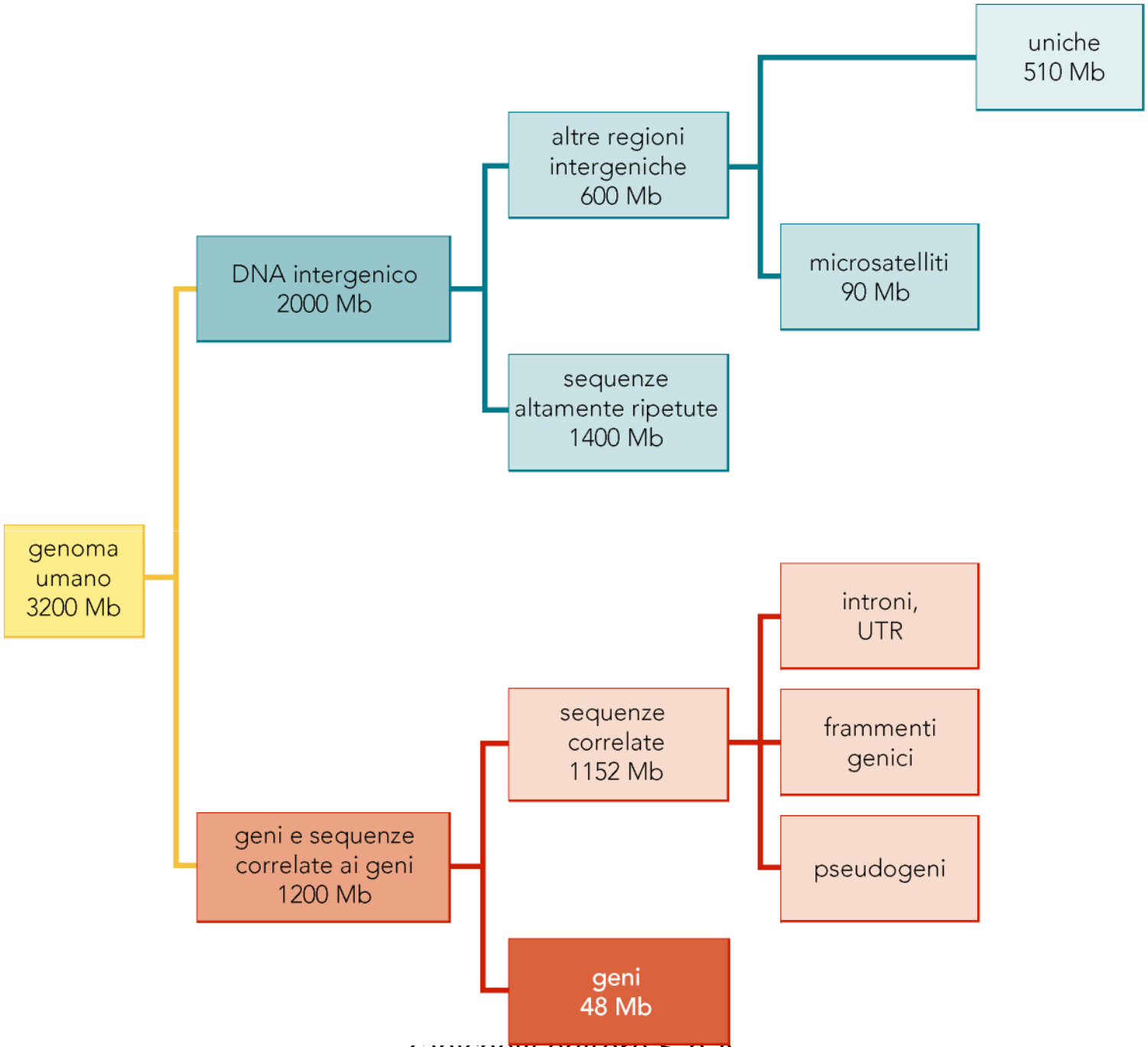
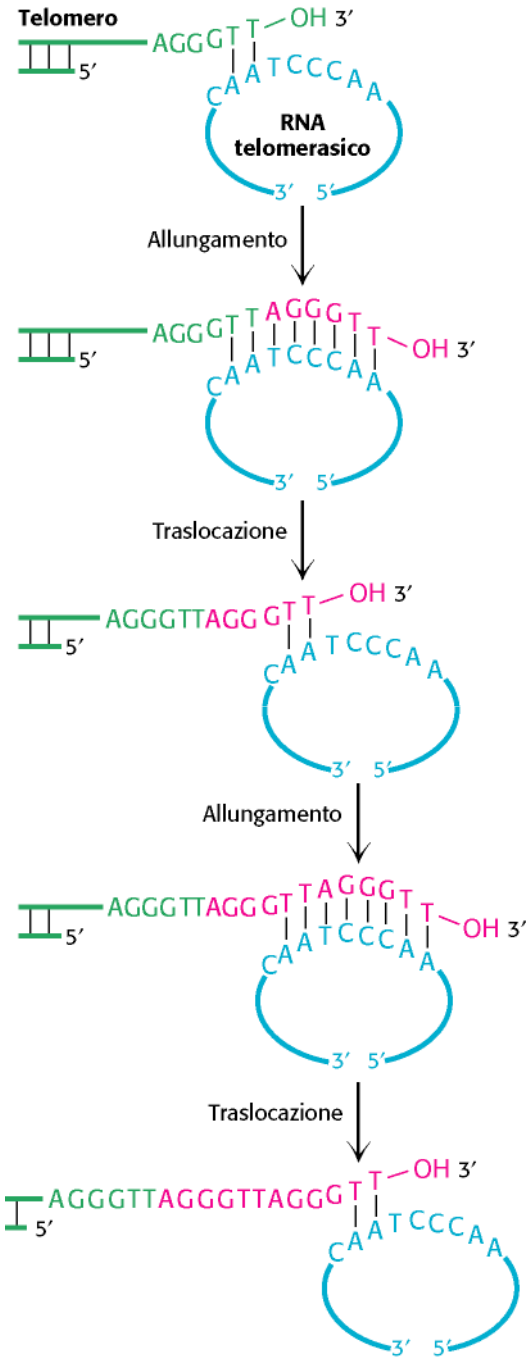
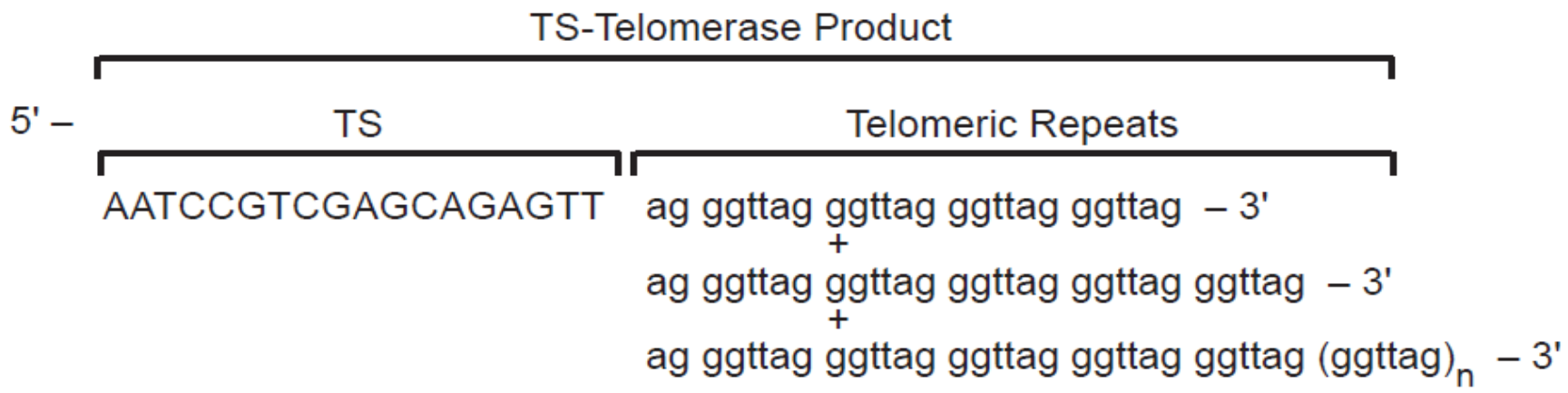
TELOMERI



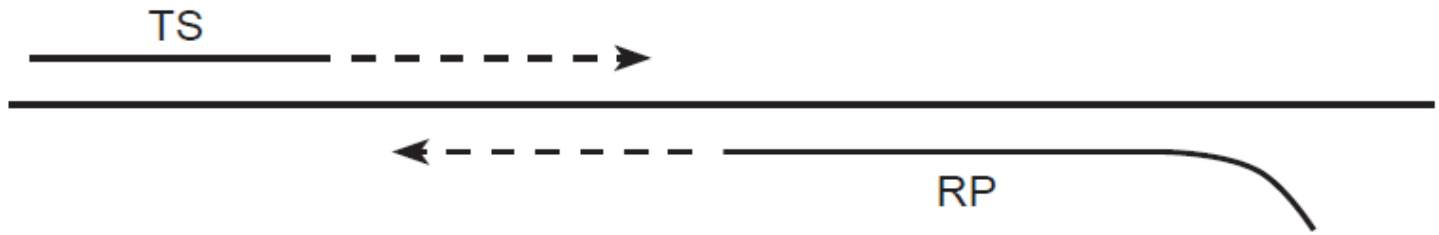
SEQUENZA TELOMERICA

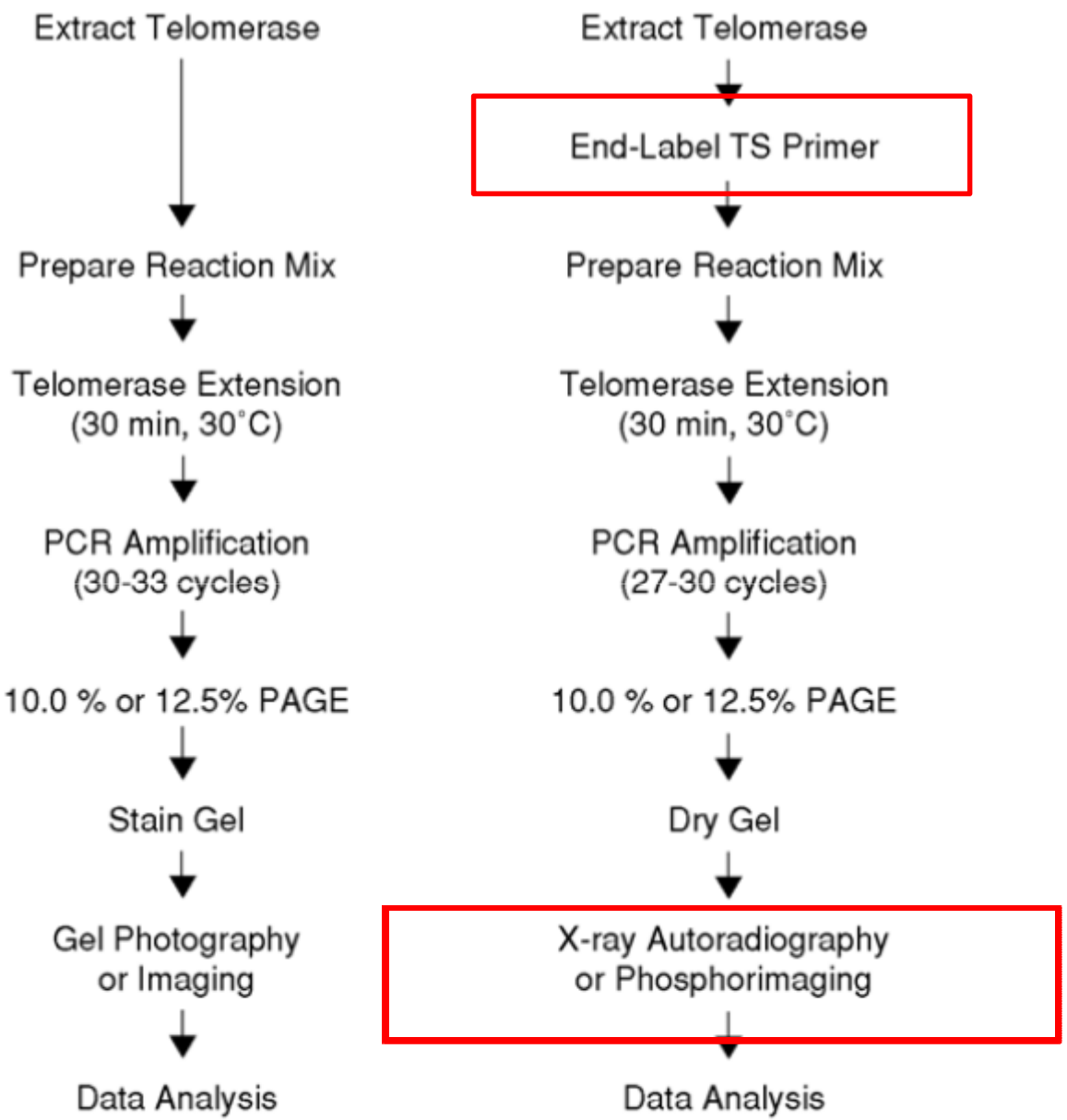
Watson et al., BIOLOGIA MOLECOLARE DEL GENE, Zanichelli editore S.p.A.

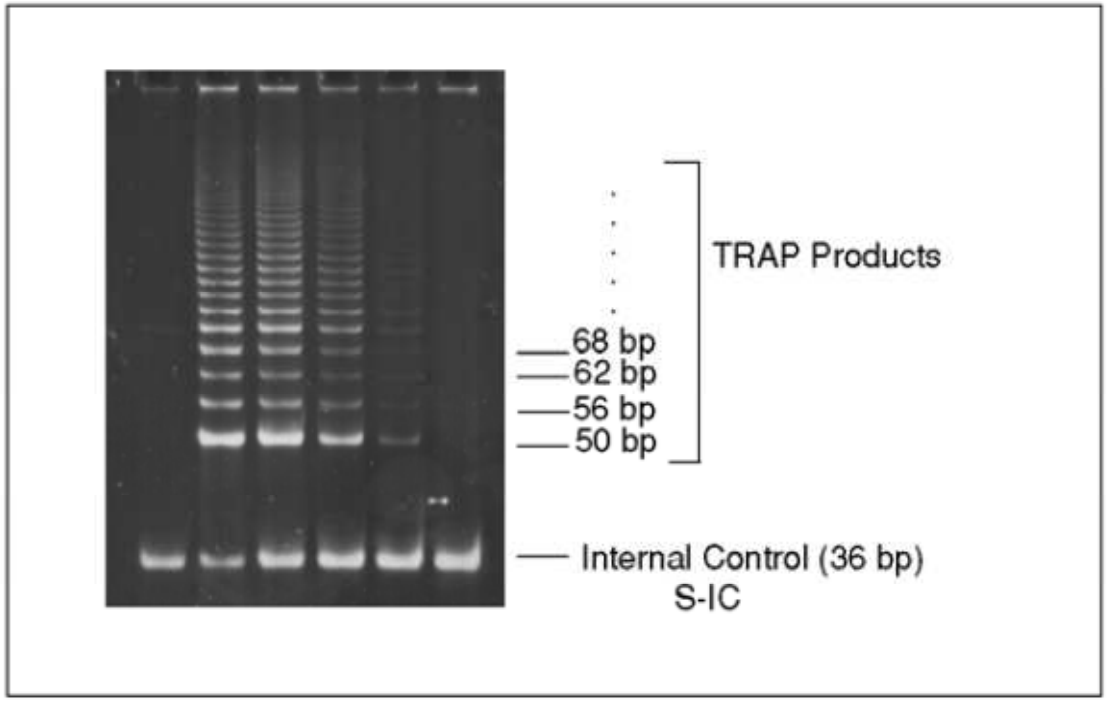




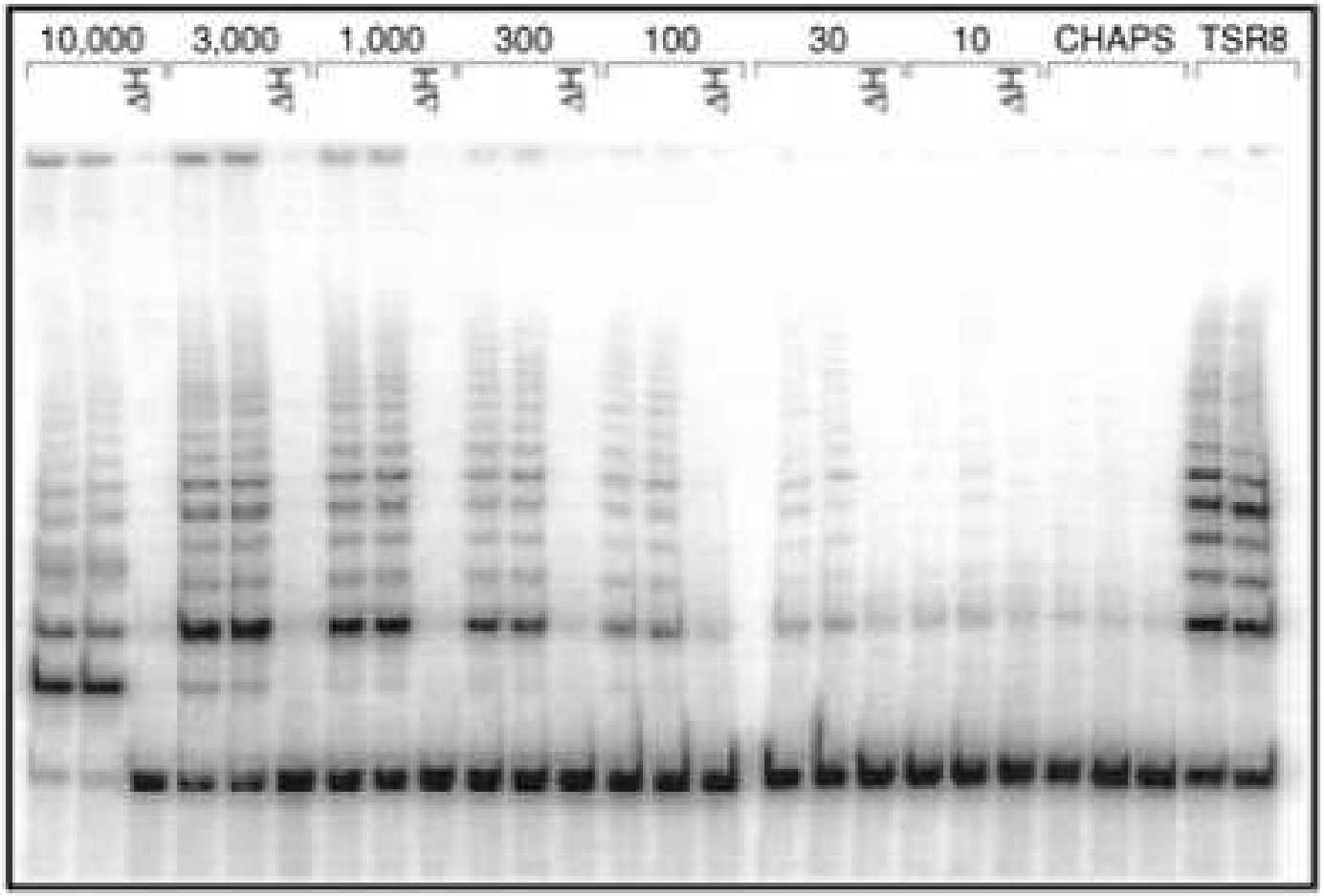
STEP 2. Amplification of TS-Telomerase Product By PCR



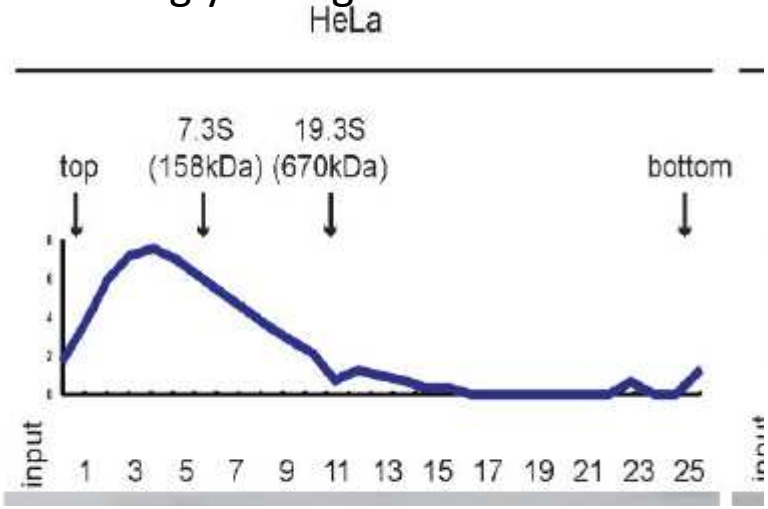

Extract Telomerase
Prepare Reaction Mix
Telomerase Extension
(30 min, 30˚C)
PCR Amplification
(30-33 cycles)
10.0 % or 12.5% PAGE
Stain Gel
Gel Photography
or Imaging
Data Analysis
Extract Telomerase
End-Label TS Primer
Prepare Reaction Mix
Telomerase Extension
(30 min, 30˚C)
PCR Amplification
(27-30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



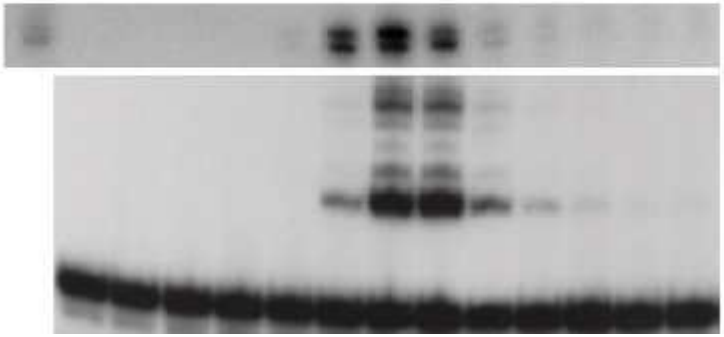
Telomeric Repeat Amplification Protocol


10,000
3,000
1,000
300
100
30
10
CHAPS
TSR8
NT
NT
NT
NT
NT
NT
NT

10-30% glycerol gradients

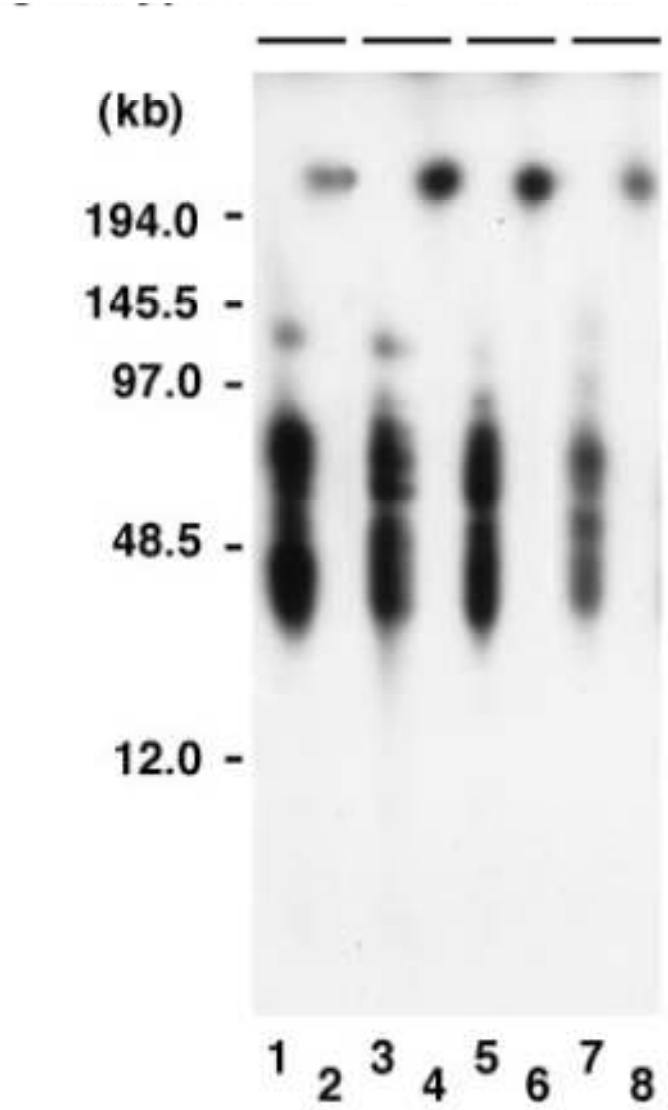


Total protein



NB: TERC

TRAP



l

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

La Telomerasi

The telomerase complex is composed of telomerase reverse transcriptase (TERT), the RNA component (TERC),
TERT enzymatically adds TTAGGG nucleotide repeats to the 3' end of telomere’s leading strand using TERC as a template.

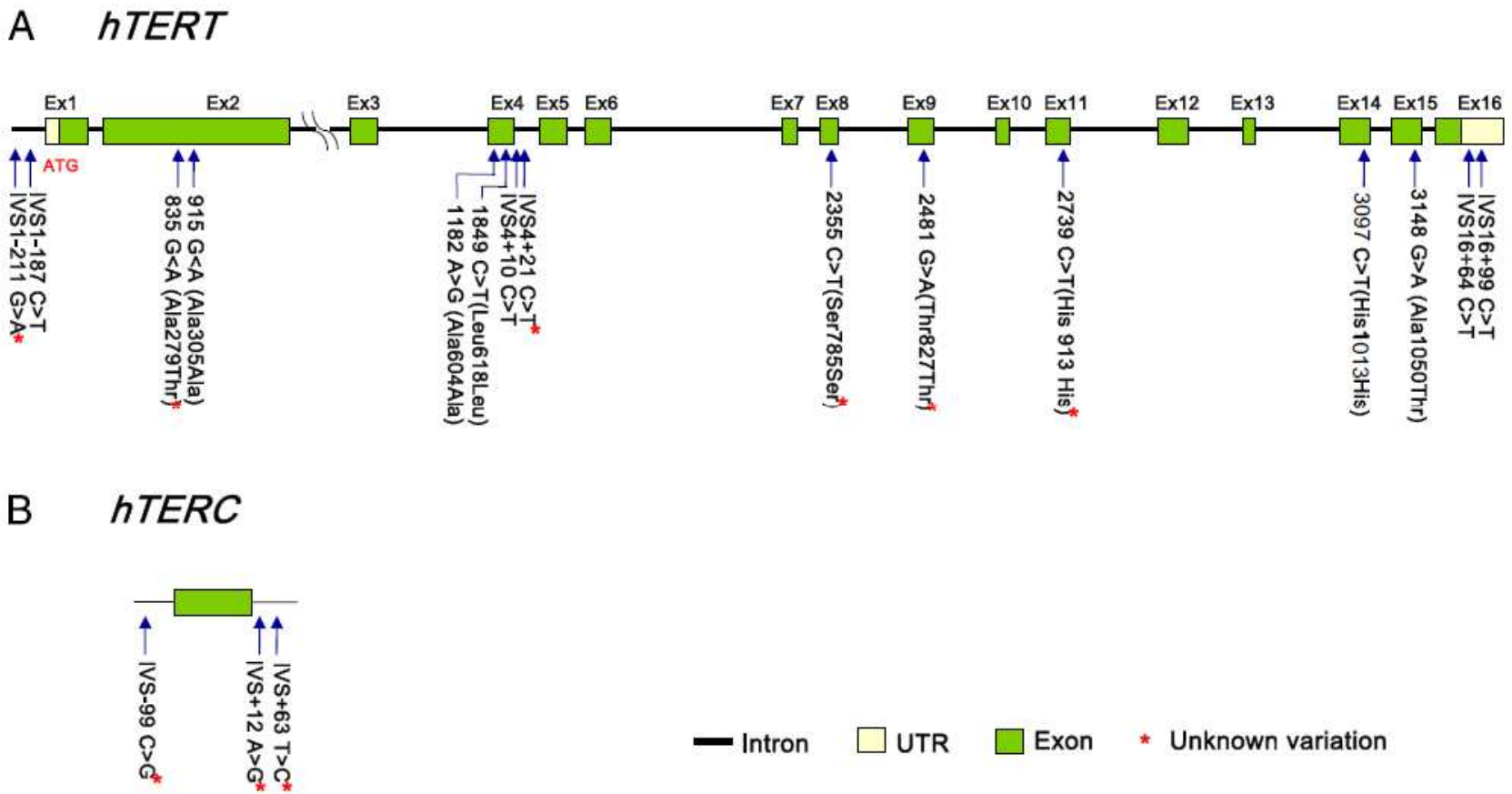
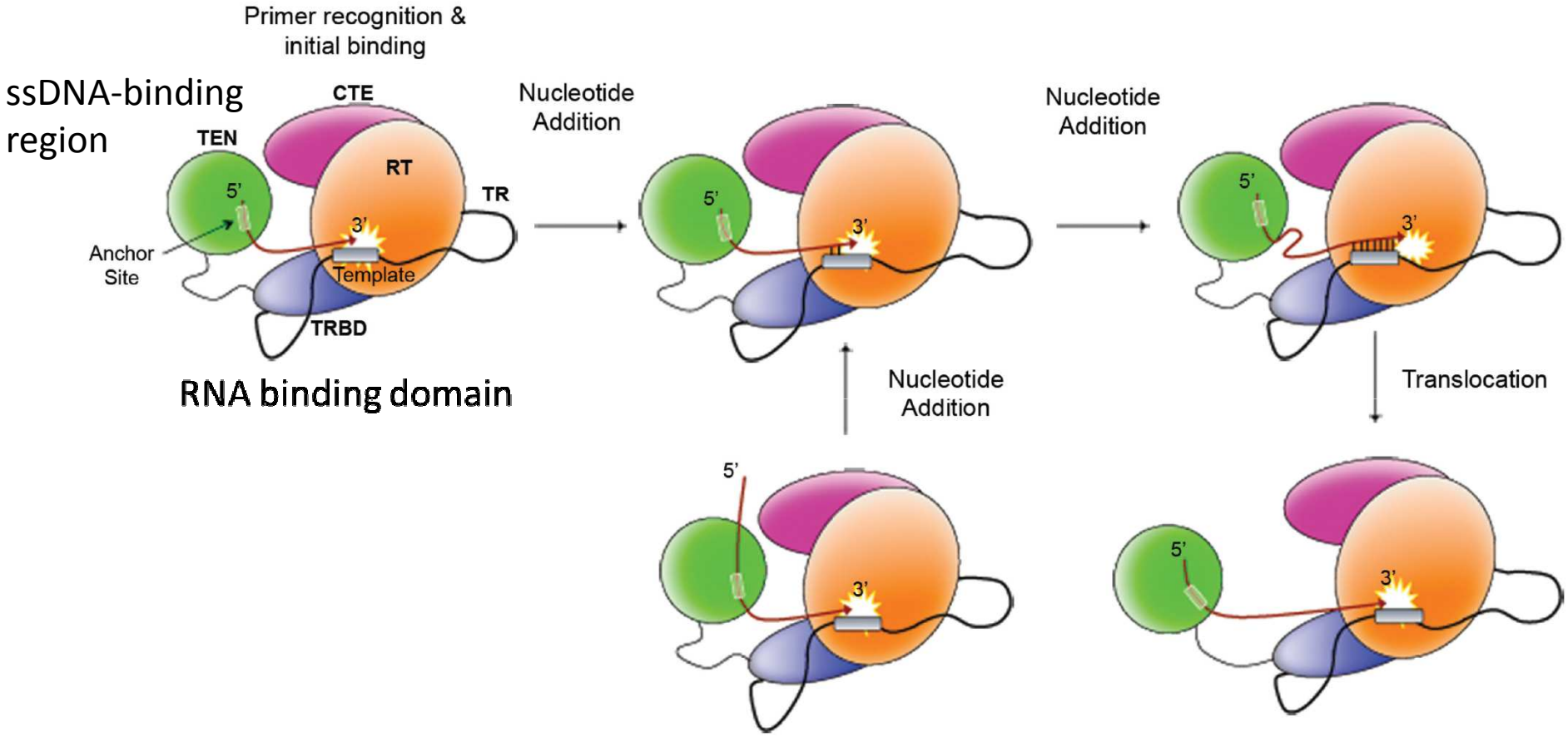
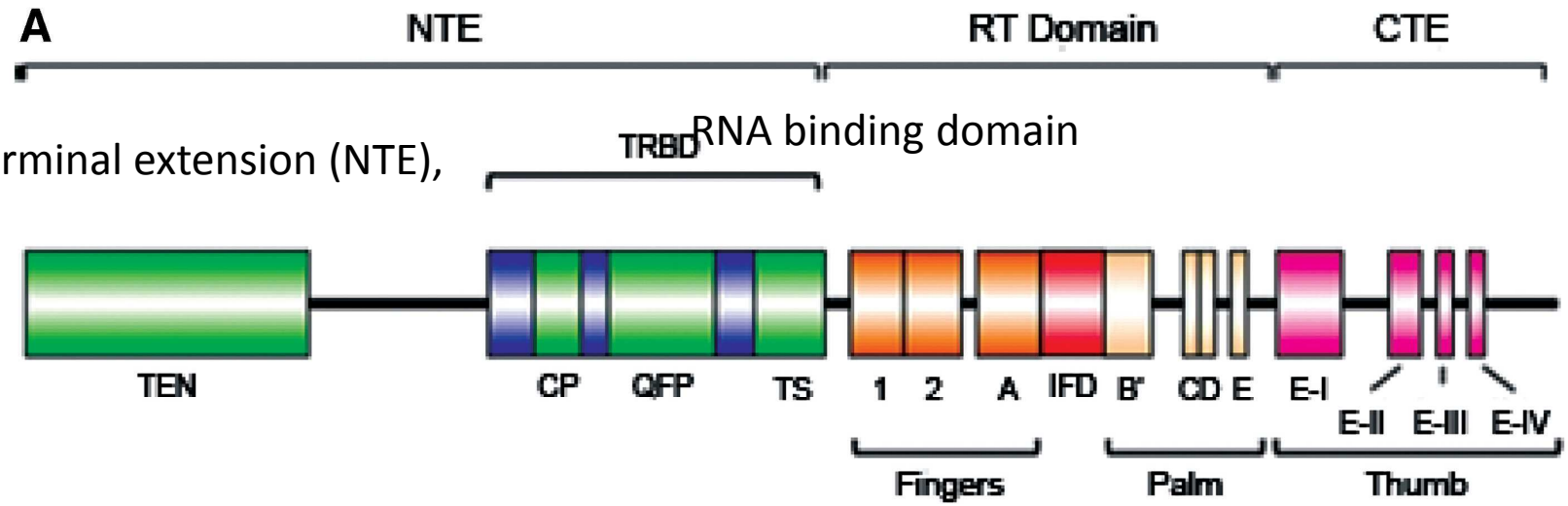
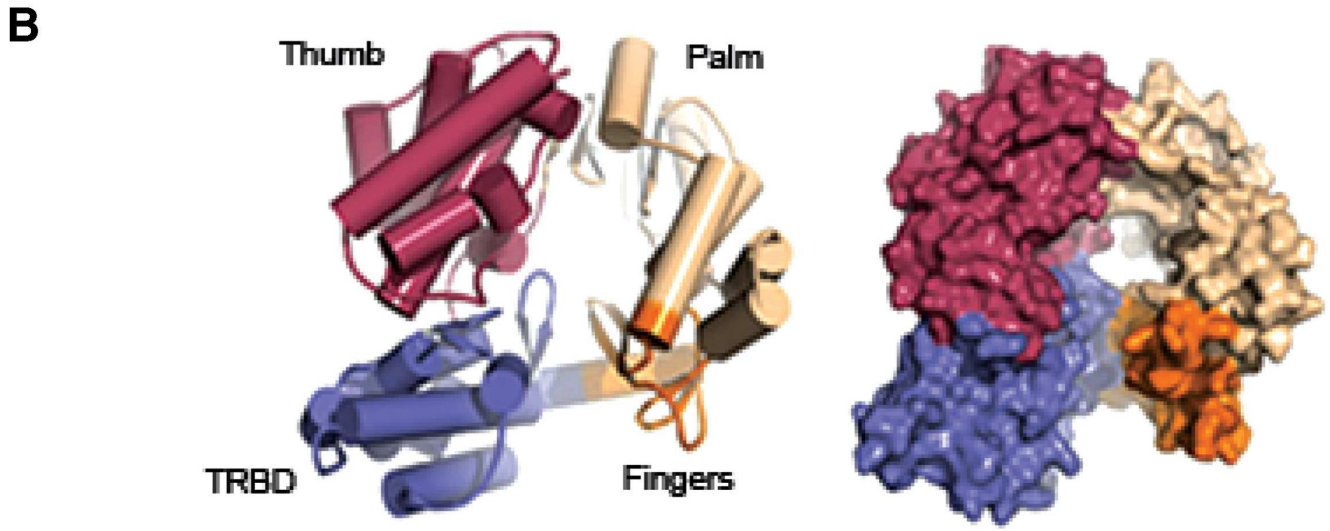


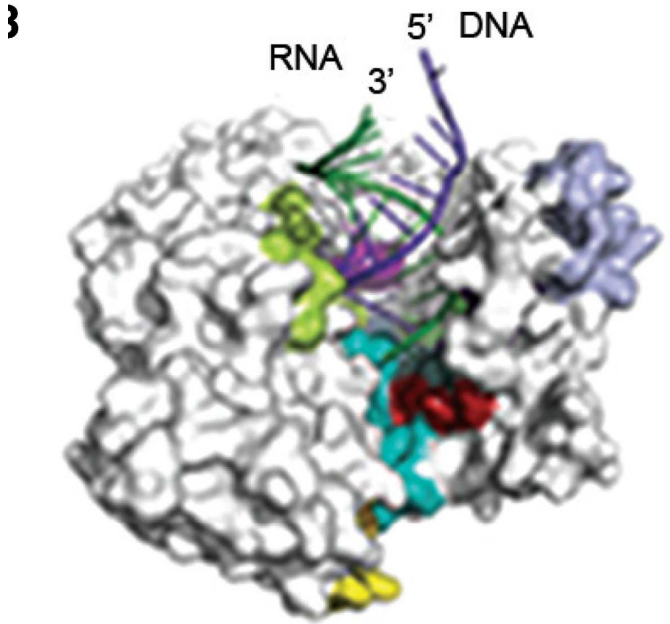
Fig. 4. Schematic representation of exons, introns, UTRs, and variants discovered. (*A*) *hTERT*. (*B*) *hTERC*. Green blocks, coding exons; white blocks, 5′ and 3′ UTRs; *previously unknown variants.

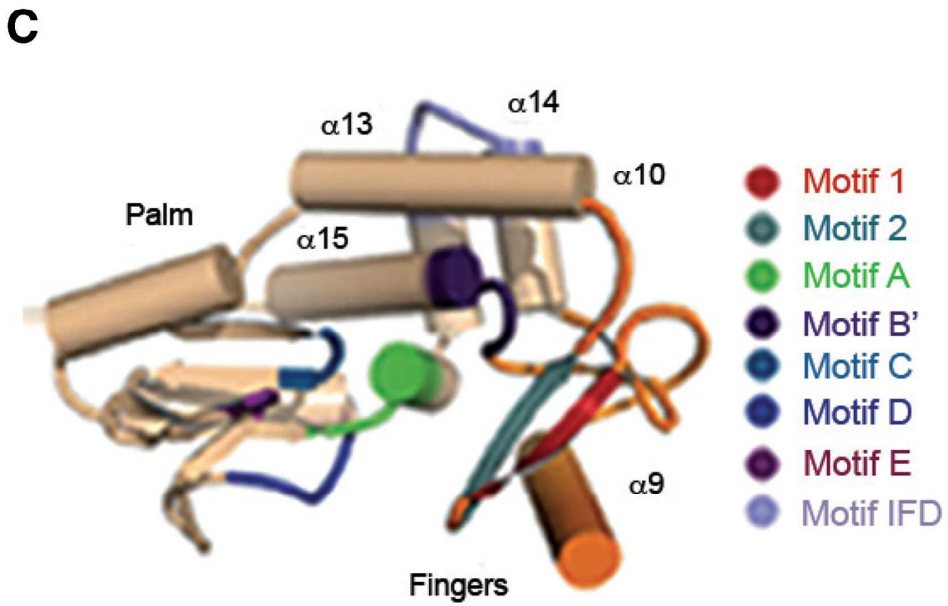

ssDNA-binding region
Primer recognition & initial binding
CTE
TEN
RT
TR
5'
3'
Anchor Site
Template
TRBD
RNA binding domain
Nucleotide Addition
Nucleotide Addition
Nucleotide Addition
Translocation

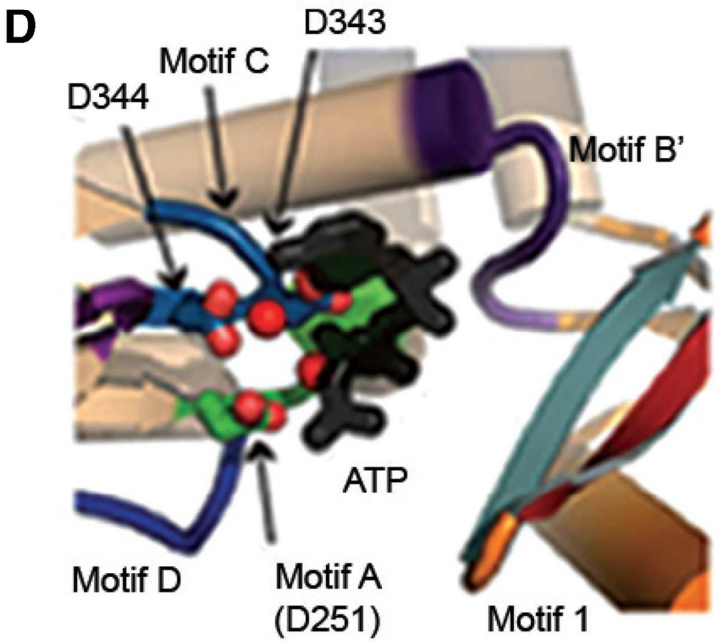


N-terminal extension (NTE), RNA binding domain

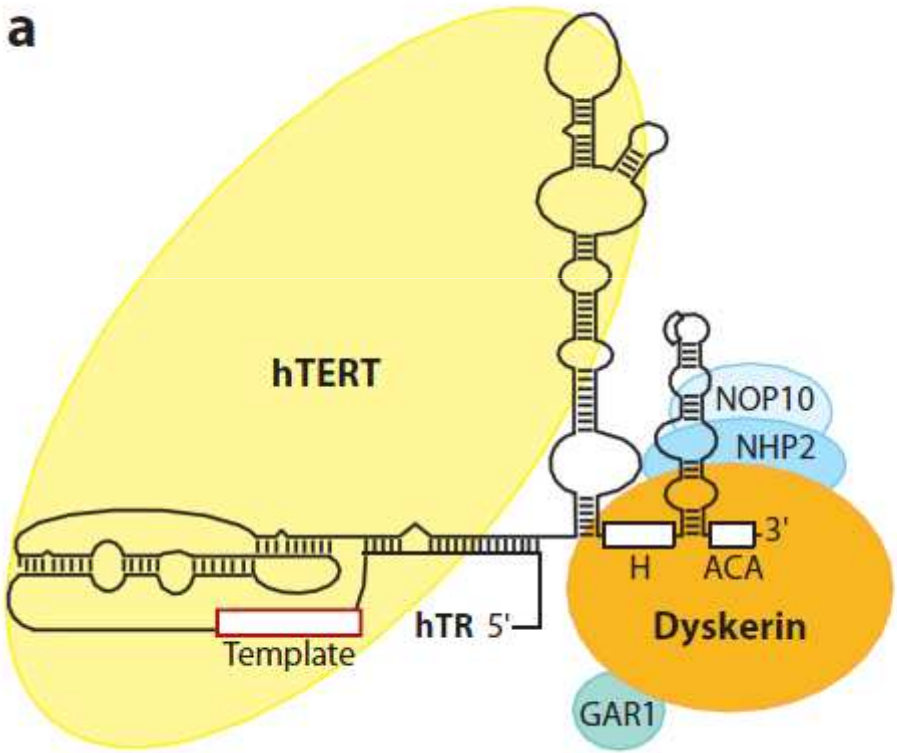



B
5' DNA
RNA
3'

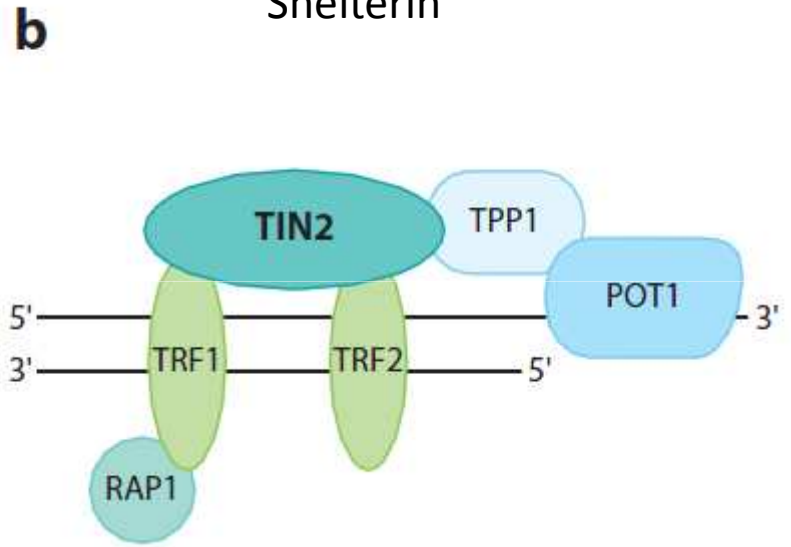

C
α13
α14
α10
Palm
α15
α9
Fingers
Motif 1
Motif 2
Motif A
Motif B'
Motif C
Motif D
Motif E
Motif IFD


D
D343
Motif C
D344
Motif B'
ATP
Motif D
Motif A
(D251)
Motif 1

Complessi macromolecolari associati al Telomero ed alla Telomerasi



Shelterin



The ribonucleoprotein dyskerin (DKC1 gene) is important for the RNA component folding and stability.
NOP10, NHP2, and GAR are other proteins that associate with the telomerase complex; these proteins are important for TERC molecule stabilization and also play a role in ribosome biogenesis and messenger RNAprocessing.

Mutations in telomerase and telomere components lead to syndromes of telomere shortening.
(a) The essential telomerase components. hTERT utilizes the template provided by hTR to add new telomeres onto the ends of chromosomes.

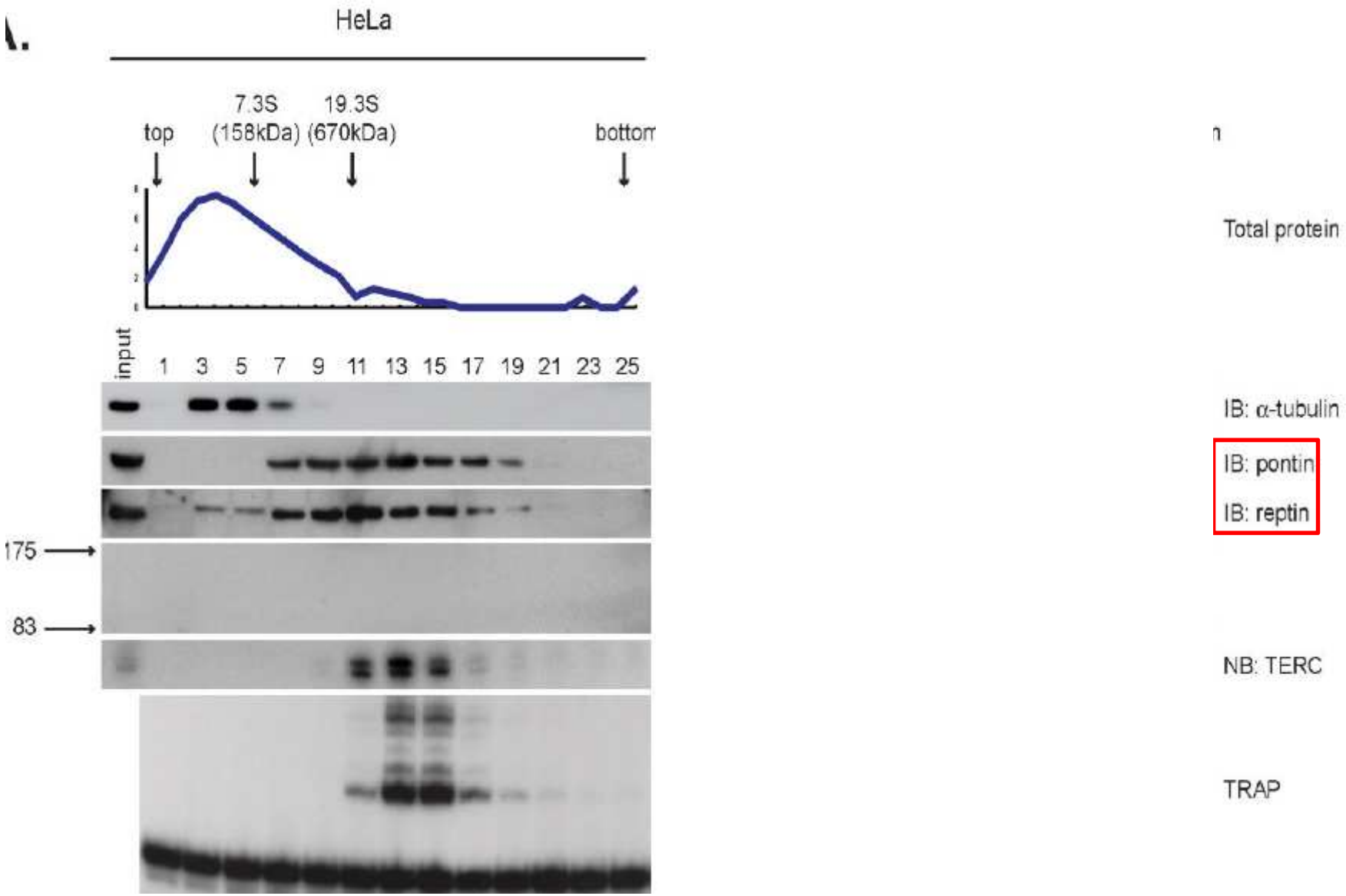
hTR is a 451-nucleotide RNA which contains a box H/ACA motif at its 3 end.
The box H/ACA motif is essential for hTR stability and for its assembly with hTERT.
These functions are mediated by the presence of the box H/ACA-binding dyskerin complex, which is composed of four proteins:
dyskerin, NOP10, NHP2 and GAR1.

Loss-of-function mutations in hTR, hTERT, DKC1, and likely NOP10
and NHP2 lead to a decrease in available telomerase dose and accelerated telomere shortening.

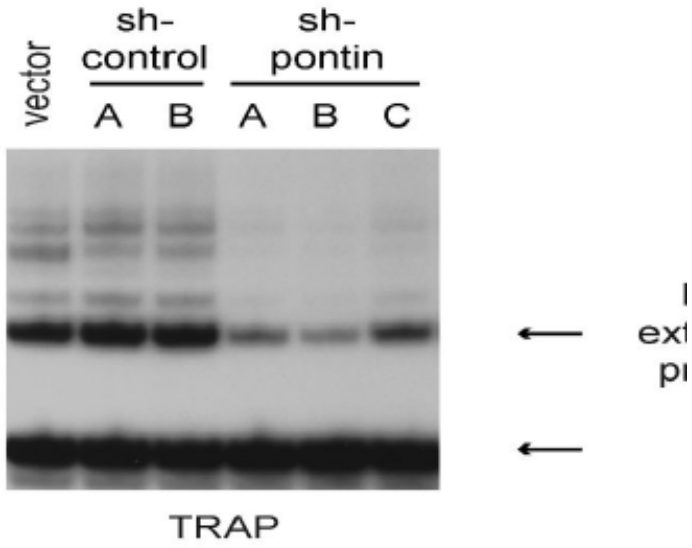
Few other proteins are known to be required for human telomerase function, significantly limiting our understanding of both telomerase regulation and mechanisms of telomerase action.

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

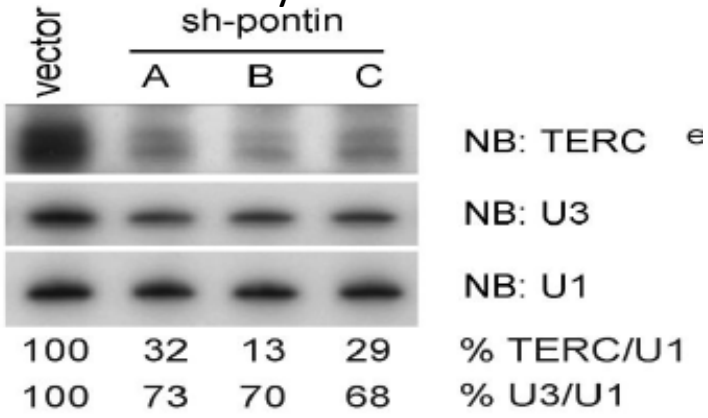
Pontin interacts with both TERT and dyskerin,


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



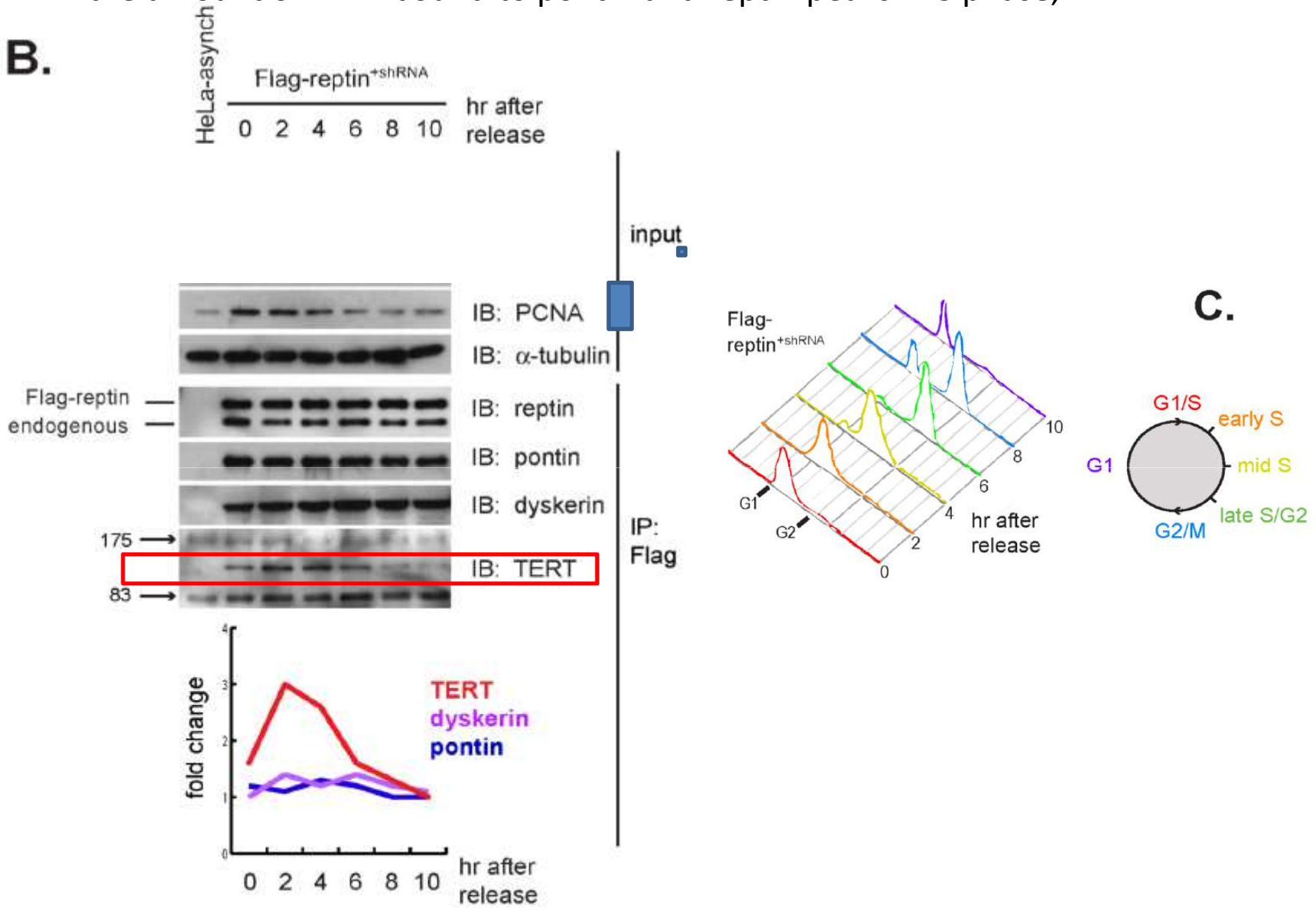
Depletion of pontin and reptin markedly impairs accumulation of the telomerase RNP, indicating an essential role in telomerase assembly.



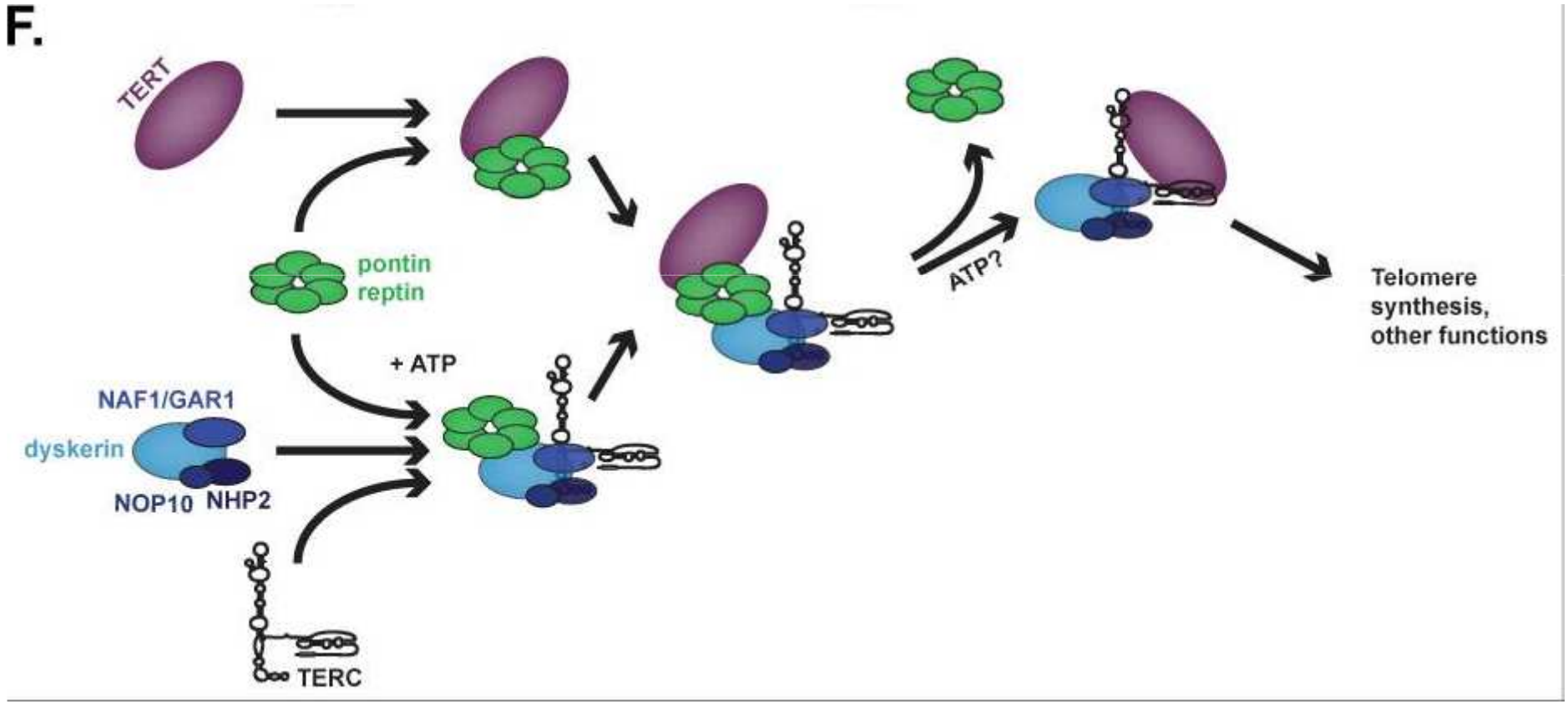
the amount of TERT bound to pontin and reptin peaks in S phase,
evidence for dynamic cell cycle-dependent regulation of TERT

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

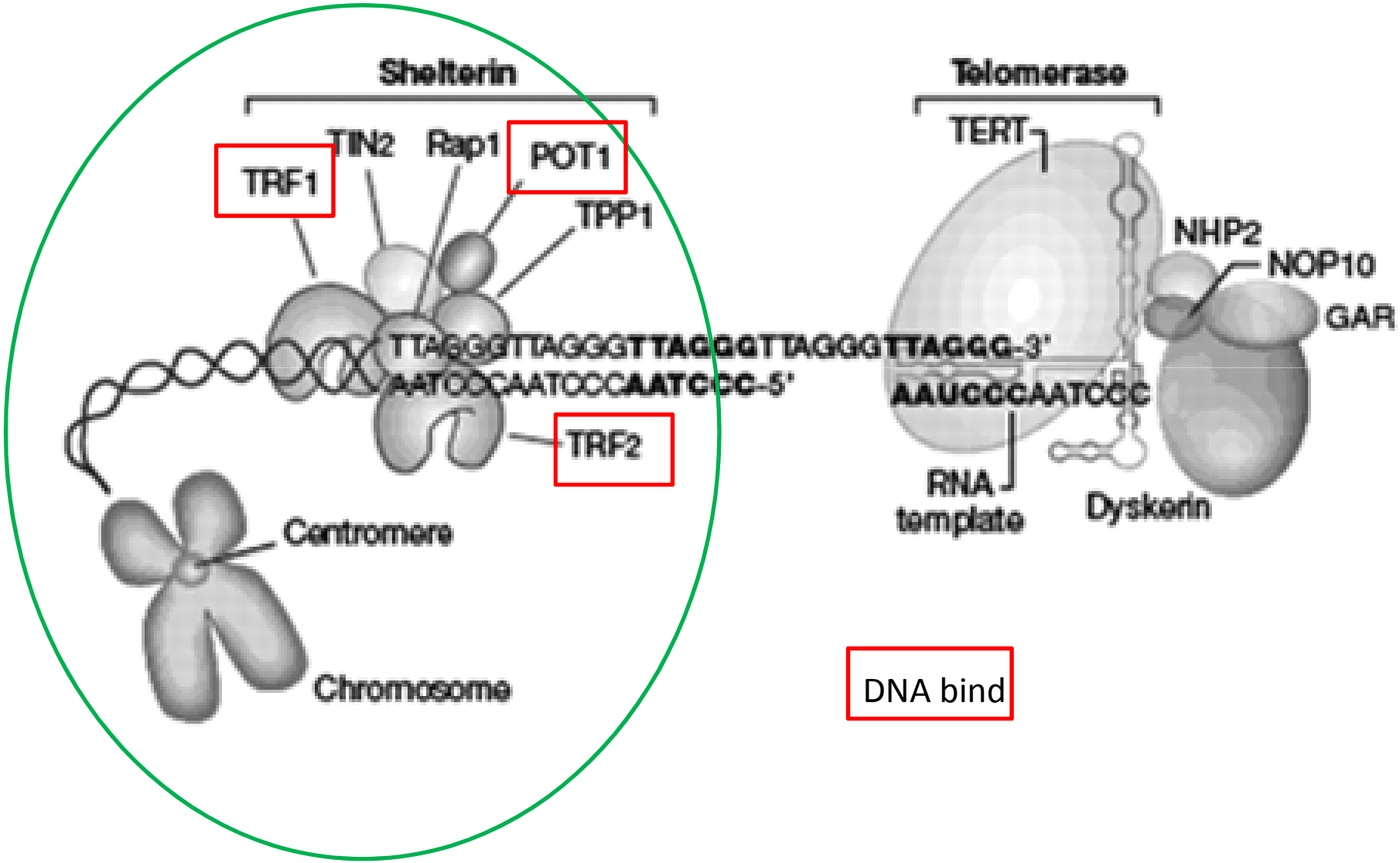
the amount of TERT bound to pontin and reptin peaks in S phase,



evidence for dynamic cell cycle-dependent regulation of TERT.


F.
TERT
pontin
reptin
+ ATP
NAF1/GAR1
dyskerin
NOP10
NHP2
TERC
ATP?
Telomere
synthesis,
other functions

Complessi macromolecolari associati al Telomero ed alla Telomerasi



Schematic representation of telomere structure and telomerase complex. Telomeres are at the extremities of chromosomes.
The telomeric 3' end terminates as a single-stranded, G-rich overhang. Telomeres are capped by a protein complex (TRF1, TRF2, TPP1, POT1, TIN2, and Rap1), collectively known as shelterin, that physically shield the DNA.

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.

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.