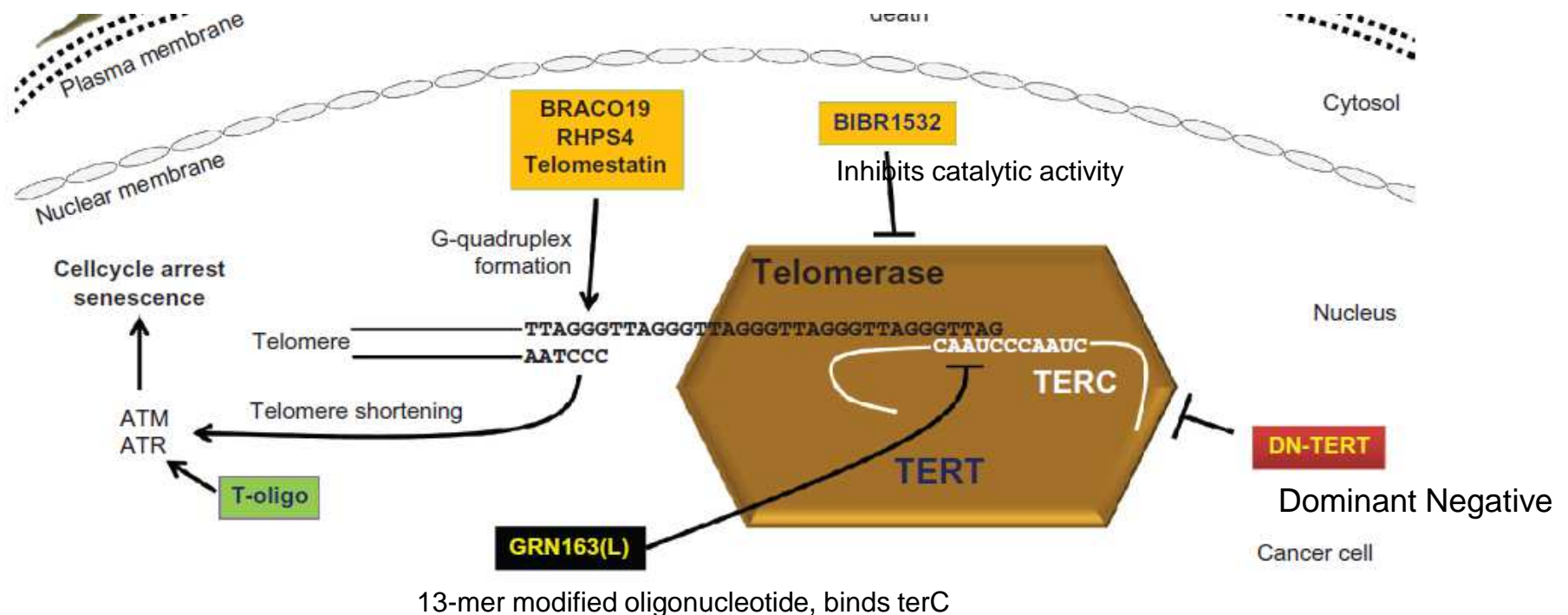


GRN163(L), DN-TERT, and BIBR1532 directly inhibit telomerase

BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

T-oligo mimics dysfunctional telomeres

Vaccination with peptides derived from TERT or introduction of TERT mRNA into dendritic cells activates T and/or B cells, which recognize and eliminate TERT-expressing cancer cell



- [EMBO Mol Med.](#) 2012
- **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.
- 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.

- Treatment of 1- and 2-year old mice with an adeno associated virus (AAV) 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.

Glossary

Chromatin remodeler: ATP driven molecular machines that alter chromatin structure by sliding, ejecting, or restructuring nucleosomes. For example, ATRX associates with histone H3 to maintain higher order chromatin structure.

DNA methyltransferase: A class of enzymes that catalyze the transfer of a methyl group to cytosine or guanine residues, altering gene expression and recruiting heterochromatin related proteins to the region.

Extrachromosomal TTAGGG repeats (ECTR): Telomeric DNA repeats which are located in the nucleus but are not associated with linear chromosomes and can form higher order structures. ECTRs are a hallmark of ALT cells.

G-quadruplex: A G-quadruplex forms when guanine residues are donors and acceptors in C–G base pairing, forming a square, planar 4 guanine tetrad. These tetrads can stack to form a four-stranded quadruplex that obstructs a moving replication fork.

Histone chaperone: Proteins that function at multiple steps during nucleosome formation.

Chaperones mediate histone transfer and ensure that nucleosomes are reassembled after DNA replication, DNA repair, and transcription. For example, ASF1 is a conserved eukaryotic histone H3/H4 chaperone that participates in the assembly and disassembly of histones H3 and H4 dimers, whereas DAXX has been shown to function as a histone chaperone of histone H3.3–H4 dimers.

Histone deacetylase (HDAC): A class of enzymes that removes acetyl groups from lysine histone lysine residues, allowing for a tighter interaction between DNA and nucleosome.

Histone H3: Histone H3 is one of four main histone proteins in the globular nucleosome, around which DNA winds to form higher order chromatin structures. H3's N-terminal tail is subject to extensive post-translational epigenetic modifications.

Homology directed repair:.

Glossary

Homology directed repair: Homology directed repair is a mechanism of double-strand DNA break repair occurring during G2 and S phase in which a homologous recombination event between sister chromatids serves as a template for repair.

Lysine methyltransferase: A histone modifying enzyme that can add up to three methyl groups to a histone lysine residue, promoting chromatin condensation, heterochromatin formation and facilitating gene expression.

Non-homologous end joining (NHEJ): NHEJ is a mechanism of double-strand DNA break repair occurring mainly during G1 phase of the cell cycle in which strand overhangs serve as a template and breaks are directly ligated together.

Shelterin: A telomere specific nucleoprotein complex consisting of 6 proteins: TRF1, TRF2, RAP1, TIN2, TPP1, and POT1. It maintains telomere structure and regulates telomere function. Of the six proteins, TRF2 binds the double stranded TTAGGG repeat DNA, whereas POT1 binds both double-stranded TTAGGG repeat DNA and the 3' overhang of single-stranded TTAGGG repeats.

Telomeric DNA: Consists of the hexameric sequence TTAGGG that is repeated in contiguous tracts at the ends of chromosomes.

Telomerase reverse transcriptase (hTERT): hTERT is the human form of the telomerase reverse transcriptase. It catalyzes the synthesis and addition of TTAGGG repeats to telomeres.

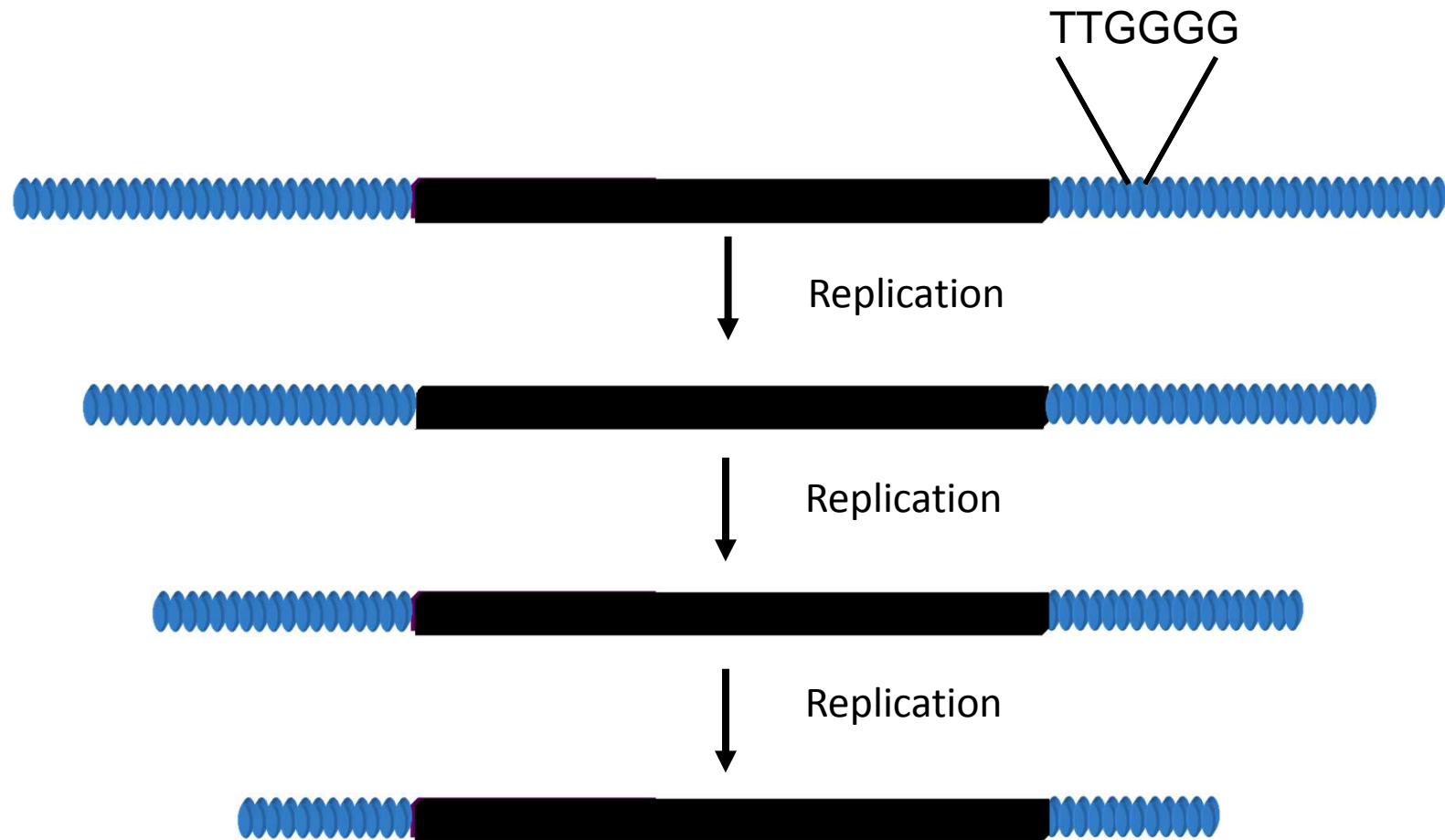
TELOMERI

In somatic cells, telomeres shorten with each division (a phenomenon termed the **end-replication problem**) to a **minimal threshold** of telomere length known as the Hayflick Limit.

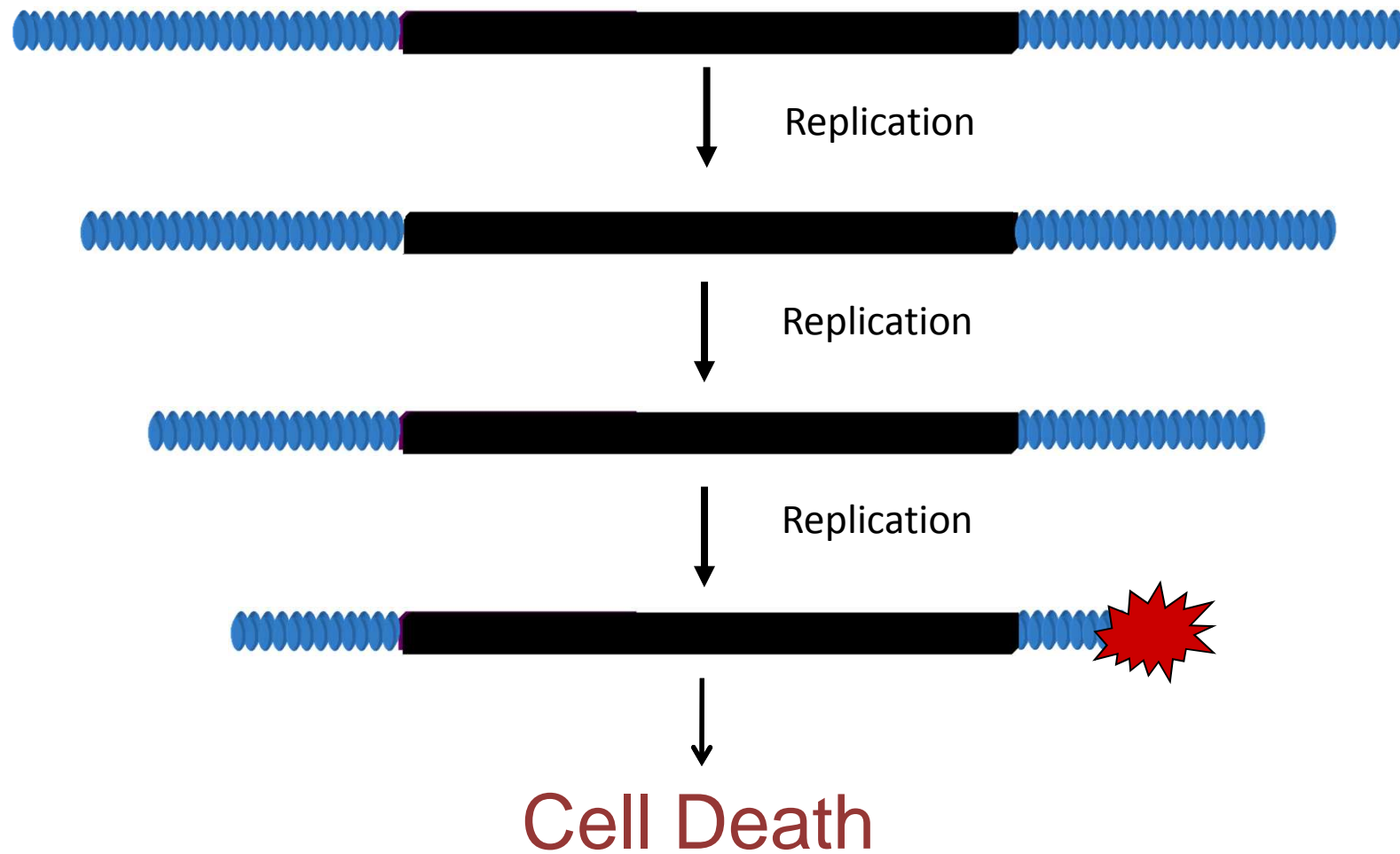
Once this threshold is breached, **telomeres lose their protective capacity** resulting in two critical outcomes [1]. Either the cell detects the threat posed by such shortened telomeres, resulting in the initiation of a p53 dependent signaling cascade that induces replicative senescence, **a state of permanent cell growth arrest**

If the cell continues to proliferate, telomere shortening will eventually kill the cell at crisis

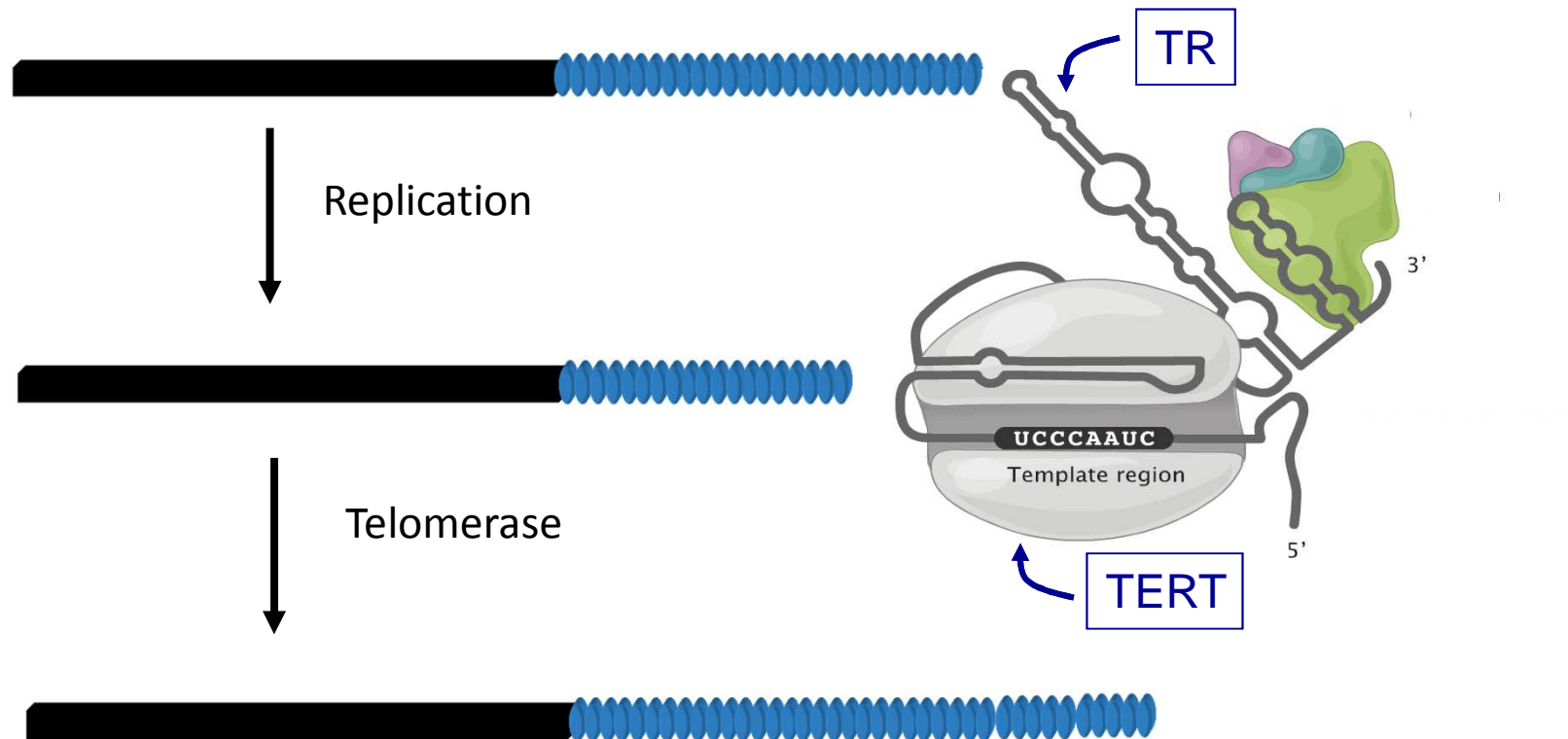
Telomeres shorten as cells divide



Telomere shortening leads to cell death



Telomerase allows telomere length equilibrium maintenance



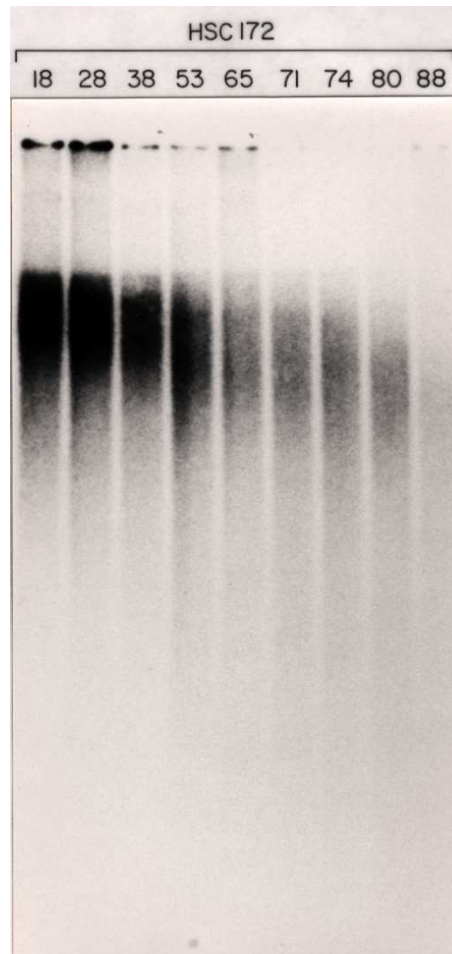
Telomere shortening in cellular senescence

Telomeres shorten during ageing of human fibroblasts

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

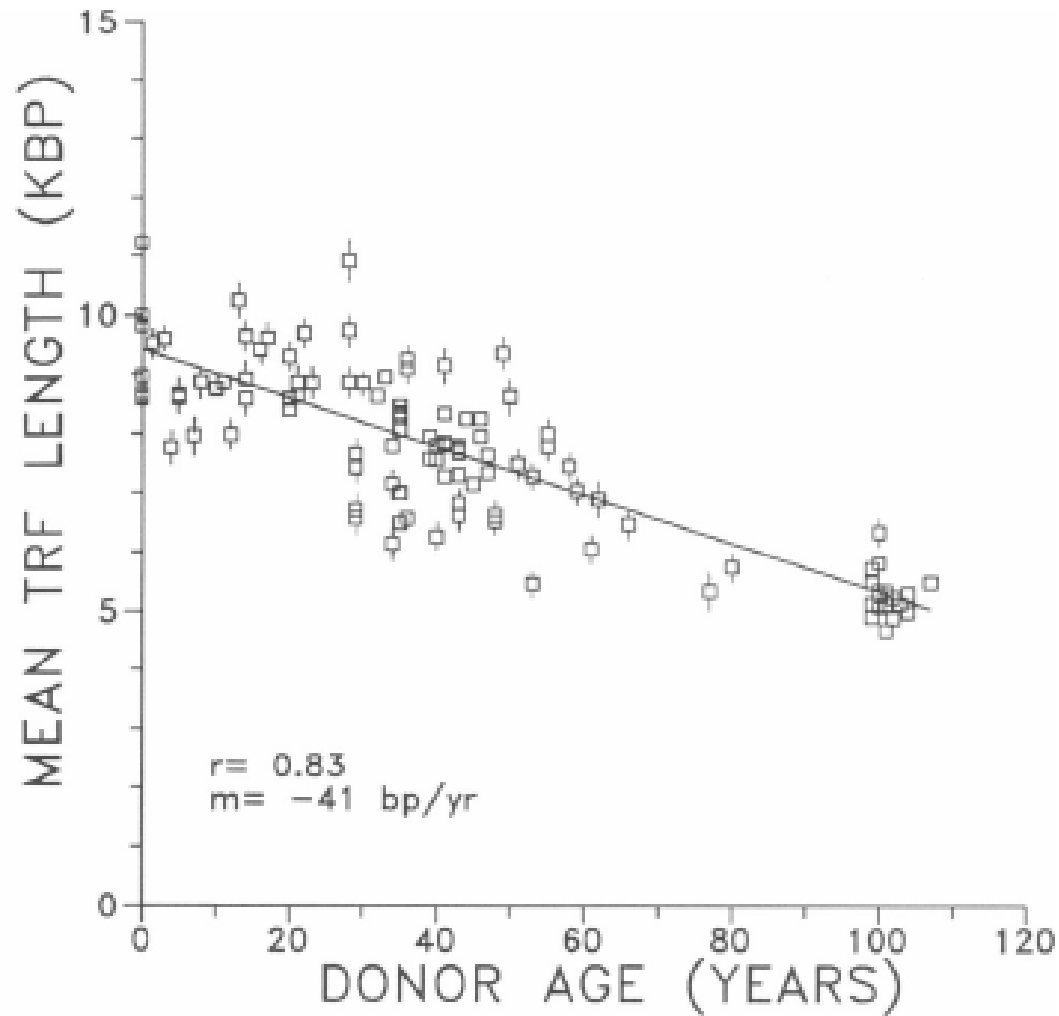
* Department of Biochemistry, McMaster University,
1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

† Cold Spring Harbor Laboratory, Cold Spring Harbor,
New York 11724, USA



Nature May 1990

Telomeres shorten with age



Telomerase is
limiting in cells

- [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.**
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- Abstract 2
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letters to nature

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, Duane Road, London W12 0NN, 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 RNA⁴. 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 hTR⁶ and a specialized reverse transcriptase (hTERT)¹². Dyskeratosis congenita is a multi-system 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 function¹⁴. Dyskeratosis congenita patients have markedly shorter telomeres than normal individuals and this is apparent from an early age¹⁵. 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 an autosomal dominant inheritance (DCR101; see Supplementary Info

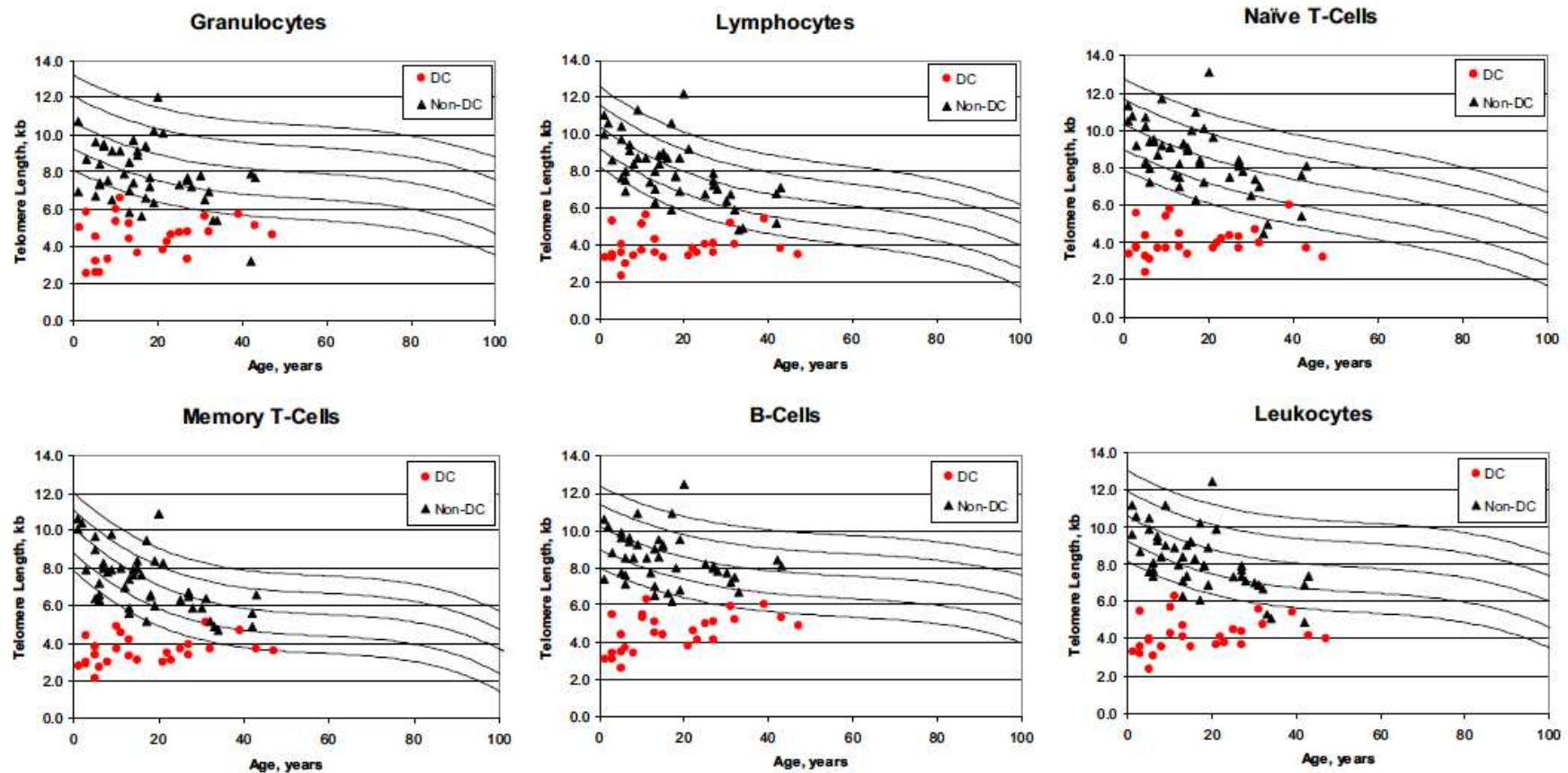
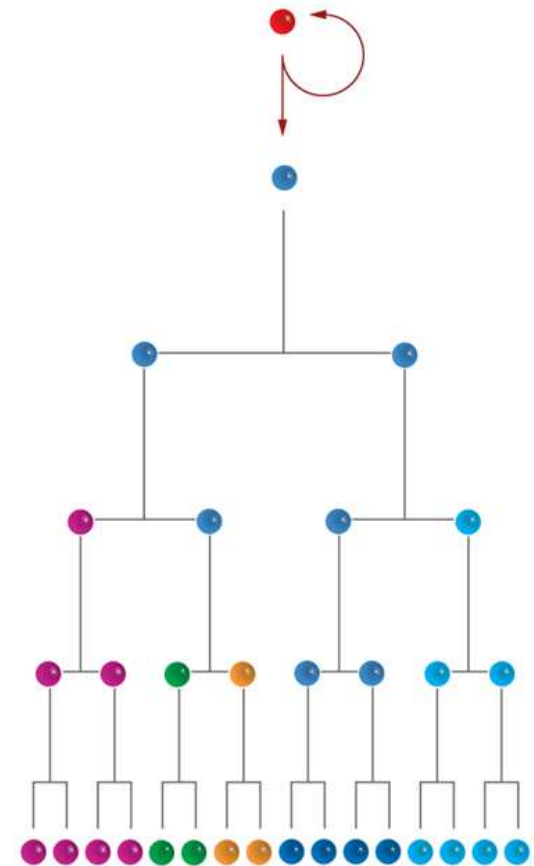


Figure 3. Telomere length according to age in dyskeratosis congenita and non-dyskeratosis congenita patients. The vertical axis represents telomere length in kilobytes. Lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal control subjects. Symbols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

Dyskeratosis congenita causes bone marrow failure

- Skin and nail problems
 - Skin hyperpigmentation
 - Rashes
 - Abnormal nail growth
- Mortality
 - Bone marrow failure
 - Cancer
 - other?



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 short telomeres, suggesting that they may be at risk for the disease. We did not iden-

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 121231, or at marmanil@jhmi.edu.

NEJM 357 p1317
(2007)

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.