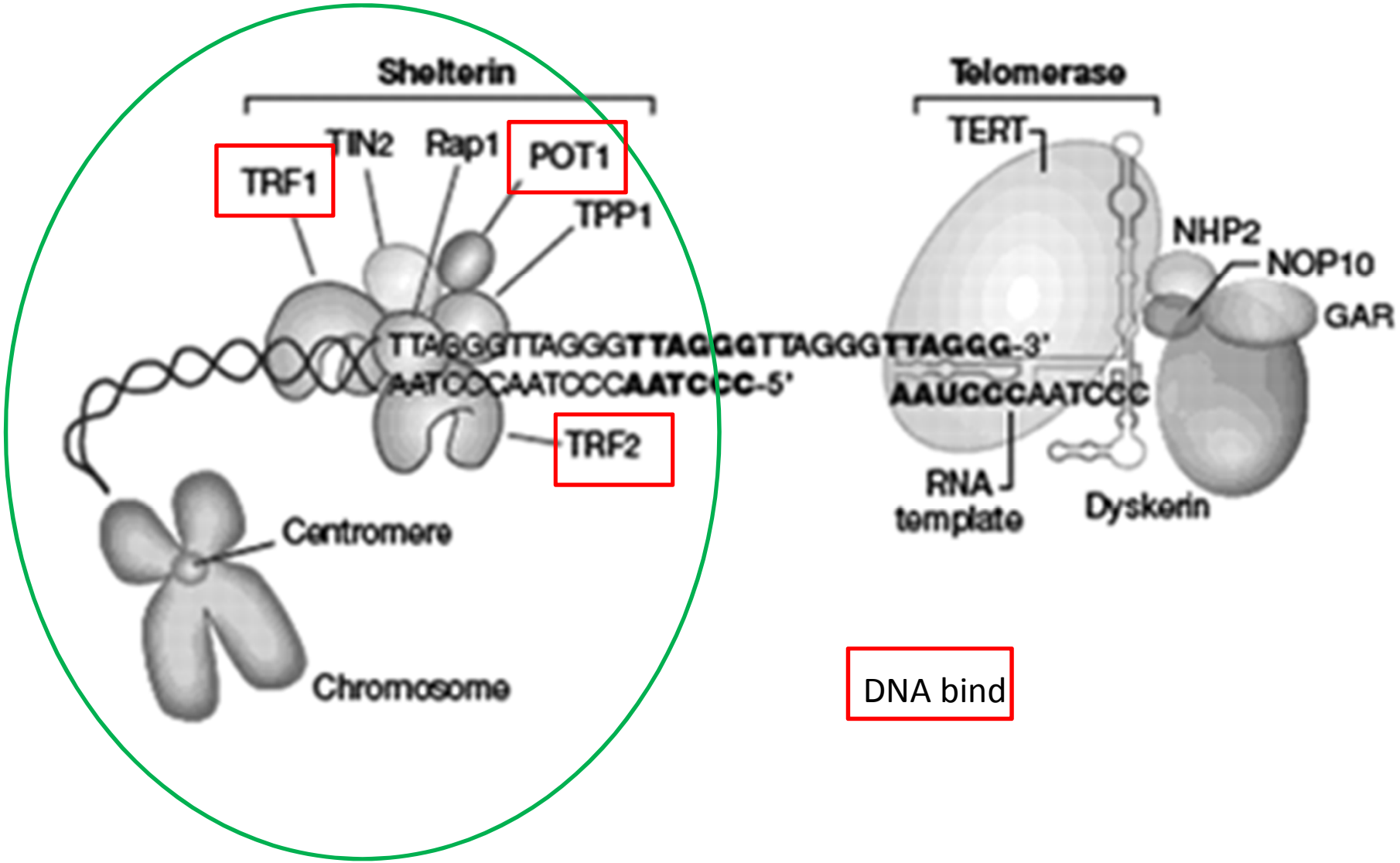
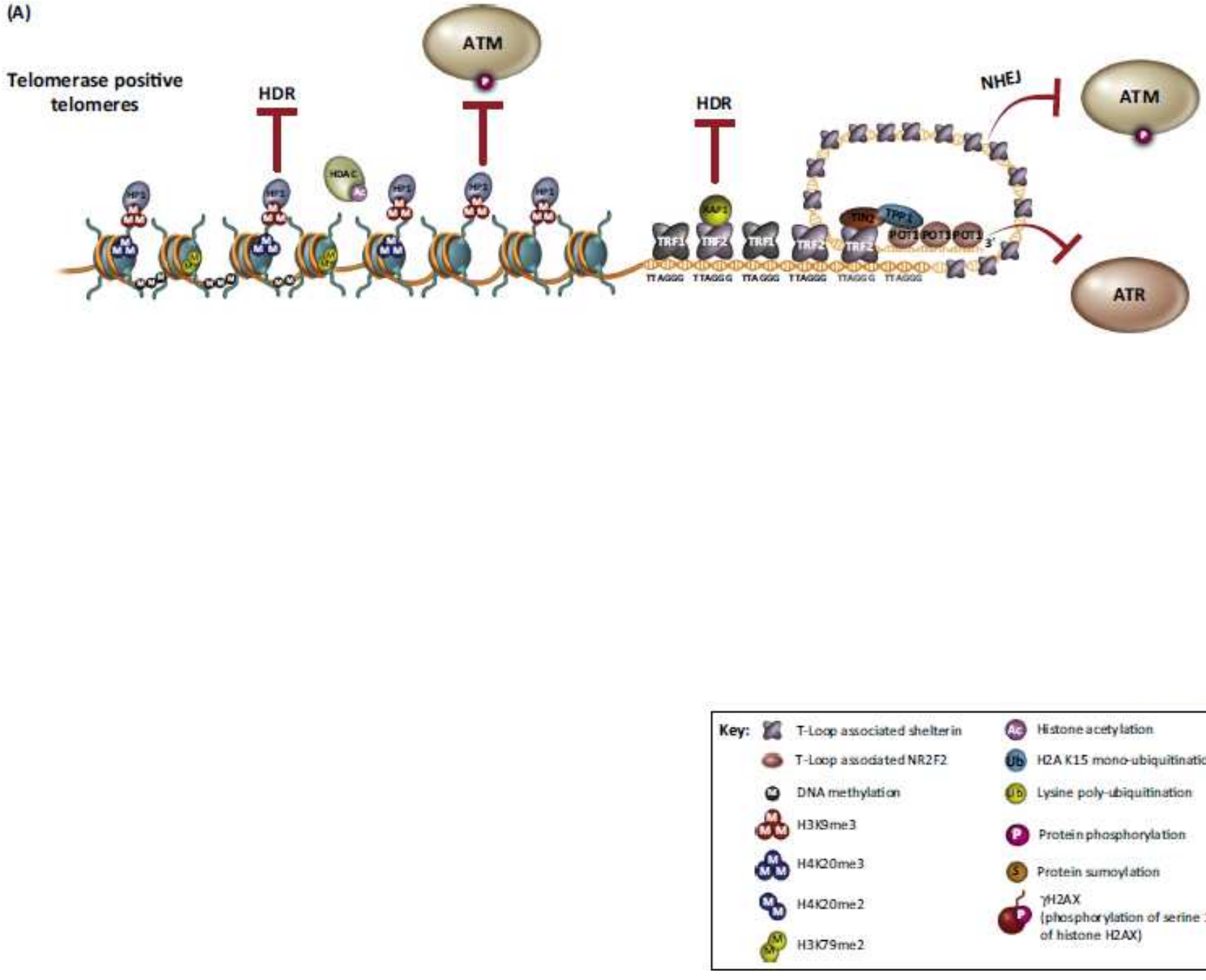
TELOMERI

Complessi macromolecolari associati al Telomero ed alla Telomerasi

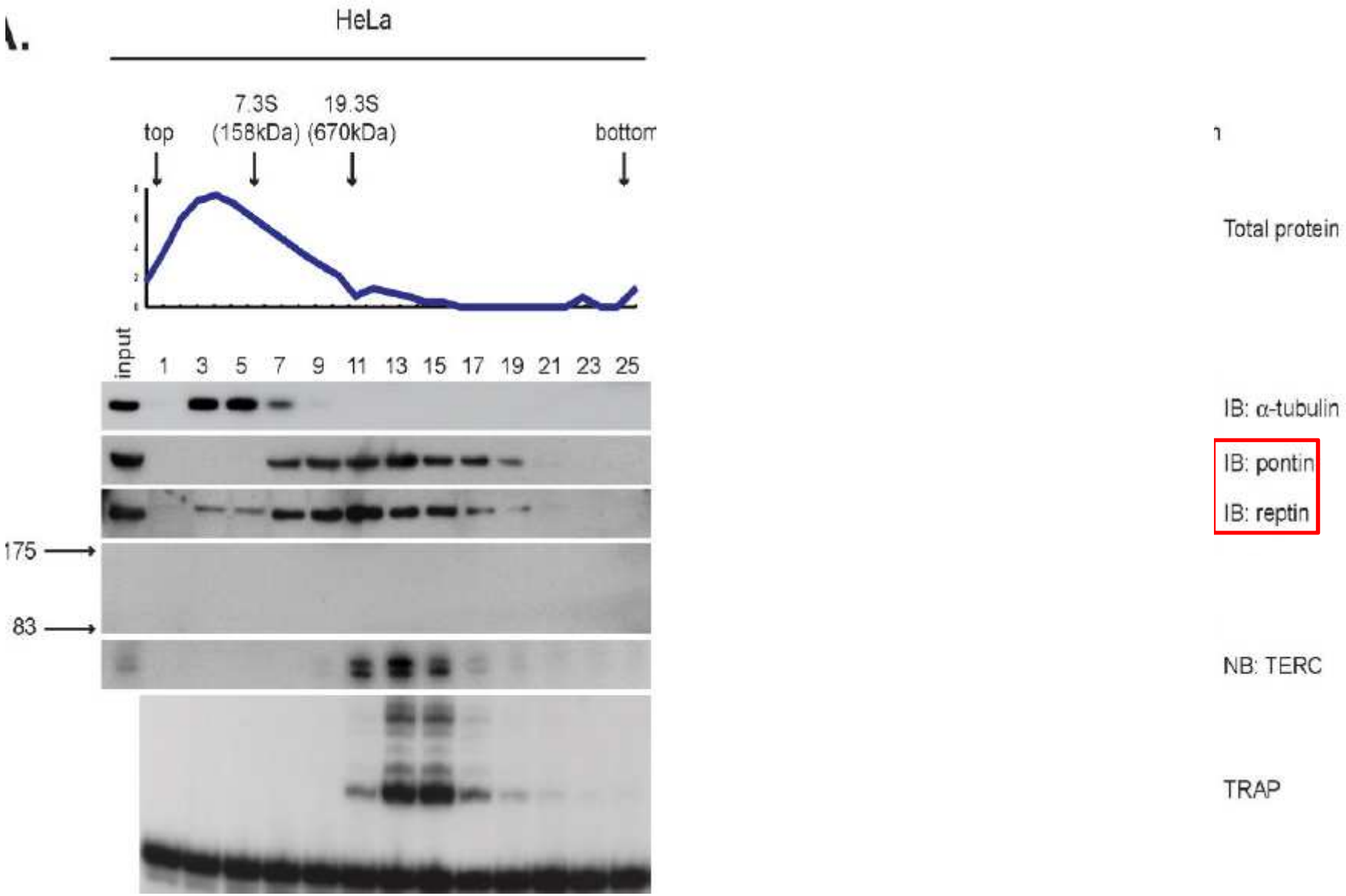



(A)
Telomerase positive telomeres
HDR
ATM
HDR
HDR
NHEJ
ATM
ATR
HDAC
Ac
TRF1
TRF2
RAP1
TIN2
TPP1
POT1
TTAGGG
Key:
T-Loop associated shelterin
T-Loop associated NR2F2
DNA methylation
H3K9me3
H4K20me3
H4K20me2
H3K79me2
Histone acetylation
H2A K15 mono-ubiquitination
Lysine poly-ubiquitination
Protein phosphorylation
Protein sumoylation
γH2AX
(phosphorylation of serine 139 of histone H2AX)

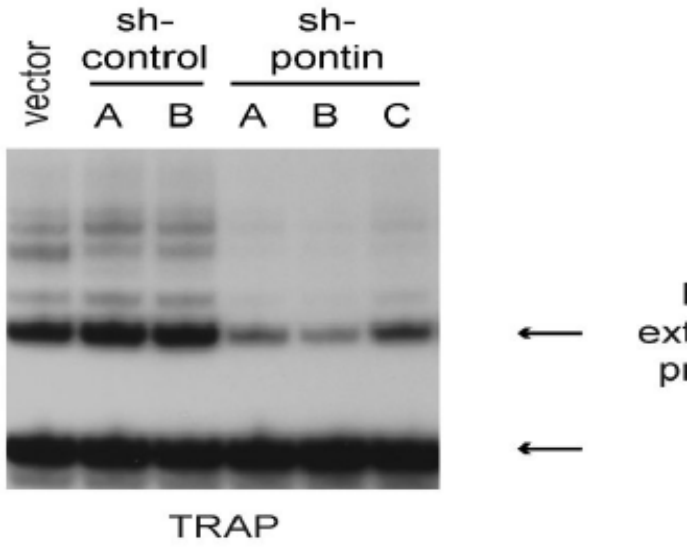
Few other proteins are known to be required for human telomerase function, significantly limiting our understanding of both telomerase regulation and mechanisms of telomerase action.

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

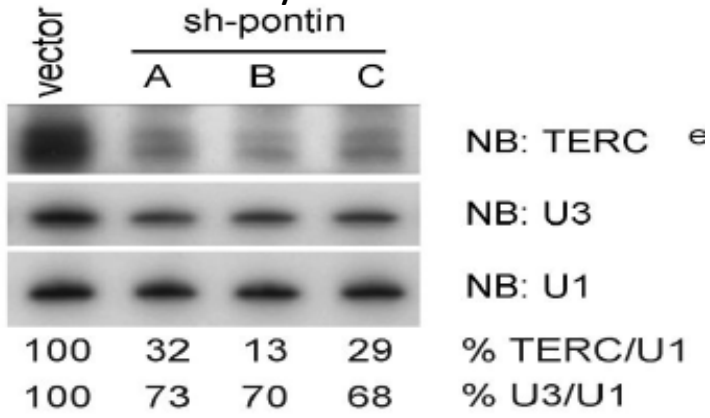
Pontin interacts with both TERT and dyskerin,


HeLa
top
7.3S (158kDa)
19.3S (670kDa)
bottom
input
1 3 5 7 9 11 13 15 17 19 21 23 25
175
83
Total protein
IB: α-tubulin
IB: pontin
IB: reptin
NB: TERC
TRAP

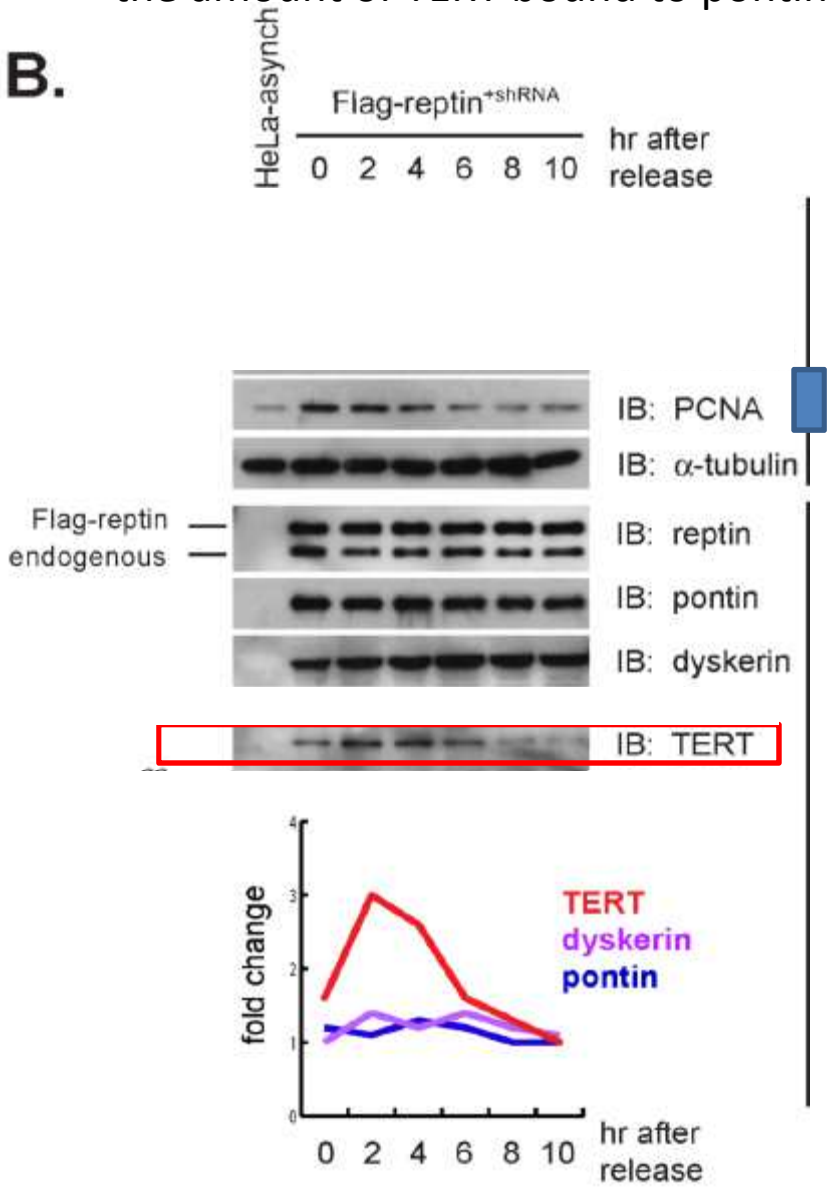
Depletion of pontin and reptin markedly impairs telomerase activity



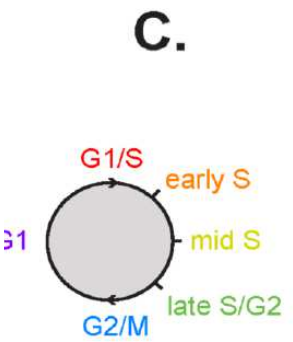
Depletion of pontin and reptin markedly impairs accumulation of the telomerase RNP, indicating an essential role in telomerase assembly.



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



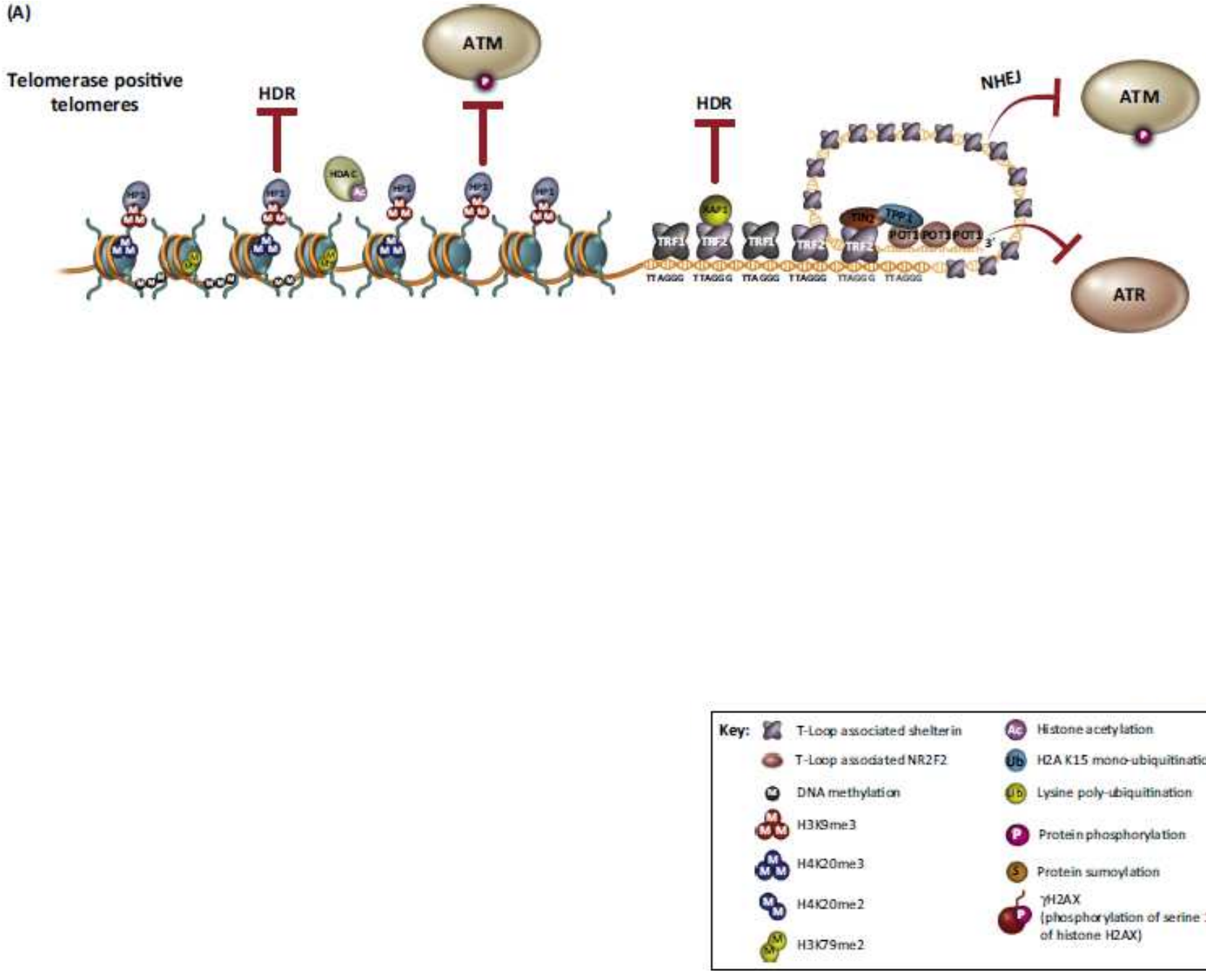
Co-immunoprecipitation of reptin with TERT and pontin over a ten hour timecourse



evidence for cell cycle-dependent regulation of TERT.

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

These findings reveal a requirement for additional enzymes in telomerase RNP biogenesis and suggest new approaches for inhibiting telomerase in human cancer.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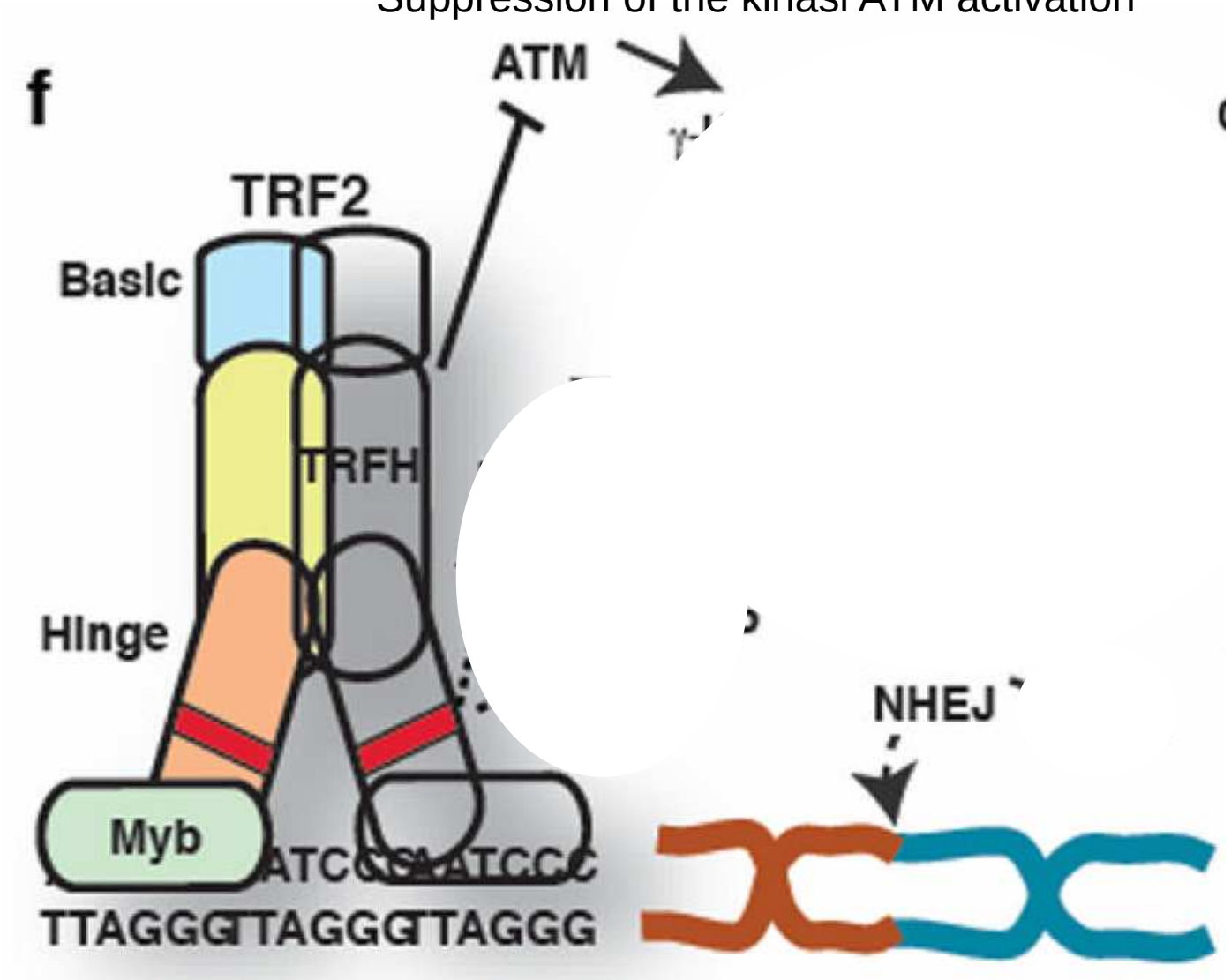
Histone acetylation
H2A K15 mono-ubiquitination
Lysine poly-ubiquitination
Protein phosphorylation
Protein sumoylation
γH2AX
(phosphorylation of serine 139 of histone H2AX)

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.

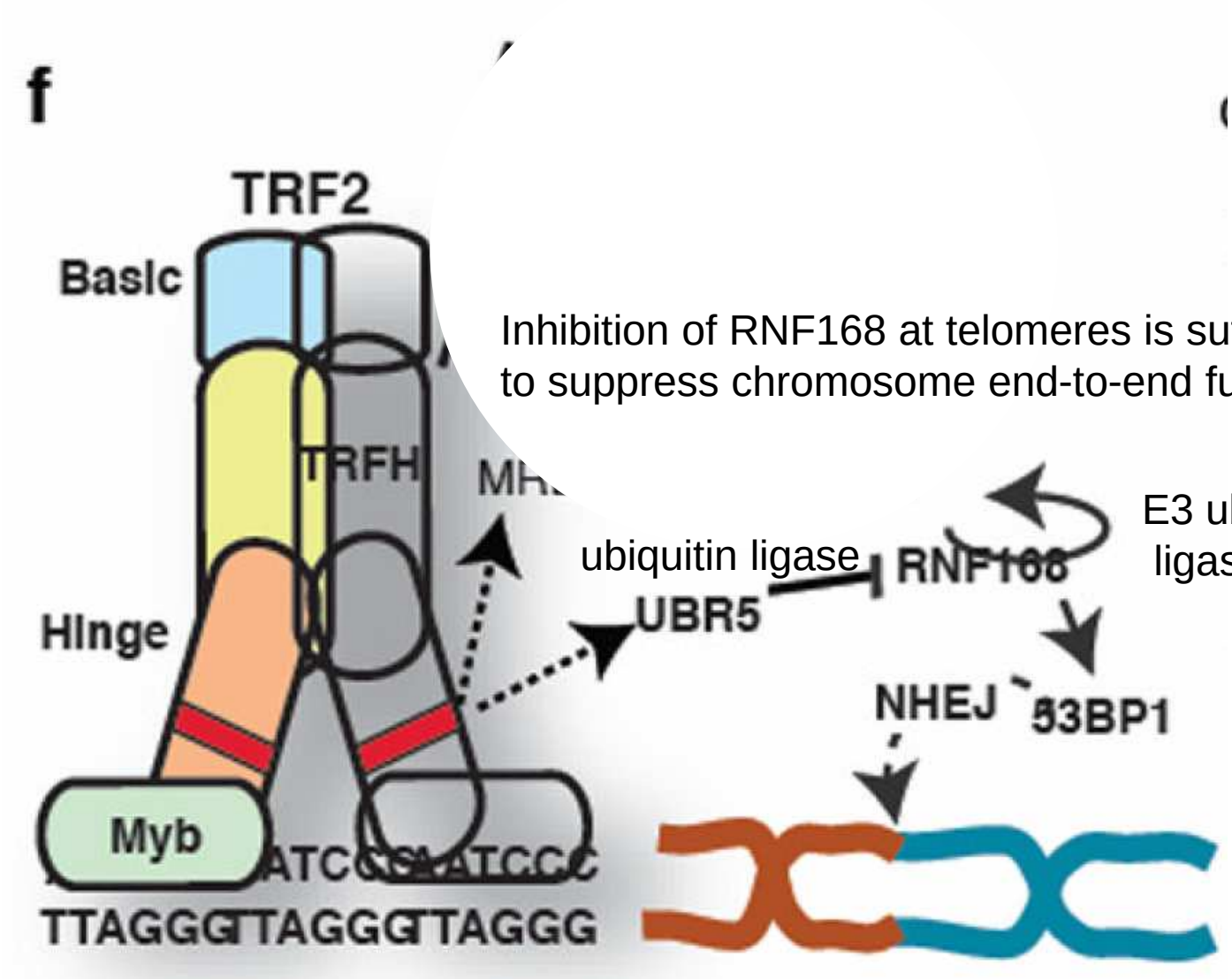
TRF2-mediated end protection:
1) the dimerization domain of TRF2 is required to inhibit ATM activation, the key initial step involved in activation of a DNA damage response.
.

mechanism for TRF2-mediated chromosome end protection

Suppression of the kinasi ATM activation



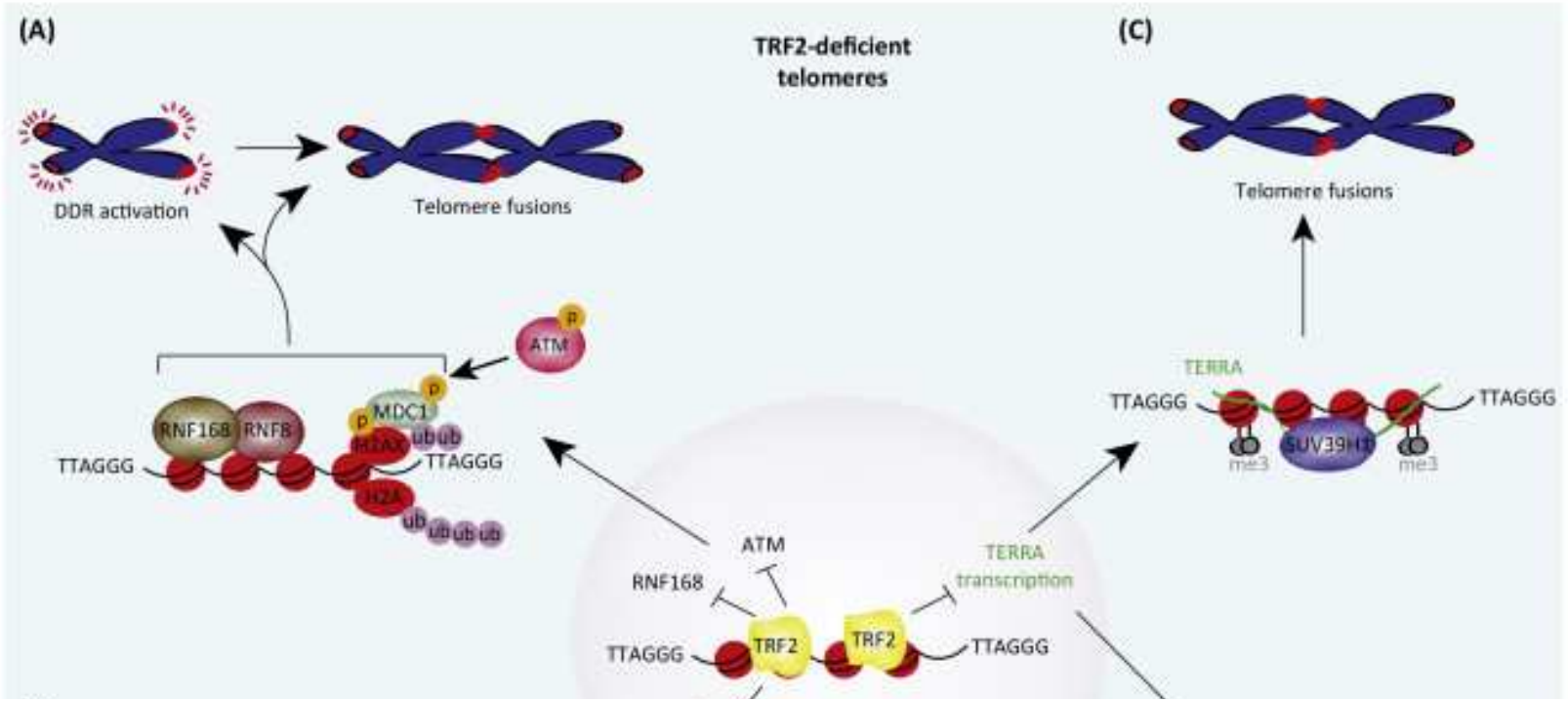
mechanism for TRF2-mediated chromosome end protection



Inhibition of RNF168 at telomeres is sufficient to suppress chromosome end-to-end fusions.

2) 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.

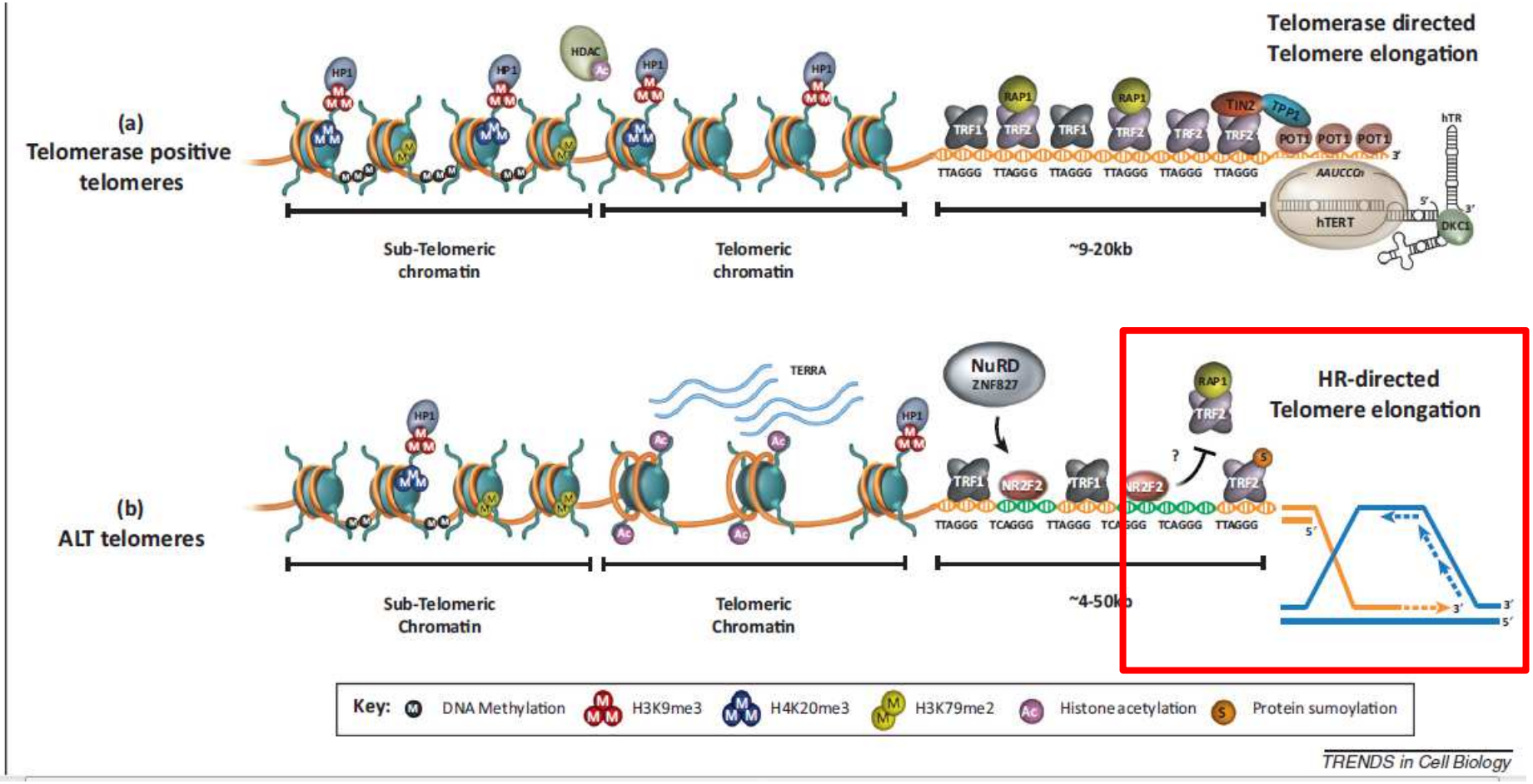


TERRA-mediated recruitment of SUV39H1 to TRF2-devoid telomeres leads to telomere fusions

TRF2 inhibits the recruitment to or activation at telomeres of ATM, RNF168 (E3 ubiquitin protein ligase) as well as the transcription of telomeric transcripts, TERRA (telomeric repeat-containing RNA).

Figure 4. TRF2 (telomere repeat-binding factor 2) inhibits end-processing pathways by regulating the chromatin environment at telomeres...

Alternative lengthening of telomeres (ALT)


(a)
Telomerase positive telomeres
Telomerase directed Telomere elongation
HDAC
HP1
RAP1
TRF1
TRF2
TIN2
TPP1
POT1
hTR
hTERT
DKC1
TTAGGG
AAUCCC
Sub-Telomeric chromatin
Telomeric chromatin
~9-20kb
(b)
ALT telomeres
TERRA
NuRD
ZNF827
NR2F2
HR-directed Telomere elongation
Sub-Telomeric Chromatin
Telomeric Chromatin
~4-50kb
Key:
DNA Methylation
H3K9me3
H4K20me3
H3K79me2
Histone acetylation
Protein sumoylation
TRENDS in Cell Biology

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

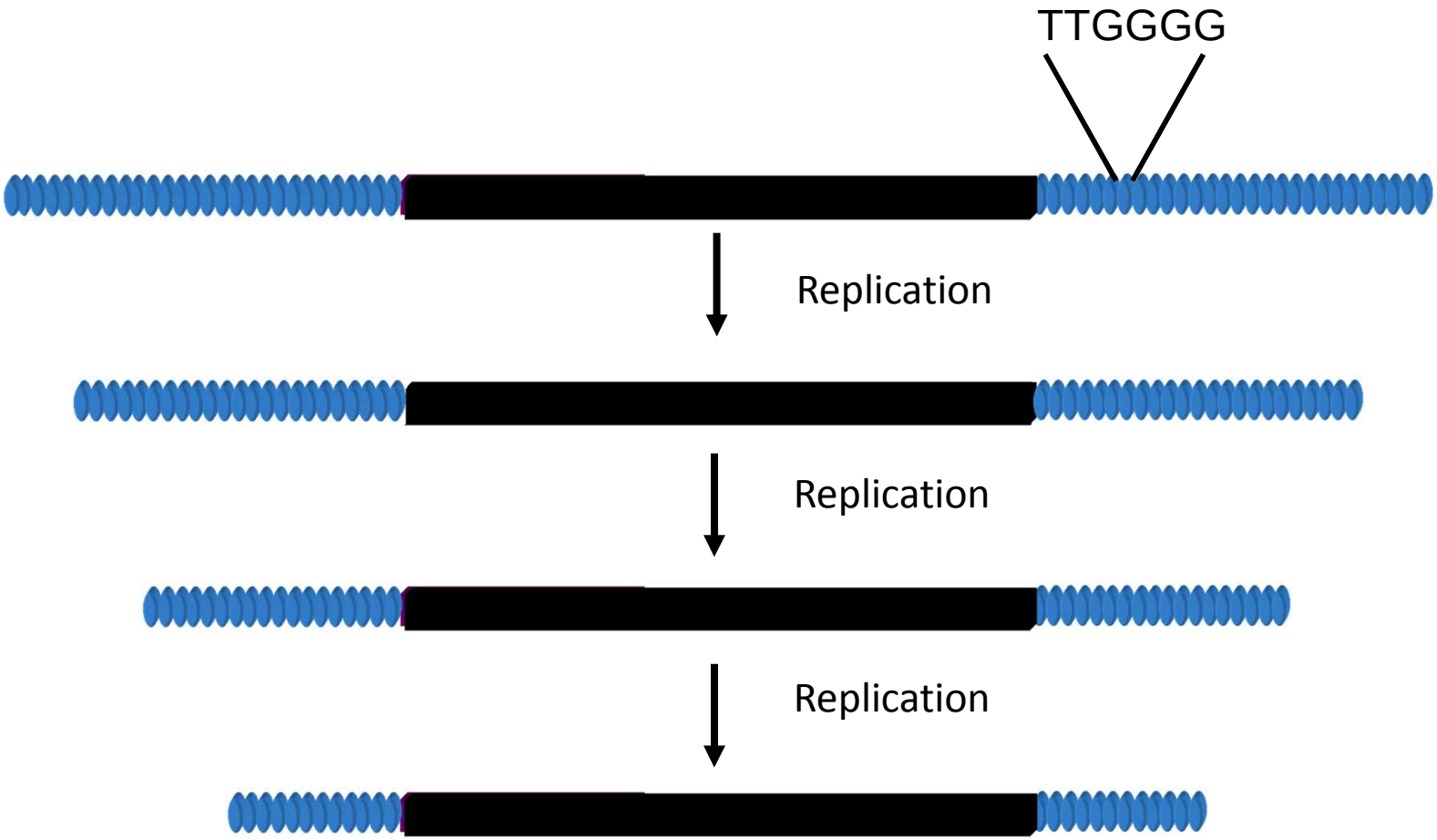
A small but significant number of cancers do so via the exchange of telomeric DNA between chromosomes by alternative lengthening of telomeres - ALT

Alternative lengthening of telomeres (ALT) telomeres are considerably longer (4–50 kb)

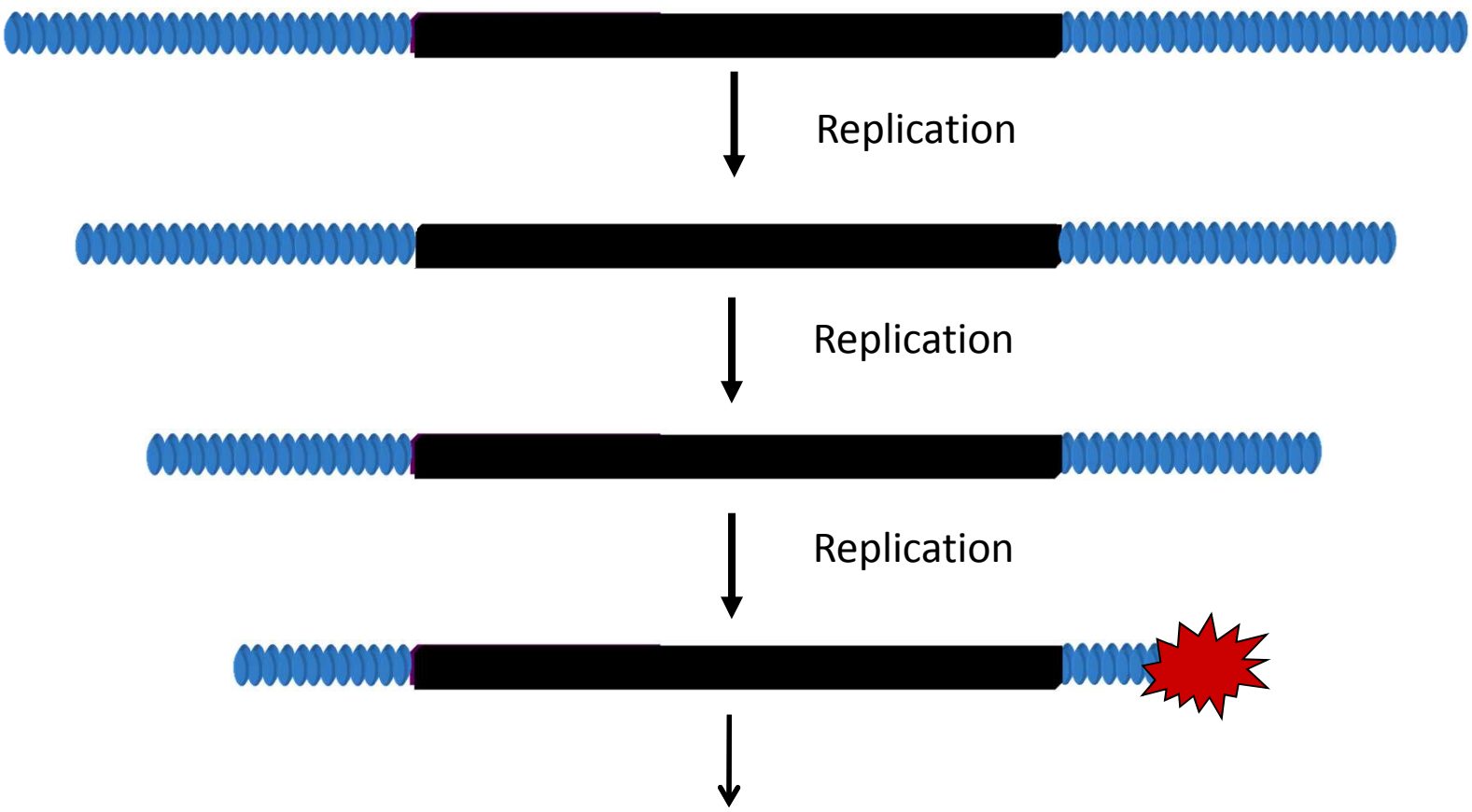
These provide a platform for the binding of proteins such as the nucleosome remodeling complex.
ALT telomeres display reduced levels of H3K9me3.
As a result, they may contain elevated levels of histone acetylation as suggested by the reduced compaction of nucleosomal arrays.

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).

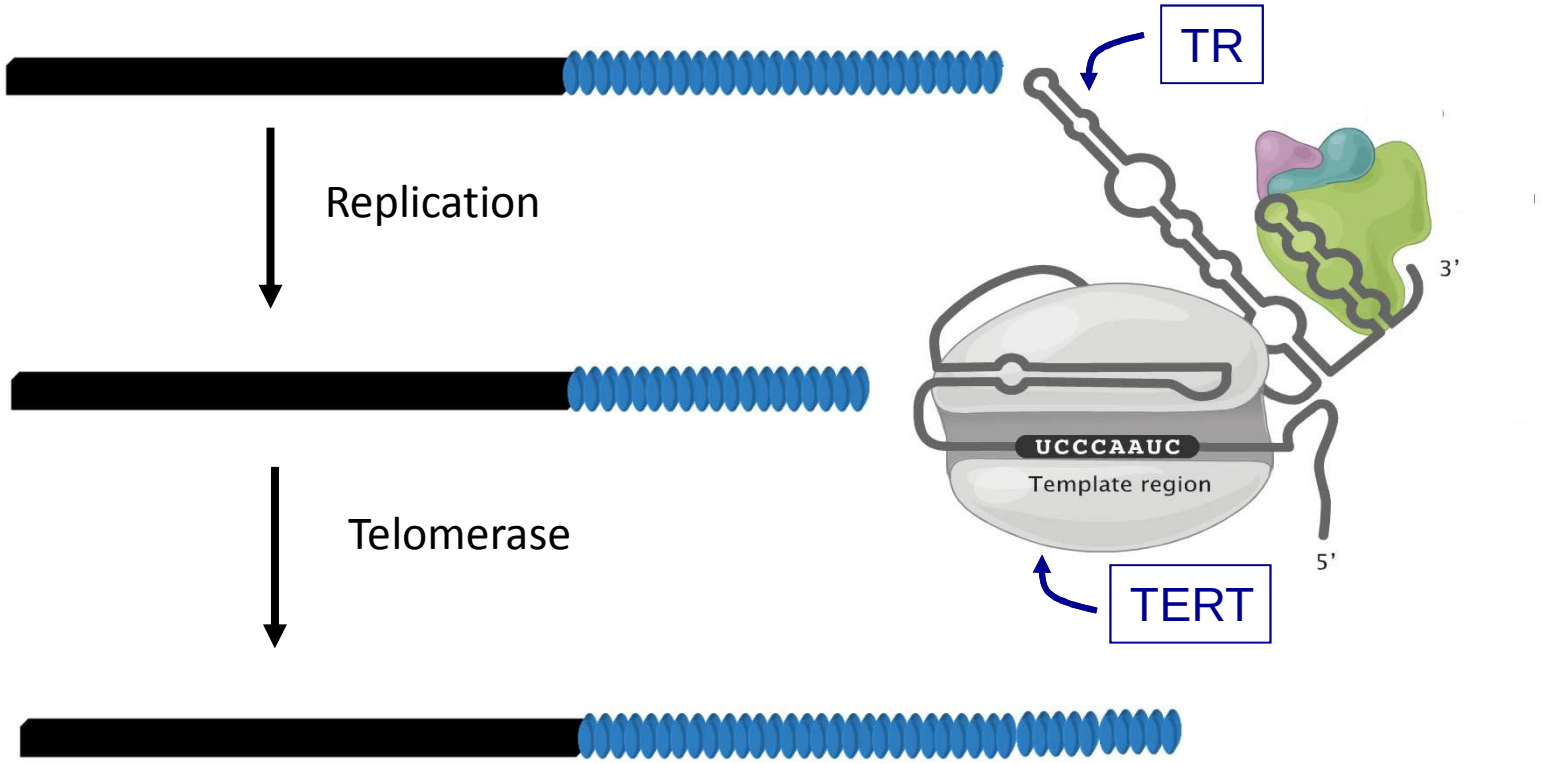
Telomeres shorten as cells divide



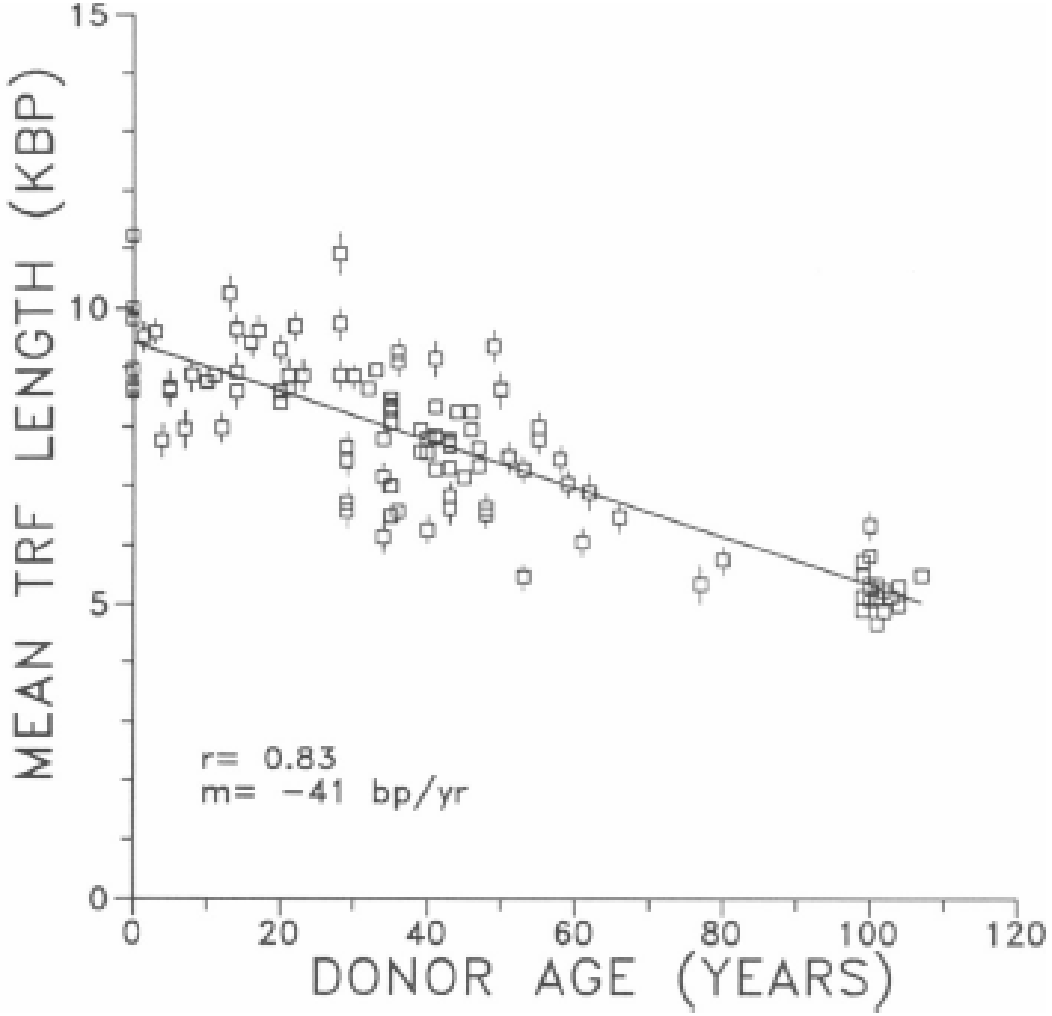
Telomere shortening leads to cell death



Telomerase allows telomere length equilibrium maintenance



Telomeres shorten with age



Telomerase is limiting in cells

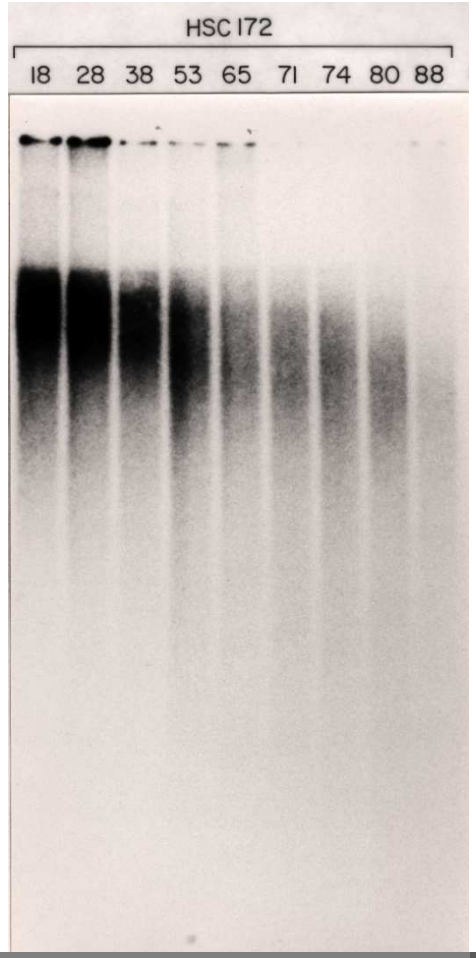
Vaziri et al. *AJHG* (1995)

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

- 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.

- 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.

.

Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer.

- 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.

EMBO Mol Med. 2012;4(8):691-704

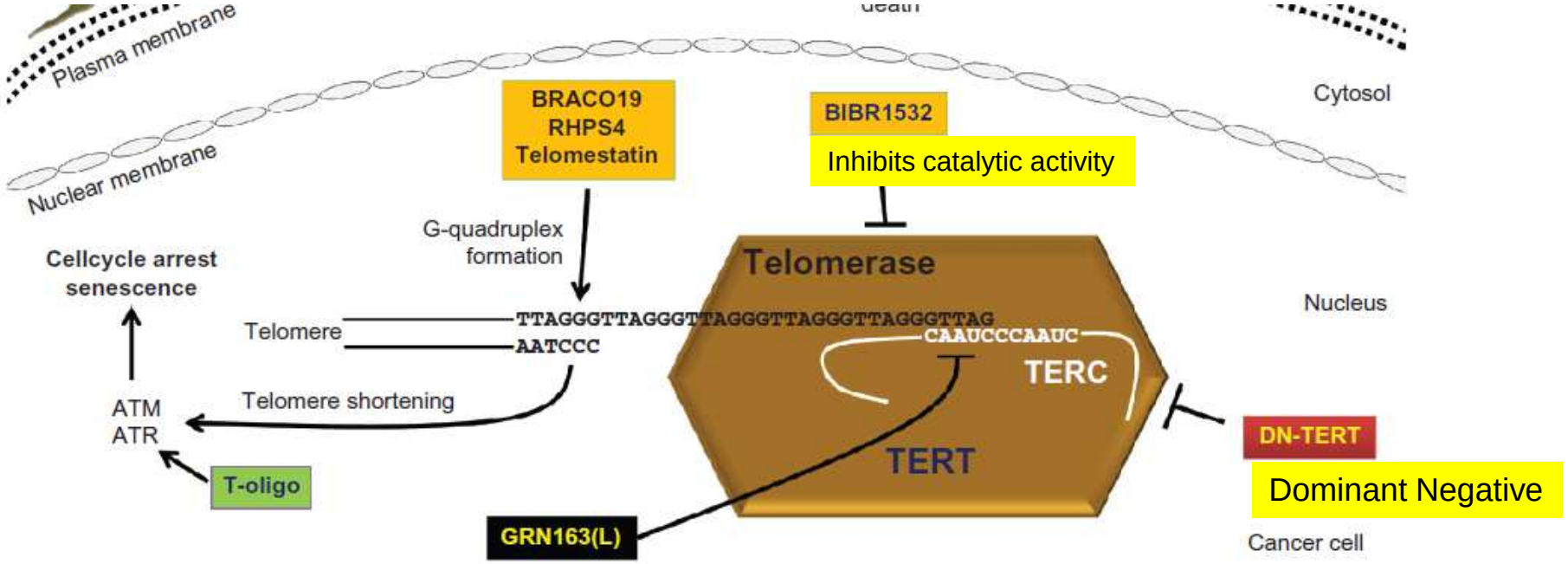
- These beneficial effects were not observed with a catalytically inactive TERT, demonstrating that they require telomerase activity.

- 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.

EMBO Mol Med. 2012;4(8):691-704

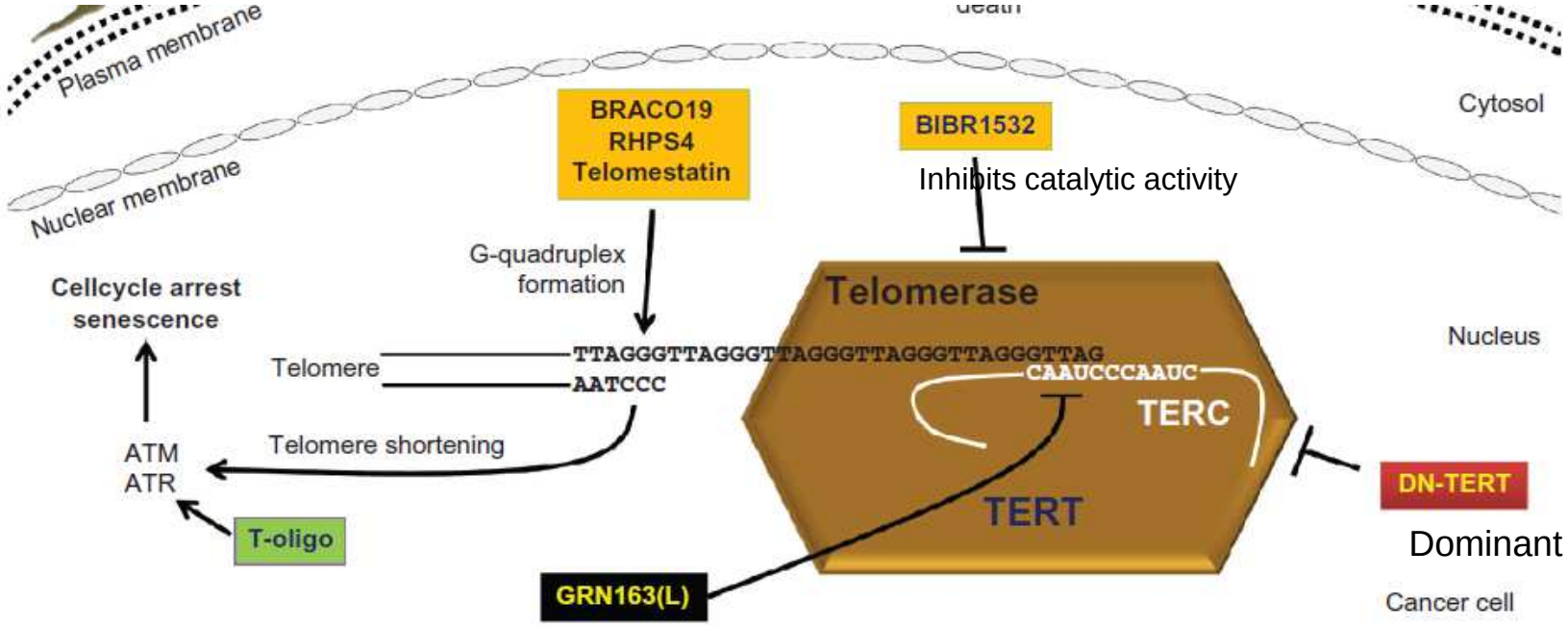
Strategie anti tumorali basate sulla inibizione della telomerasi

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



BRACO19, RHPS4, and telomestatin promote G-quadruplex formation

T-oligo mimics dysfunctional telomeres



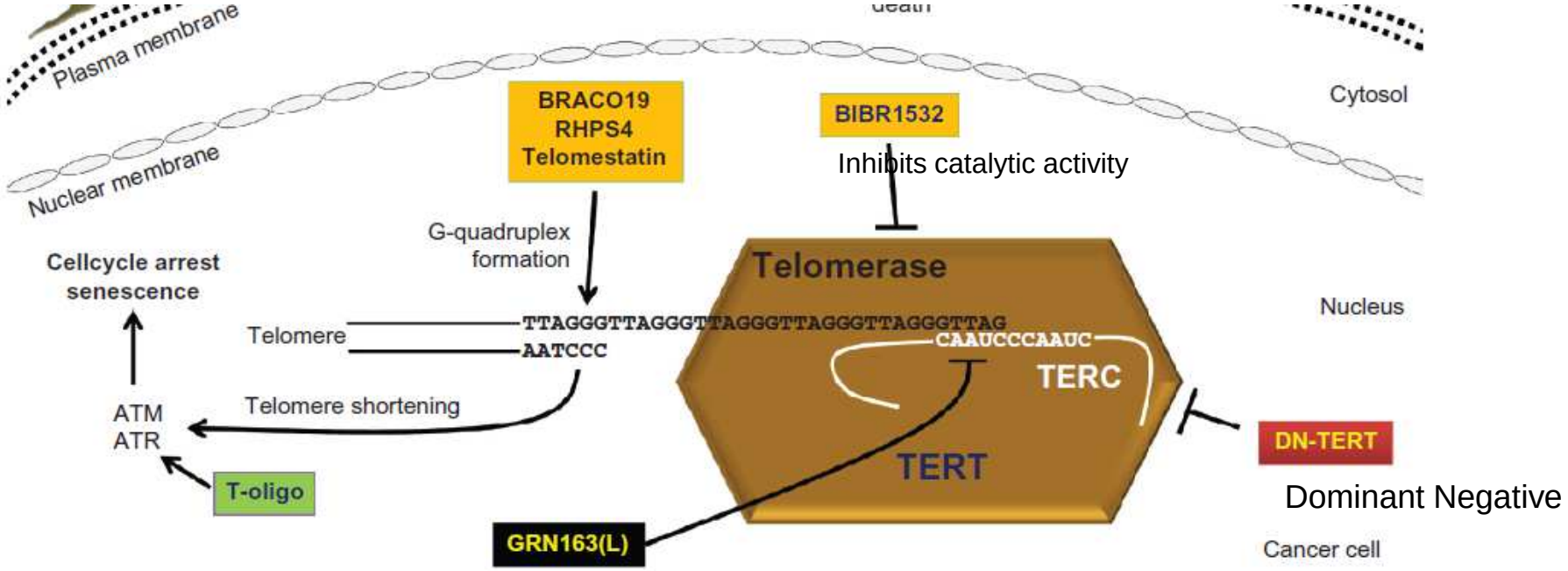
13-mer modified oligonucleotide, binds terC

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



13-mer modified oligonucleotide, binds terC

MUTAZIONI e 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

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.

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.

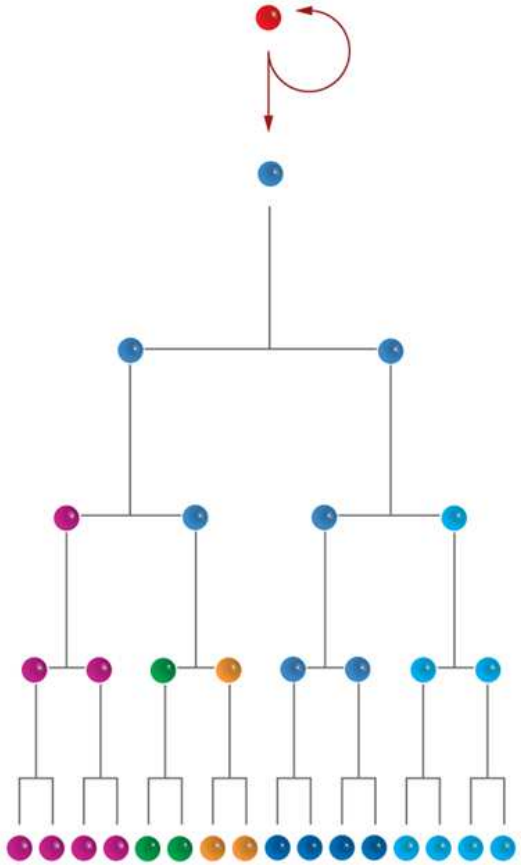
Among the families on the dyskeratosis congenita registry at the Hammersmith Hospital is a large family from Iowa, USA, with a mild form of dyskeratosis congenita and a[illegible] inheritance (DCR101; see Supplementary Info[illegible]

Nature 2001

Dyskeratosis congentia causes bone marrow failure

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

- Mortality
  - Bone marrow failure
  - Cancer
  - other?



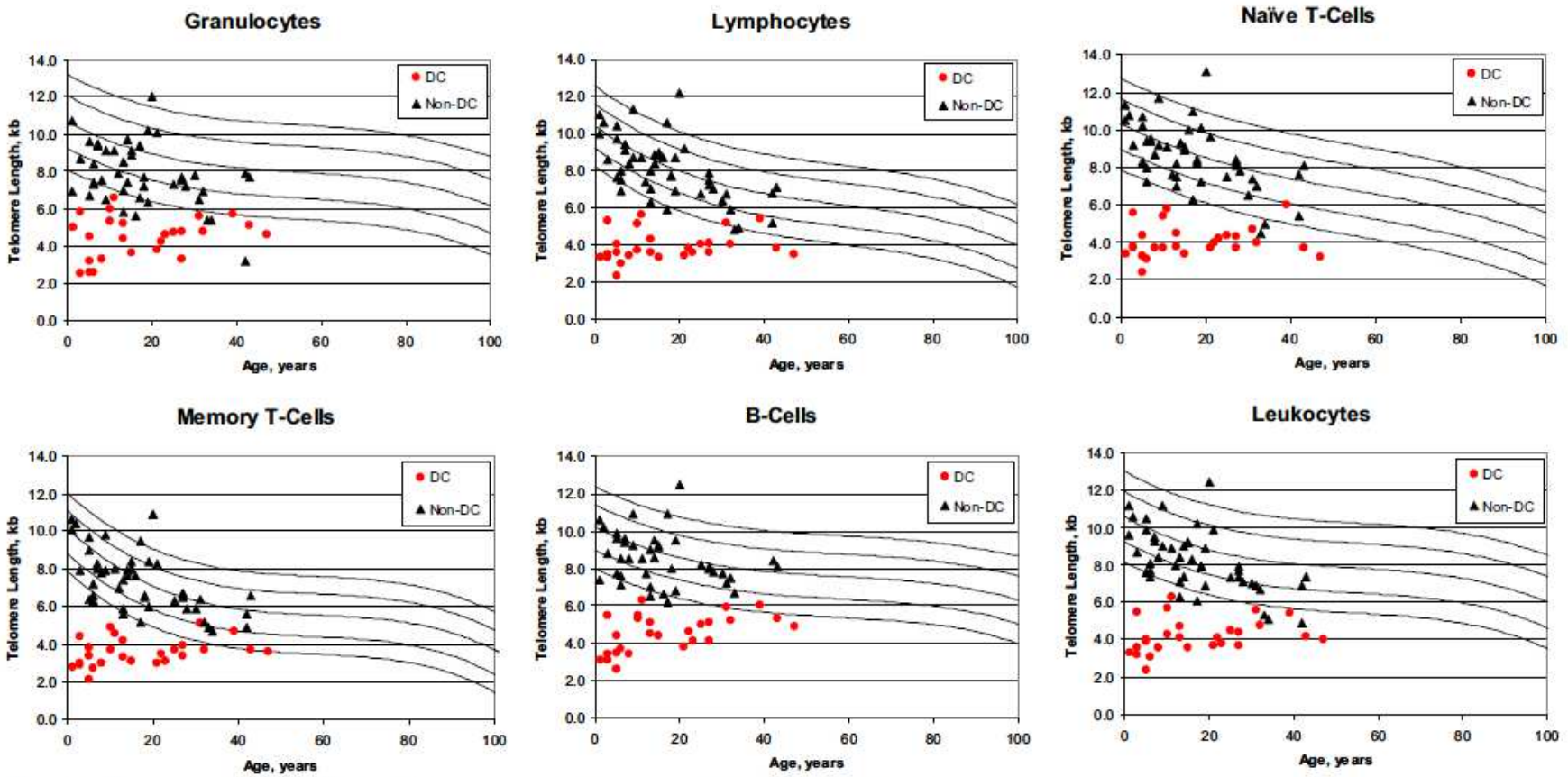


Figure 3. Telomere length according to age in dyskeratosis congenita and non–dyskeratosis congenita patients. The vertical axis represents telomere length in kilobytes. Lines in the figures indicate the first, tenth, 50th, 90th, and 99th percentiles of results from 400 normal control subjects. Symbols 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)

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.

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 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 2

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 30 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.