TELOMERI E COMPLESSI DEL TELOMERO

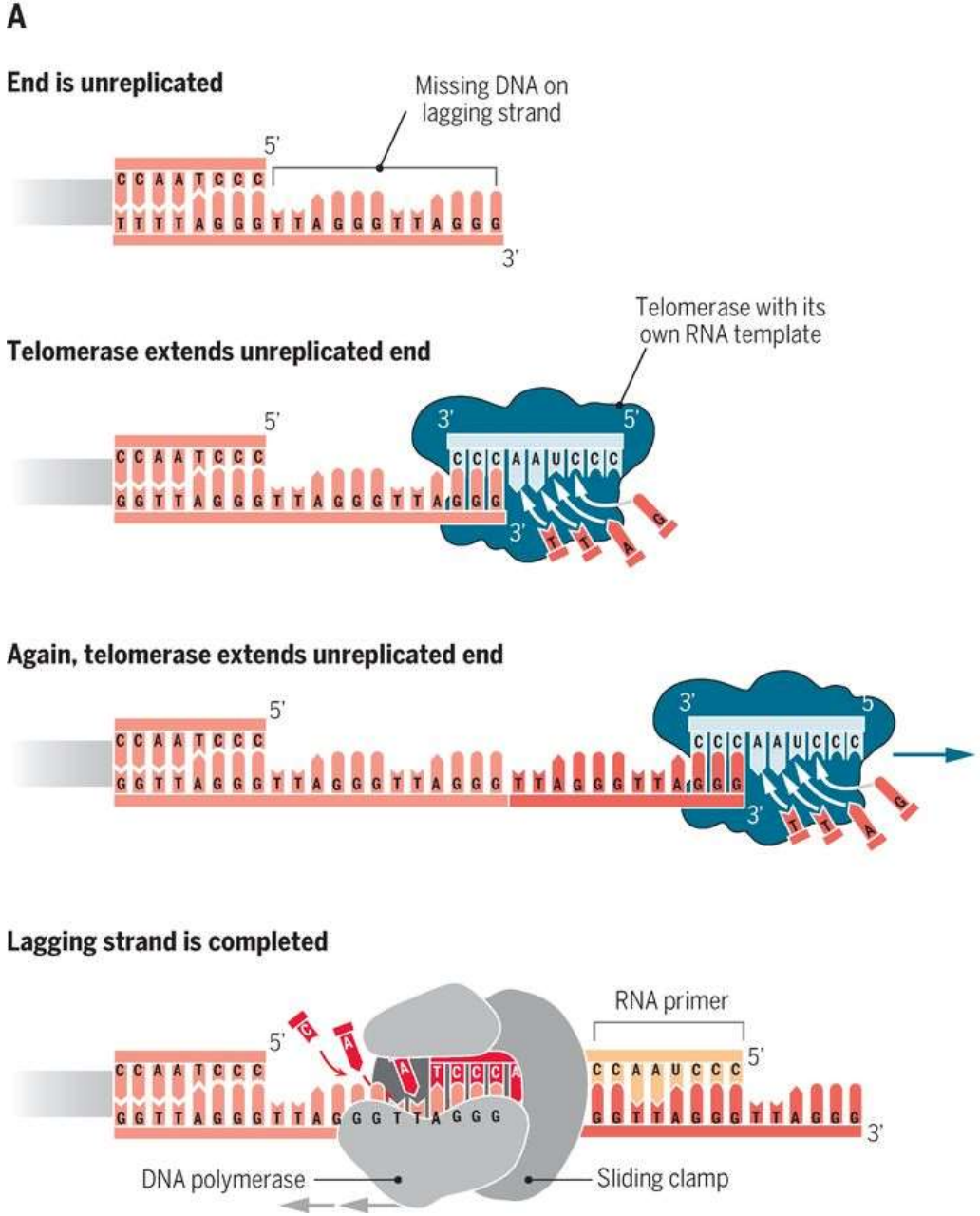
Telomeres are the terminal nucleoprotein structures located at the ends of eukaryotic chromosomes.

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

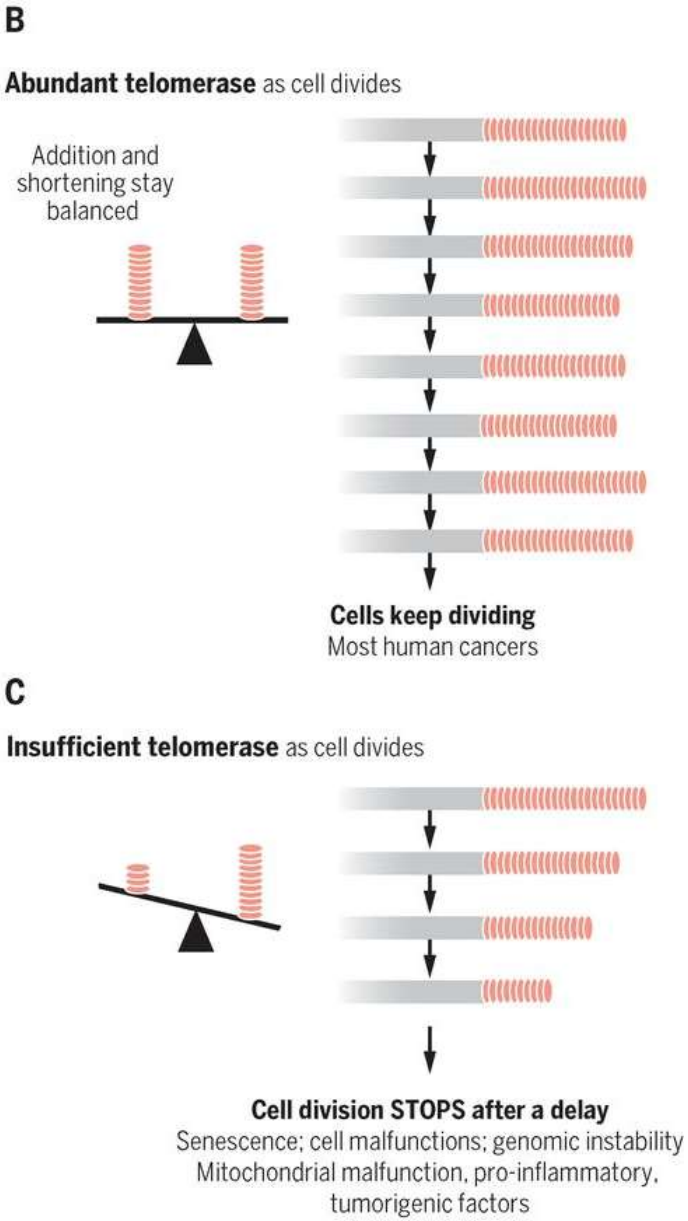
Long-term maintenance of telomeric DNA length requires telomerase.



Published by AAAS

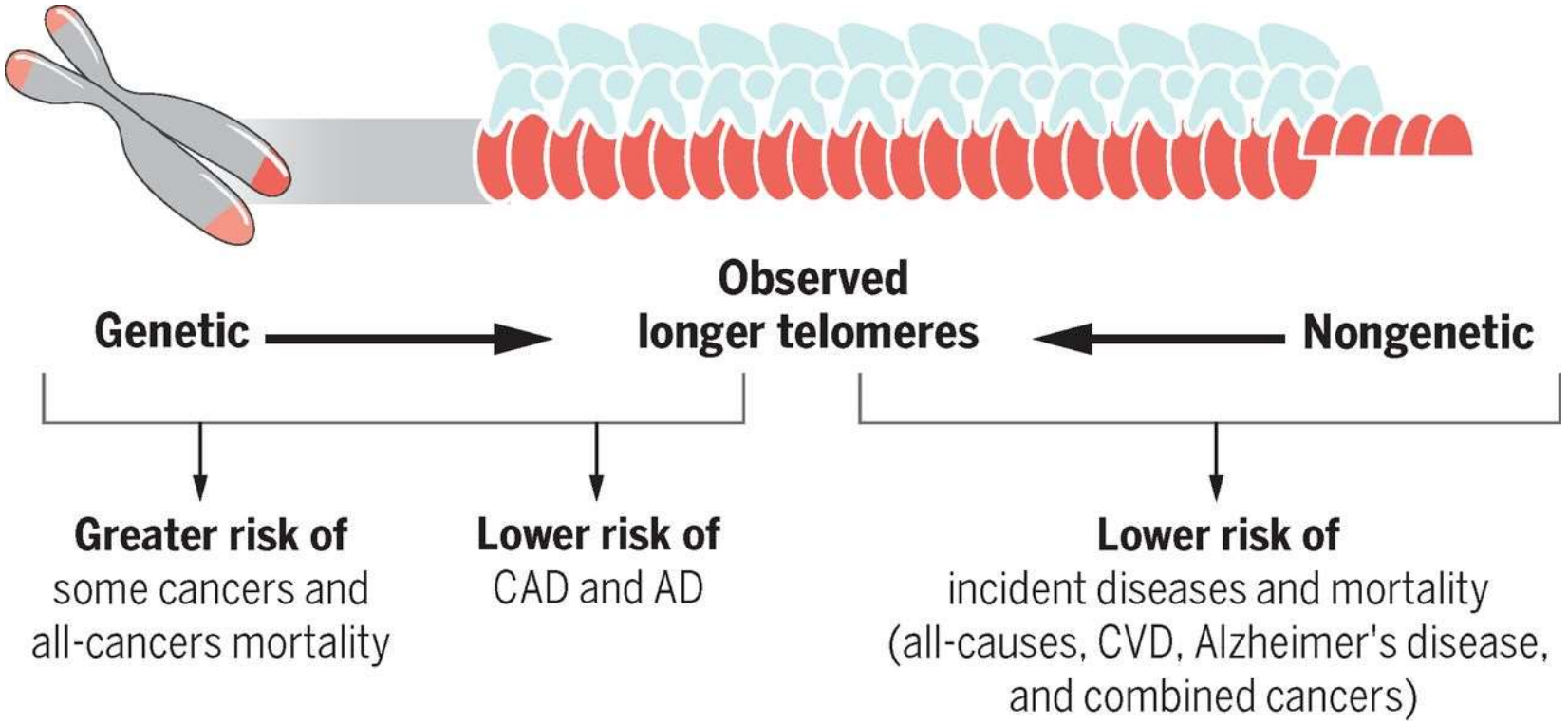
Elizabeth H. Blackburn et al. Science 2015;350:1193-1198

Long-term maintenance of telomeric DNA length requires telomerase.



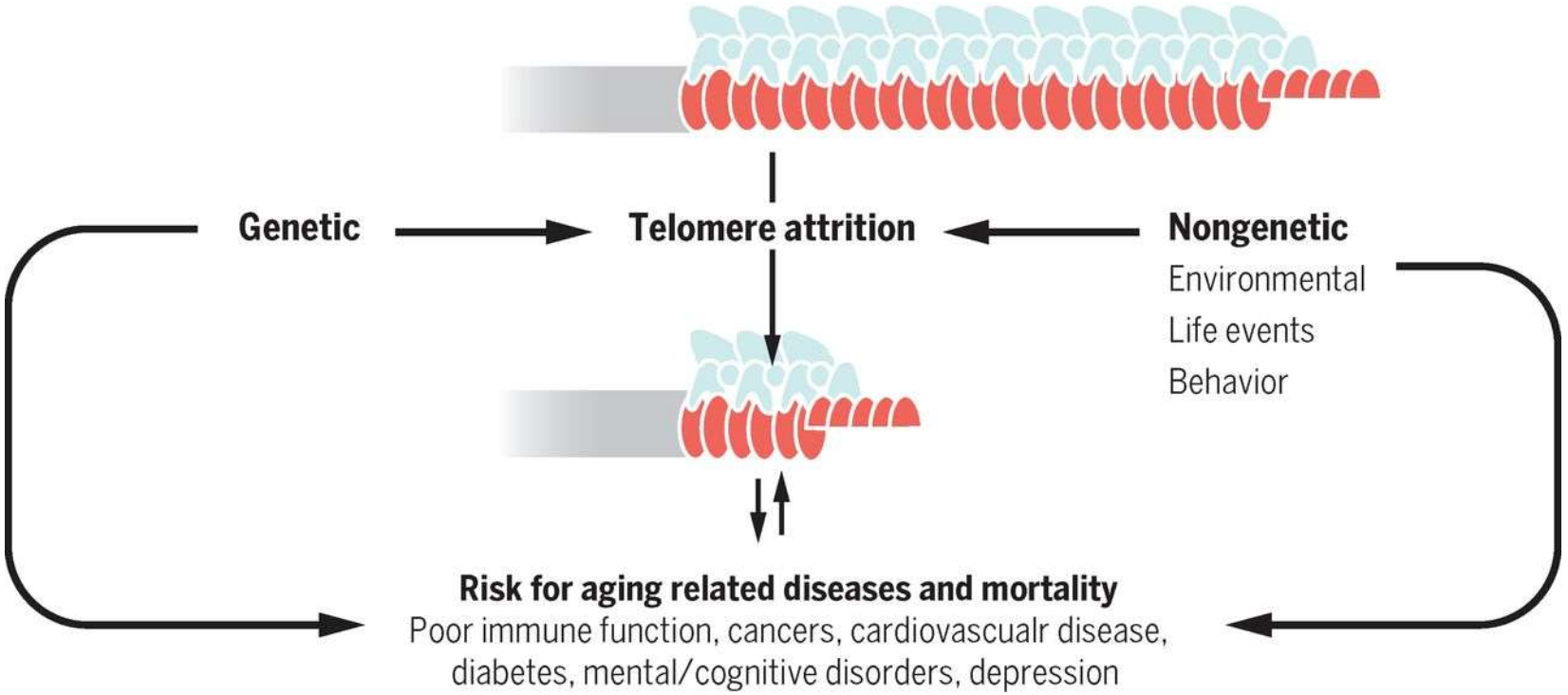
Published by AAAS **Elizabeth H. Blackburn et al. Science 2015;350:1193-1198**

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

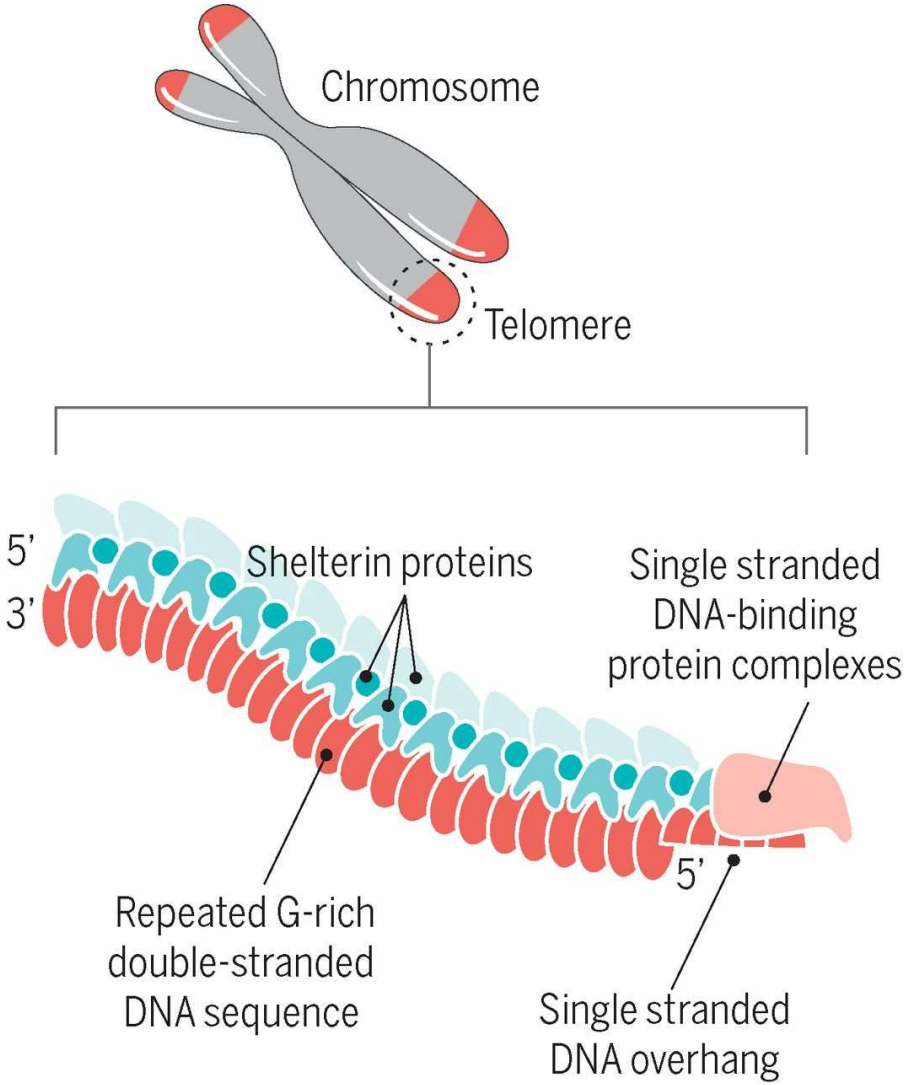
IL DNA Telomerico e le sue strutture alternative

SEQUENZA TELOMERICA

5–15 kb in humans, ~48 kb in mice

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

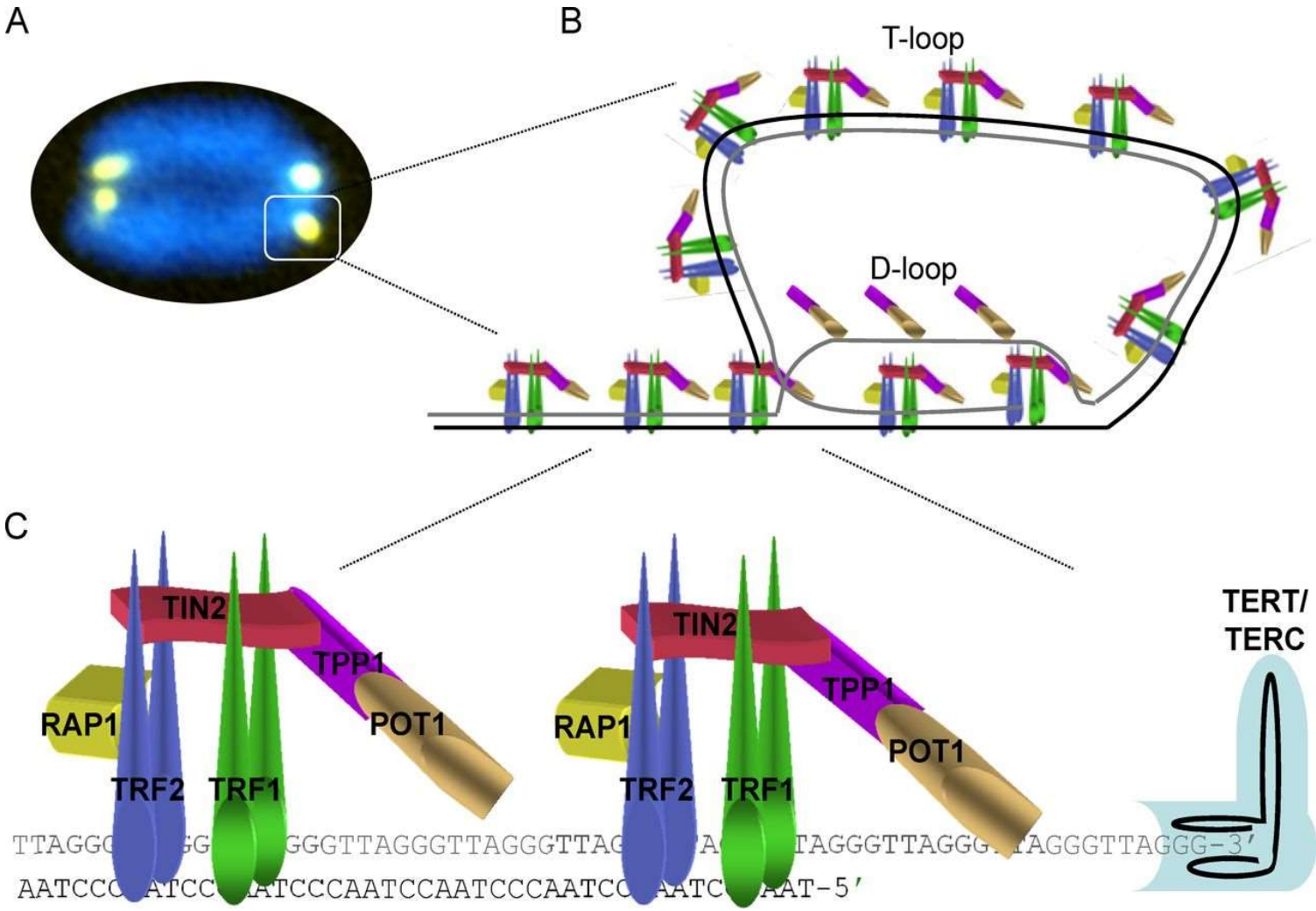
Telomere general structure.



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.

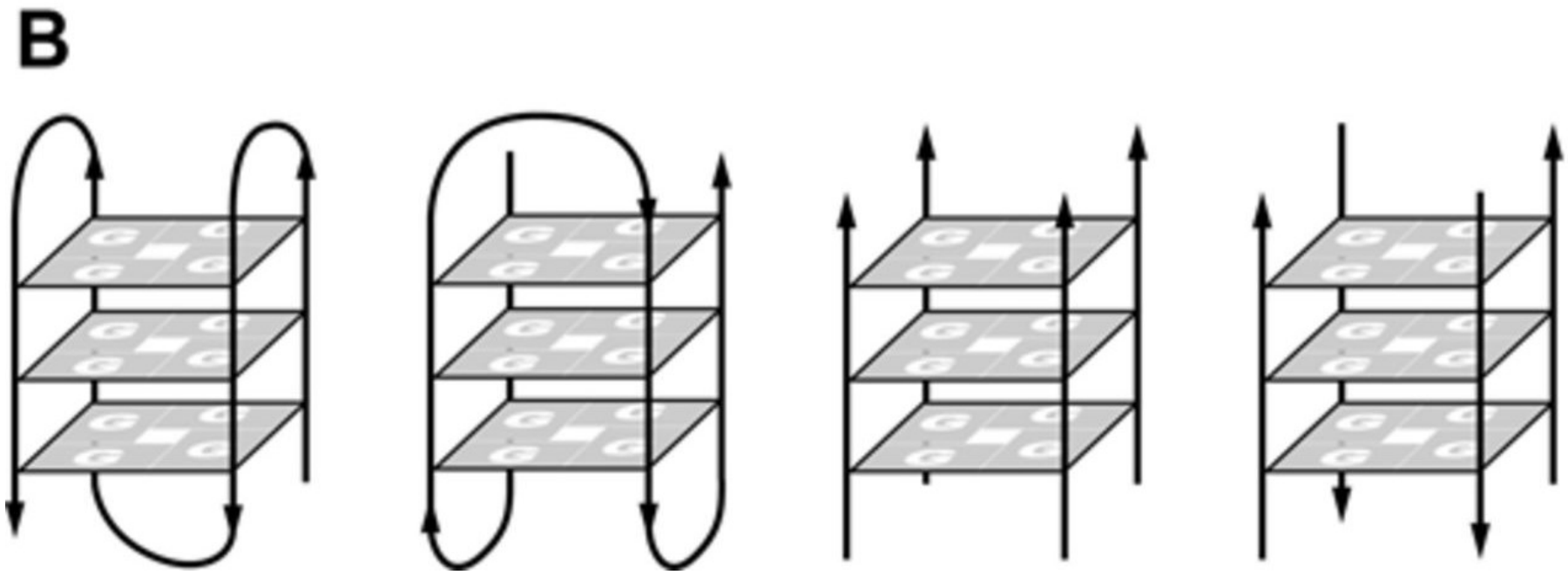
The shelterin complex and the structure of telomeres.



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

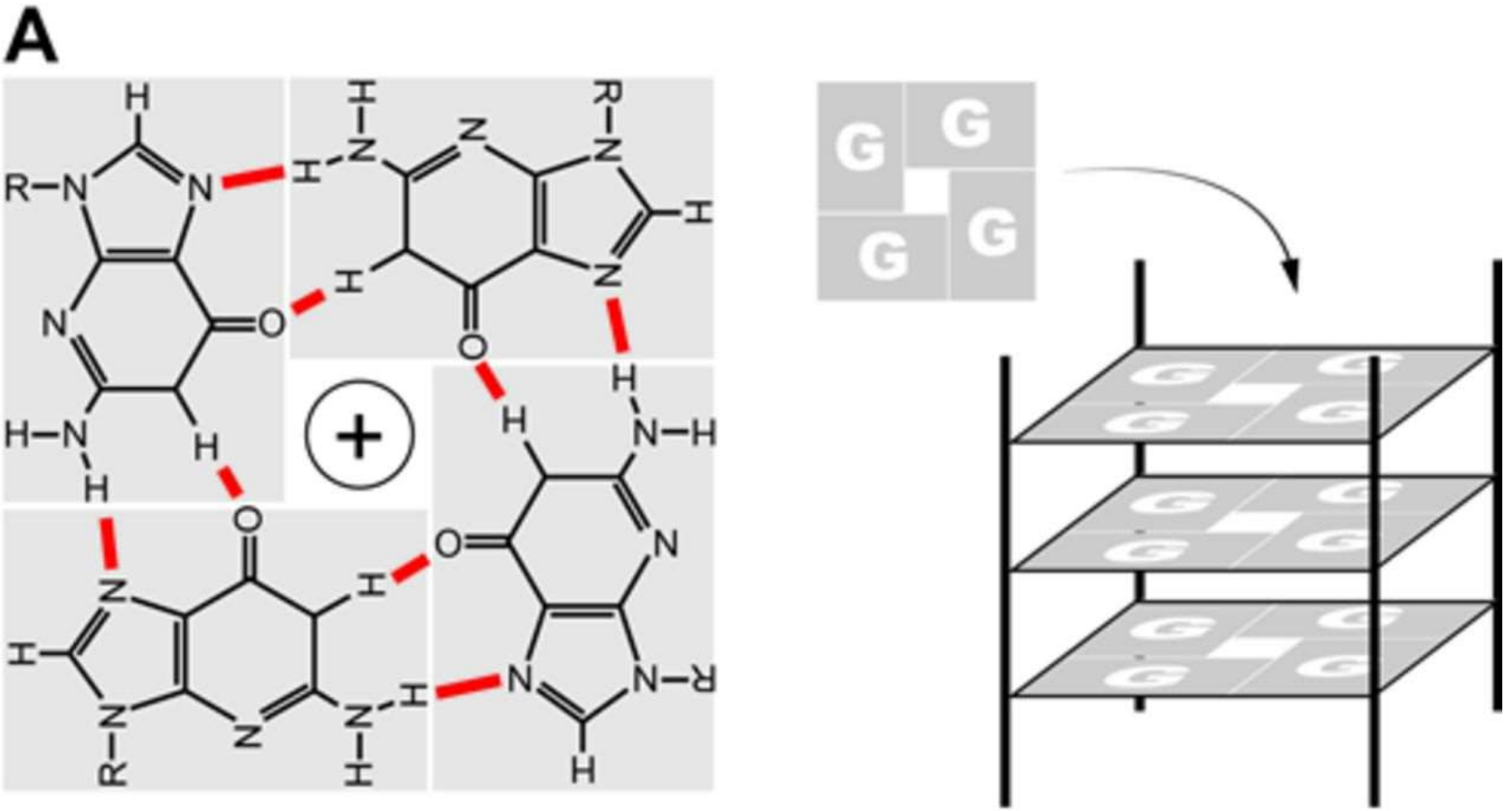
JCB

Structure of G-quadruplexes.



Daniela Rhodes, and Hans J. Lipps Nucl. Acids Res. 2015;nar.gkv862

Structure of G-quadruplexes.



Daniela Rhodes, and Hans J. Lipps Nucl. Acids Res. 2015;nar.gkv862

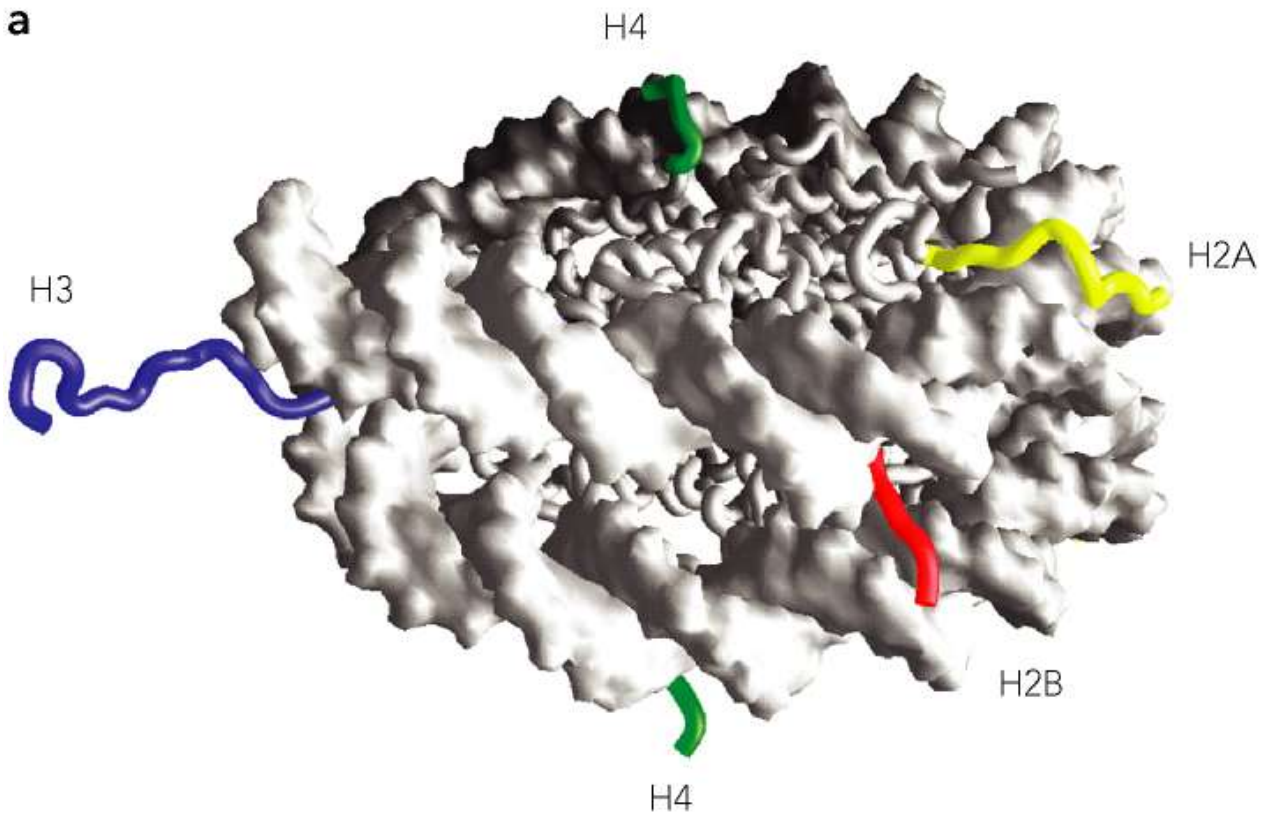
La cromatina telomerica e la sua modificazione

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

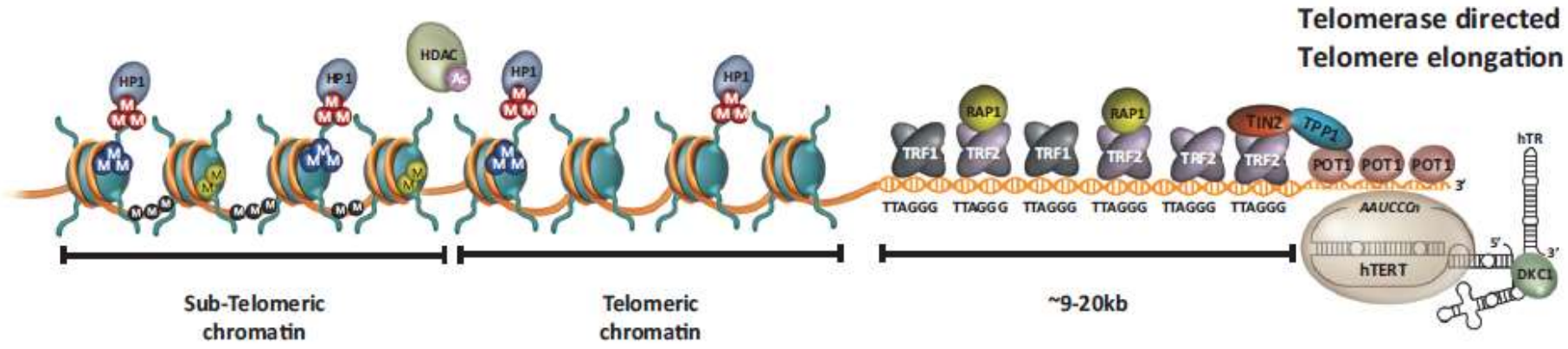
Major histone modifications *found in telomeres are*

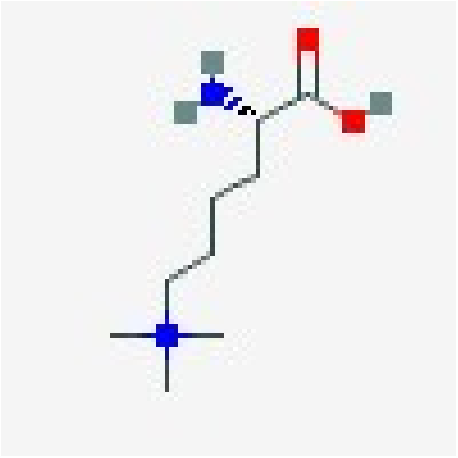
*-H3K9 and H4K20 trimethylatio*n

-low abundance of acetylated H3 and H4

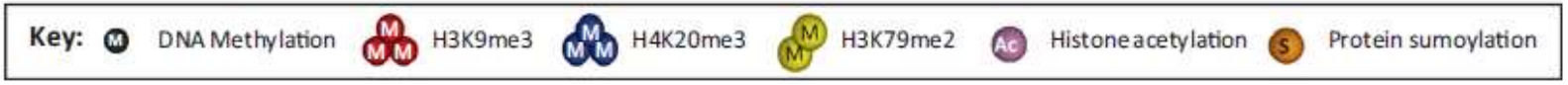


Telomeres in germ and stem cells

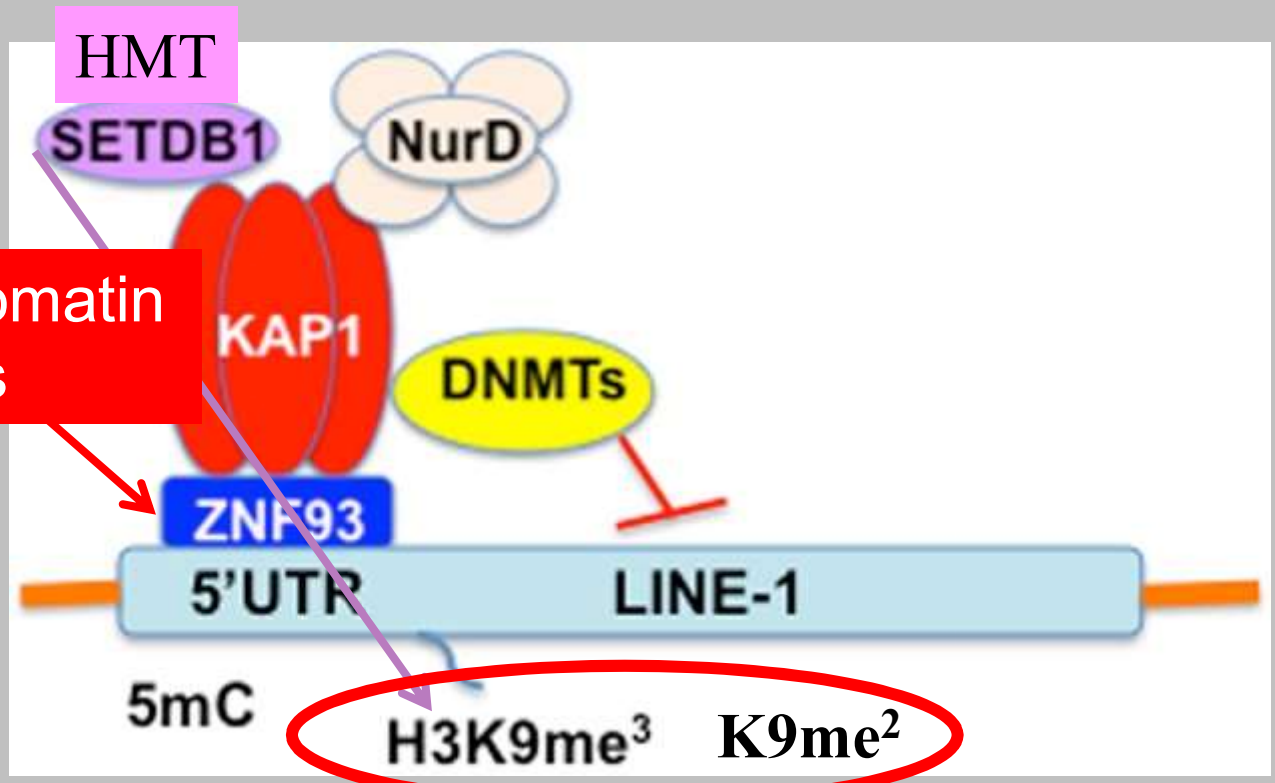




Kme3 =trimetillisina



Negative Control of the transcriptional activity of L1 in ES cells



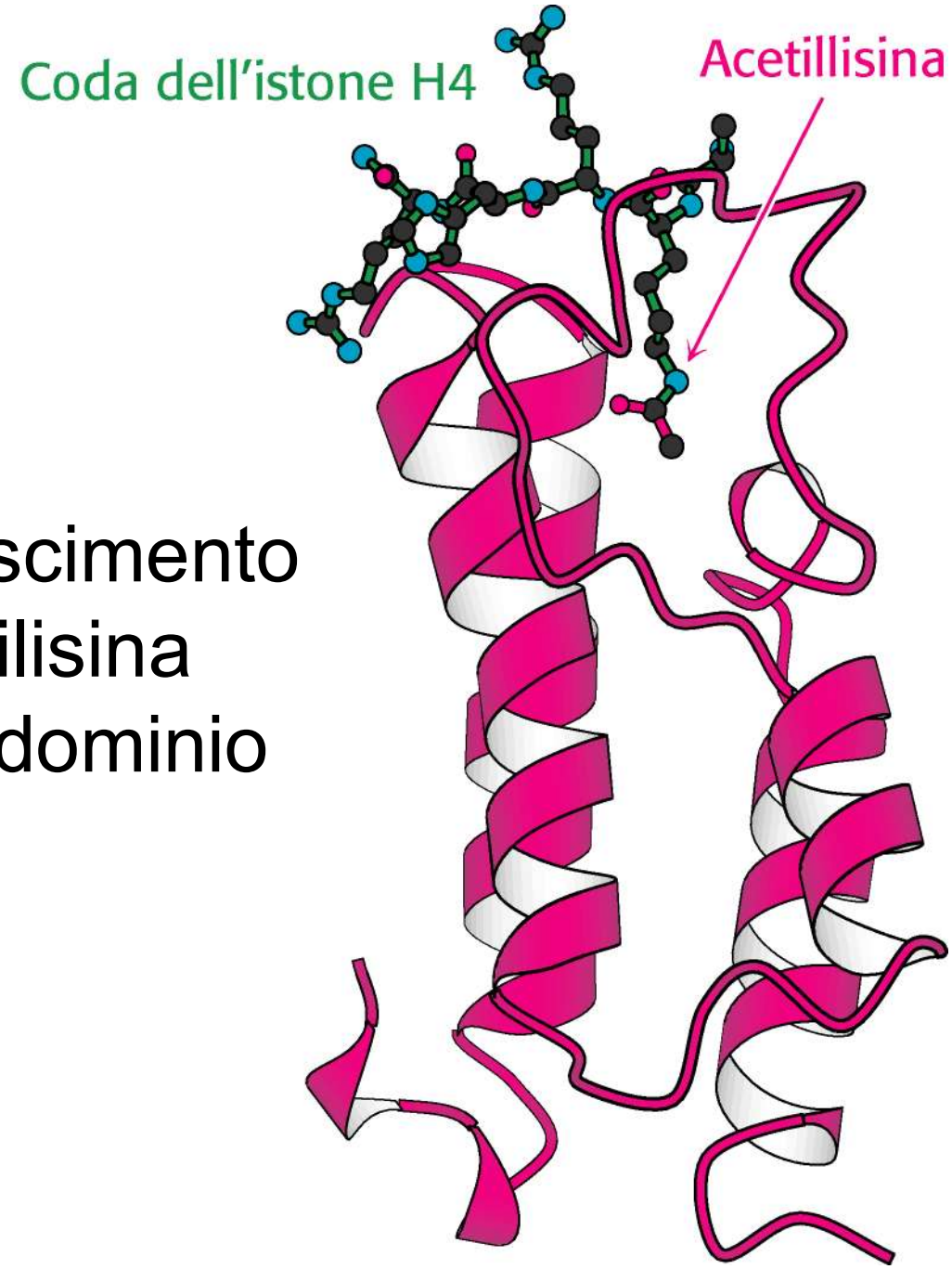
heterochromatin complexes

Riconoscimento

HMT istone metiltransferasi

Un enzima deacitilante specifico: SIRT6

Riconoscimento acetilisina bromodominio



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

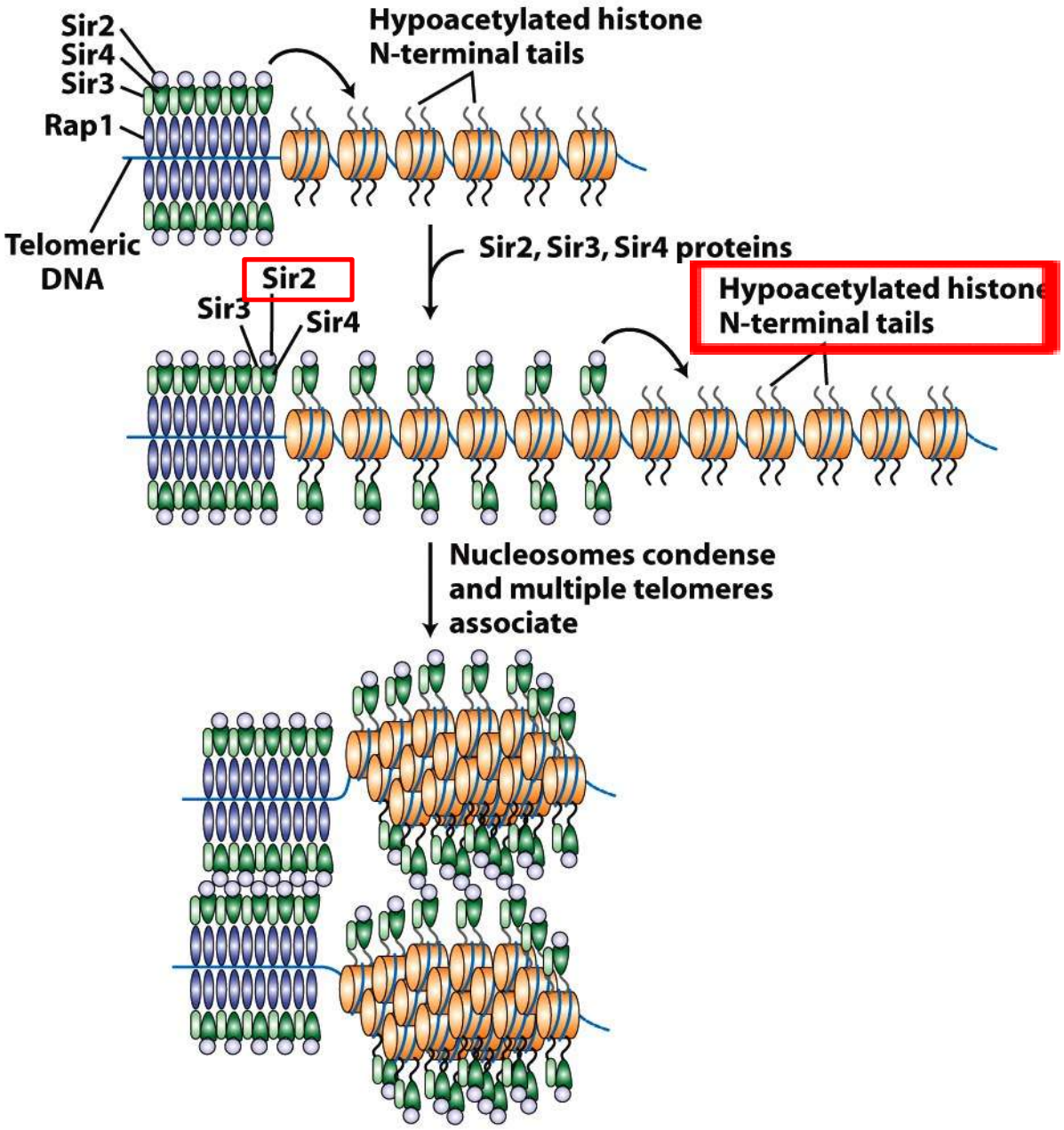
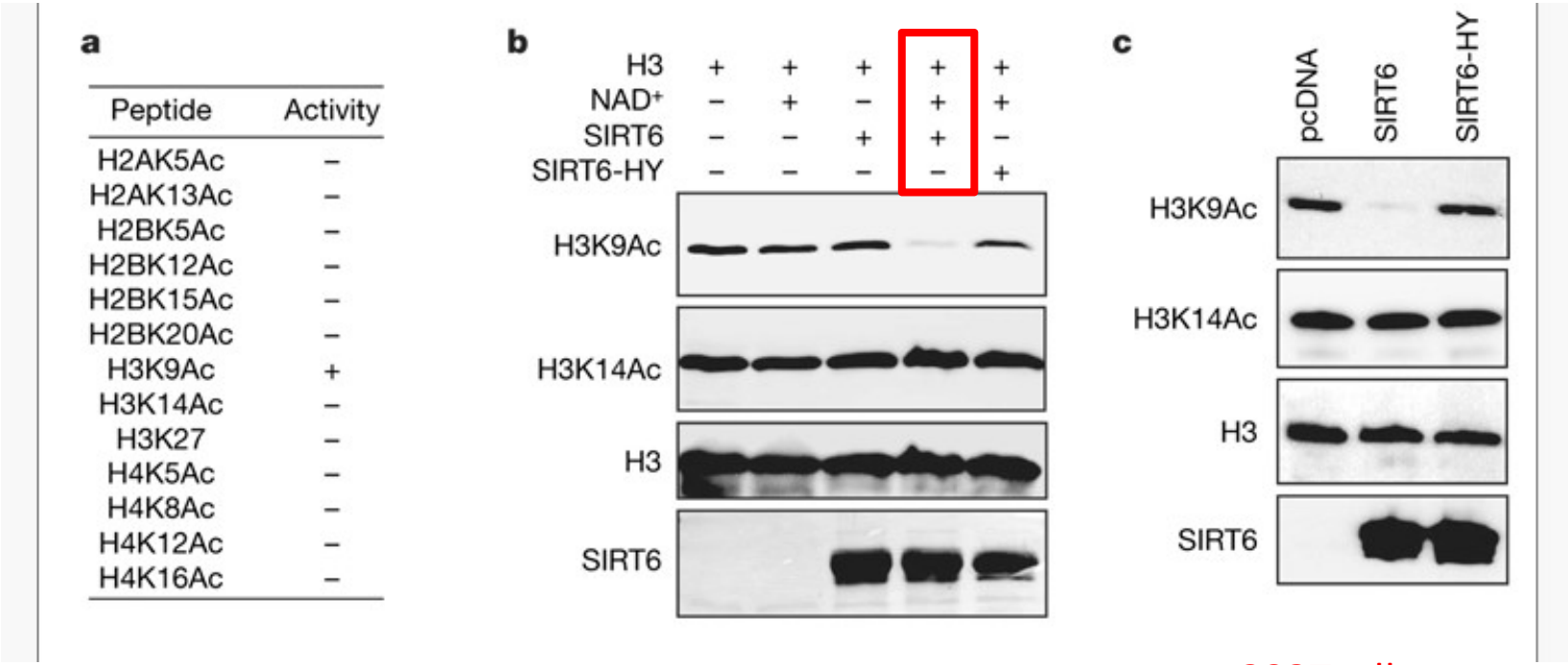


Figure 7-35
Molecular Cell Biology, Sixth Edition

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



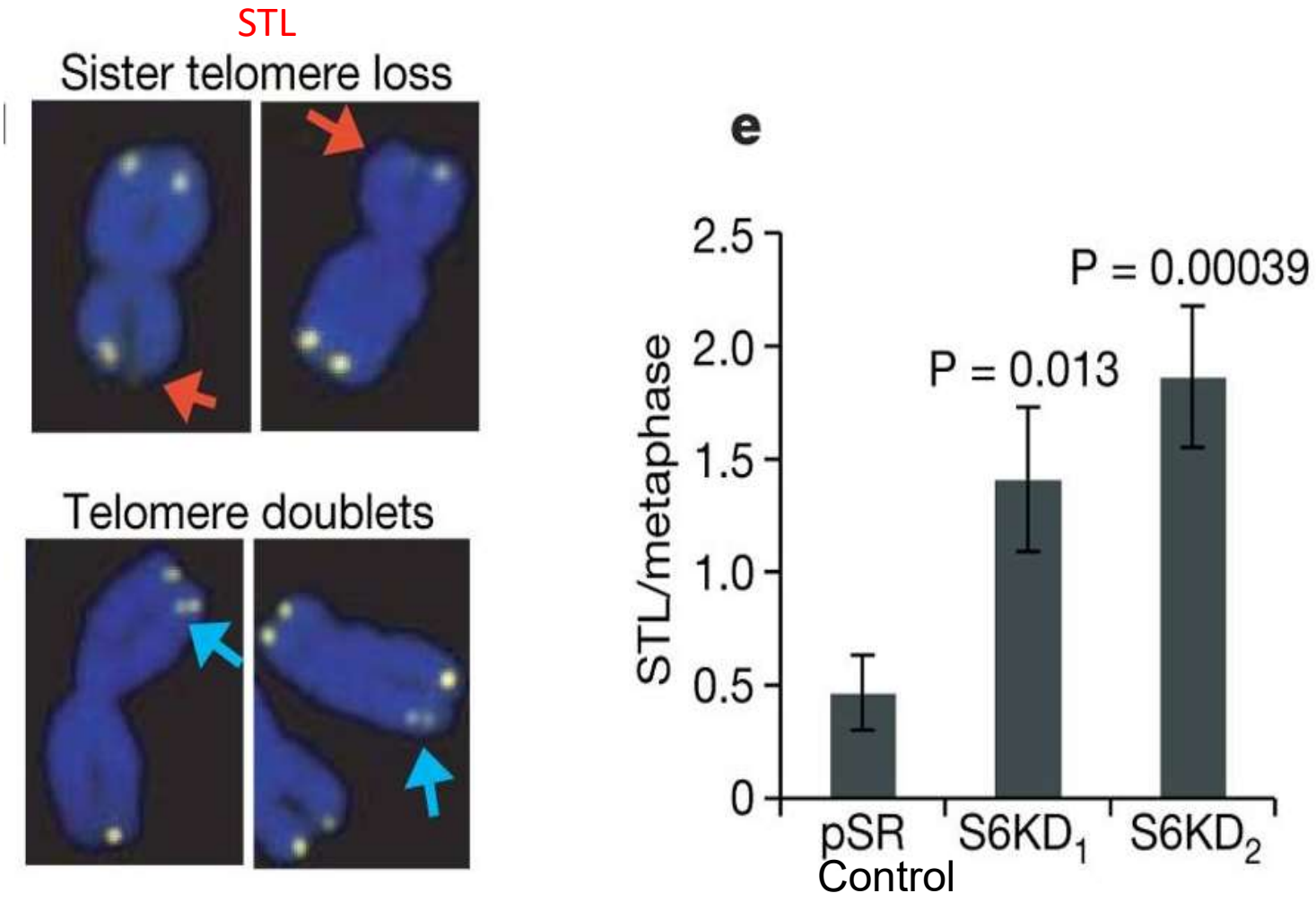
histone tail peptides

full-length histone H3

293Tcells overexpressing SIRT6

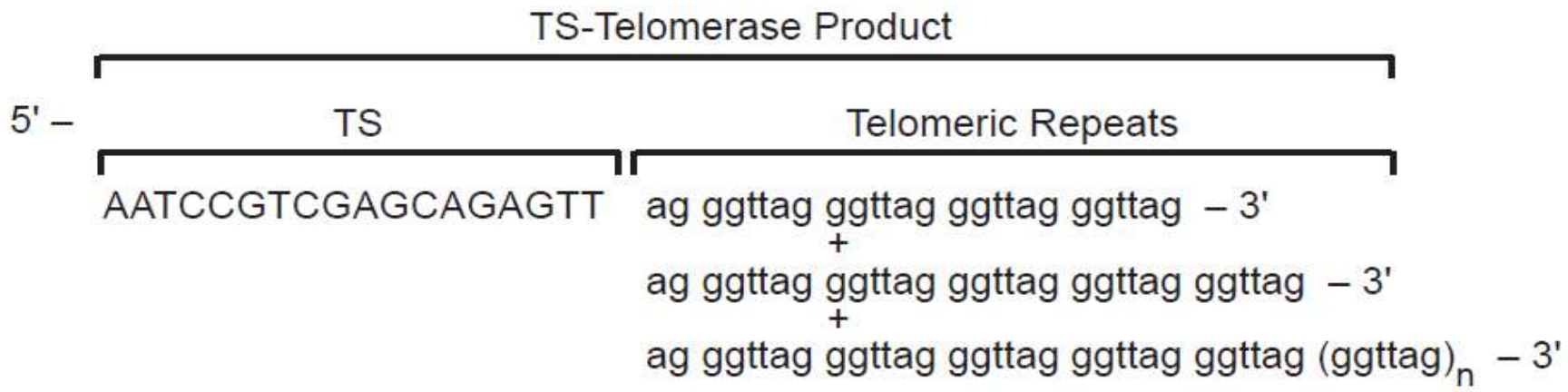
SIRT6-HY: *catalytic H133Y SIRT6 mutant protein*

SIRT6 knockdown (S6KD) cells

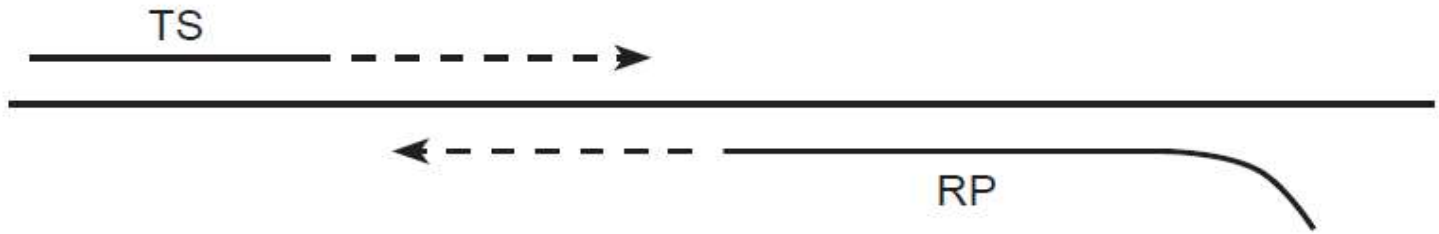


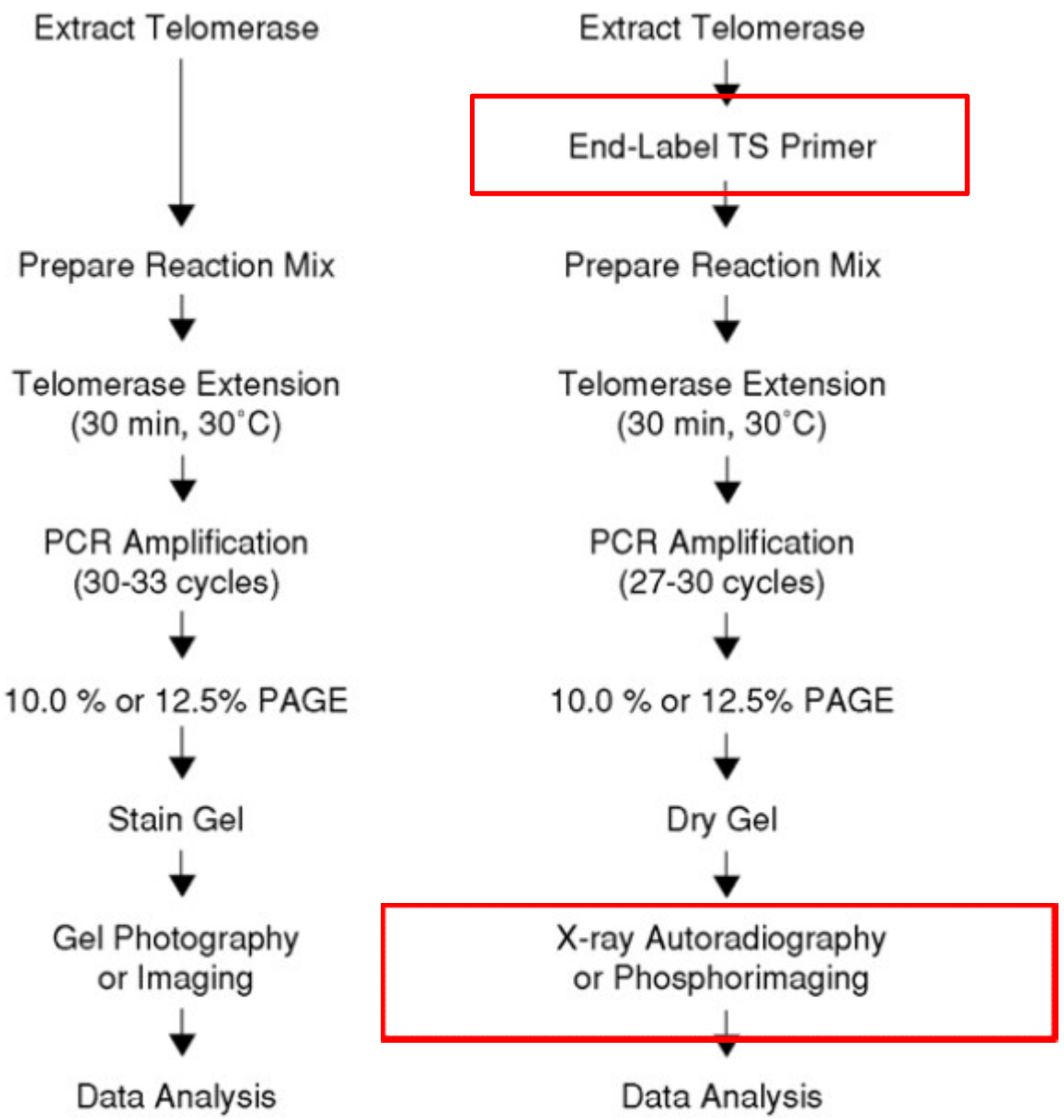
d, Representative S6KD metaphases showing aberrant telomere signals. Red arrows, sister telomere loss; blue arrows, telomere doublets. e, Quantification of sister telomere loss

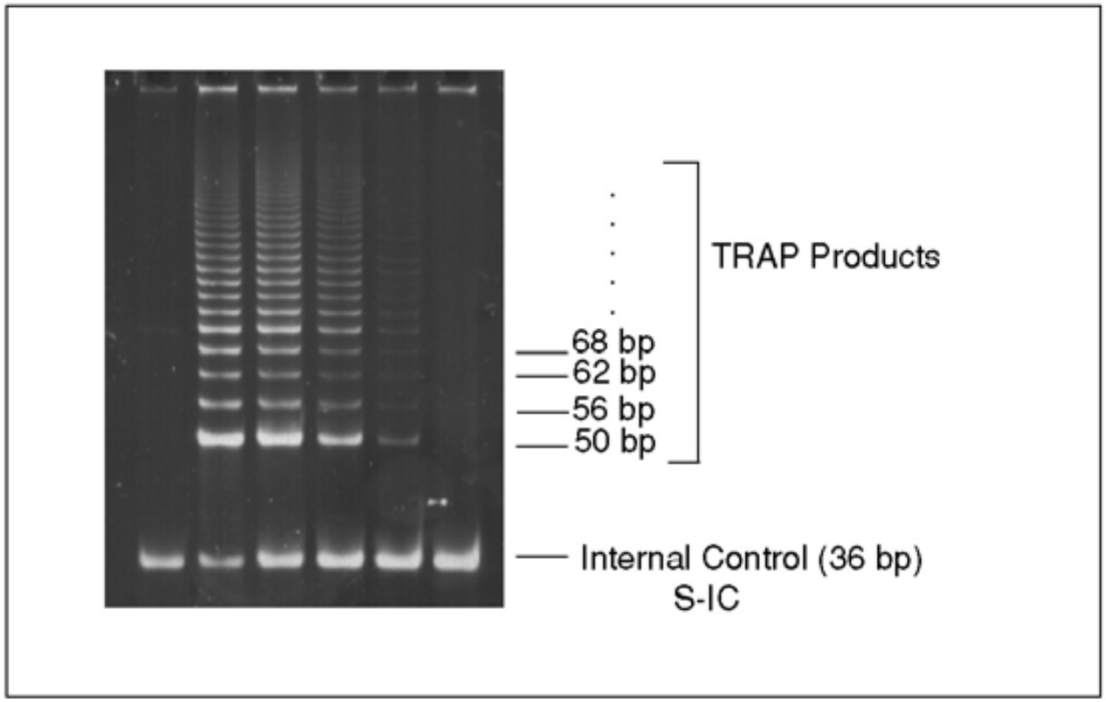
Metodi per lo studio dell’attività telomerasica



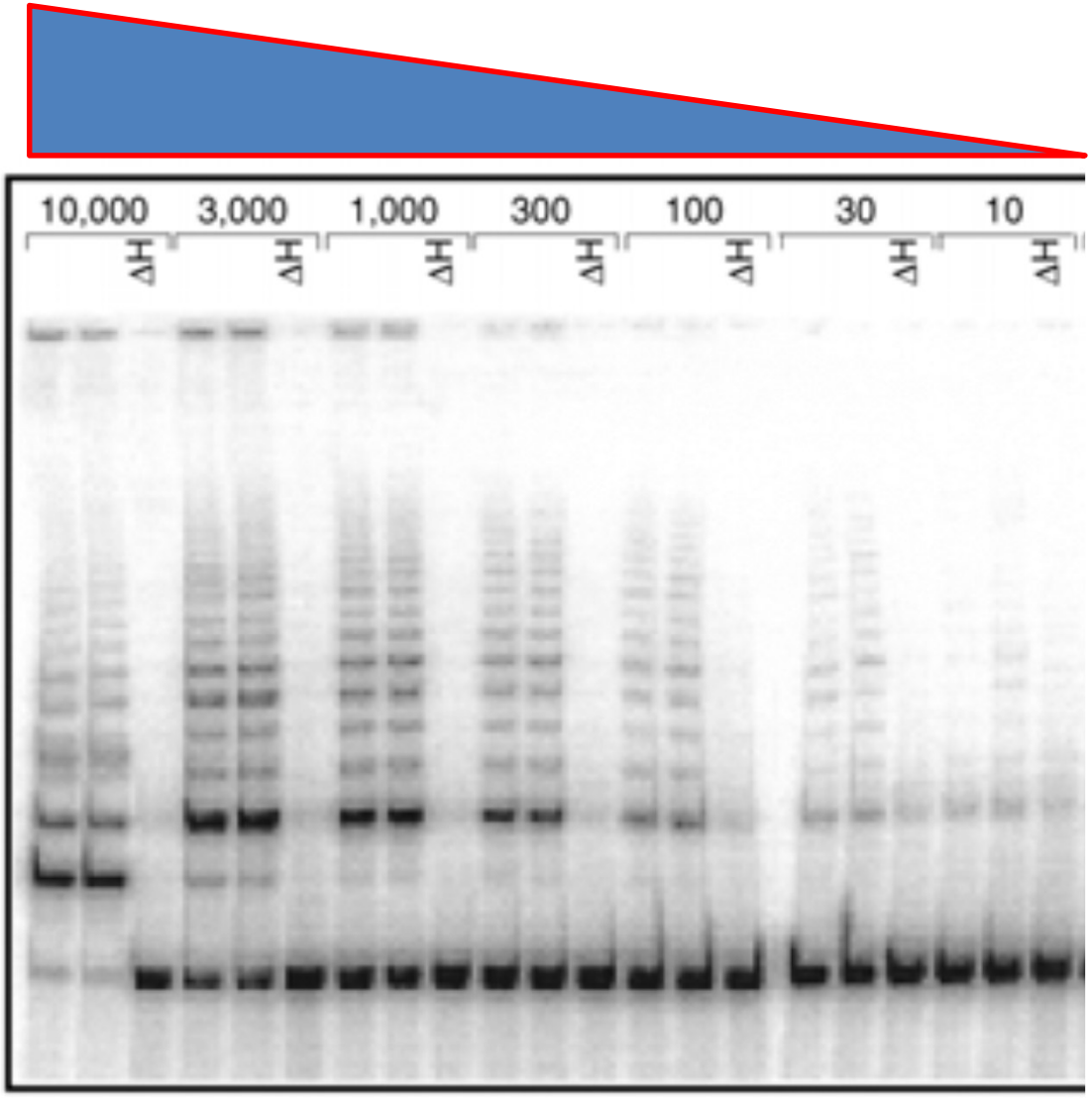
STEP 2. Amplification of TS-Telomerase Product By PCR




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

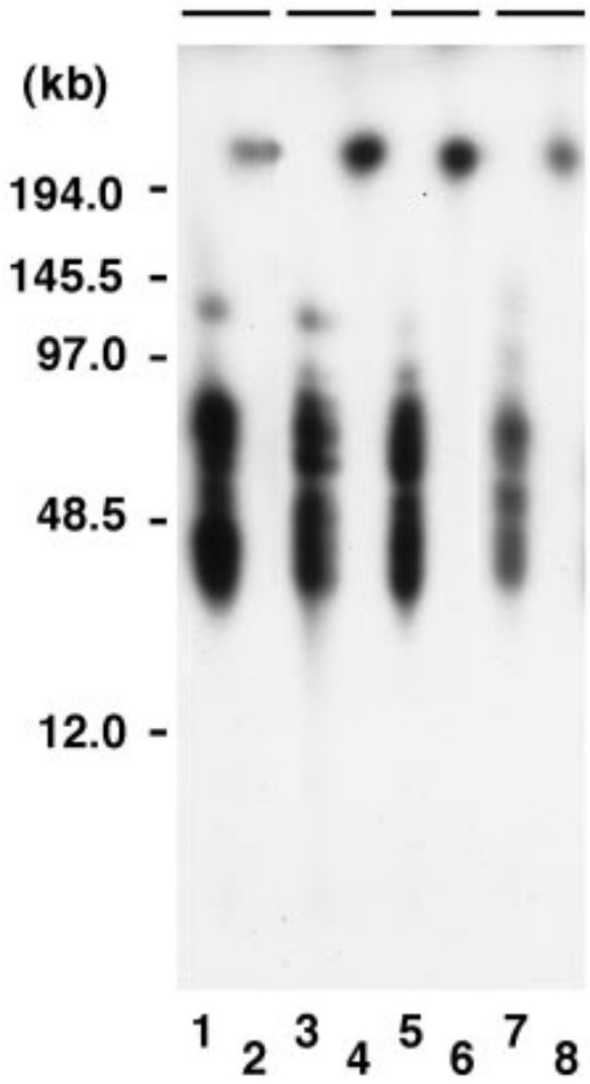


Telomeric Repeat Amplification Protocol


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

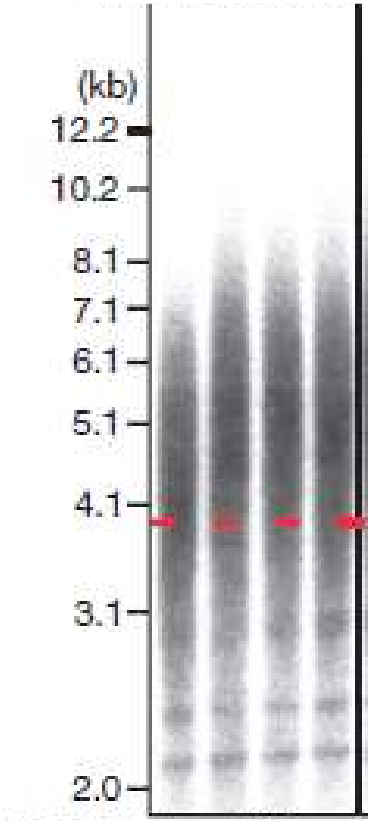
Metodi per lo studio dello stato dei telomeri

DNA TELOMERICO



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

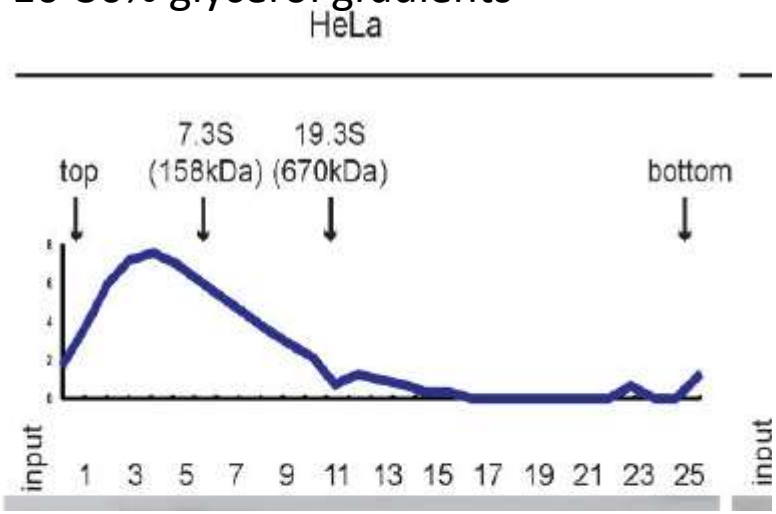
DNA TELOMERICO



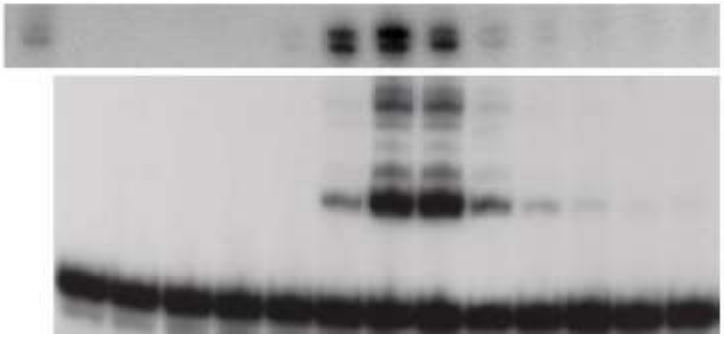
Complessi macromolecolari associati al Telomero: funzioni

COMPLESSI TELOMERICI

10-30% glycerol gradients



Total protein

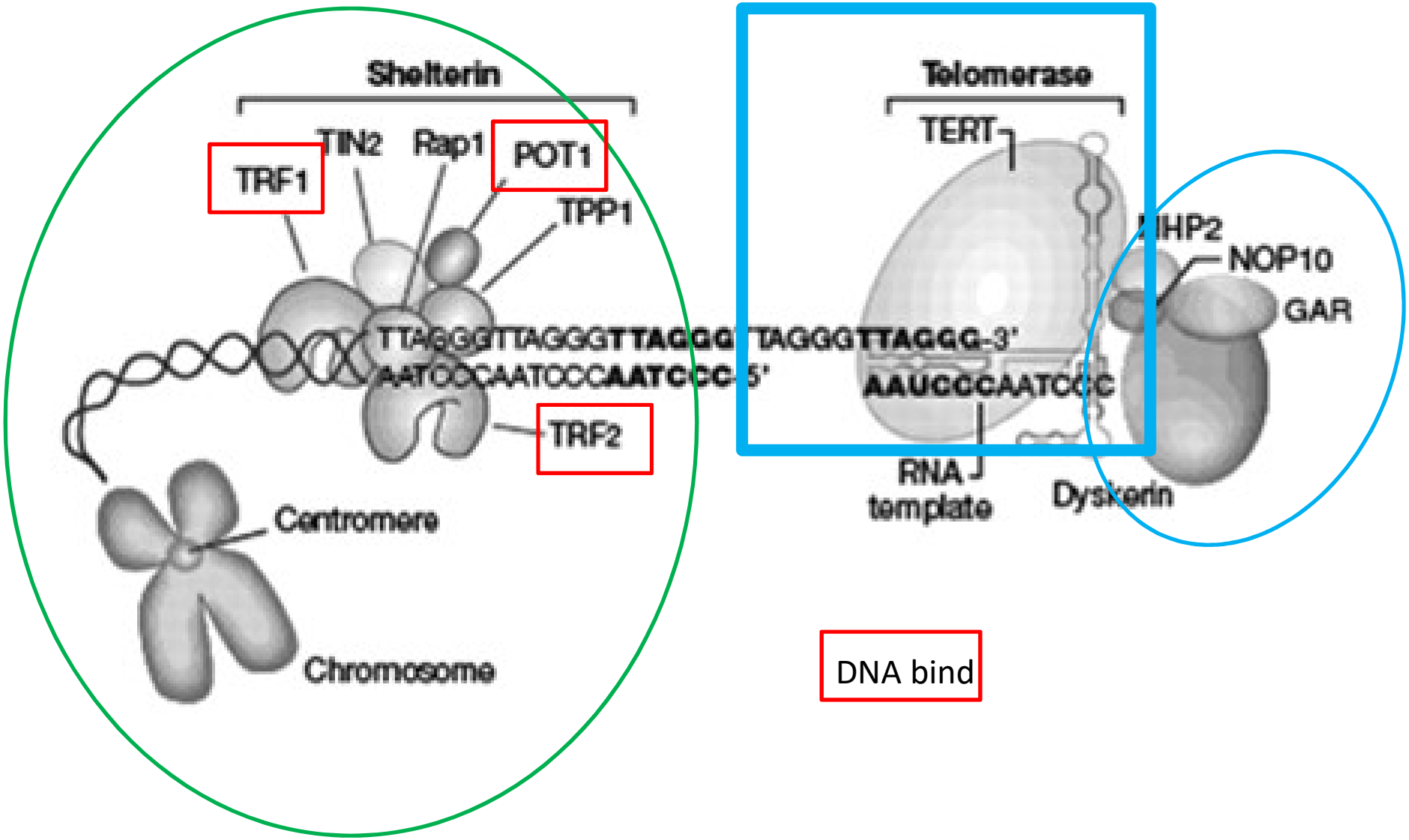


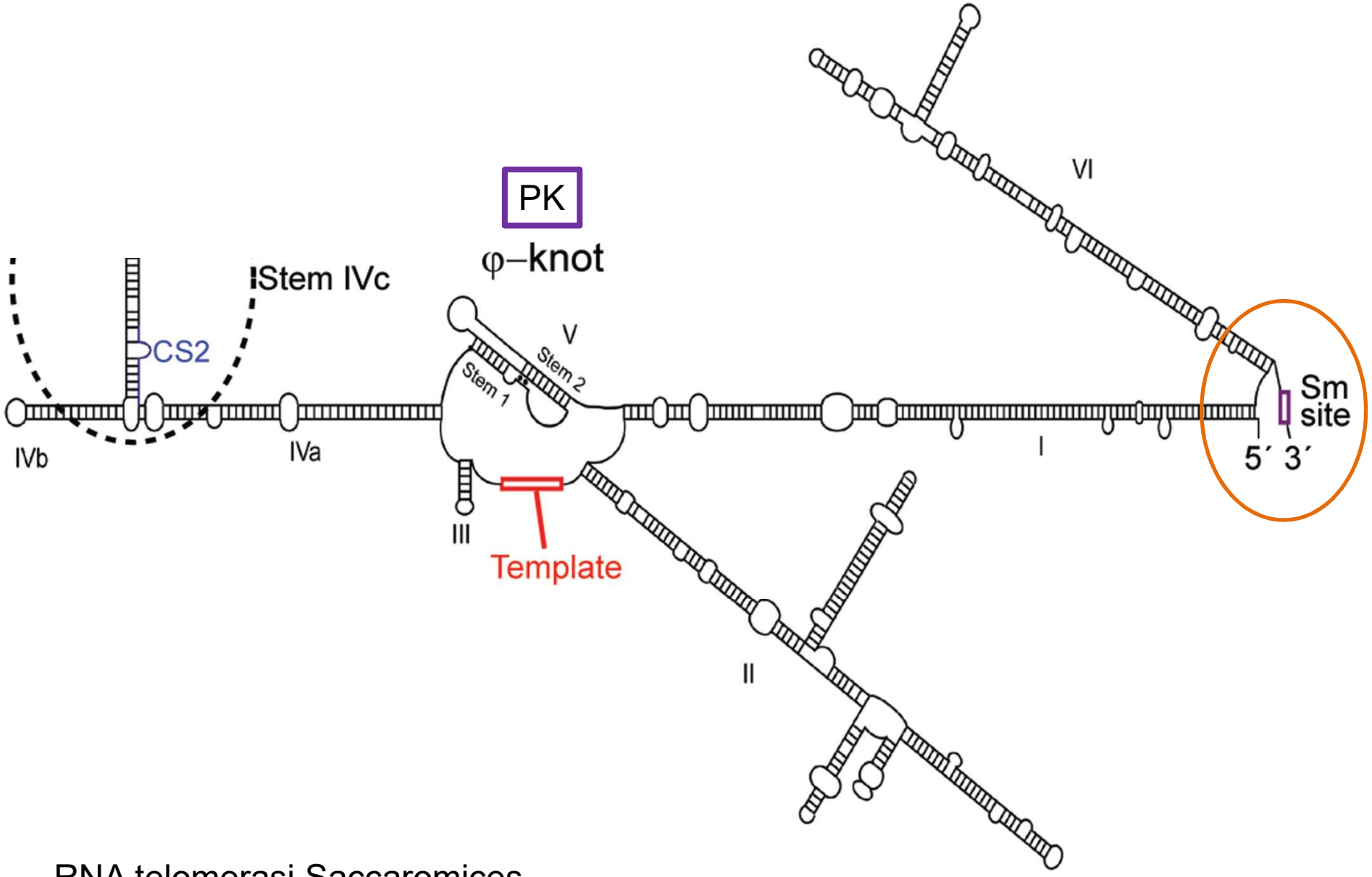
NB: TERC

TRAP

Telomeric Repeat Amplification Protocol

Complessi macromolecolari associati al Telomero ed alla Telomerasi





RNA telomerasi Saccaromices

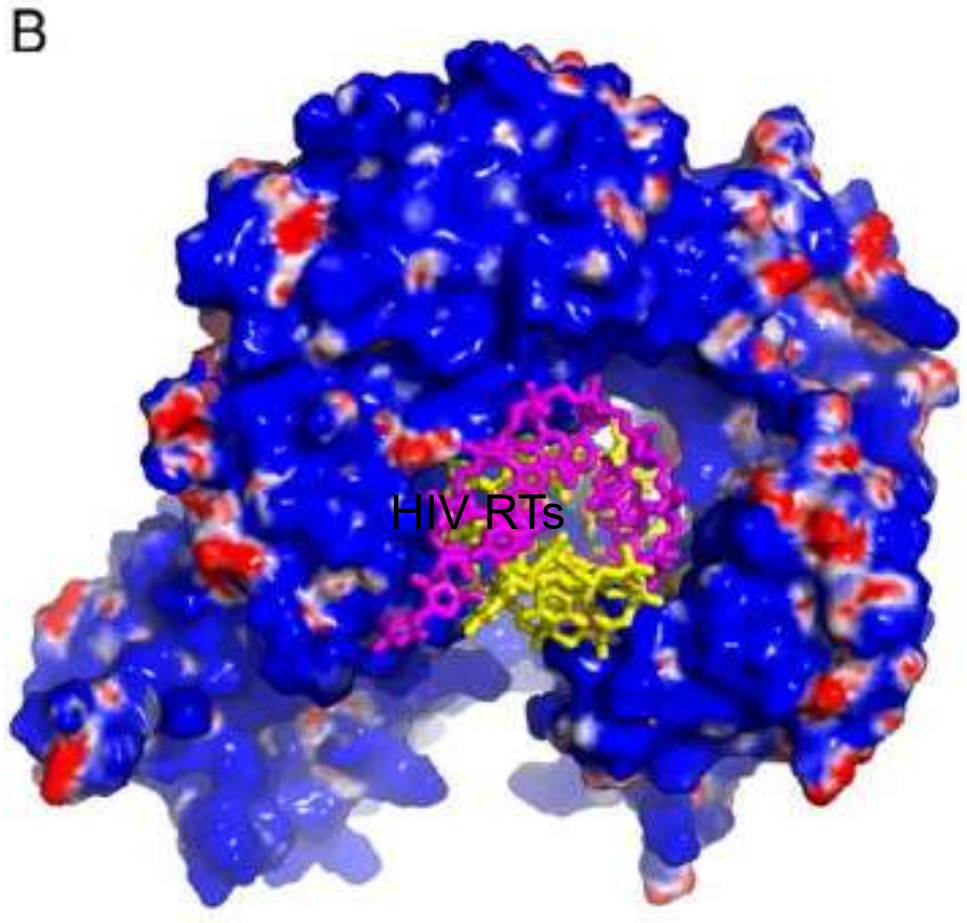
hTR is a 451-nucleotide RNA.

The 3’ end is essential for hTR stability and for its assembly with hTERT.

These functions are mediated by the dyskerin complex composed of four proteins:

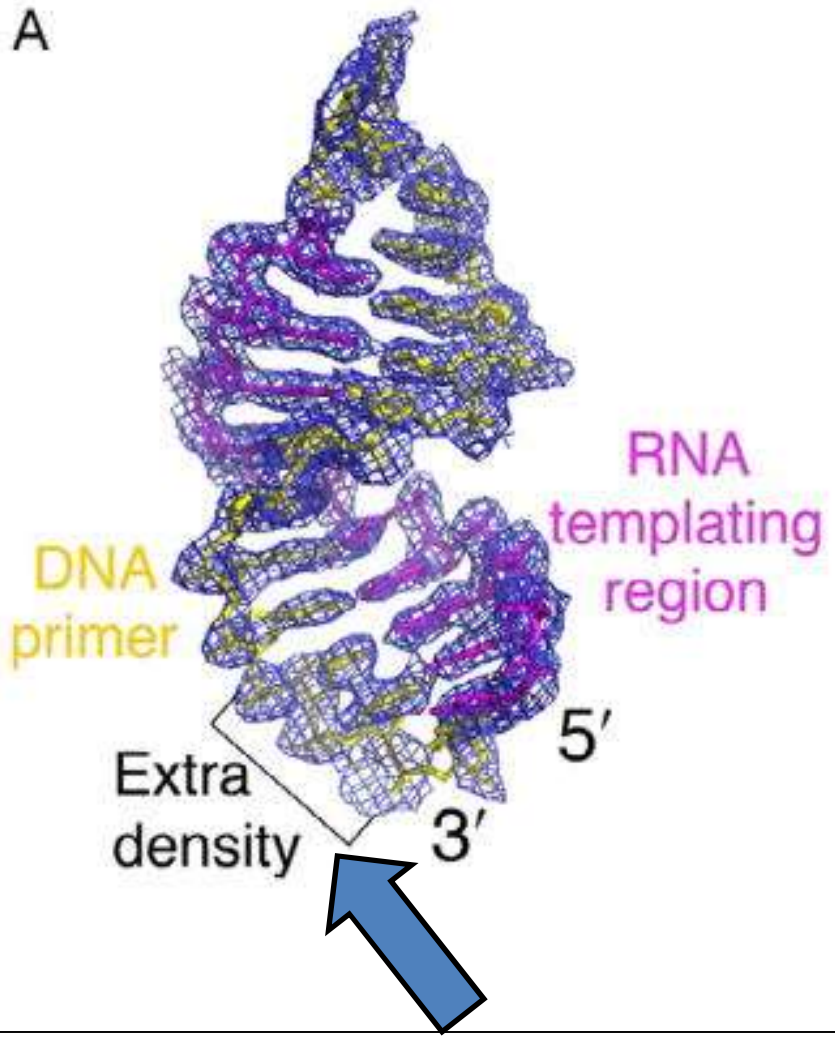
dyskerin, NOP10, NHP2 and GAR1

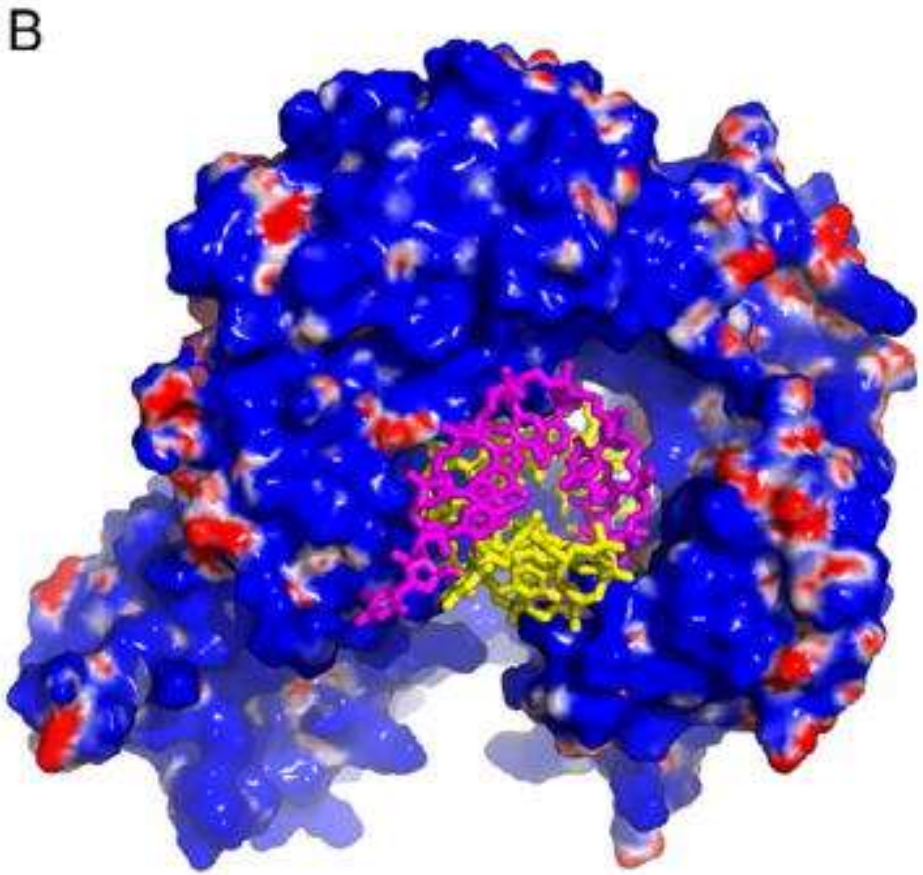
RNA (magenta stick)–DNA (yellow stick) hairpin co-crystallized with tcTERT



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

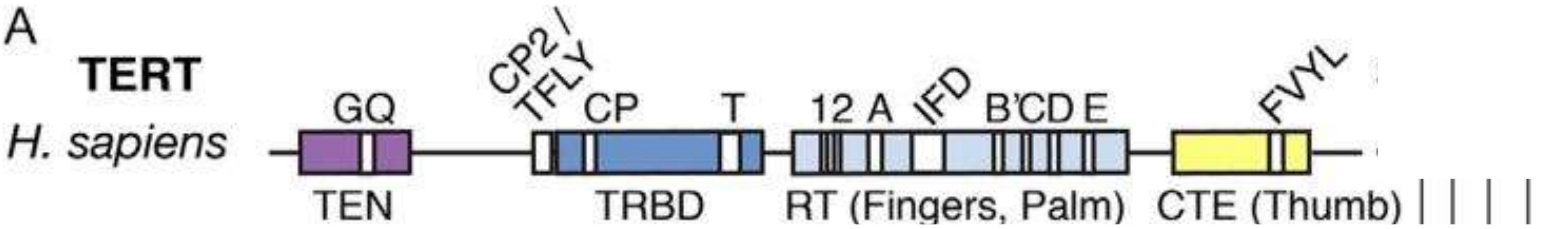
RNA (magenta stick)–DNA (yellow stick) hairpin co-crystallized with tcTERT

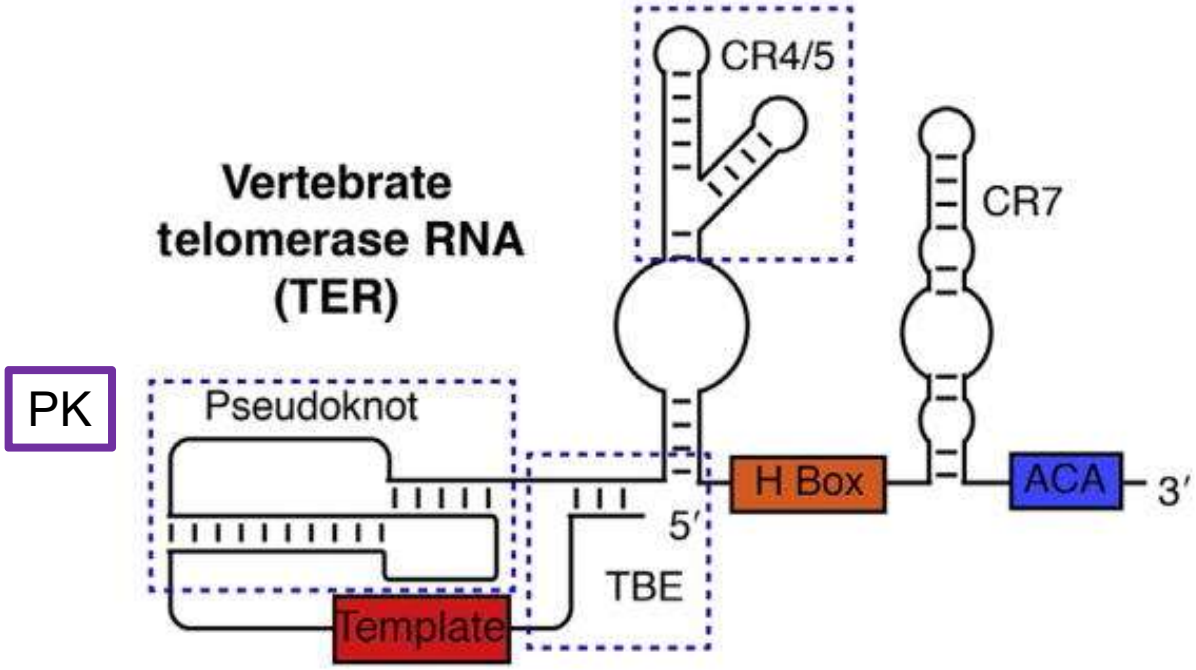




Binding Similar to HIV RTs!

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

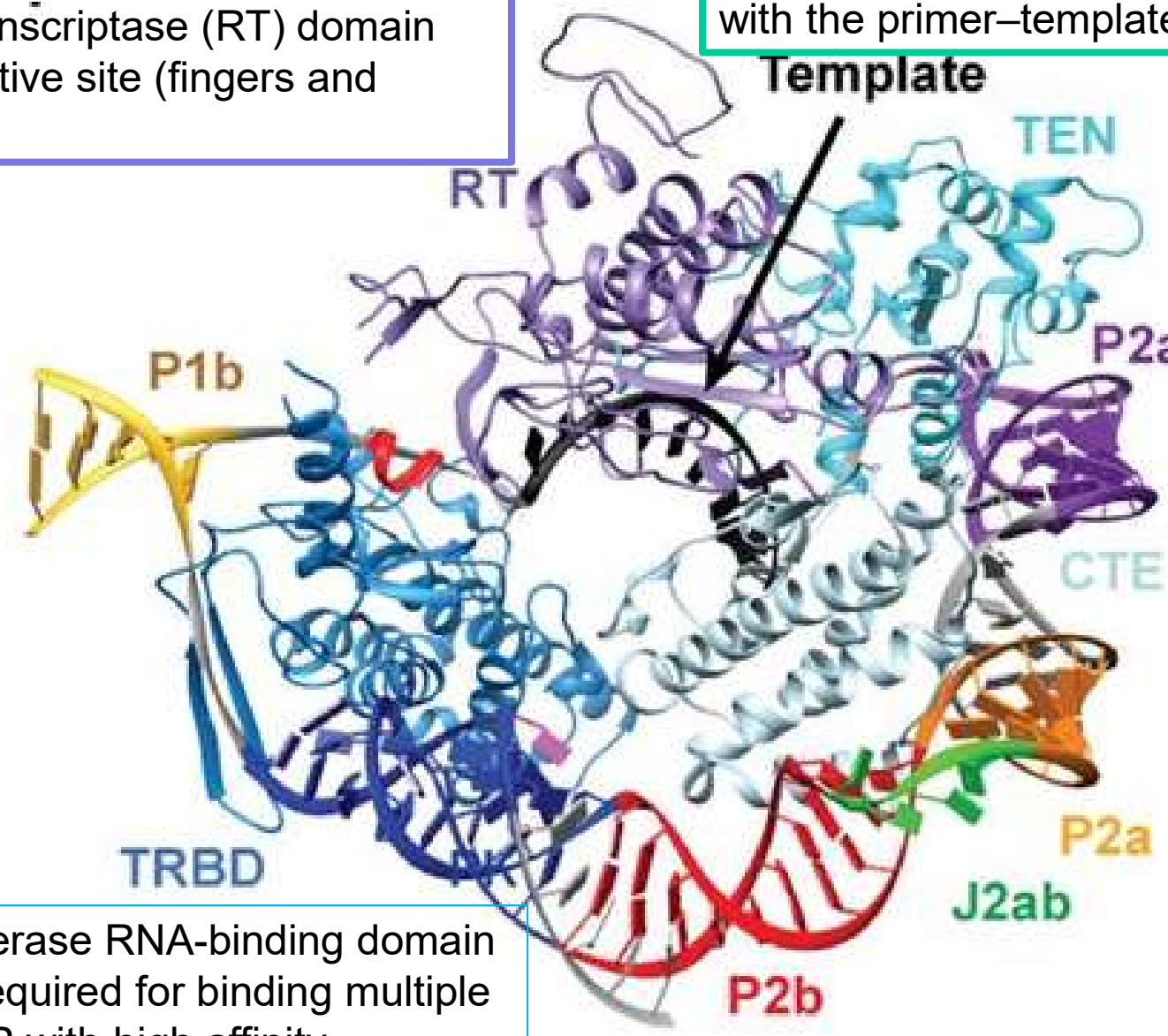

A
TERT
H. sapiens
GQ
CP2 / TFLY
CP
T
12 A
IFD
B'CD E
FVYL
TEN
TRBD
RT (Fingers, Palm)
CTE (Thumb)


Vertebrate telomerase RNA (TER)
CR4/5
CR7
PK
Pseudoknot
H Box
ACA
3′
5′
TBE
Template

Models of human TERT–t/PK.

reverse transcriptase (RT) domain enzyme active site (fingers and palm)

N-terminal (TEN) domain interacts with the primer–template duplex

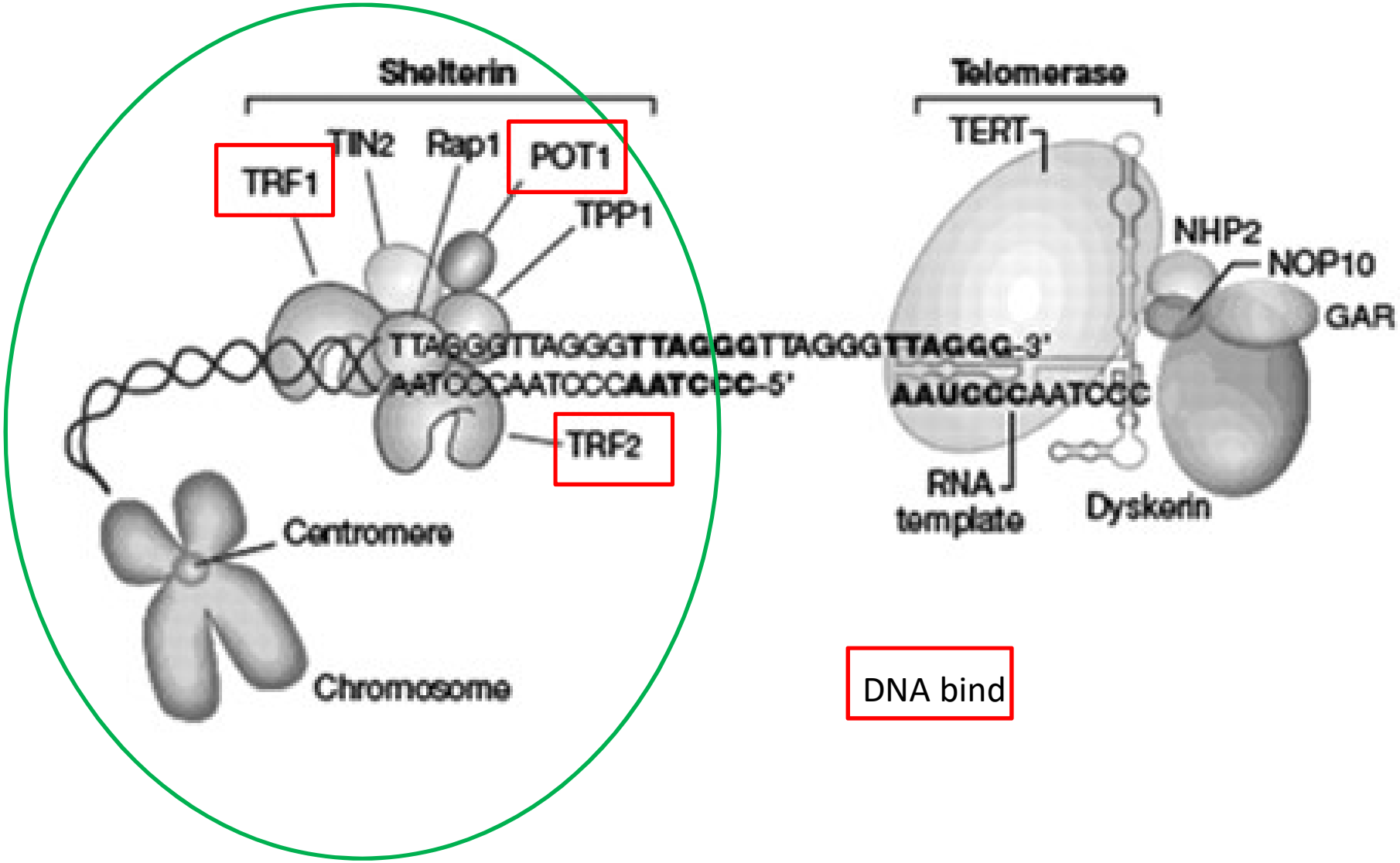


C-terminal extension (CTE) (thumb) processivity

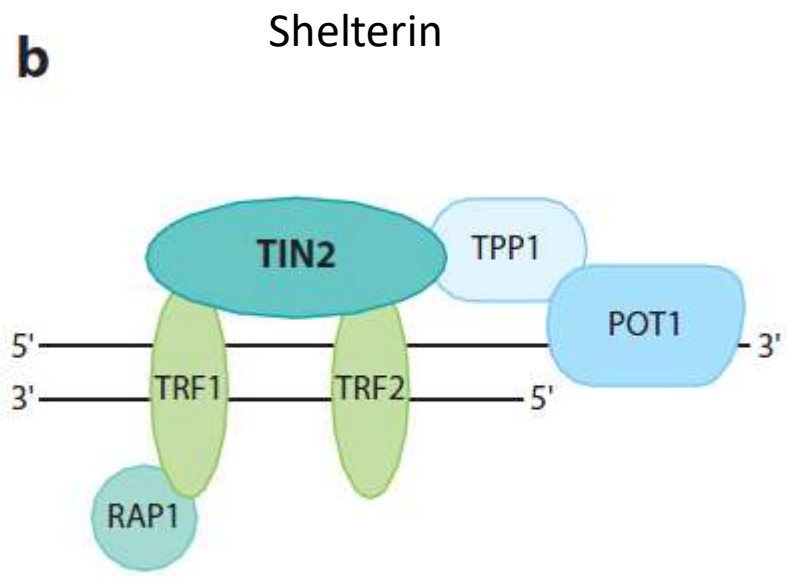
the telomerase RNA-binding domain (TRBD) required for binding multiple sites of TR with high affinity

Yaqiang Wang et al. PNAS 2016;113:E5125-E5134

Complessi macromolecolari associati al Telomero ed alla Telomerasi



Complessi macromolecolari associati al Telomero

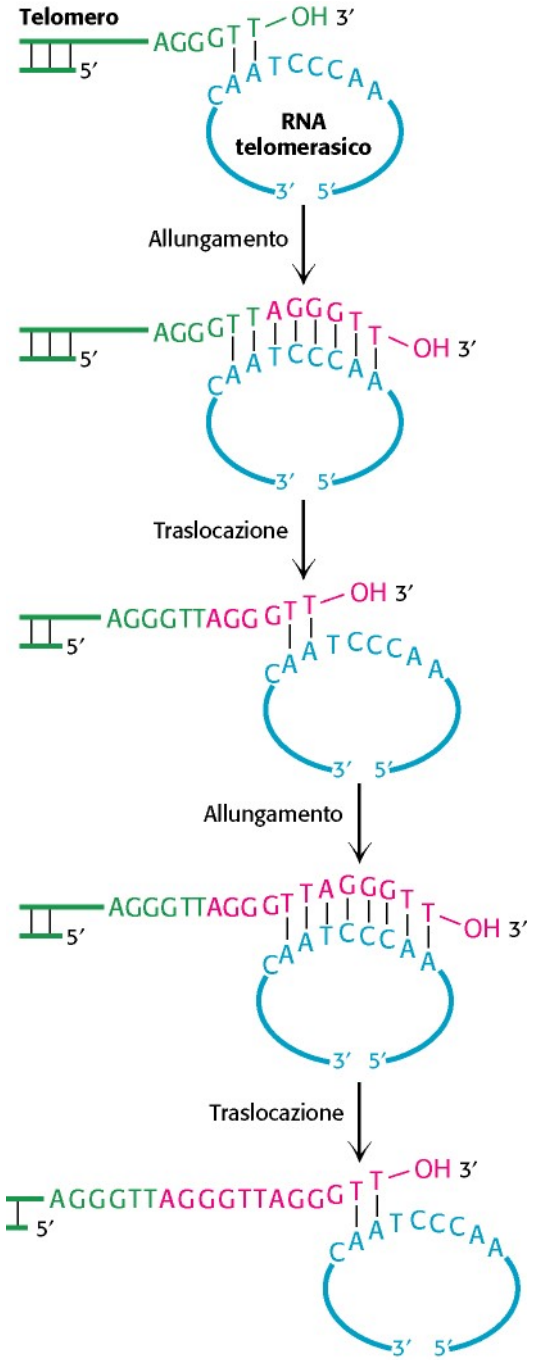


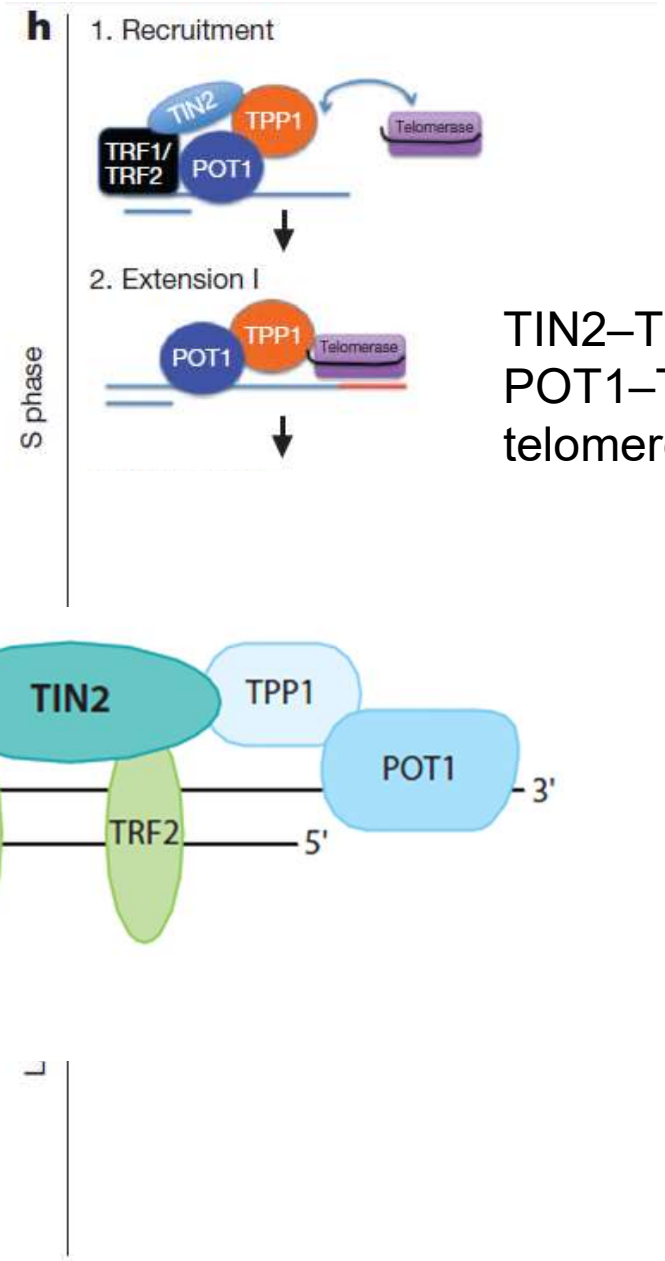
Telomeres are coated by a group of at least six proteins, collectively called **shelterin**
Three proteins, TRF1, TRF2, and POT1 (single-stranded repeats) directly recognize and bind to TTAGGG repeats
TIN2 TPP1, and Rap1, interconnect the telomere-binding proteins to form the entire complex

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

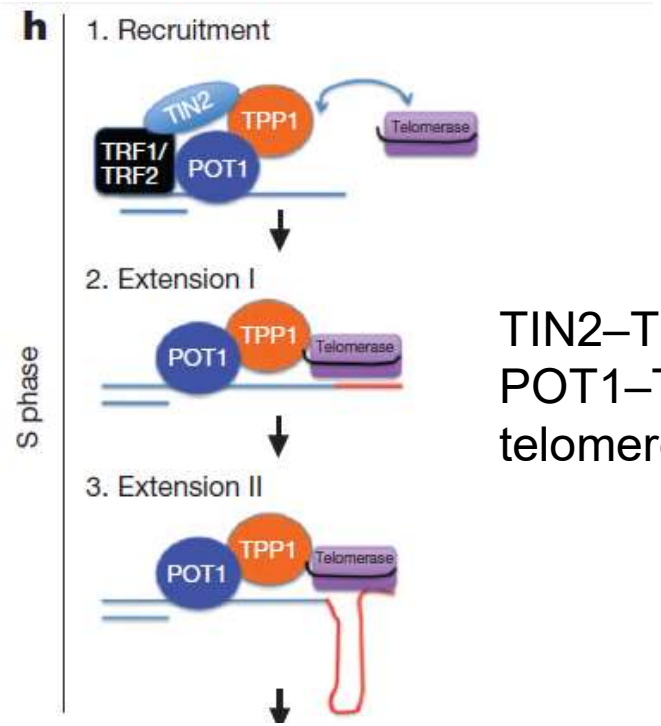
L’allungamento del telomero modello riassuntivo

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

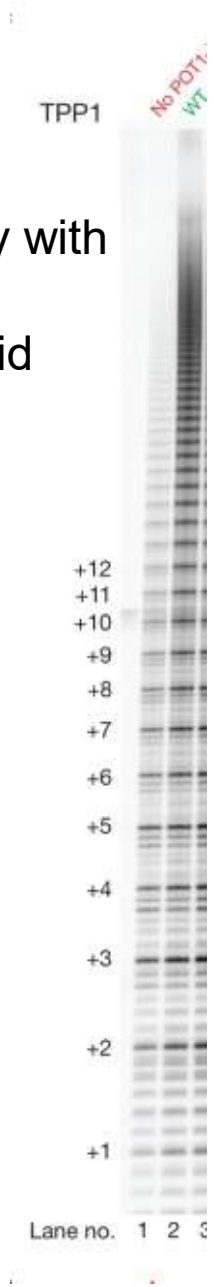
Telomere synthesis involves trafficking of telomerase and telomerase is thought to be recruited to telomeres through interactions with telomere-binding proteins.

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

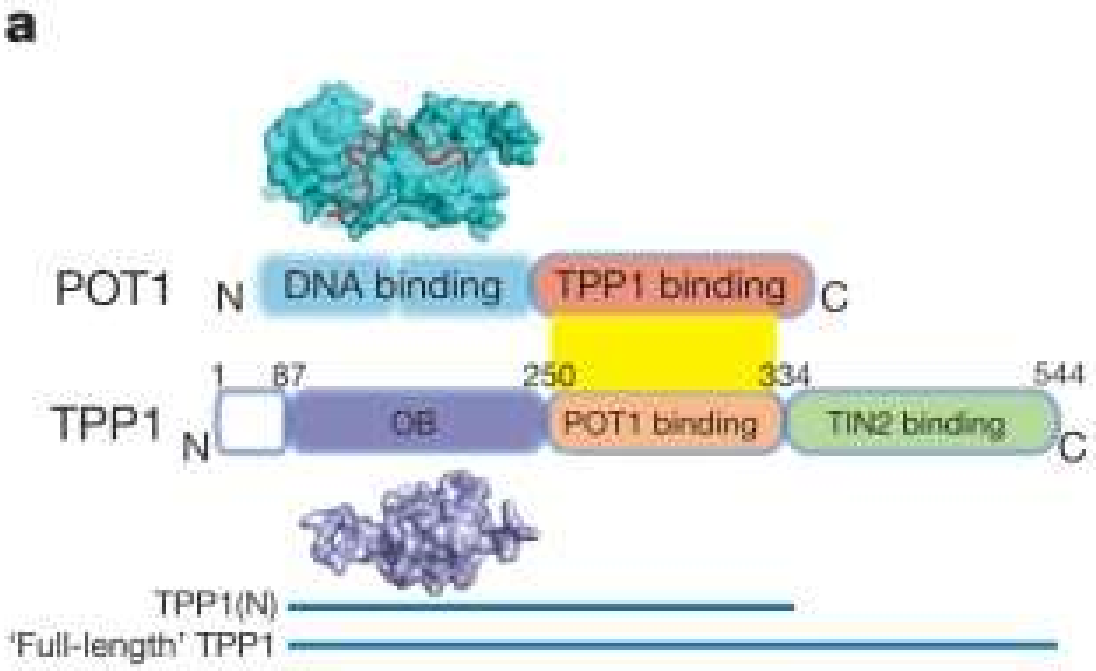
The TPP1 OB-fold domain is sufficient to recruit telomerase to a heterologous chromatin locus.
A minimal TPP1 OB-fold inhibits telomere maintenance by blocking access of telomerase to its binding site at telomeres.

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.

Direct telomerase activity assay with primer of lysates from cells co-transfected with a TR plasmid and POT1, TPP1 MUTANTS and TERT.



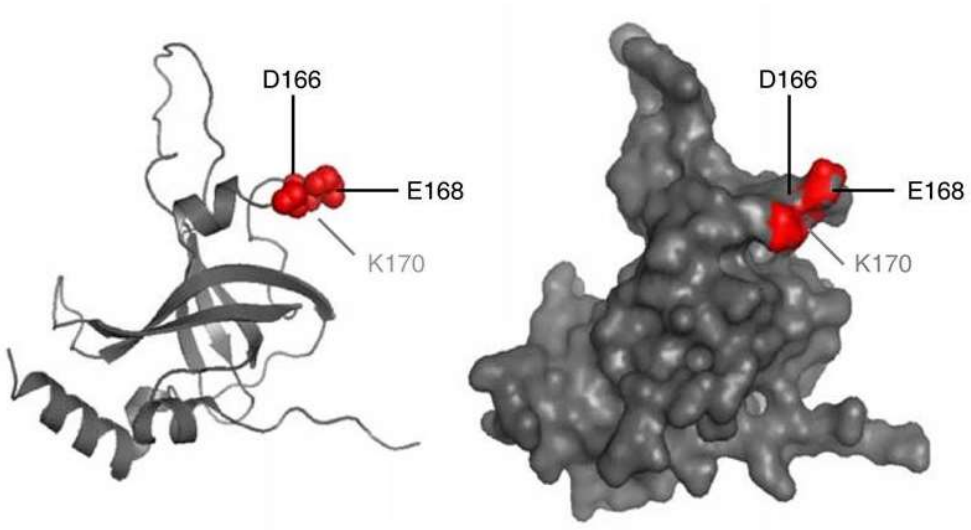
J Nandakumar *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature11648



nature

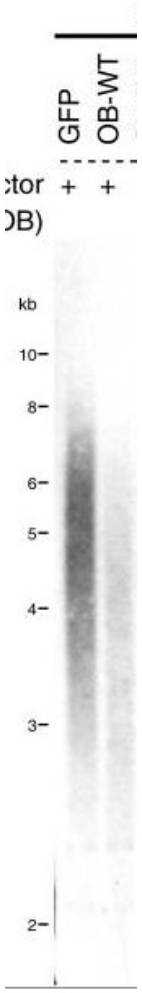
J Nandakumar *et al. Nature* **000**, 1-5 (2012) doi:10.1038/nature11648

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

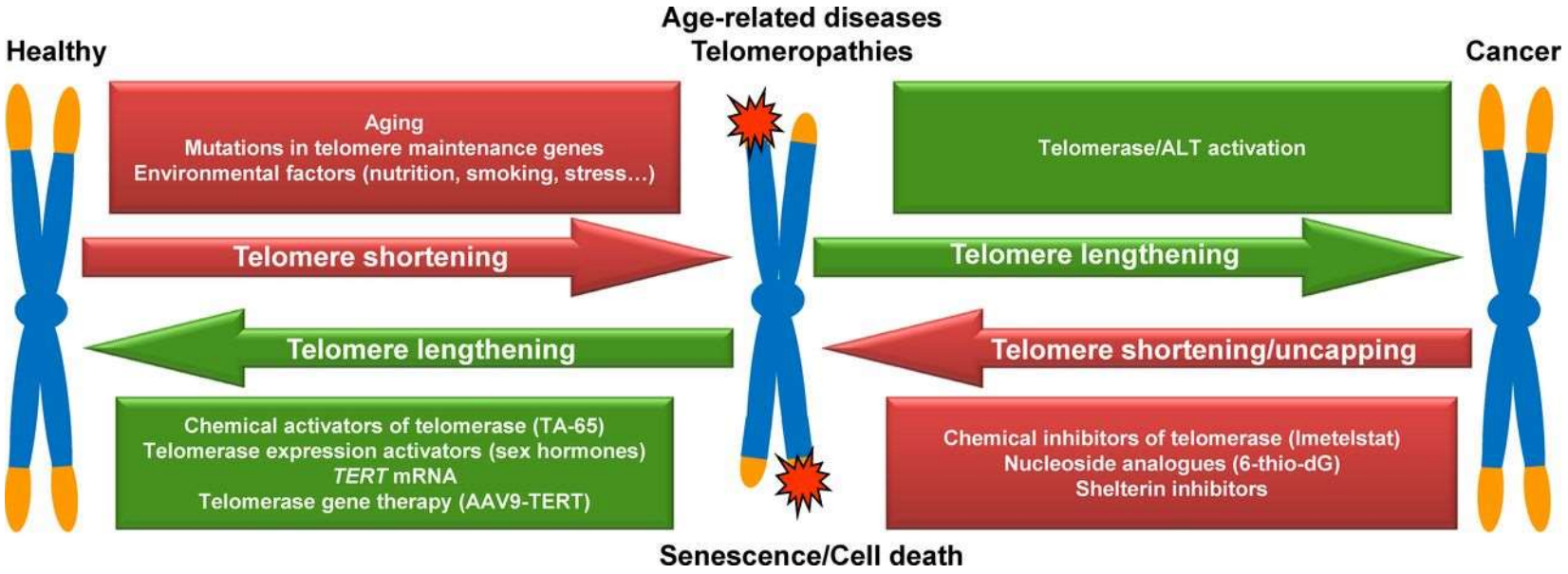


Structural representation of TPP1-OB domain (PDB 2i46).
Residues required for telomerase interaction shown in red

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



Natural factors and therapeutic interventions affecting telomere-mediated diseases.



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

JCB