TELOMERI E COMPLESSI DEL TELOMERO

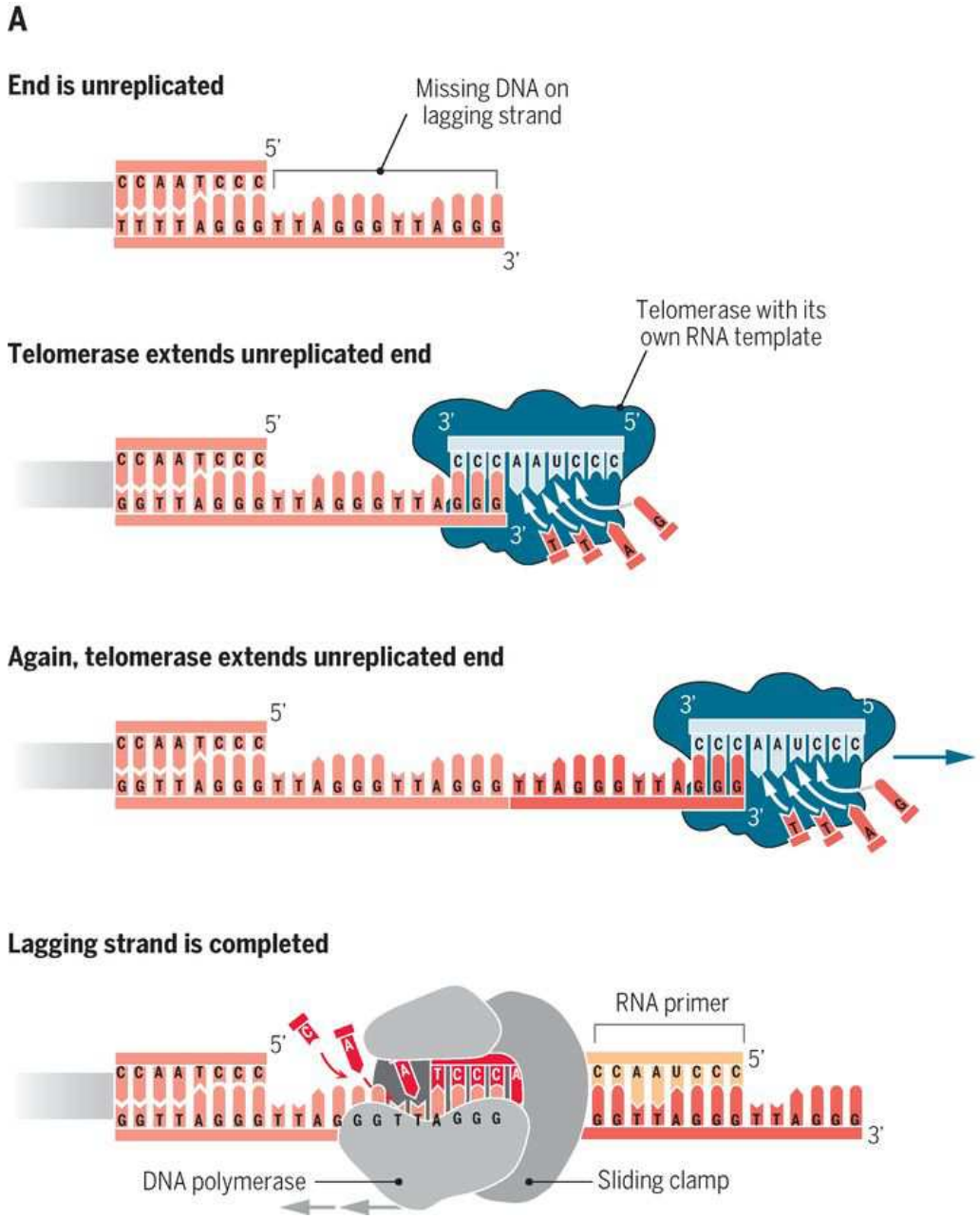
Telomeres are the terminal nucleoprotein structures located at the ends of eukaryotic chromosomes.

These structures function as

A guardians of genome stability by limiting unwanted DNA repair activity at chromosome ends, and in human cells,

B by controlling the total number of times a cell can divide, thereby limiting the accumulation of genomic instability in actively cycling cells

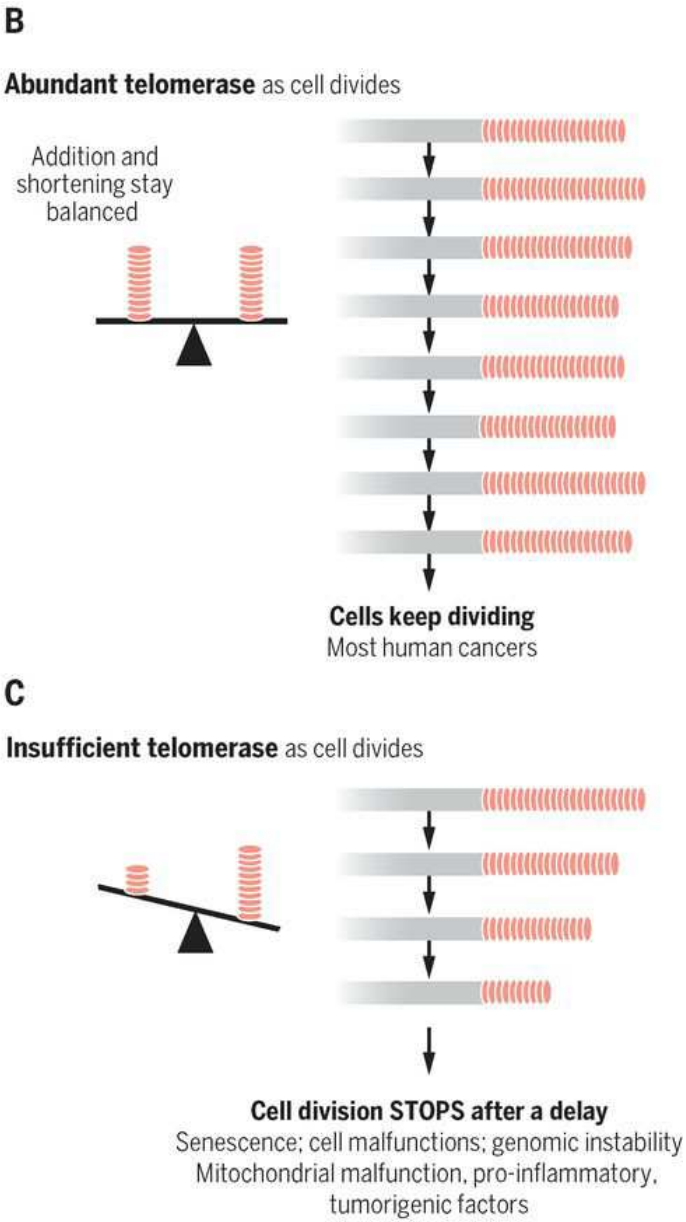
Long-term maintenance of telomeric DNA length requires telomerase.



Published by AAAS

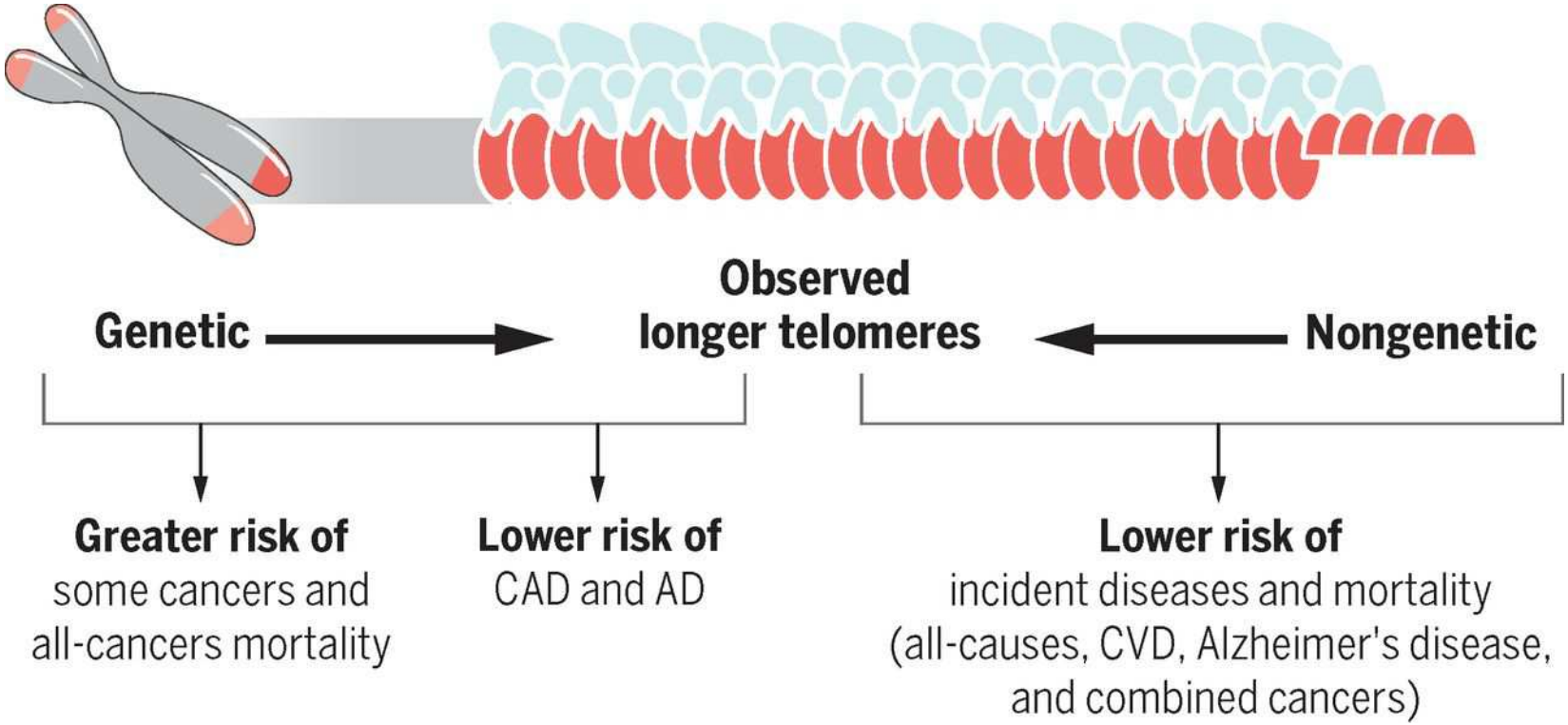
Elizabeth H. Blackburn et al. Science 2015;350:1193-1198

Long-term maintenance of telomeric DNA length requires telomerase.



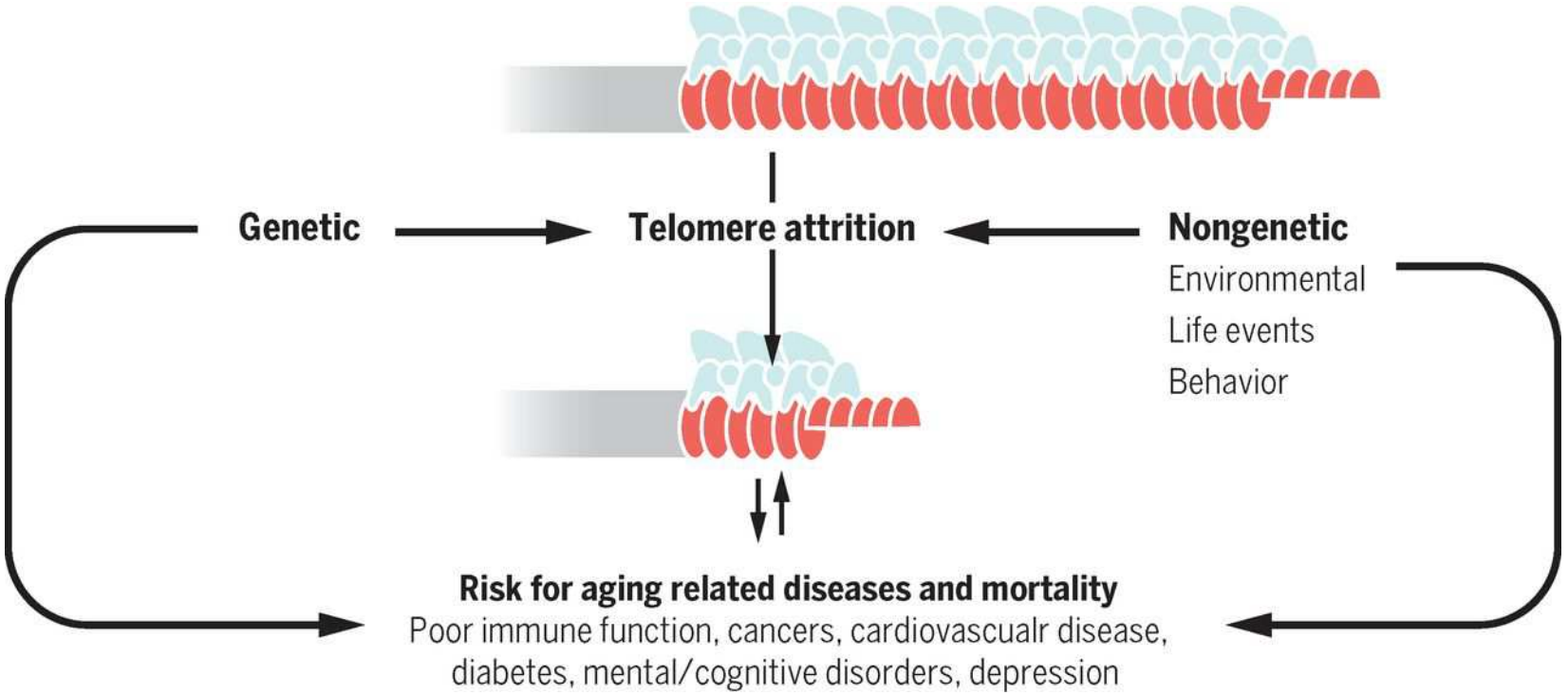
Published by AAAS **Elizabeth H. Blackburn et al. Science 2015;350:1193-1198**

Different inputs to telomere maintenance have disease-specific consequences.



Elizabeth H. Blackburn et al. Science 2015;350:1193-1198

Relationship of telomere attrition to human aging-related diseases.



Elizabeth H. Blackburn et al. Science 2015;350:1193-1198

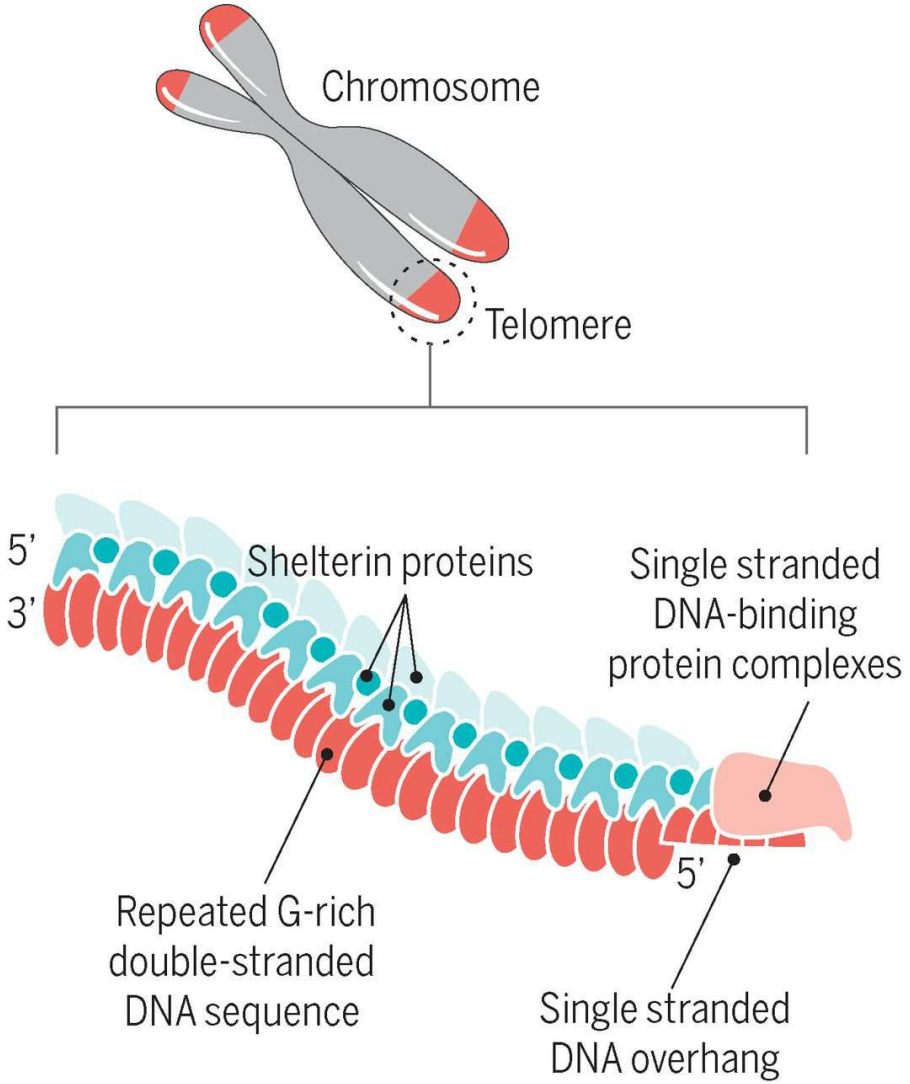
IL DNA Telomerico e le sue strutture alternative

SEQUENZA TELOMERICA

5–15 kb in humans, ~48 kb in mice

Watson et al., BIOLOGIA MOLECOLARE DEL GENE, Zanichelli editore S.p.A.

Telomere general structure.

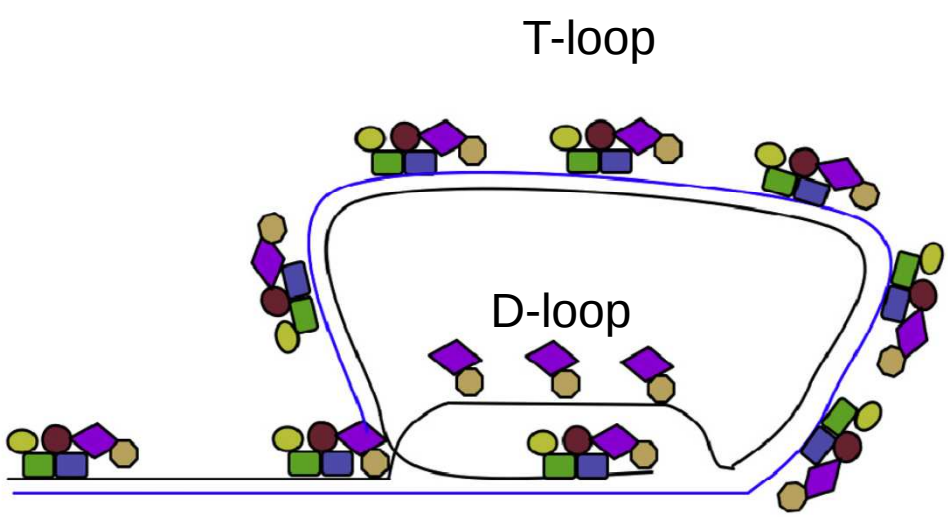


Elizabeth H. Blackburn et al. Science 2015;350:1193-1198

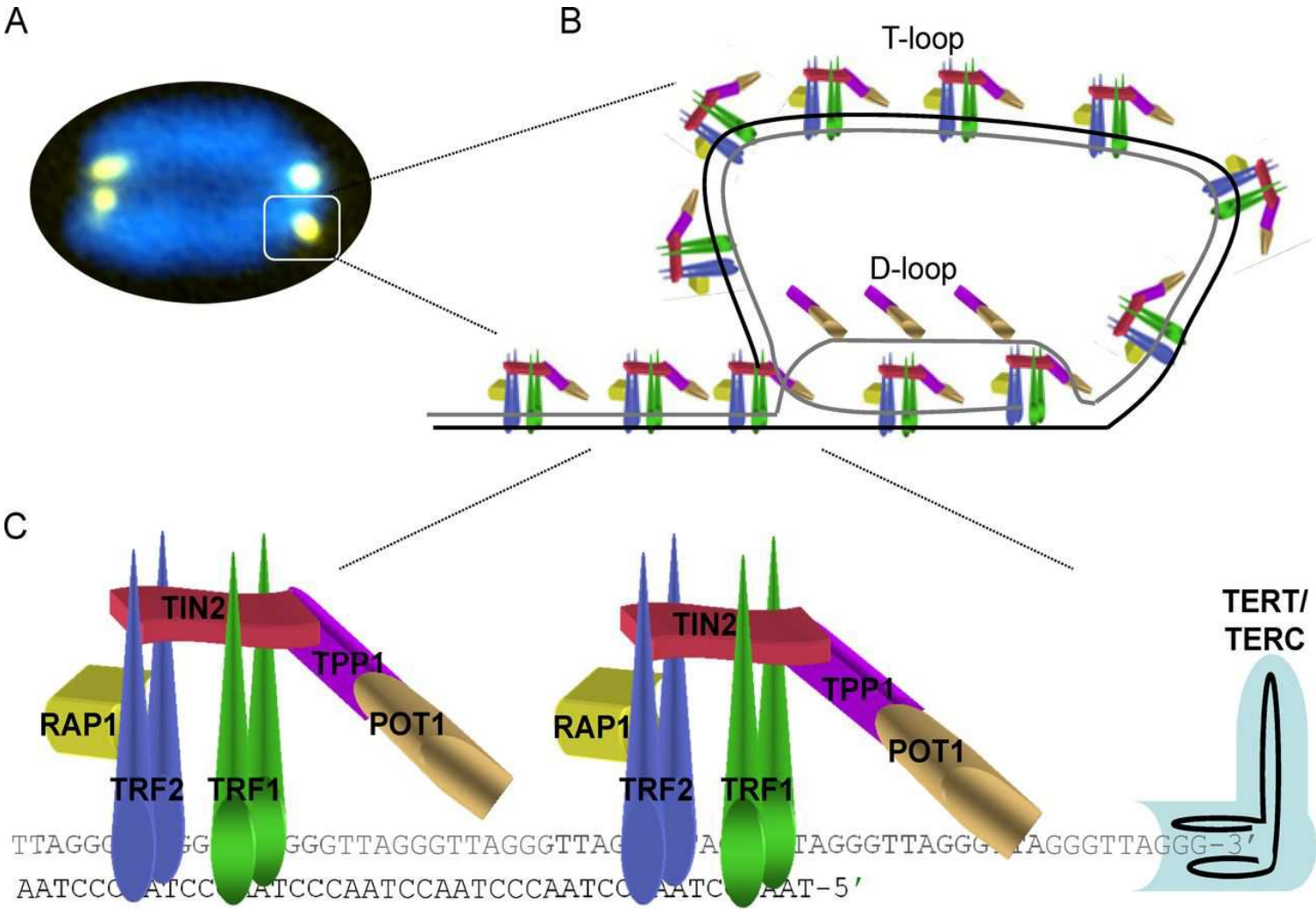
The single-stranded 3′ overhang folds back into the telomeric DNA, invades the double-helix, and anneals with the C-rich strand, forming a loop known as T-loop, thus hiding the very ends of chromosomal DNA.

TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3′

AATCCCAATCCCAATCCCAATCCAATCCCAATCCCAATCCCAATC-5′



The shelterin complex and the structure of telomeres.

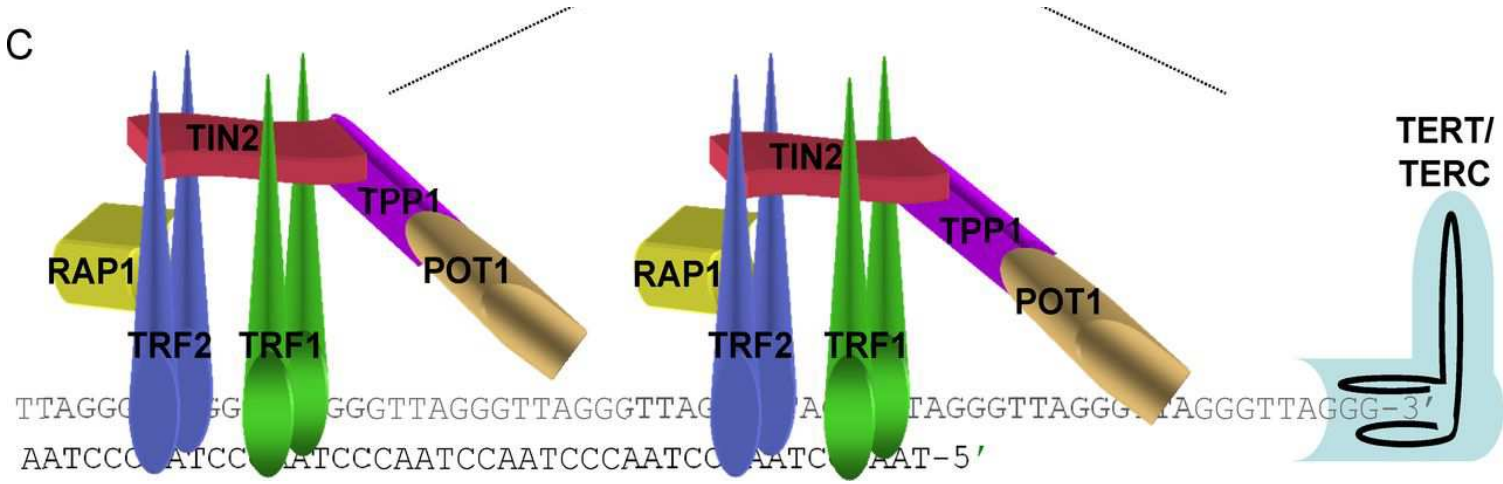


Paula Martínez, and Maria A. Blasco J Cell Biol
doi:10.1083/jcb.201610111

JCB

The shelterin complex and the structure of telomeres.

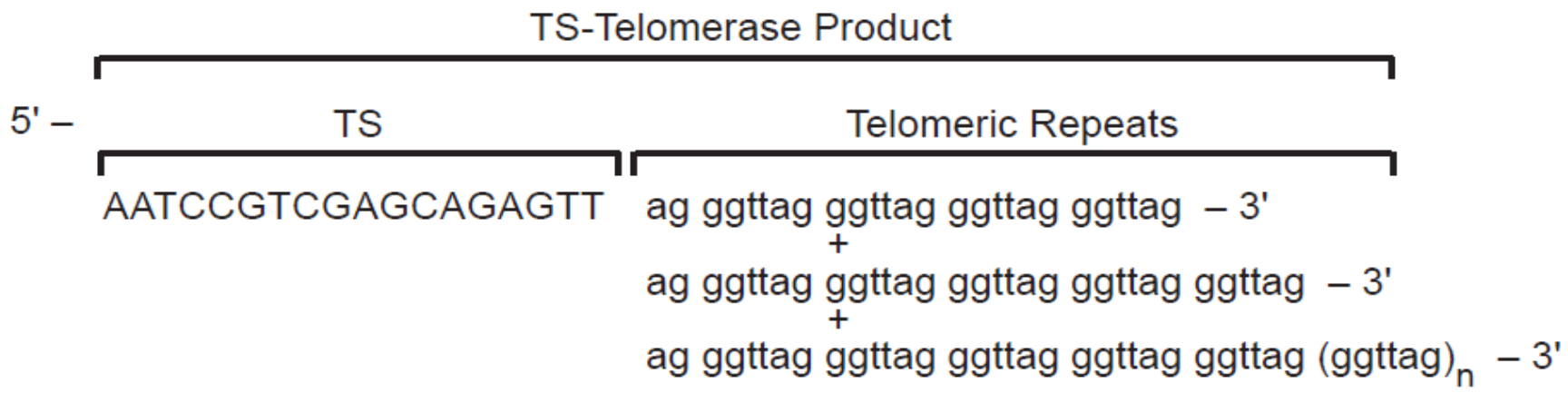
telomeric repeat binding factor 1 (TRF1)
telomeric repeat binding factor 2 (TRF2)
repressor-activator protein 1 (RAP1)
protection of telomeres protein 1 (POT1)
POT1-TIN2 organizing protein (TPP1)
TIN2



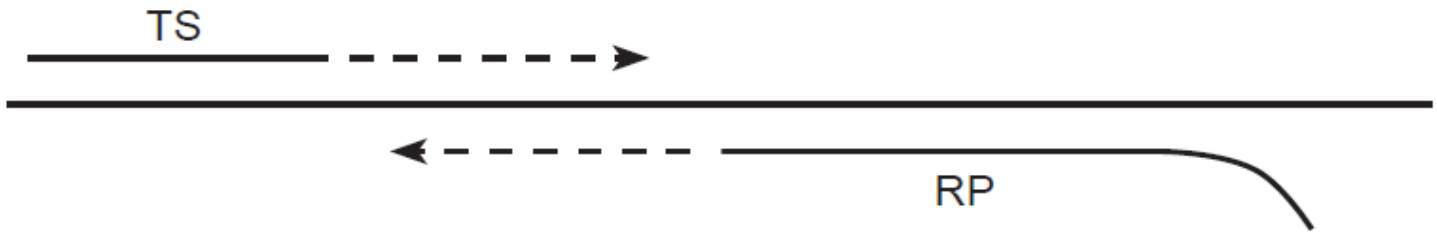
Paula Martínez, and Maria A. Blasco J Cell Biol
doi:10.1083/jcb.201610111

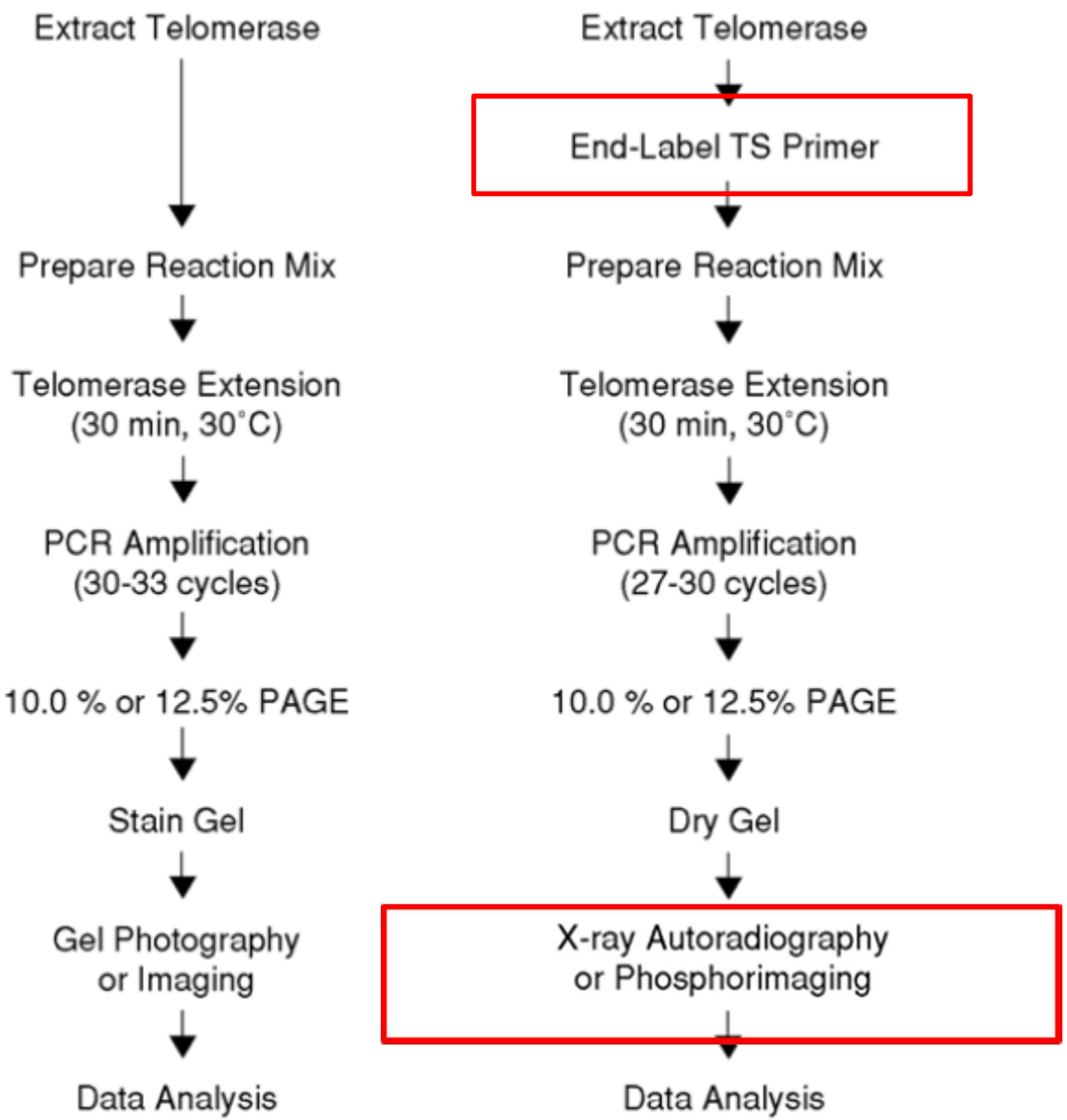
JCB

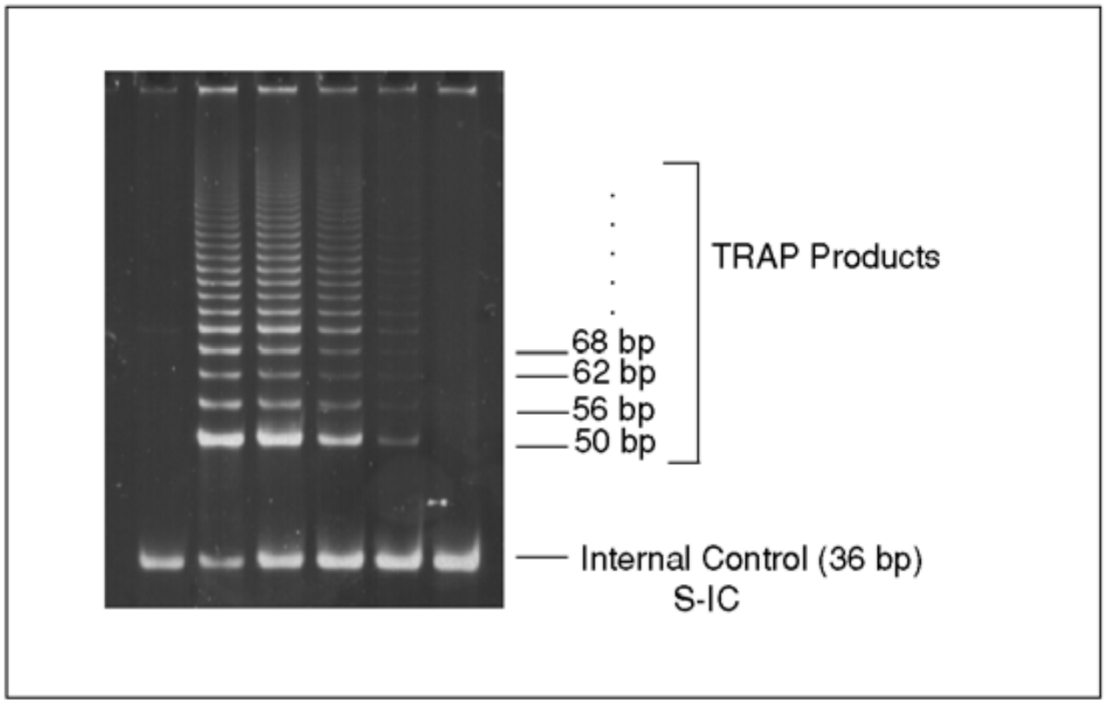
Metodi per lo studio dell’attività telomerasica



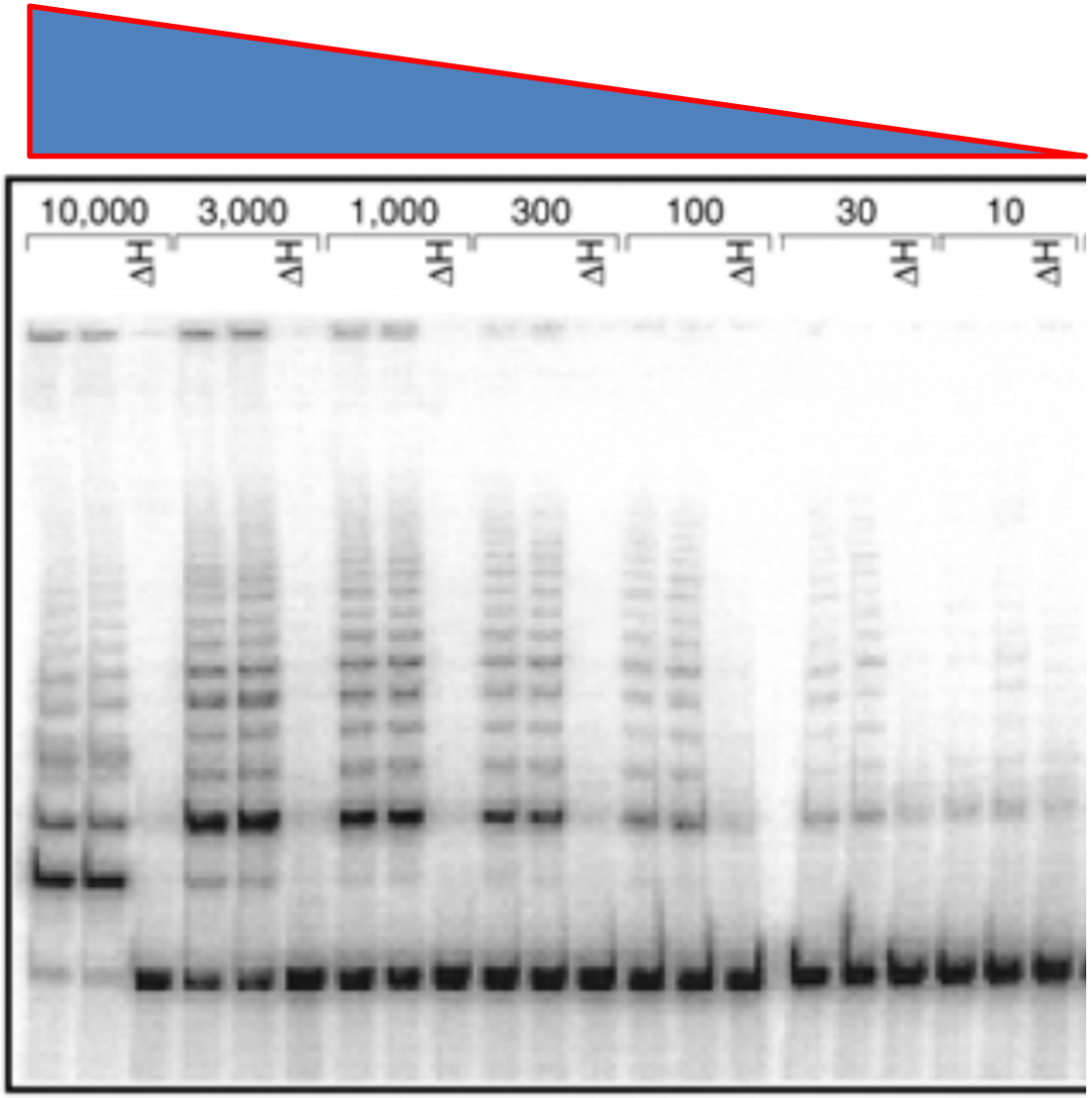
STEP 2. Amplification of TS-Telomerase Product By PCR




Extract Telomerase
Prepare Reaction Mix
Telomerase Extension
(30 min, 30˚C)
PCR Amplification
(30-33 cycles)
10.0 % or 12.5% PAGE
Stain Gel
Gel Photography
or Imaging
Data Analysis
Extract Telomerase
End-Label TS Primer
Prepare Reaction Mix
Telomerase Extension
(30 min, 30˚C)
PCR Amplification
(27-30 cycles)
10.0 % or 12.5% PAGE
Dry Gel
X-ray Autoradiography
or Phosphorimaging
Data Analysis

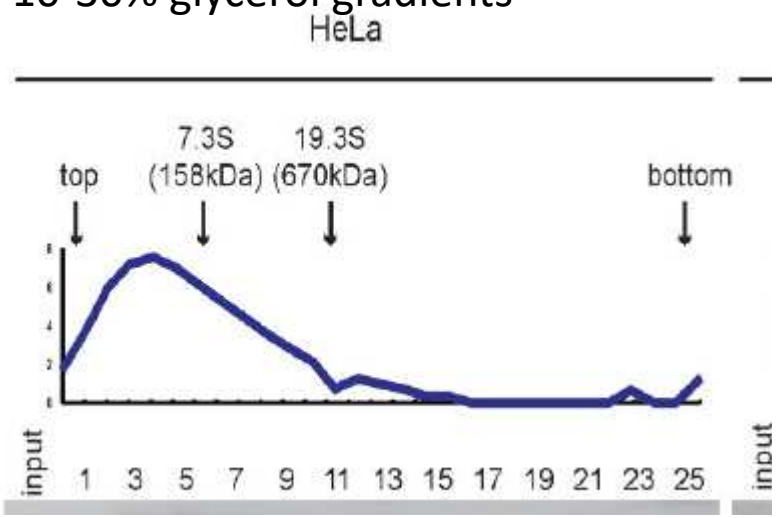


Telomeric Repeat Amplification Protocol

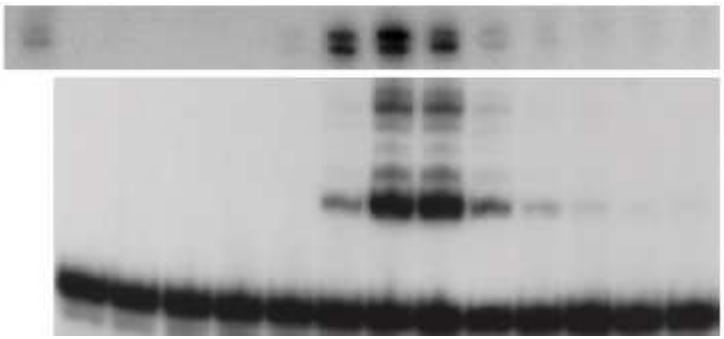

10,000
3,000
1,000
300
100
30
10
ΔH
ΔH
ΔH
ΔH
ΔH
ΔH
ΔH

COMPLESSI TELOMERICI

10-30% glycerol gradients



Total protein



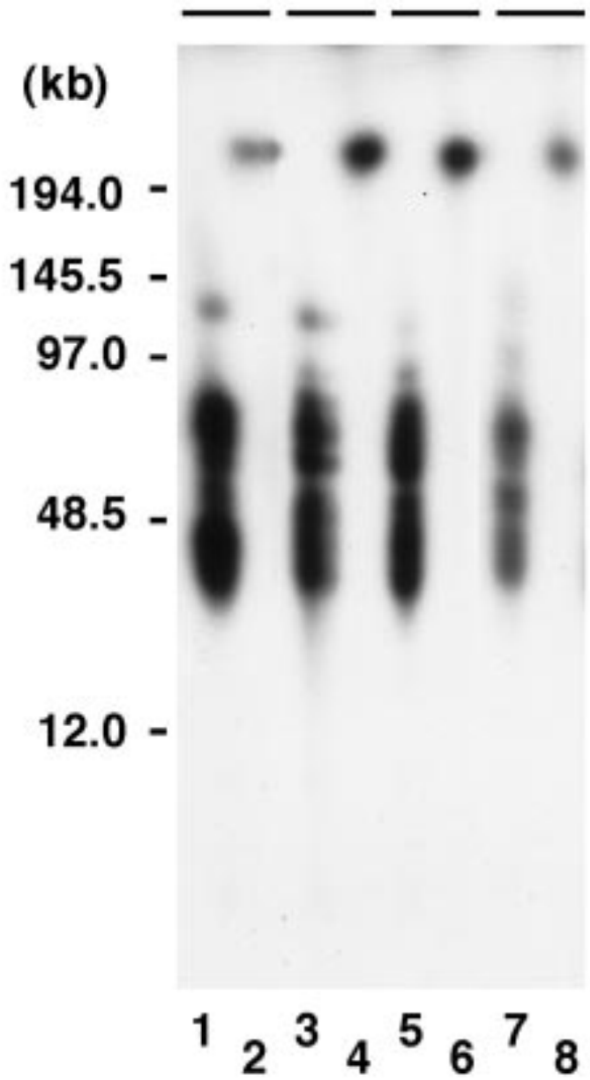
NB: TERC

TRAP

Telomeric Repeat Amplification Protocol

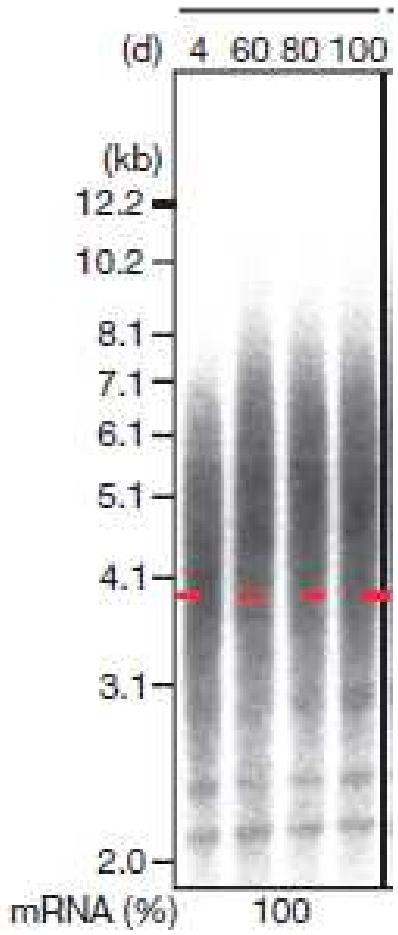
Metodi per lo studio dello stato dei telomeri

DNA TELOMERICO



digested with RsaI and Hinf - Odd lanes
pulse-field gel electrophoresis
hybridized with the telomeric specific [TTAGGG]3 probe

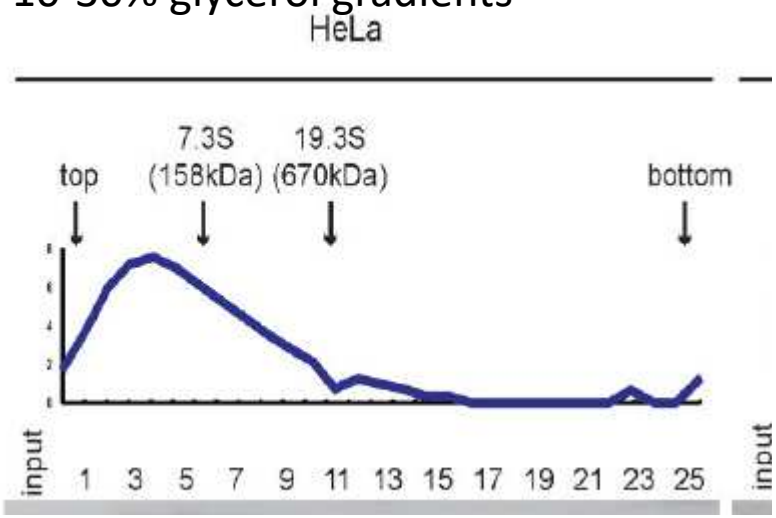
DNA TELOMERICO



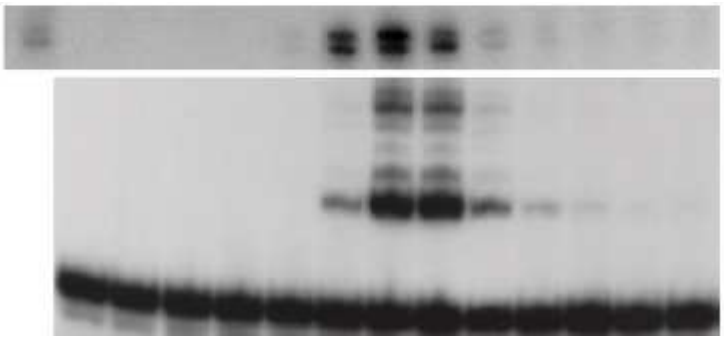
Complessi macromolecolari associati al Telomero: funzioni

COMPLESSI TELOMERICI

10-30% glycerol gradients



Total protein

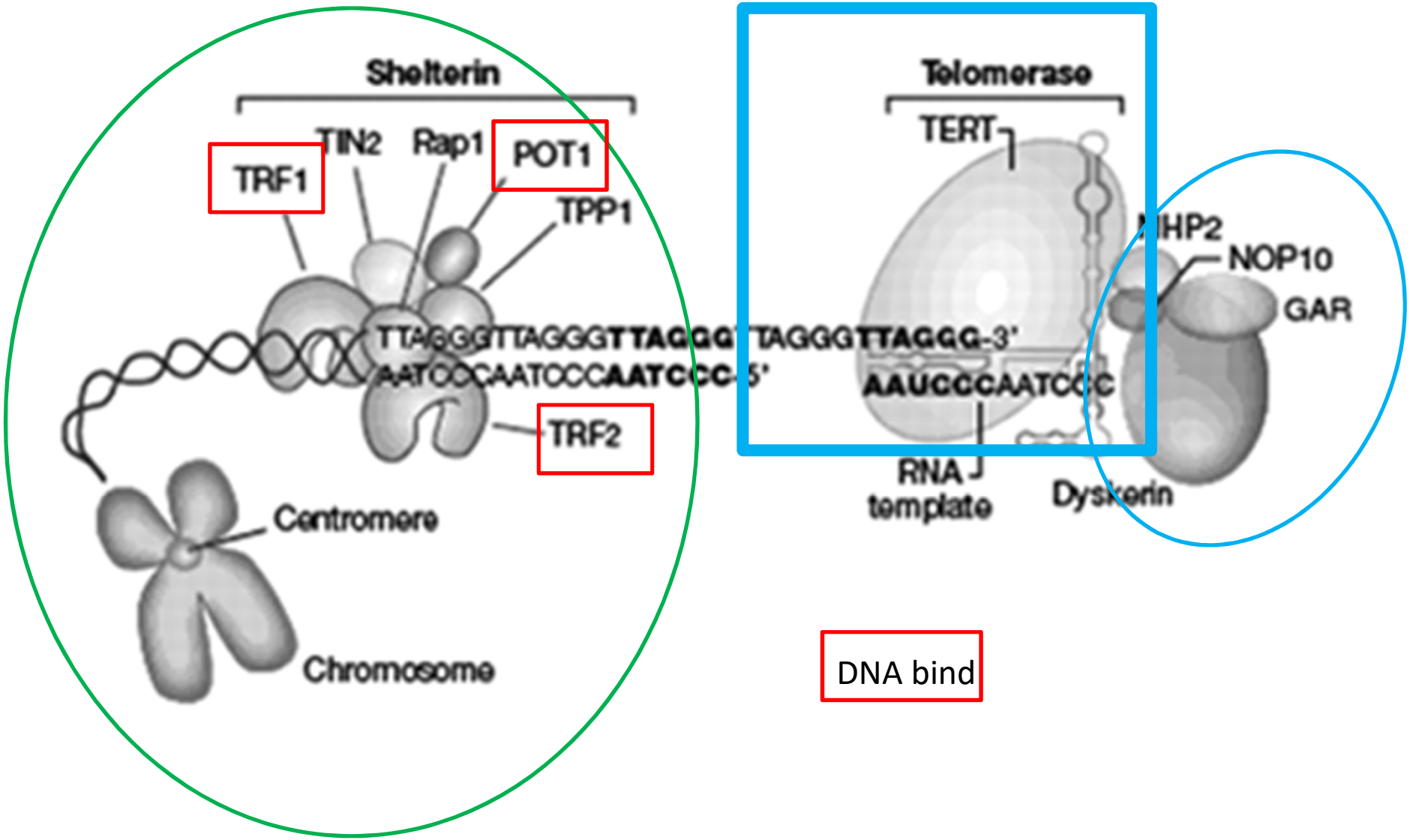


NB: TERC

TRAP

Telomeric Repeat Amplification Protocol

Complessi macromolecolari associati al Telomero ed alla Telomerasi

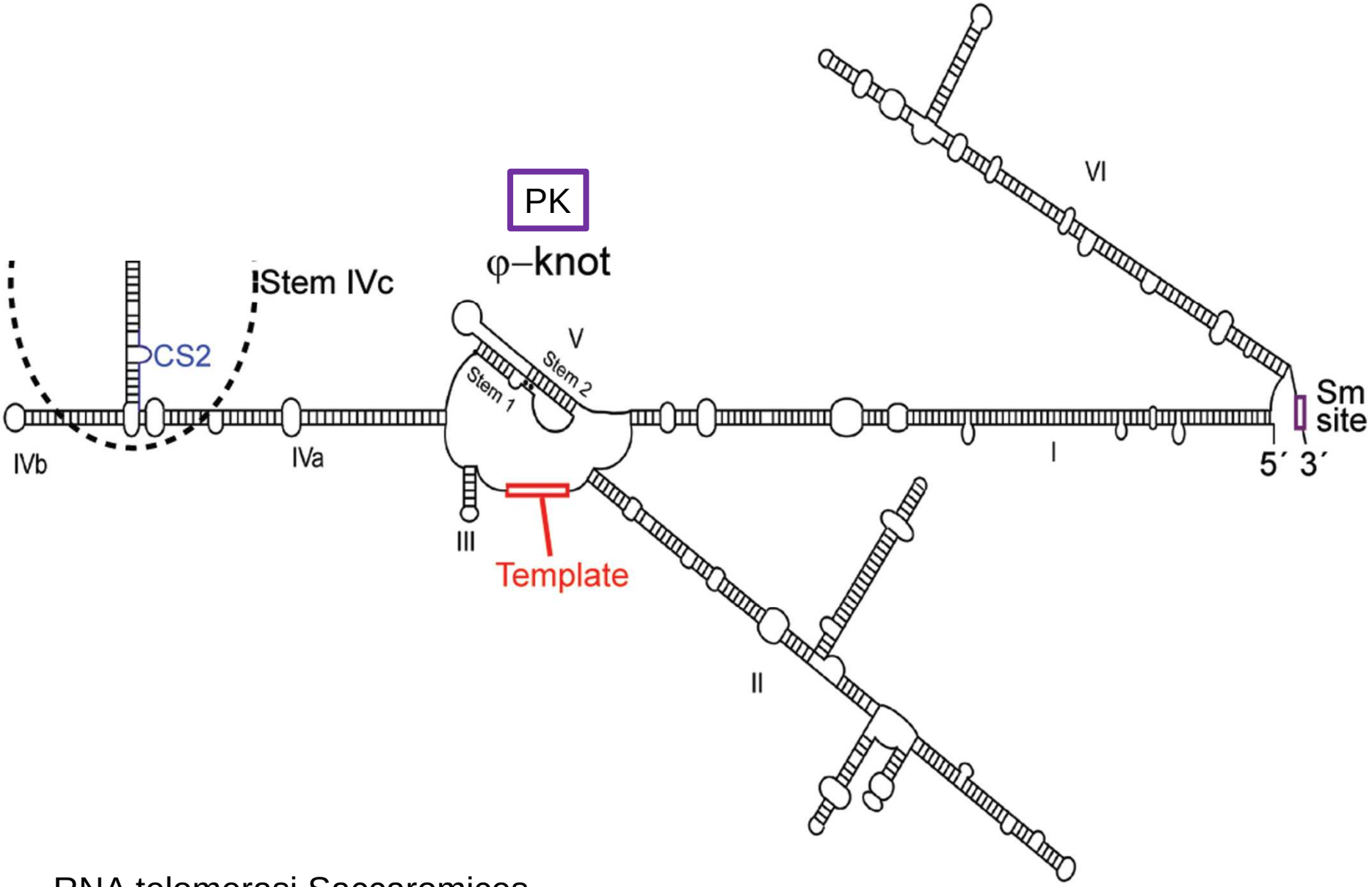


RNA TEMPLATO DELLA TELOMERASI

hTR is a 451-nucleotide RNA

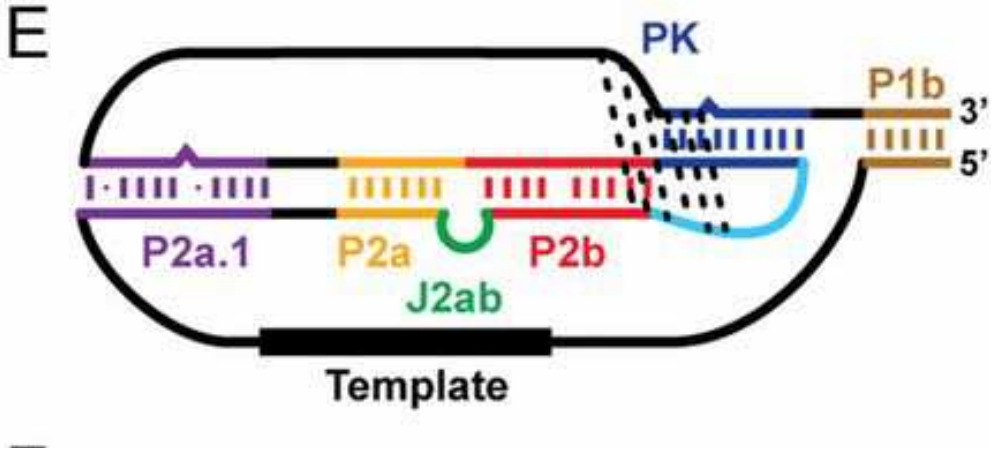
contains a box H/ACA motif at its 3 end essential for hTR stability and for its assembly with hTERT mediated by the presence of the box H/ACA-binding dyskerin complex, which is composed of four proteins:

dyskerin, NOP10, NHP2 and GAR1.

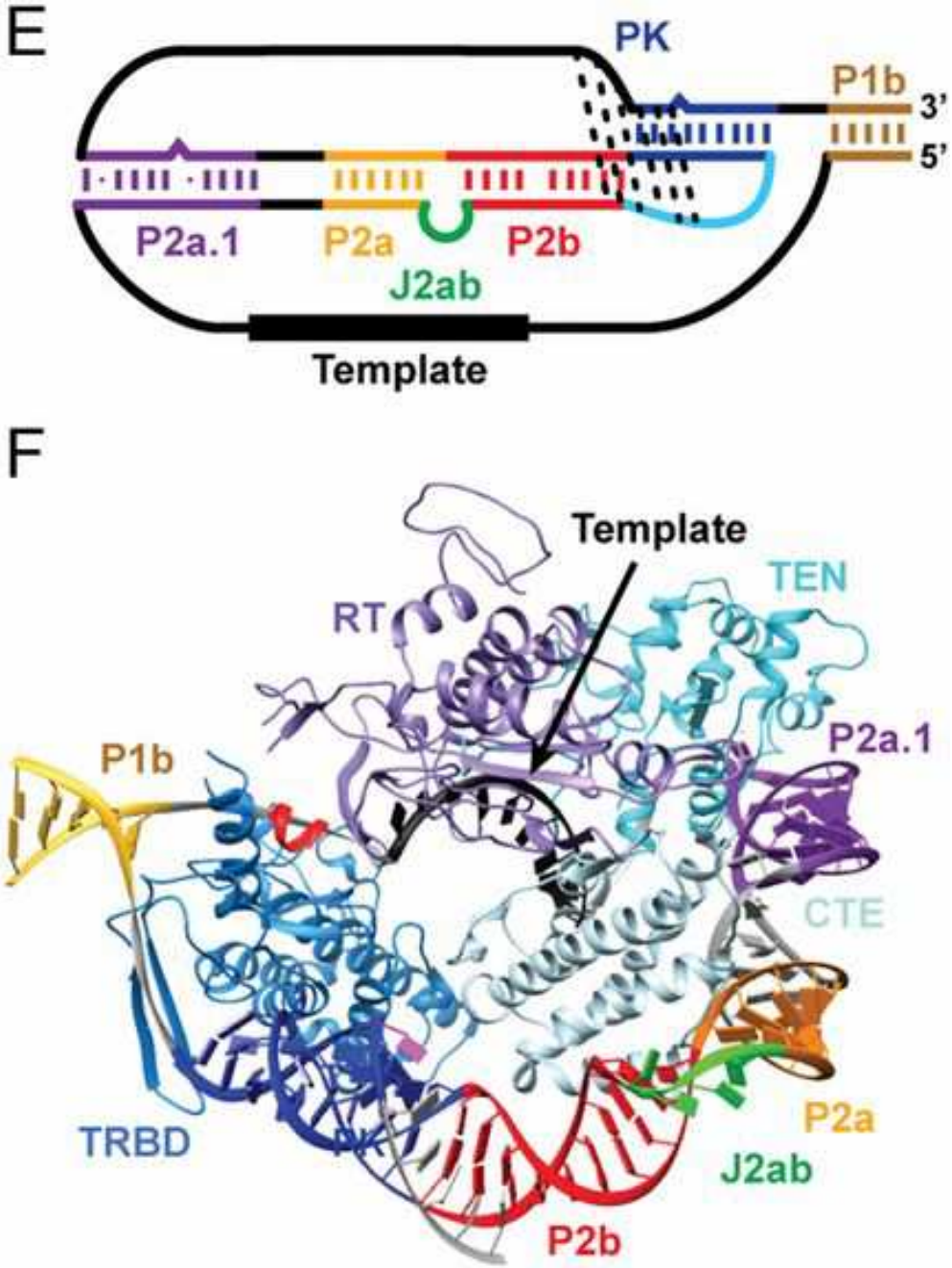


RNA telomerasi Saccaromices

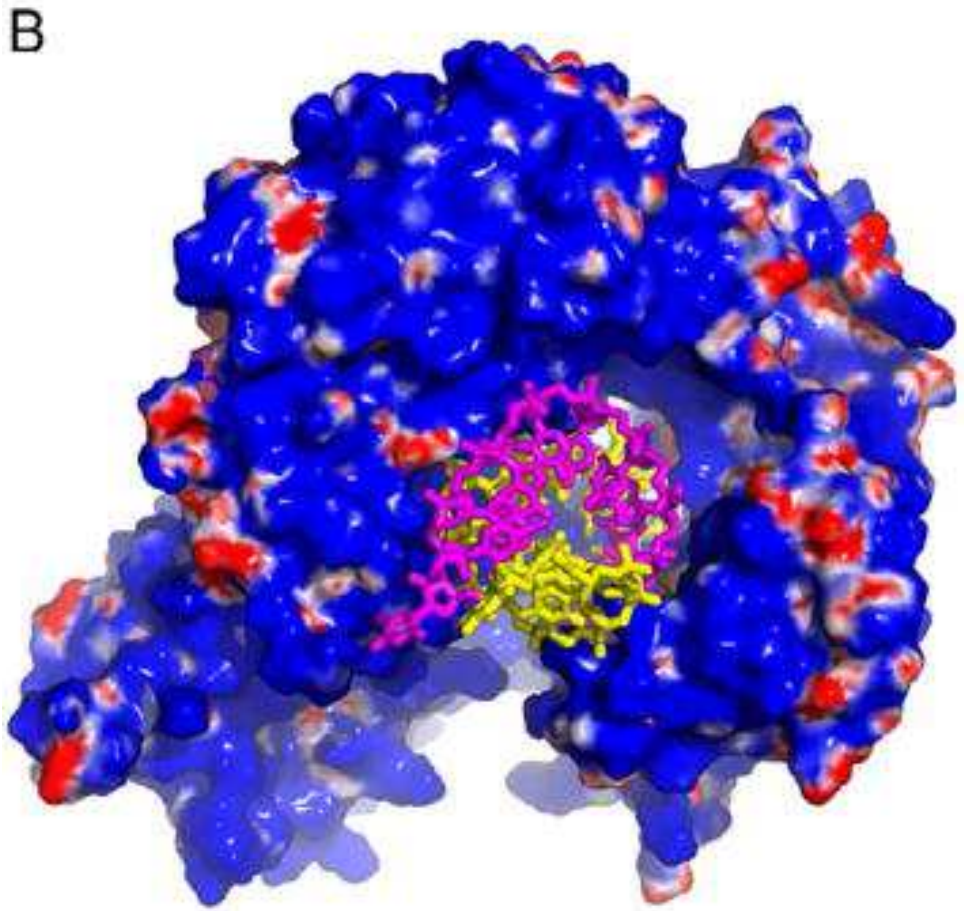
human TERT–t/PK.



human TERT–t/PK.

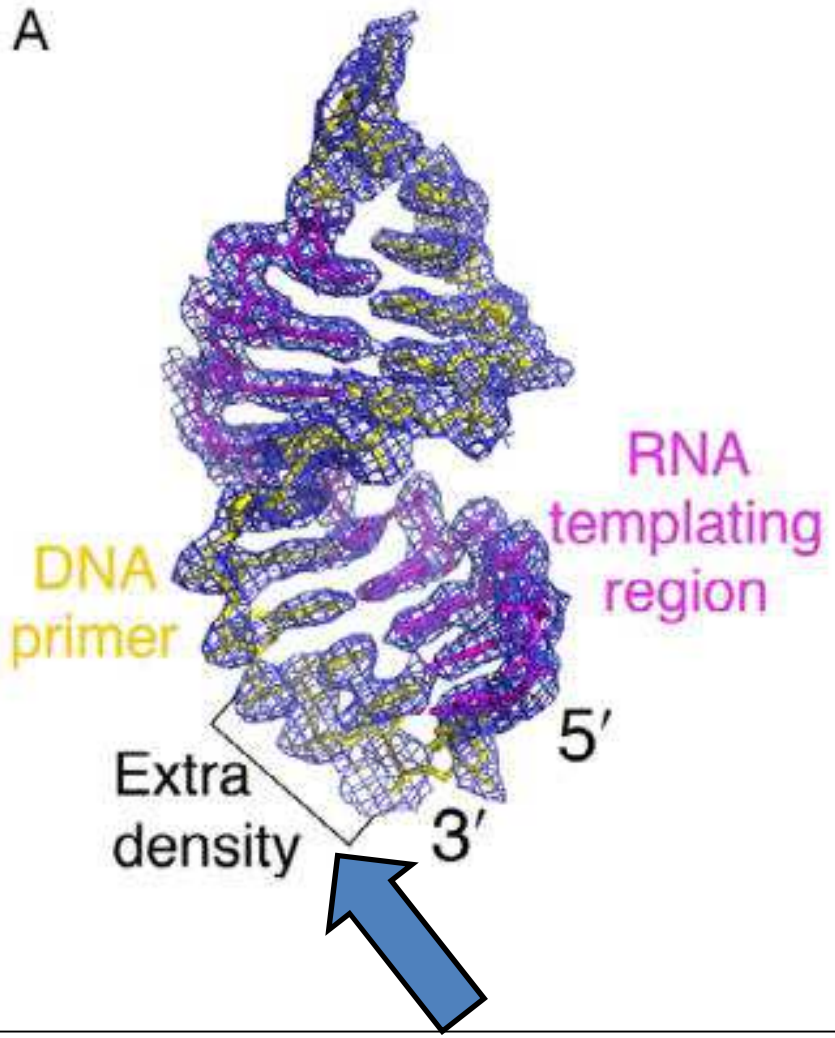


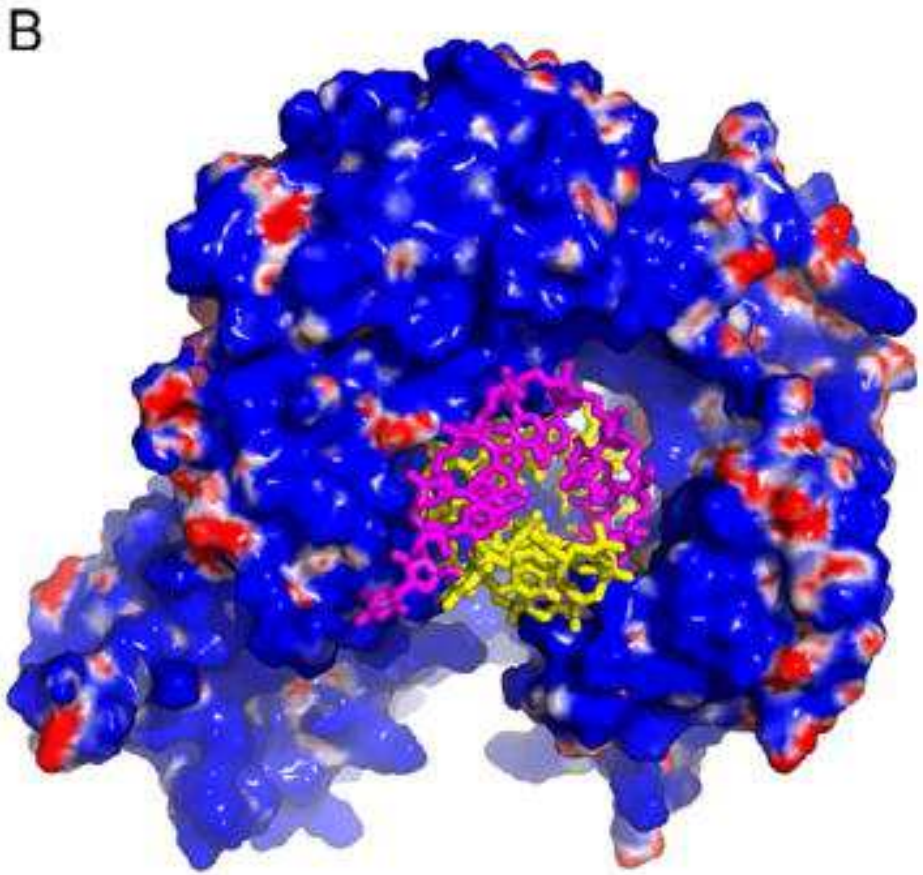
RNA (magenta stick)–DNA (yellow stick) hairpin cocrystallized with tcTERT



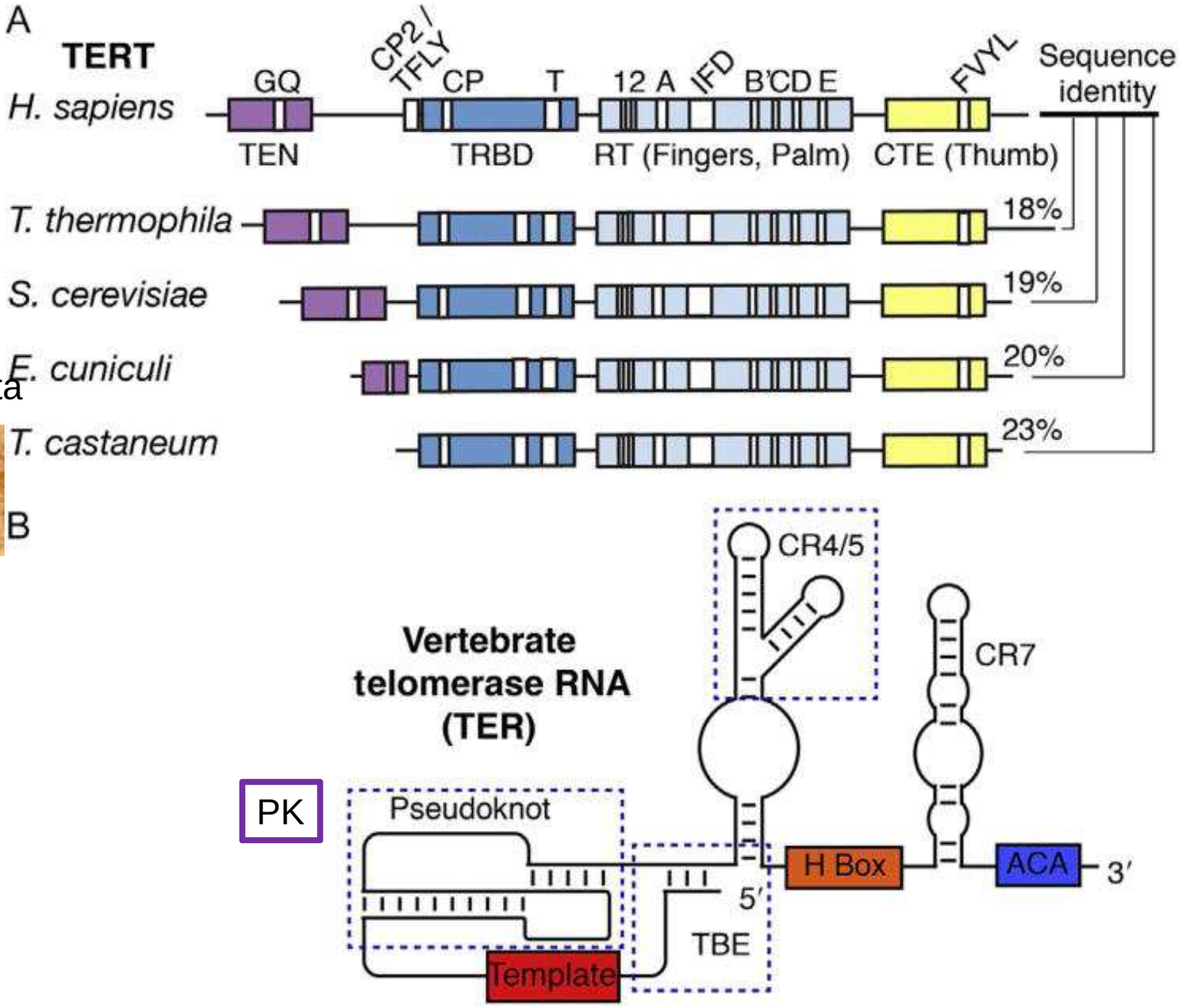
tcTERT surface charge representation,
the RNA–DNA hybrid (stick) docked in the
interior cavity of the TERT ring

RNA (magenta stick)–DNA (yellow stick) hairpin cocrystallized with tcTERT





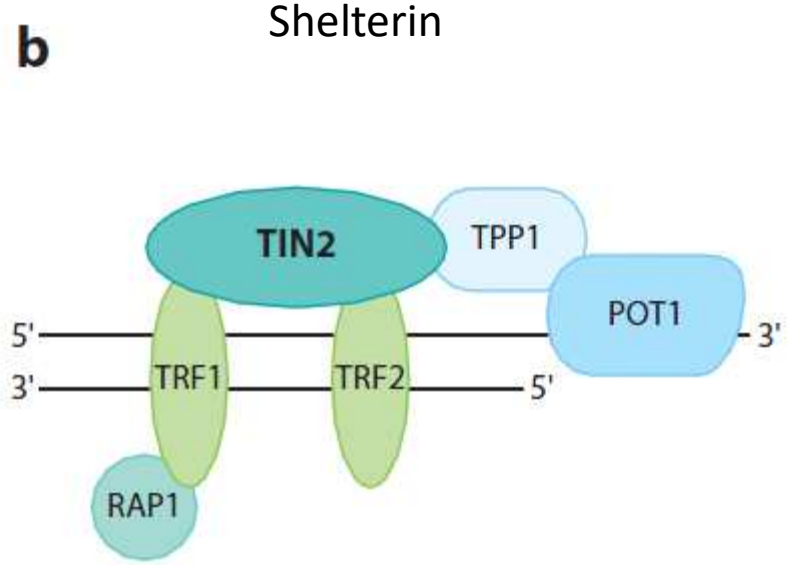
three additional nucleotides at the 3′-end of the telomeric DNA !!



Fungo parassita

Complessi macromolecolari associati al Telomero

Shelterin

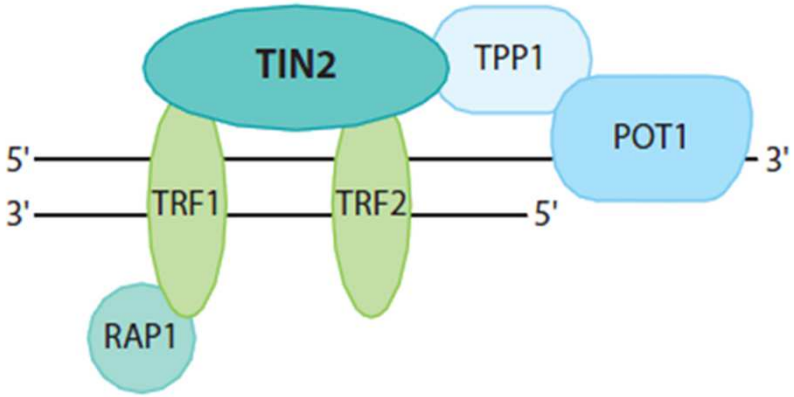


Telomeres are coated by a group of at least six proteins, collectively called **shelterin**
Three proteins, TRF1, TRF2, and POT1(single-stranded repeats) directly recognize and bind to TTAGGG repeats
TIN2 TPP1, and Rap1, interconnect the telomere-binding proteins to form the entire complex

Shelterin serves as a signal that allows the cellular DNArepair machinery to distinguish telomeres from DNA double-stranded breaks

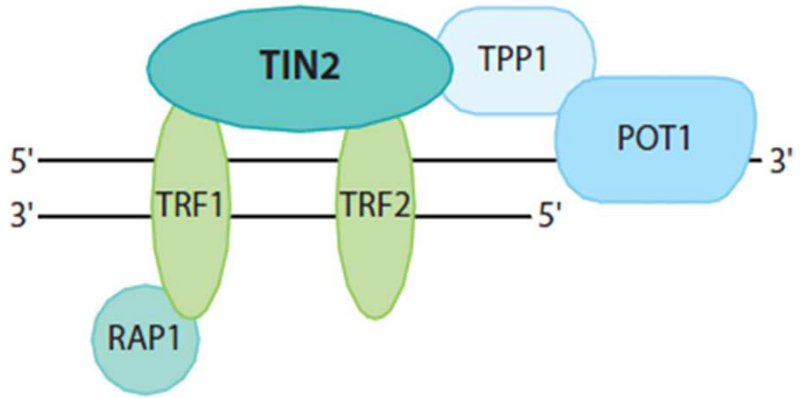
MUTATIONS IN TELOMERIC PROTEINS AND CANCER

Protein	Cancer(s)
Shelterin	
TRF1/TRF2	Gastric
POT1	Leukemia (CLL) Melanoma Glioma
TPP1	DC Melanoma
TIN2	DC
RAP1	Melanoma



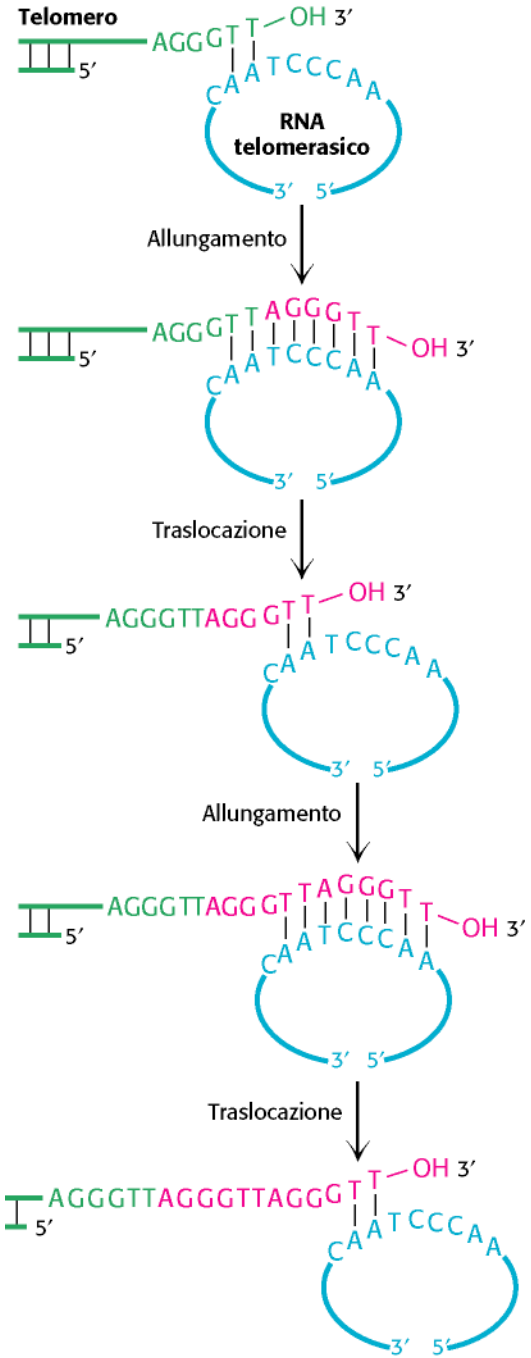
MUTATIONS IN TELOMERIC PROTEINS AND CANCER

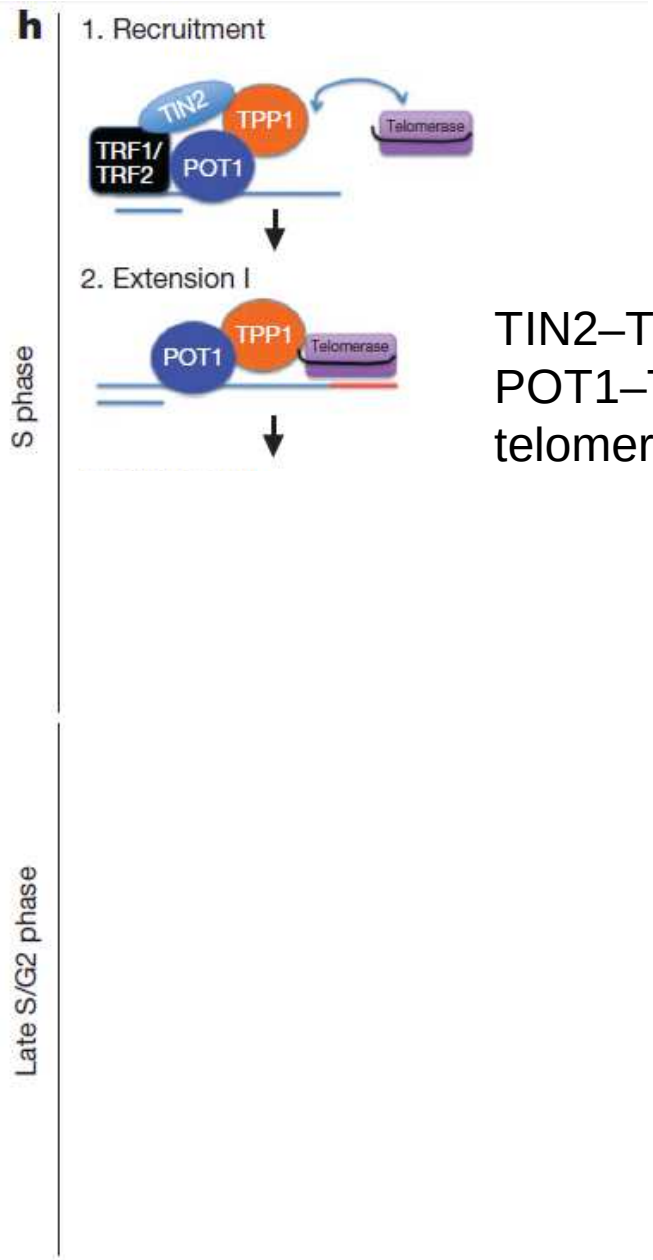
Protein	Cancer(s)
Shelterin	
TRF1/TRF2	Gastric
POT1	Leukemia (CLL) Melanoma Glioma
TPP1	DC Melanoma
TIN2	DC
RAP1	Melanoma
Telomere elongation	
TERT	Glioma Bladder Thyroid Melanoma Breast/ovarian
TERC	MDS



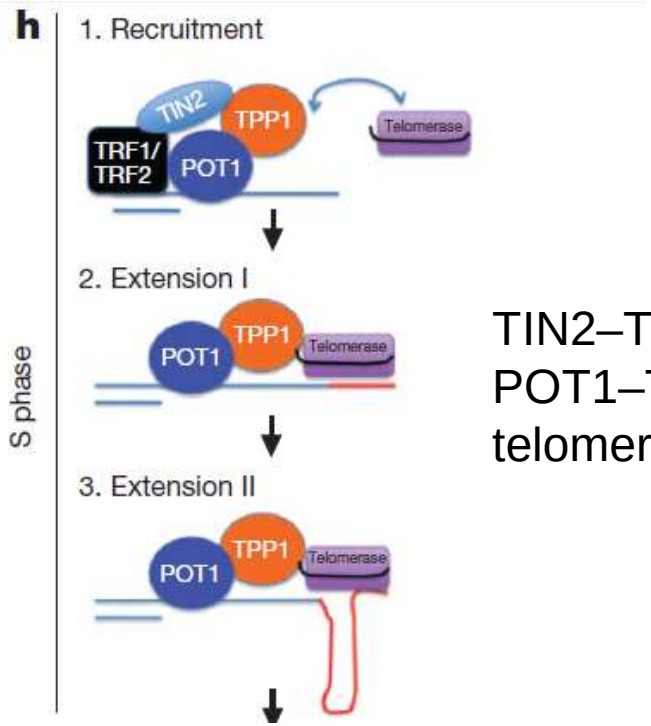
L’allungamento del telomero modello riassuntivo

During every cell division, telomeres are potentially shortened by 50–200 bp due to the end replication problem





TIN2–TPP1 recruits telomerase and POT1–TPP1 promotes processive telomere elongation



TIN2–TPP1 recruits telomerase and POT1–TPP1 promotes processive telomere elongation

IL RECLUTAMENTO DELLA TELOMERASI

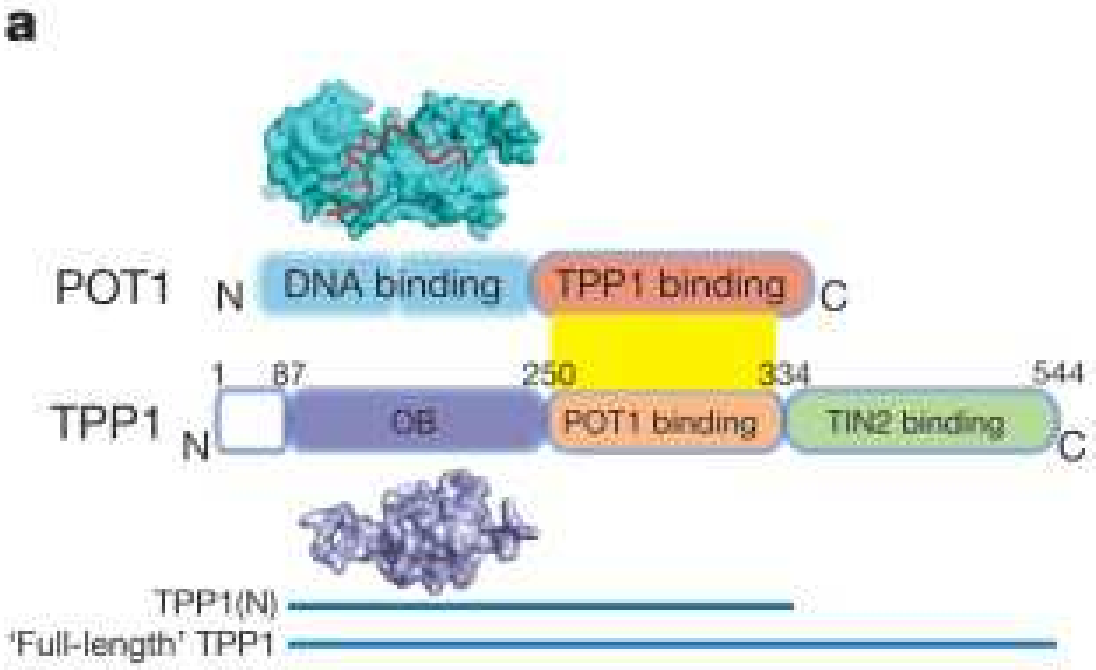
TPP1 recruits telomerase to telomeres

Telomere synthesis involves trafficking of telomerase and telomerase is thought to be recruited to telomeres through interactions with telomere-binding proteins.

The OB-fold domain of the telomere-binding protein TPP1 recruits telomerase to telomeres through an association with the telomerase reverse transcriptase, TERT.

The TPP1 OB-fold domain is sufficient to recruit telomerase to a heterologous chromatin locus.
A minimal TPP1 OB-fold inhibits telomere maintenance by blocking access of telomerase to its binding site at telomeres.

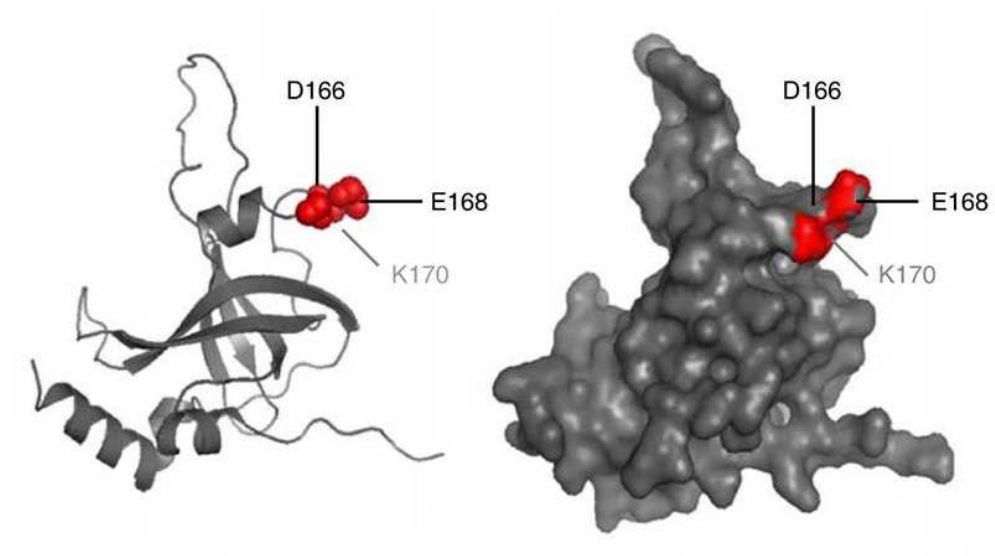
A specific loop residues within the TPP1 OB-fold is necessary for association with critical residues in TER Telomerase, including those mutated in pulmonary fibrosis patients, which defines the interface required for telomerase-TPP1 interaction.



nature

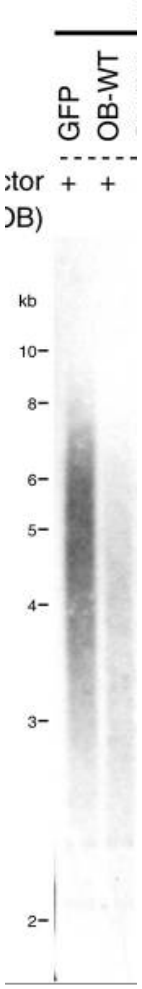
J Nandakumar *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature11648

the OB-fold domain of the telomere-binding protein TPP1 recruits telomerase to telomeres through an association with the telomerase reverse transcriptase, TERT

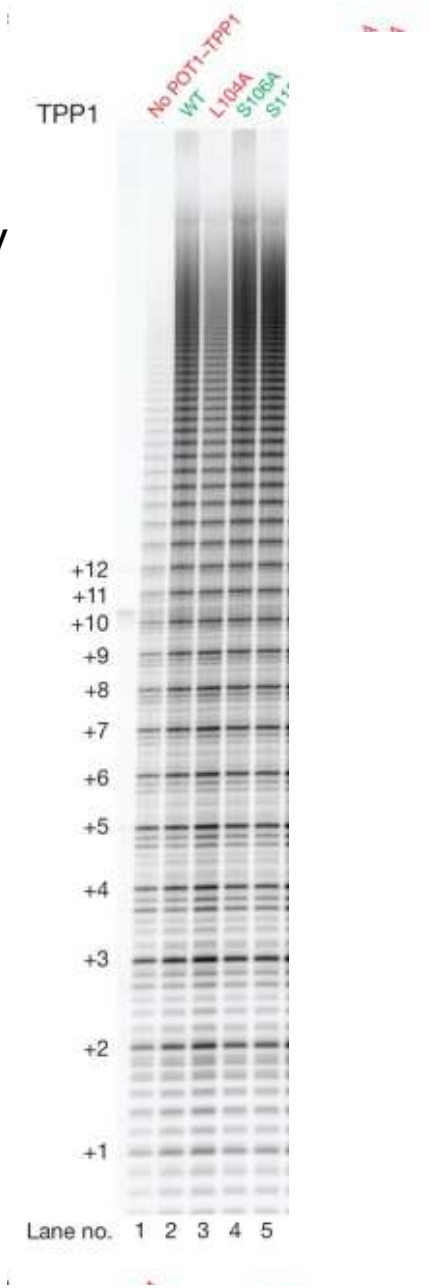


Structural representation of TPP1-OB domain (PDB 2i46).
Residues required for telomerase interaction shown in red

TPP1-OB inhibits telomere length maintenance by telomerase and blocks endogenous telomerase recruitment

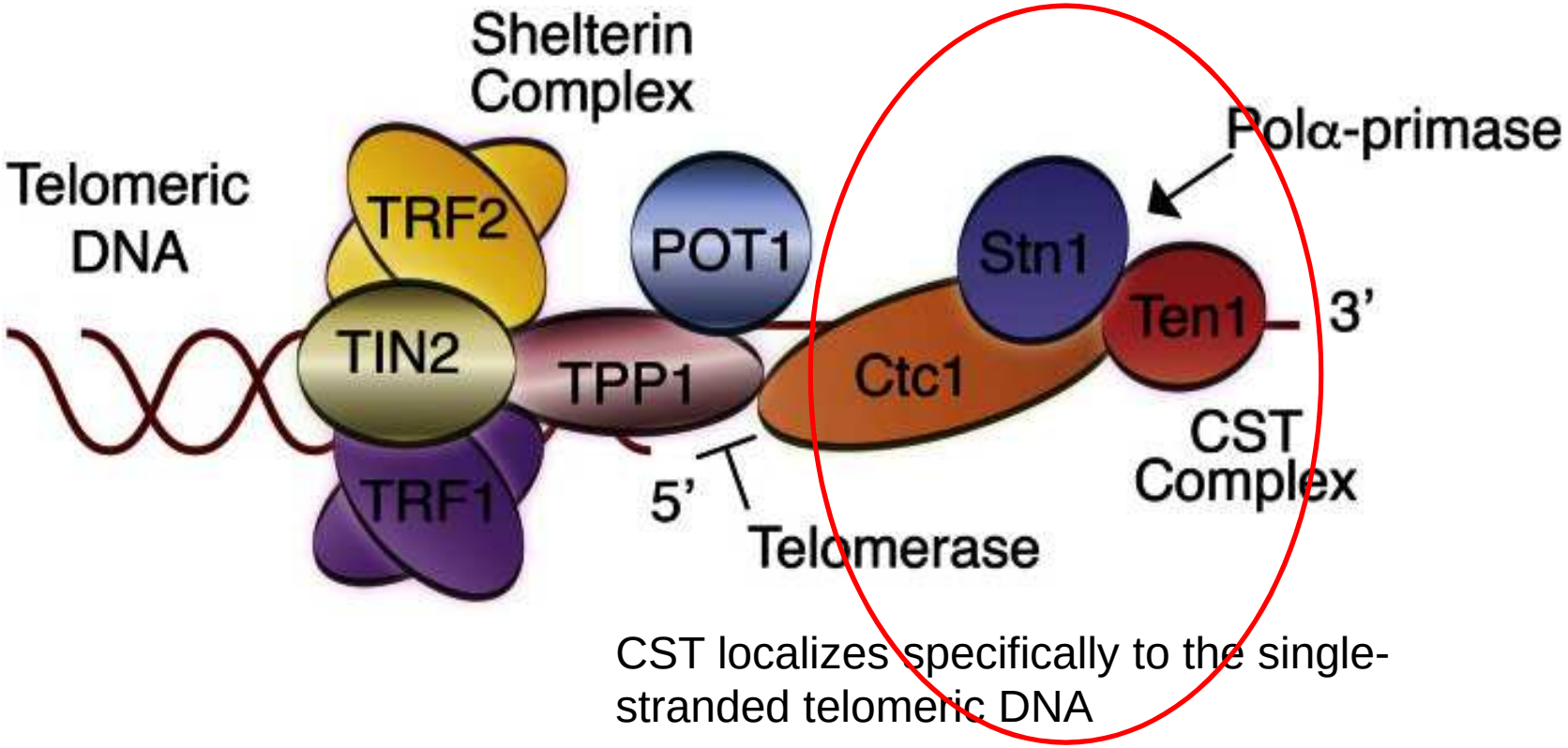


Direct telomerase activity assay of lysates from cells co-transfected with
TR plasmid
TERT
POT1



nature

J Nandakumar *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature11648

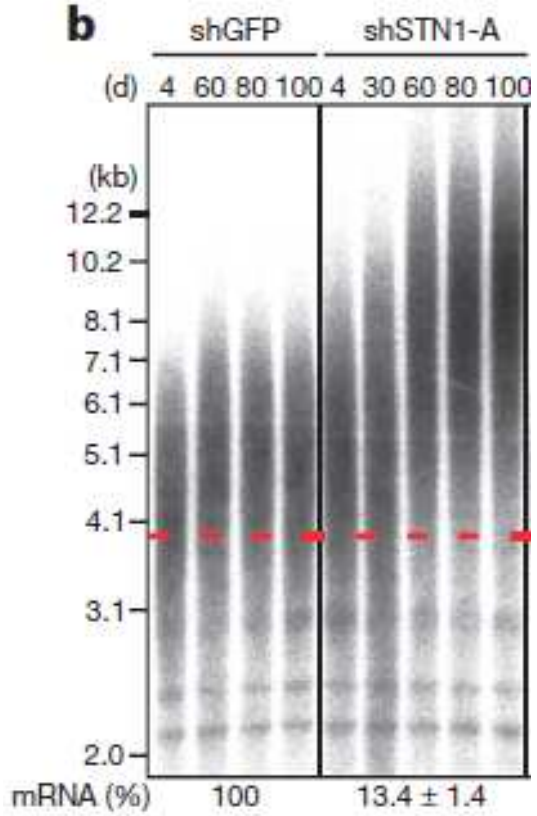


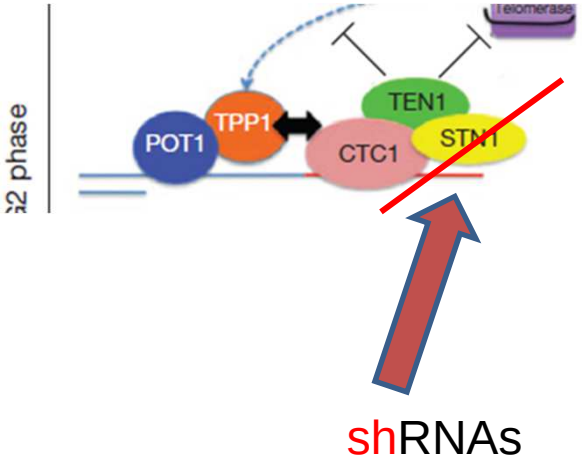
CST localizes specifically to the single-stranded telomeric DNA

chromosome end capping and telomere length regulation

The CST complex limits telomere elongation

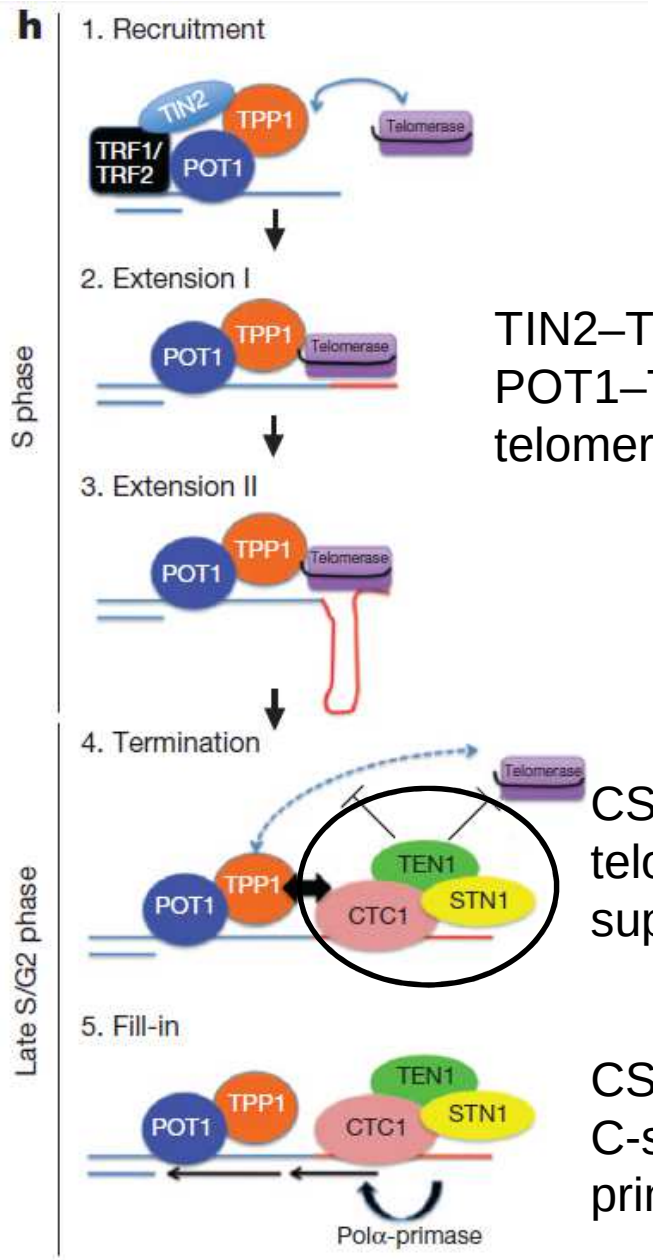
HT1080
human cancer cells





shRNAs

The CST complex is a terminator of telomerase activity



TIN2–TPP1 recruits telomerase and POT1–TPP1 promotes processive telomere elongation

CST binds to the telomerase-extended telomere end
suppressing telomerase access.

CST promotes fill-in synthesis of the C-strand stimulating DNA polymerase a-primase

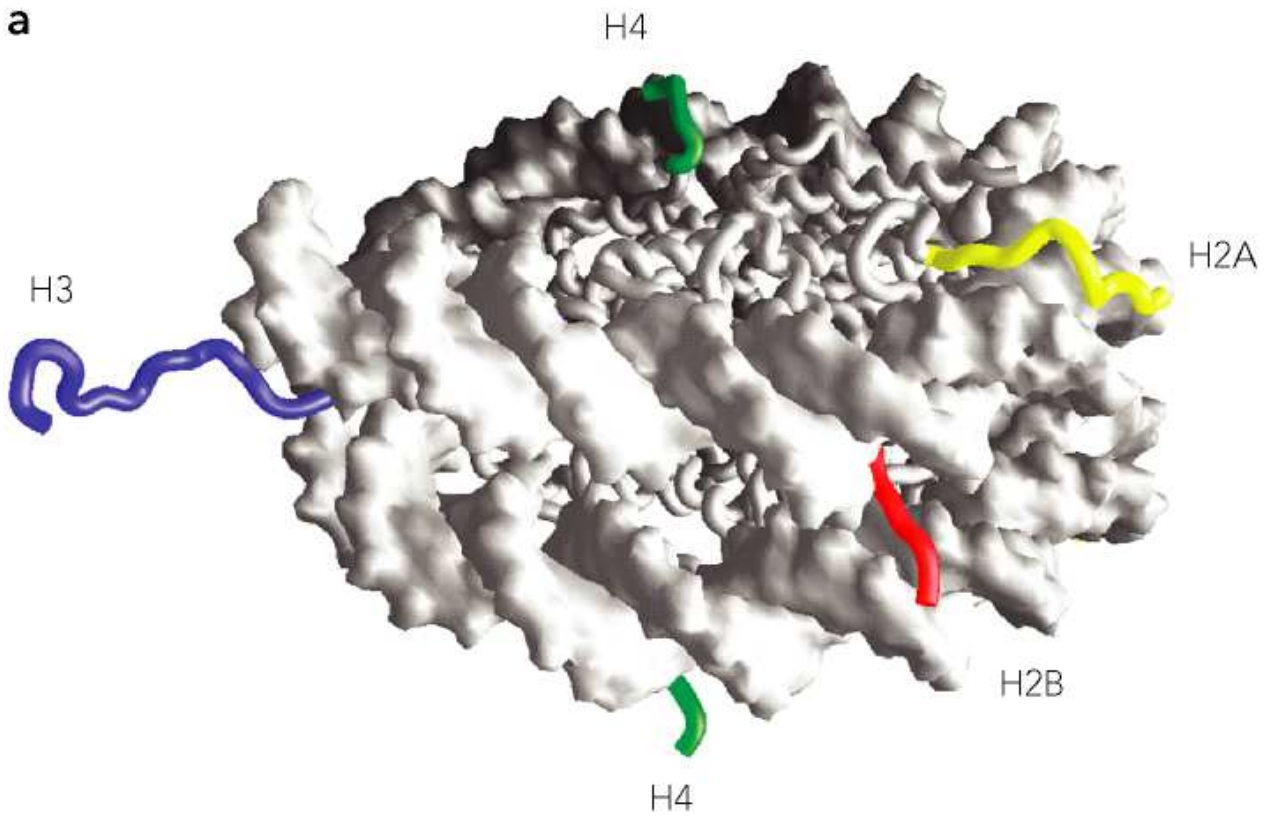
La cromatina telomerica e la sua modificazione

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

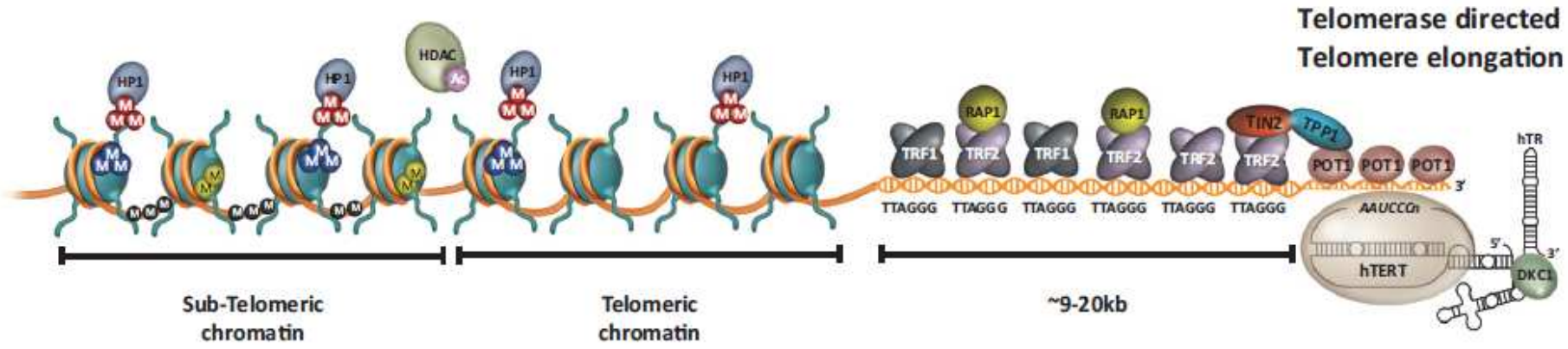
Major histone modifications *found in telomeres are*

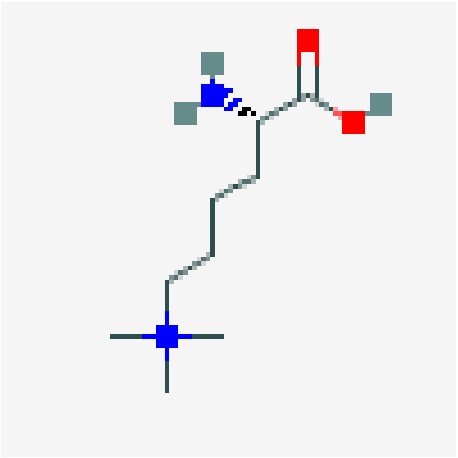
*-H3K9 and H4K20 trimethylatio*n

-low abundance of acetylated H3 and H4

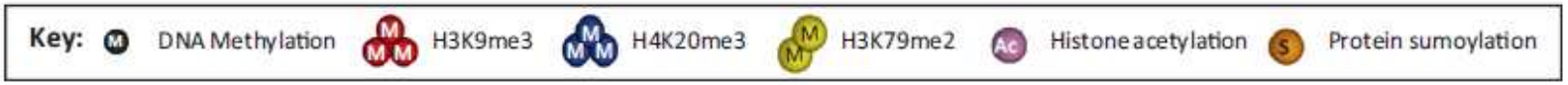


Telomeres in germ and stem cells





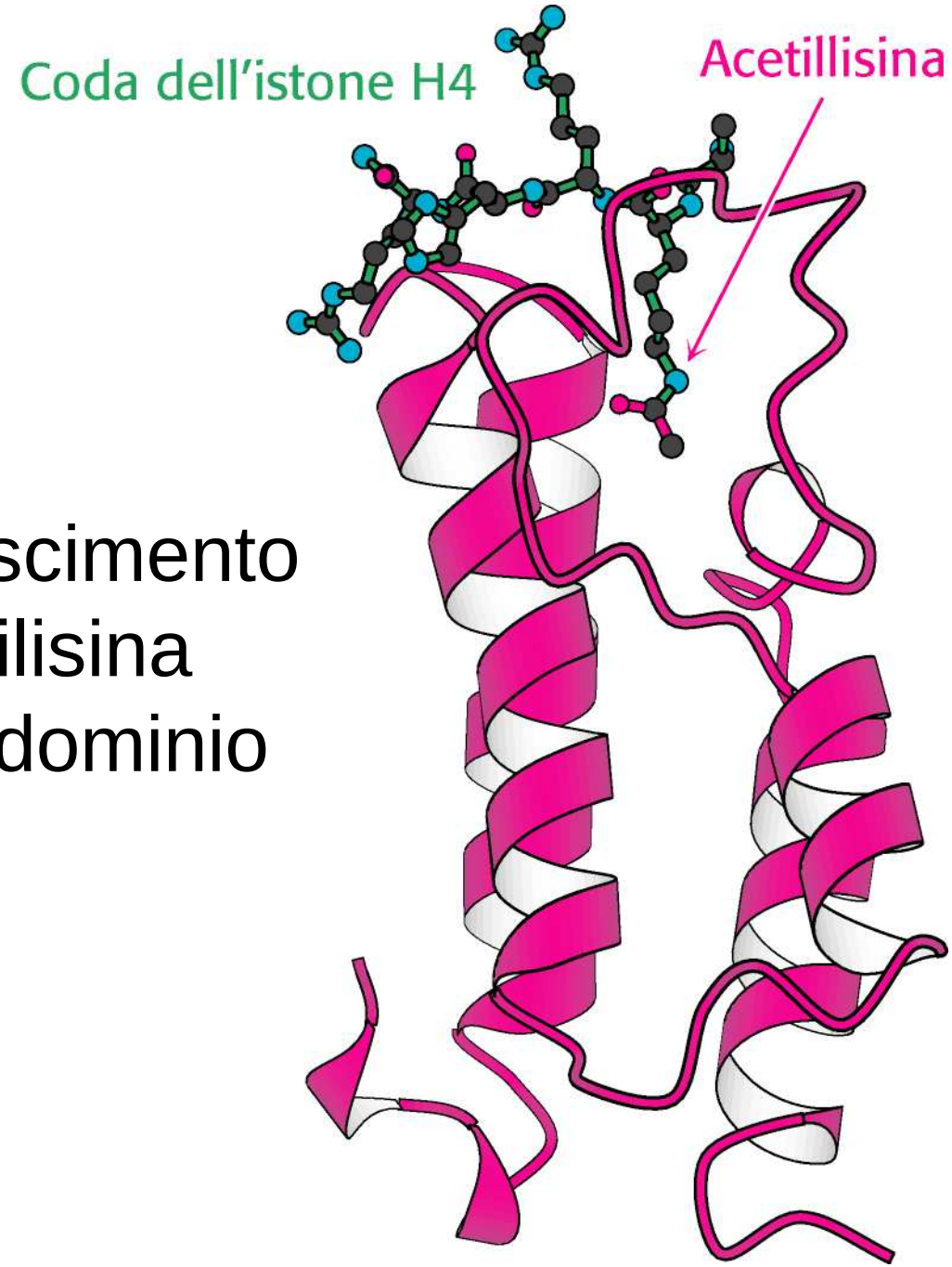
Kme3 =trimetillisina



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

Riconoscimento acetilisina bromodominio



Un enzima deacitilante specifico: SIRT6

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

The Sir2 deacetylase regulates chromatin silencing and lifespan in Saccharomyces cerevisiae.

In mice, 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 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

SIRT6 contributes to the propagation of a specialized chromatin state at mammalian telomeres,

Deacetilation is required for proper telomere metabolism and function.

chromatin regulation by SIRT6 is linked to telomere maintenance and a human premature ageing syndrome

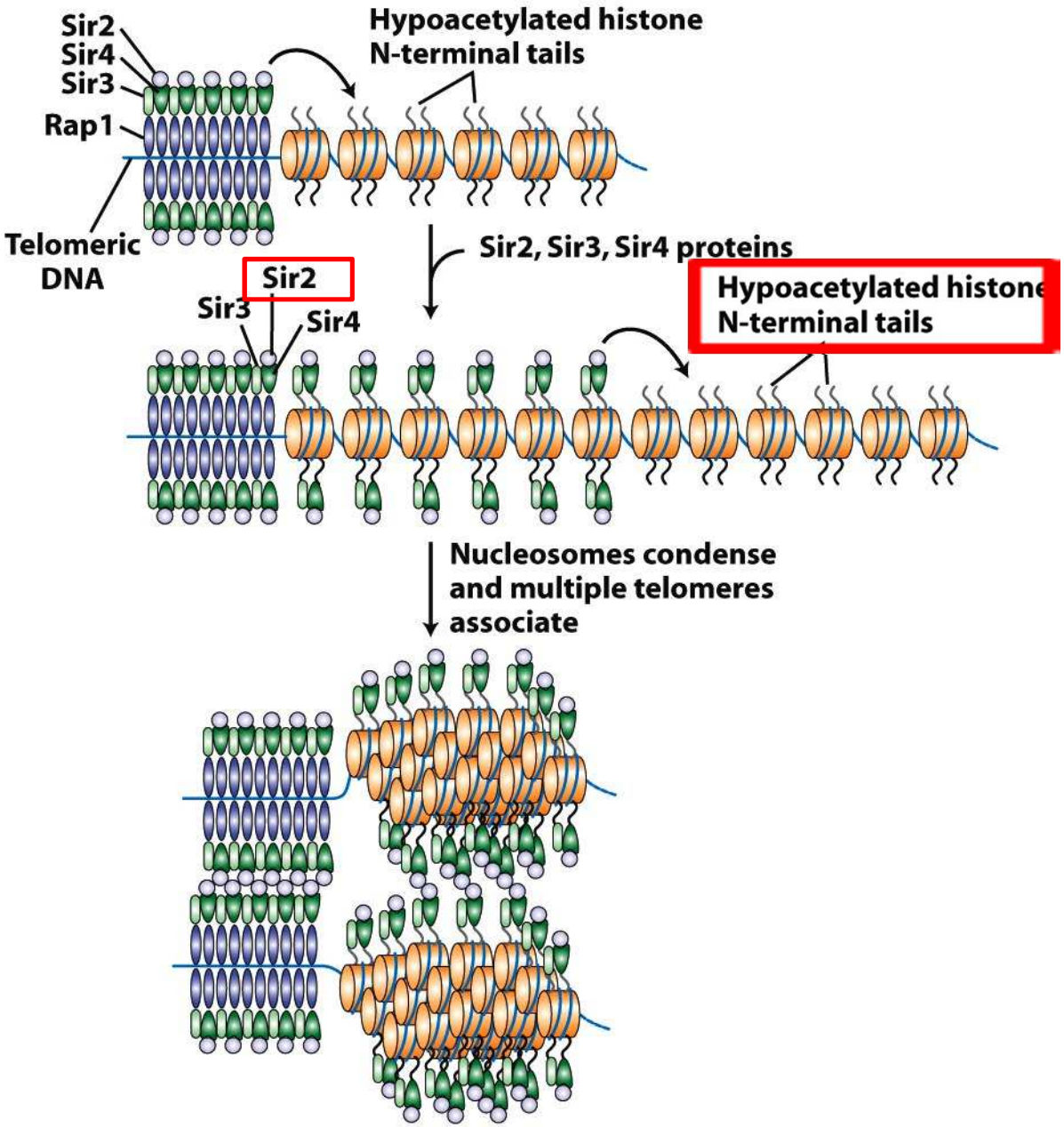
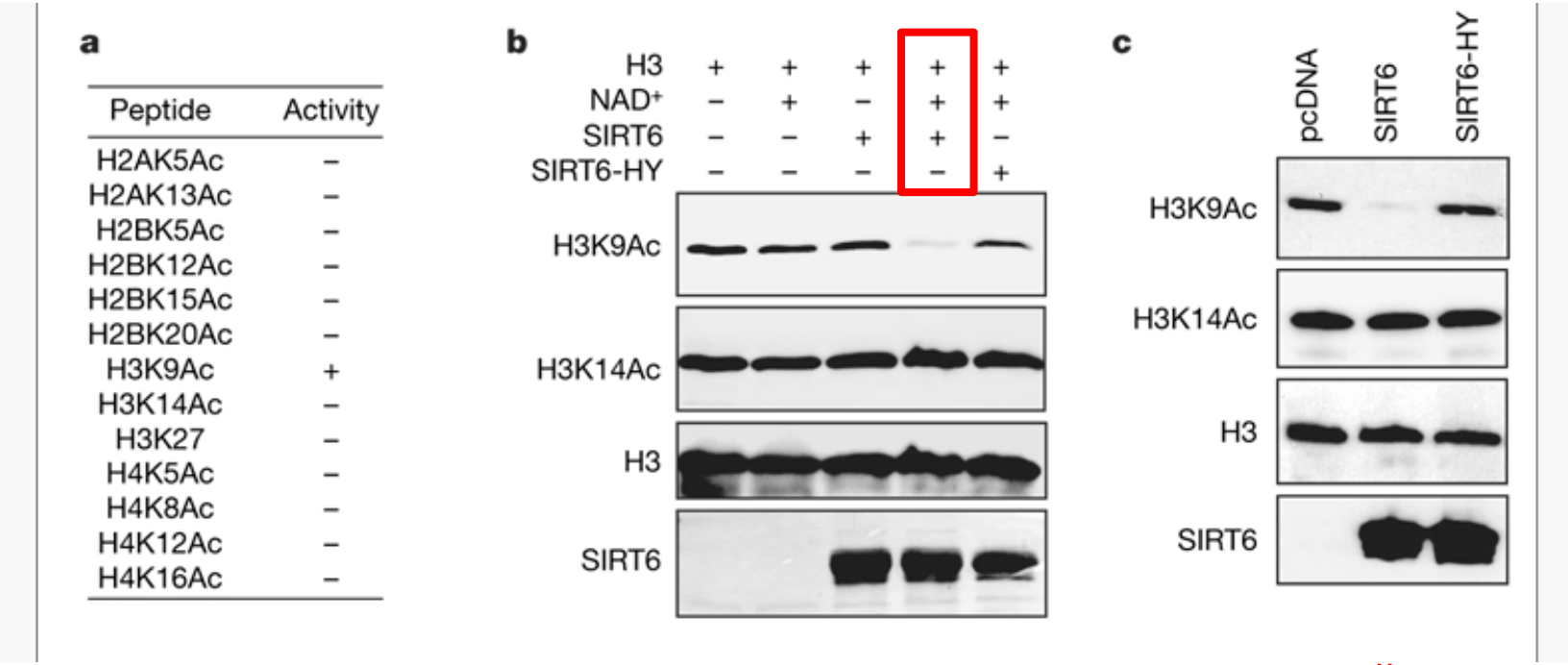


Figure 7-35
Molecular Cell Biology, Sixth Edition

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



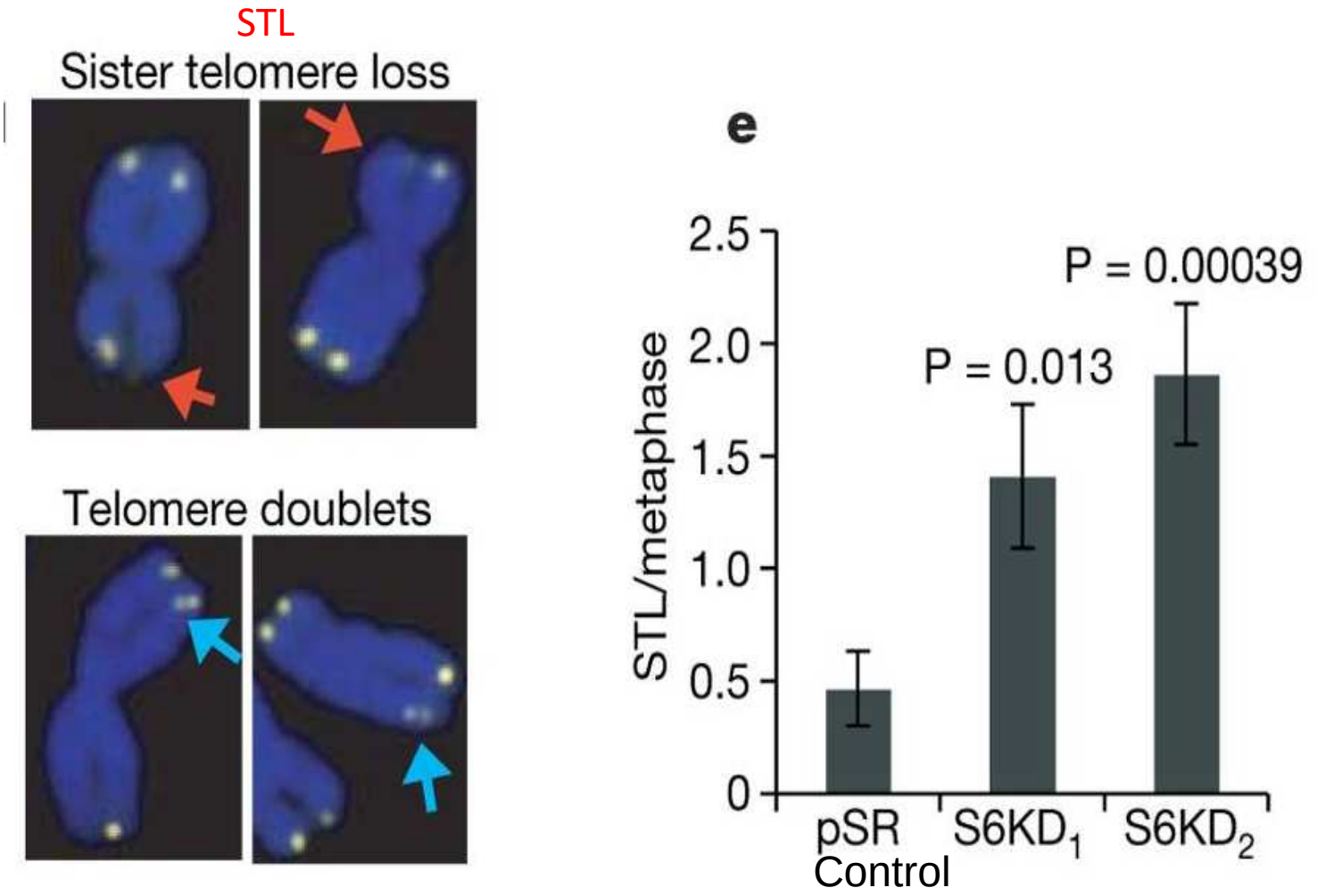
histone tail peptides

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

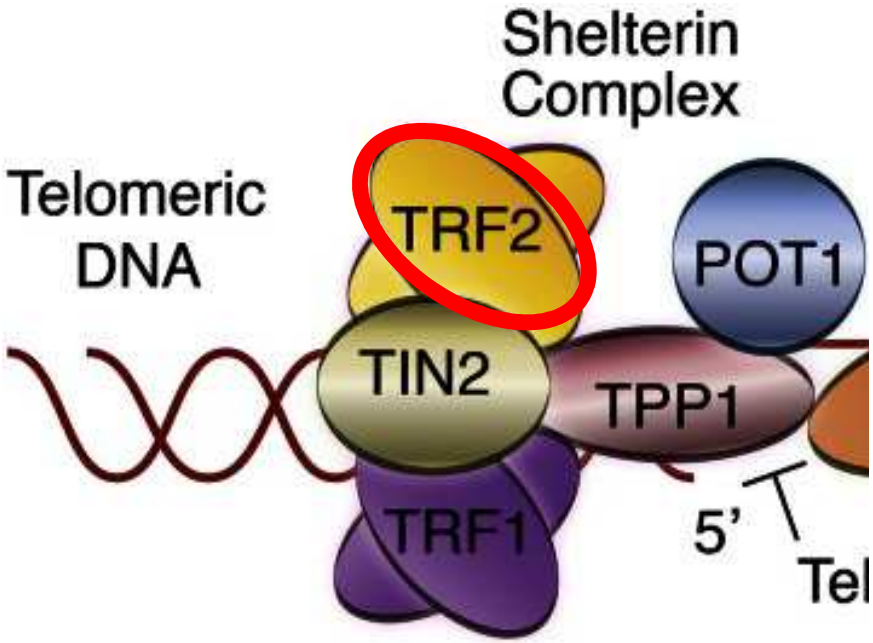
SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

SIRT6 associates specifically with telomeres

SIRT6 depletion leads to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence.

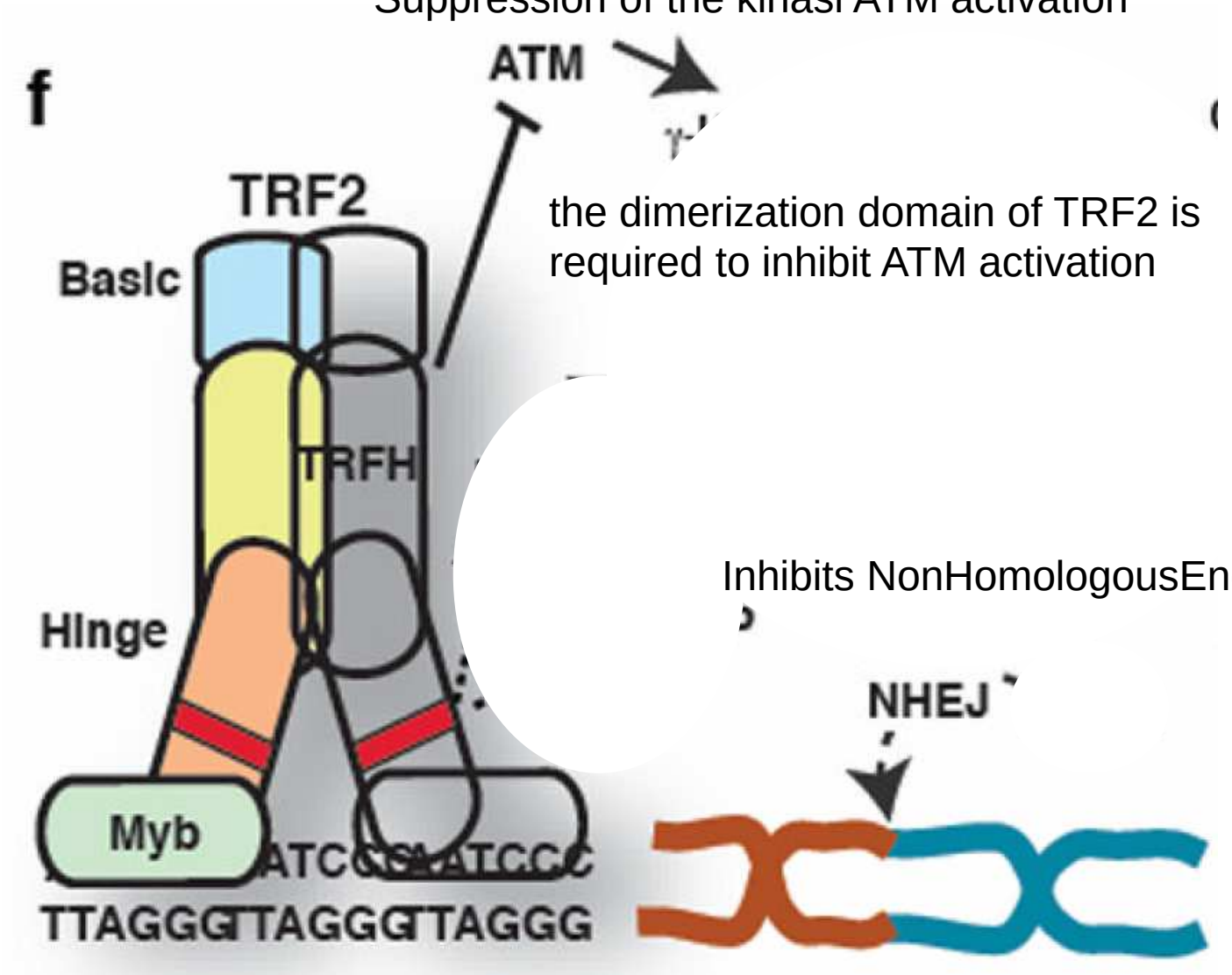
SIRT6-depleted cells exhibit abnormal telomere structures

PROTEZIONE DEL TELOMERO


Shelterin
Complex
Telomeric
DNA
TRF2
POT1
TIN2
TPP1
TRF1
5’
Tel

mechanism for TRF2-mediated chromosome end protection

Suppression of the kinasi ATM activation



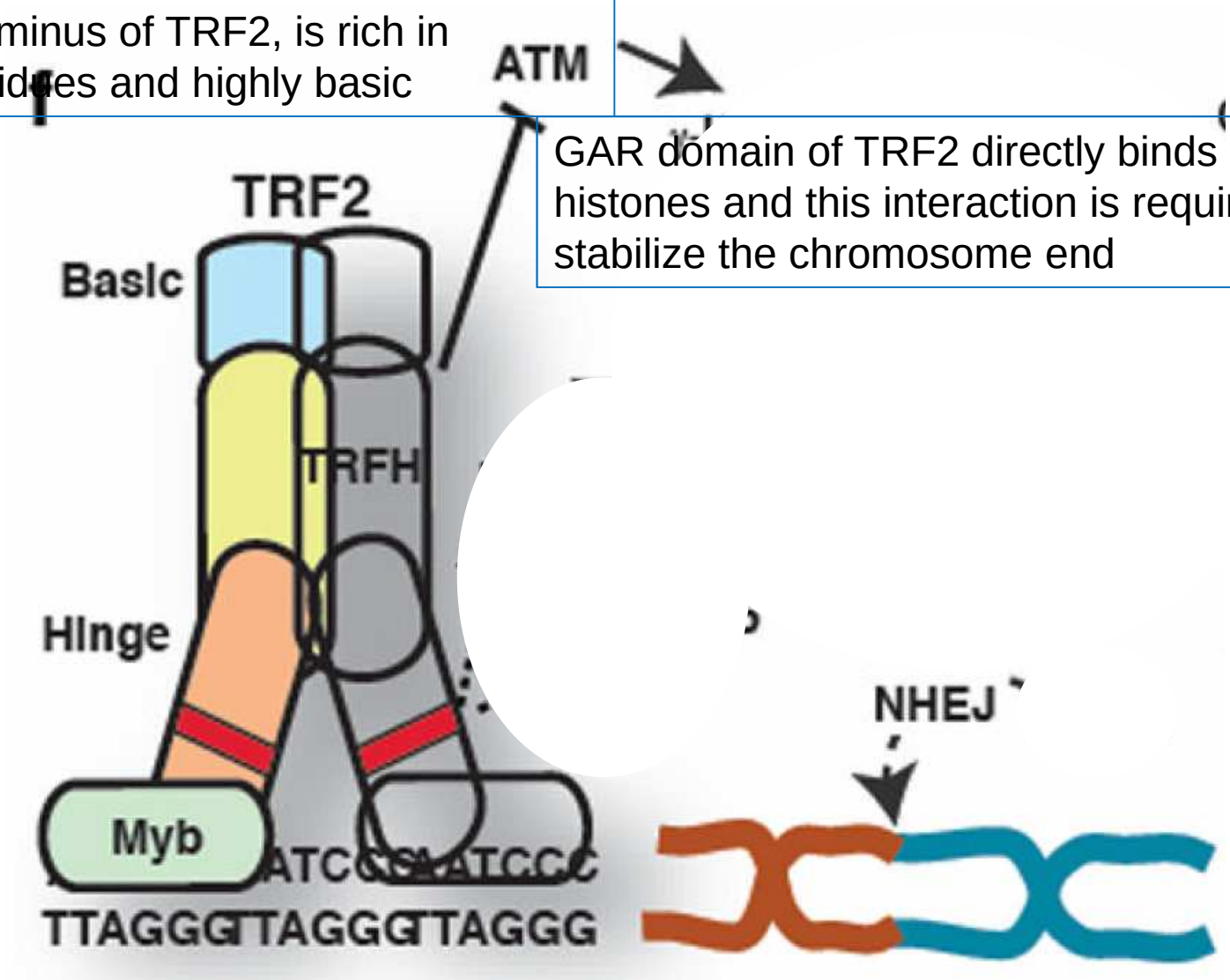
the dimerization domain of TRF2 is required to inhibit ATM activation

Inhibits NonHomologousEndJoining

mechanism for TRF2-mediated chromosome end protection

The GAR/Basic domain, which is located at the N terminus of TRF2, is rich in Gly/Arg residues and highly basic

GAR domain of TRF2 directly binds to core histones and this interaction is required to stabilize the chromosome end



Telomeric nucleosomes

Telomeric nucleosomes are hypersensitive to micrococcal nuclease.

Reconstituted nucleosomes on TTAGGG repeats show higher mobility than on other sequences.

Telomeric chromatin is enriched for heterochromatin modification, such as tri-methylation of H3K9 and H4K20, and loss of these marks affects telomere length regulation.

Direct binding of the GAR domain of TRF2 and core histones.

In vitro binding assay for the GAR domain of TRF2 and core histones.

Core histones:
mono-nucleosomes were purified using from HeLa cell nuclei digested with micrococcal nuclease-
mono-nucleosome peaks were collected and partially digested with trypsin to generate the **tailless** histones

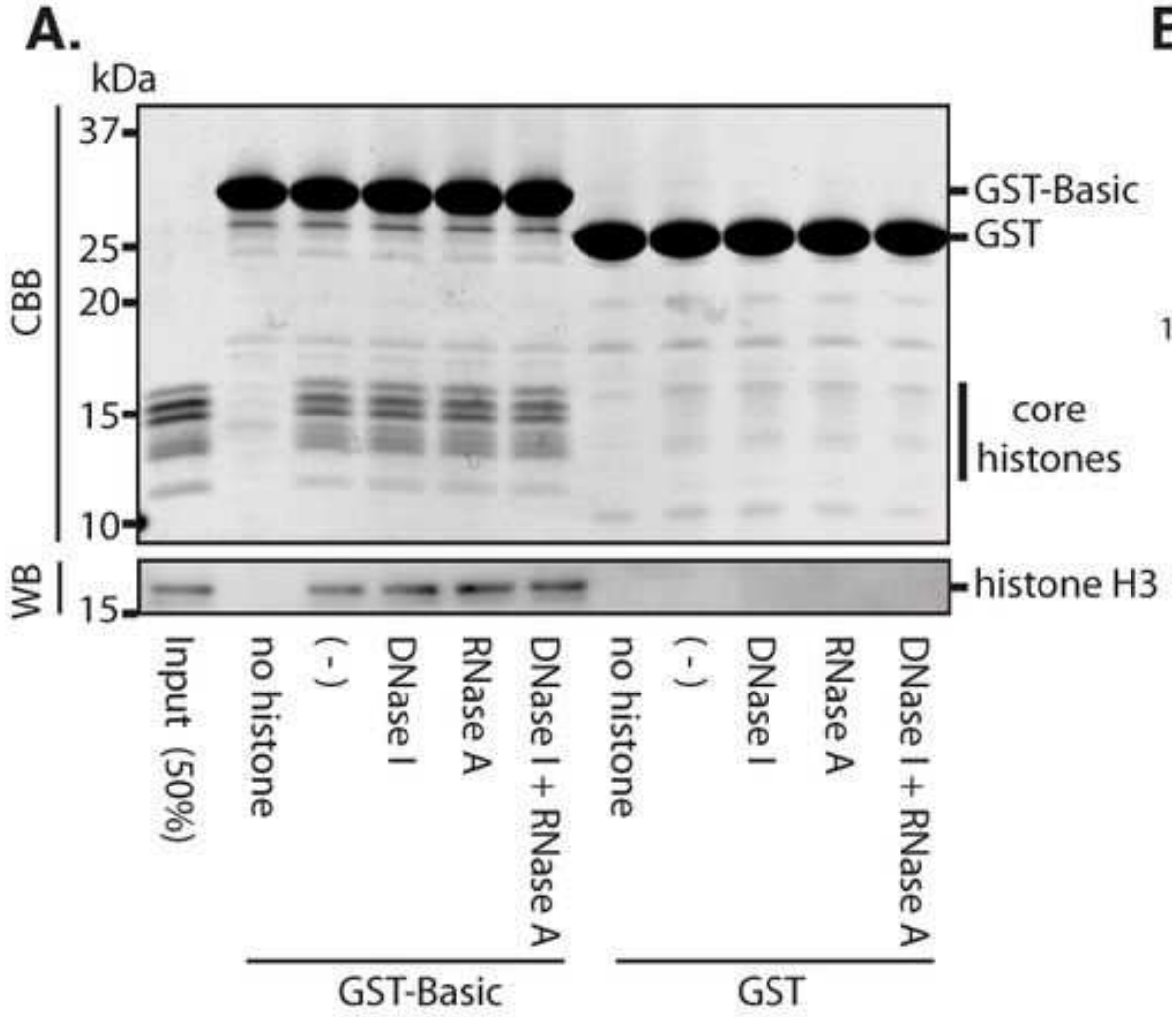
Recombinant GST-fused TRF2

GAR domain (GST-Basic) and GST protein (GST) were captured by glutathione-conjugated beads and incubated with core histones purified from HeLa cells.

Beads were washed extensively and then subjected to SDS-PAGE.

Direct binding of the GAR domain of TRF2 and core histones.

Coomassie Brilliant Blue



GAR domain fused with GST

Akimitsu Konishi et al. J. Biol. Chem. 2016;291:20798-20810

jbc

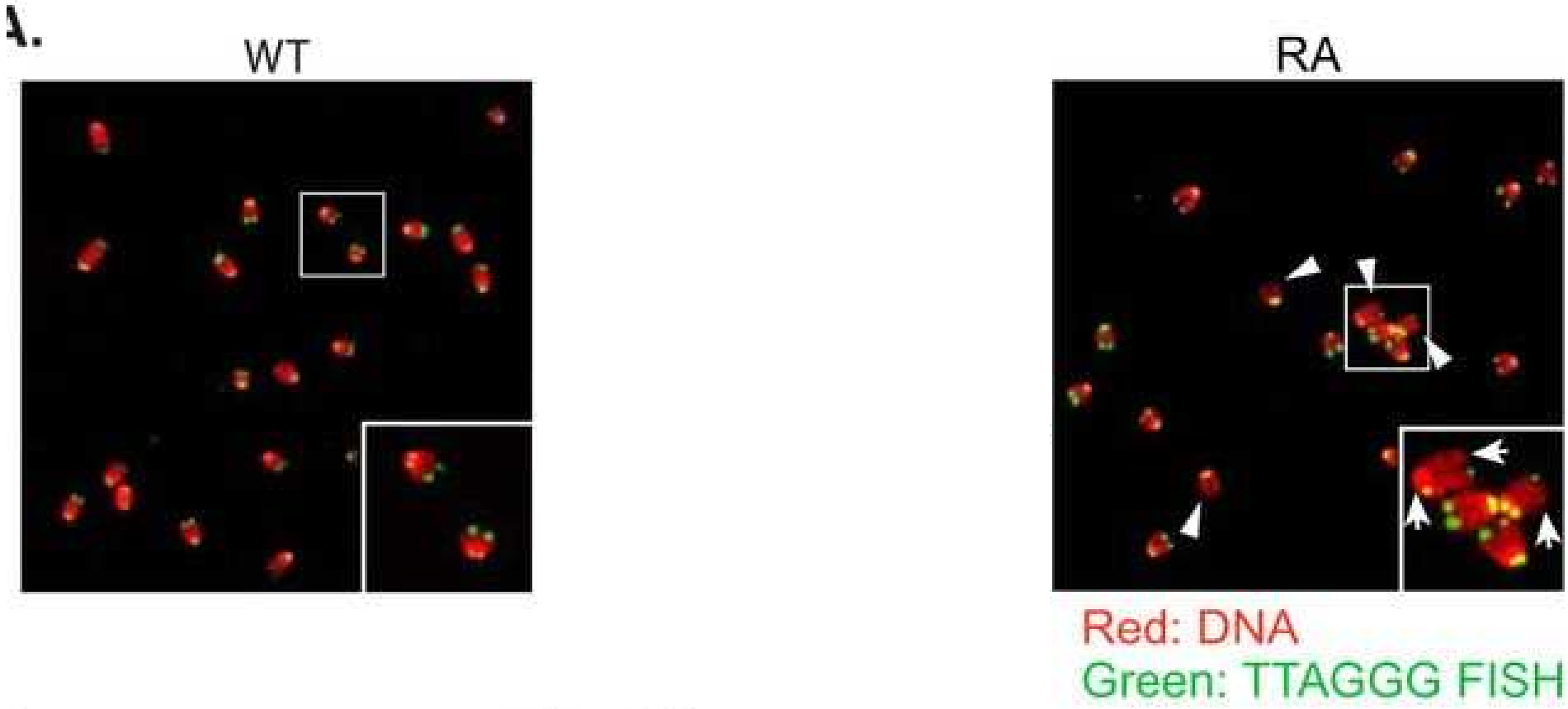
Direct binding of the GAR domain of TRF2 and core histones.

latency-associated nuclear antigen (LANA)
viral element essential for the DNA replication and genome maintenance during latency

Akimitsu Konishi et al. J. Biol. Chem. 2016;291:20798-20810

jbc

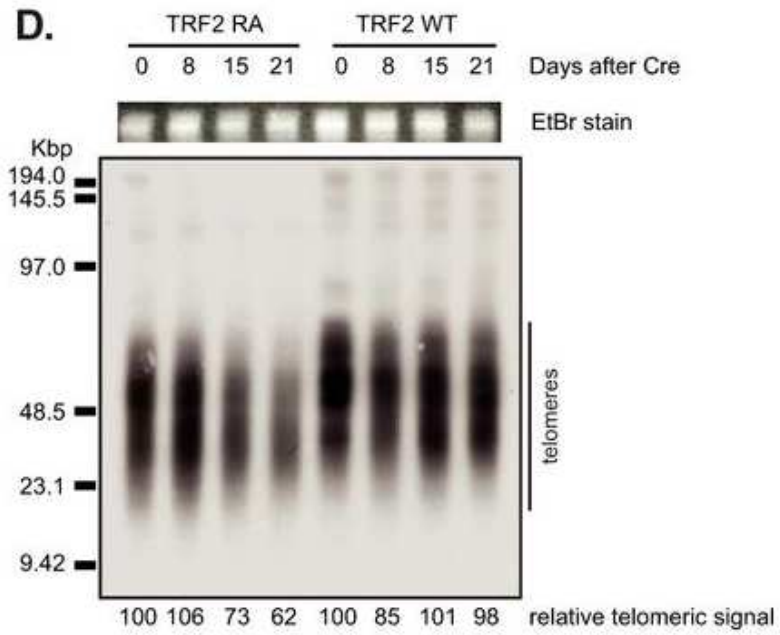
Rapid telomere DNA loss by loss of histone binding of TRF2.



Akimitsu Konishi et al. J. Biol. Chem. 2016;291:20798-20810

jbc

Rapid telomere DNA loss and t-circle generation by loss of histone binding of TRF2.



Akimitsu Konishi et al. J. Biol. Chem. 2016;291:20798-20810