Telomeres and telomerases:
why should we care about the ends of chromosomes

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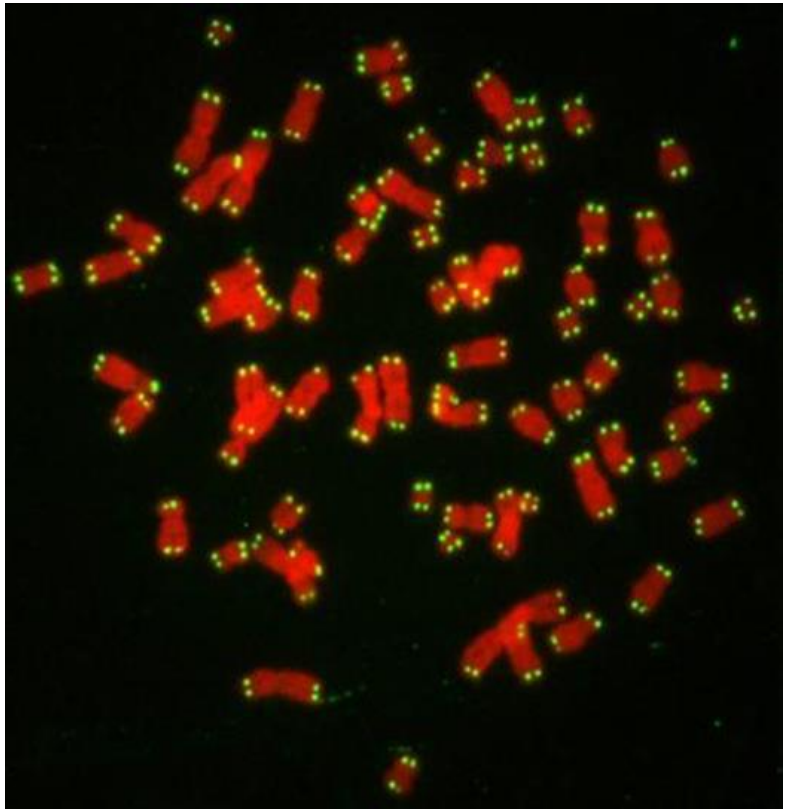
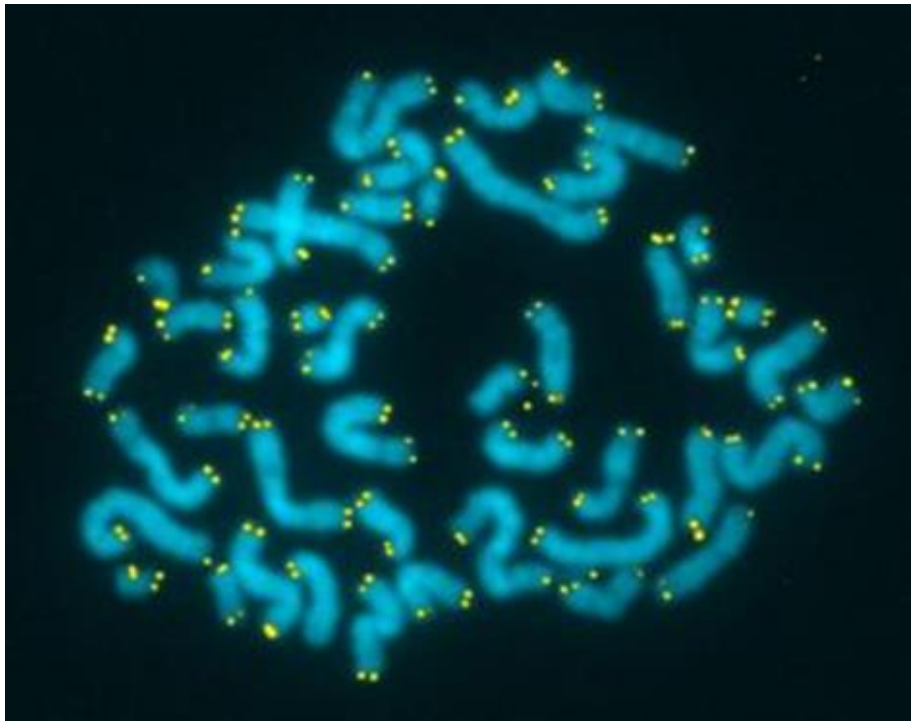
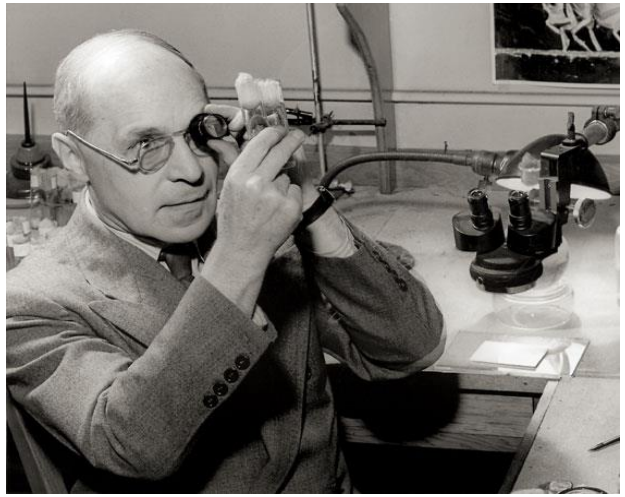
Outline:

1. **Principles of telomere biology and how these were found**
2. **Telomeres, telomerase, cancer and ageing**
3. **Telomere syndromes**
4. **Telomeres and telomerases in plants – what we can learn from our sessile companions**







H. J. Muller (1938) - Drosophila + X-rays
Chromosome ends differ from the rest of the chromosome – broken chromosomes tend to fuse, but not chromosomes with terminal parts. Specific „terminal genes“ with a protective function??

a term „**telomere**“(Greek words „*Telos*“ and „*Meros*“) – terminal+part

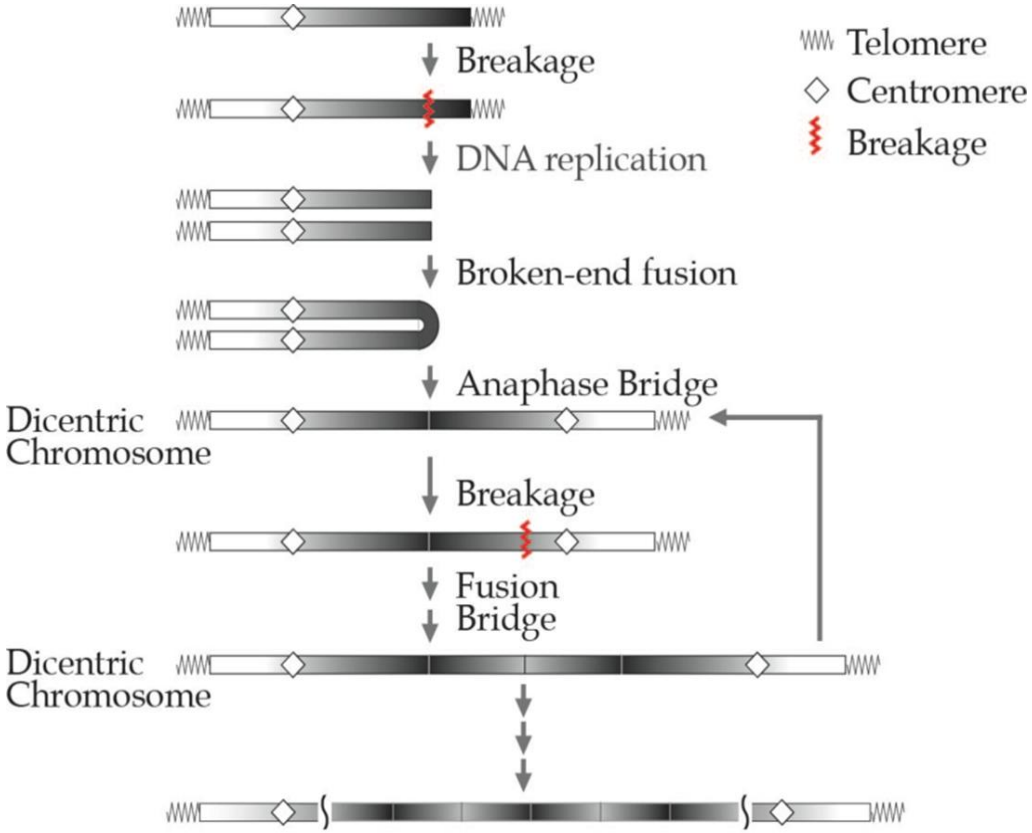
Nobel prize for X-ray mutagenesis

Barbara McClintock (1938-41) – maize, „**BFB cycle**“ (breakage-fusion-bridge)

terminal breaks do not tend to fuse

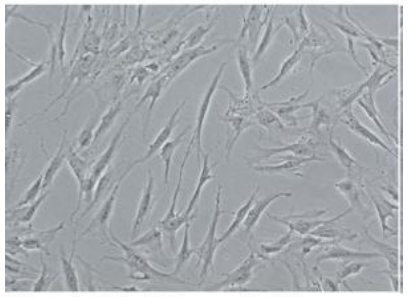
Are chromosome ends protected against fusion?

Muller and McClintock first defined so called **END-PROTECTION problem** solved by telomeres

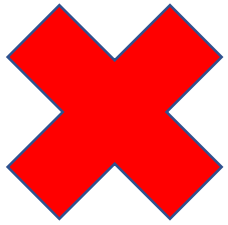


Human cells are not immortal ! ..... *Hayflick a Moorhead, 1961*

A. Human Foreskin Fibroblasts passage 28 (senescent cells)

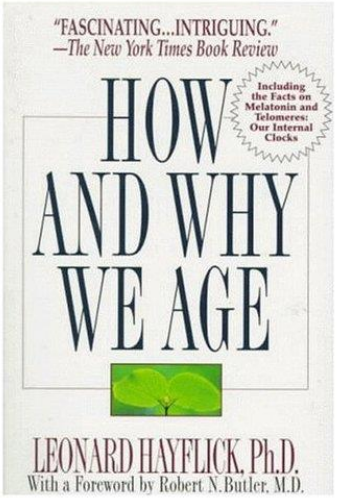


B. Human Foreskin Fibroblasts passage 5 (control)

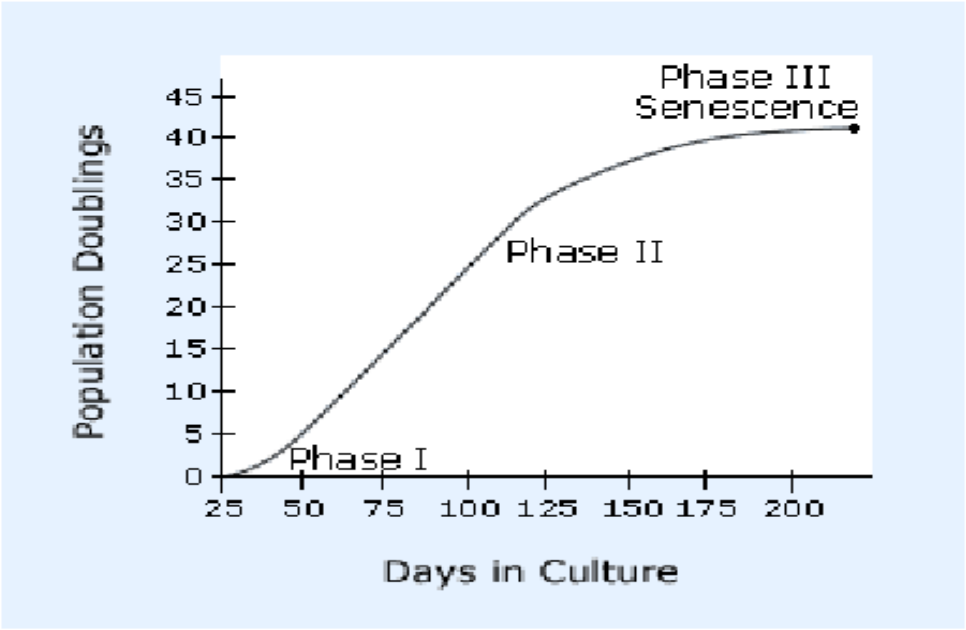


Alexis Carrel (1873-1944, Nobel Prize 1912):

- He derived a tissue culture from embryonic chicken heart and maintained it for 20 yrs:
- **animal cells are immortal in cell culture**

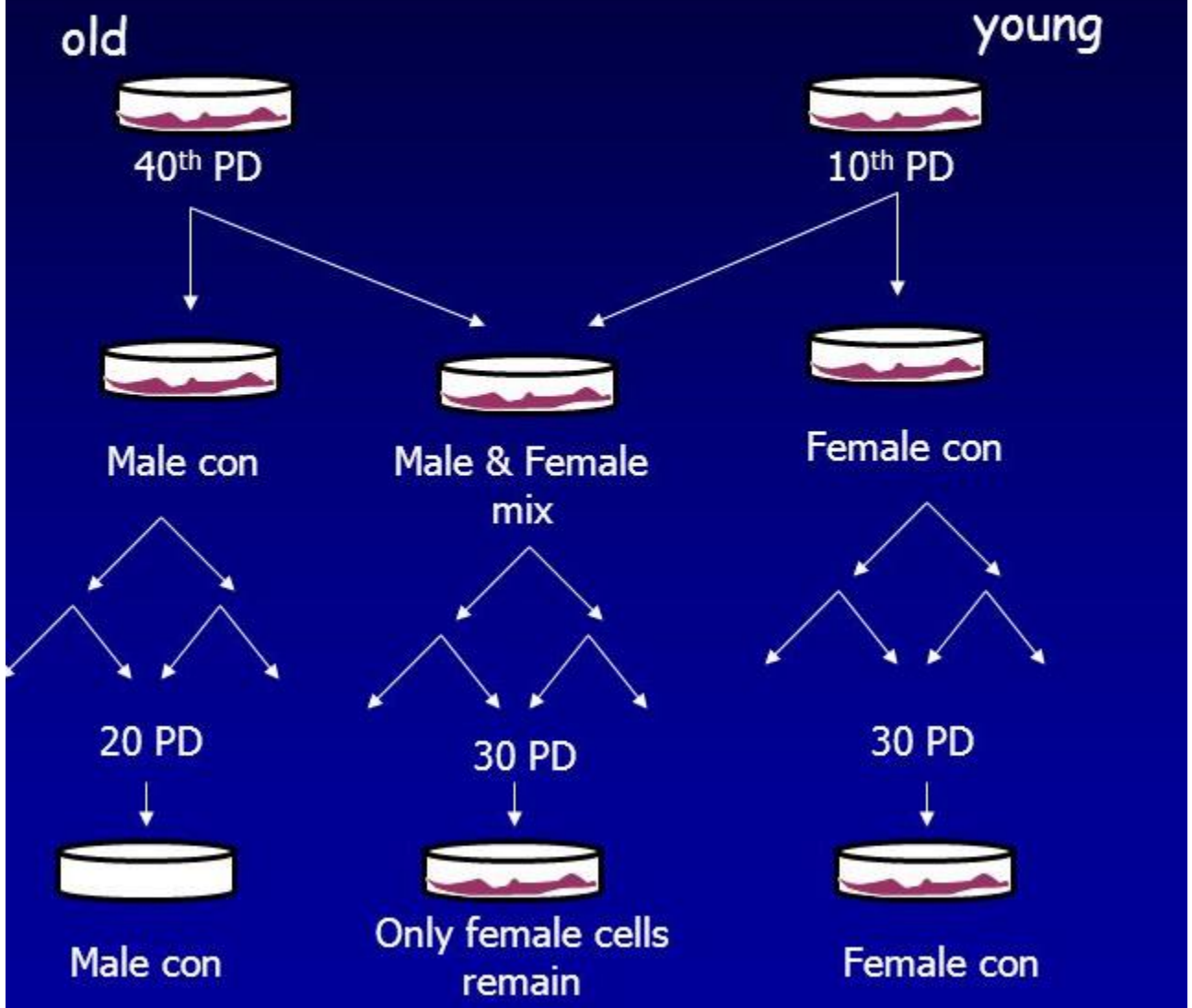


on the importance of having a good centrifuge ... (When preparing a growth medium from a tissue homogenate)



Explanation of Carrels “immortal” culture:
The daily feeding of nutrient was continually introducing new living cells to the alleged immortal culture

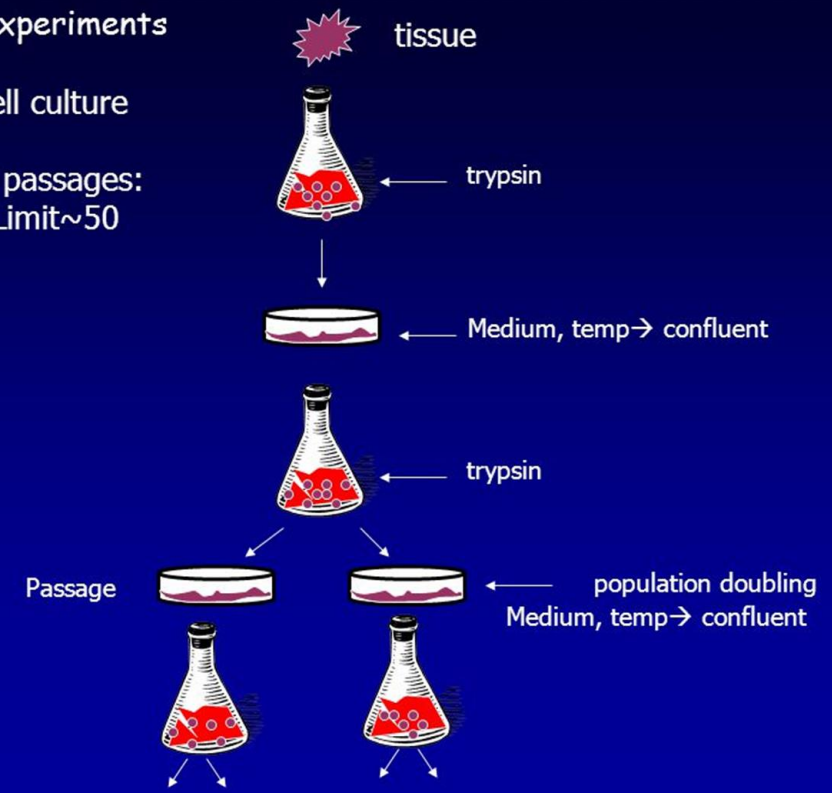
Hayflick Experiments: Male & Female Fetal Cells

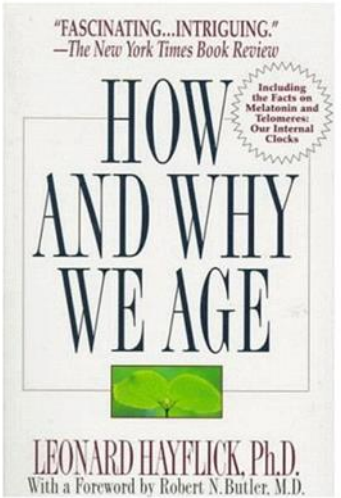


Hayflick's experiments

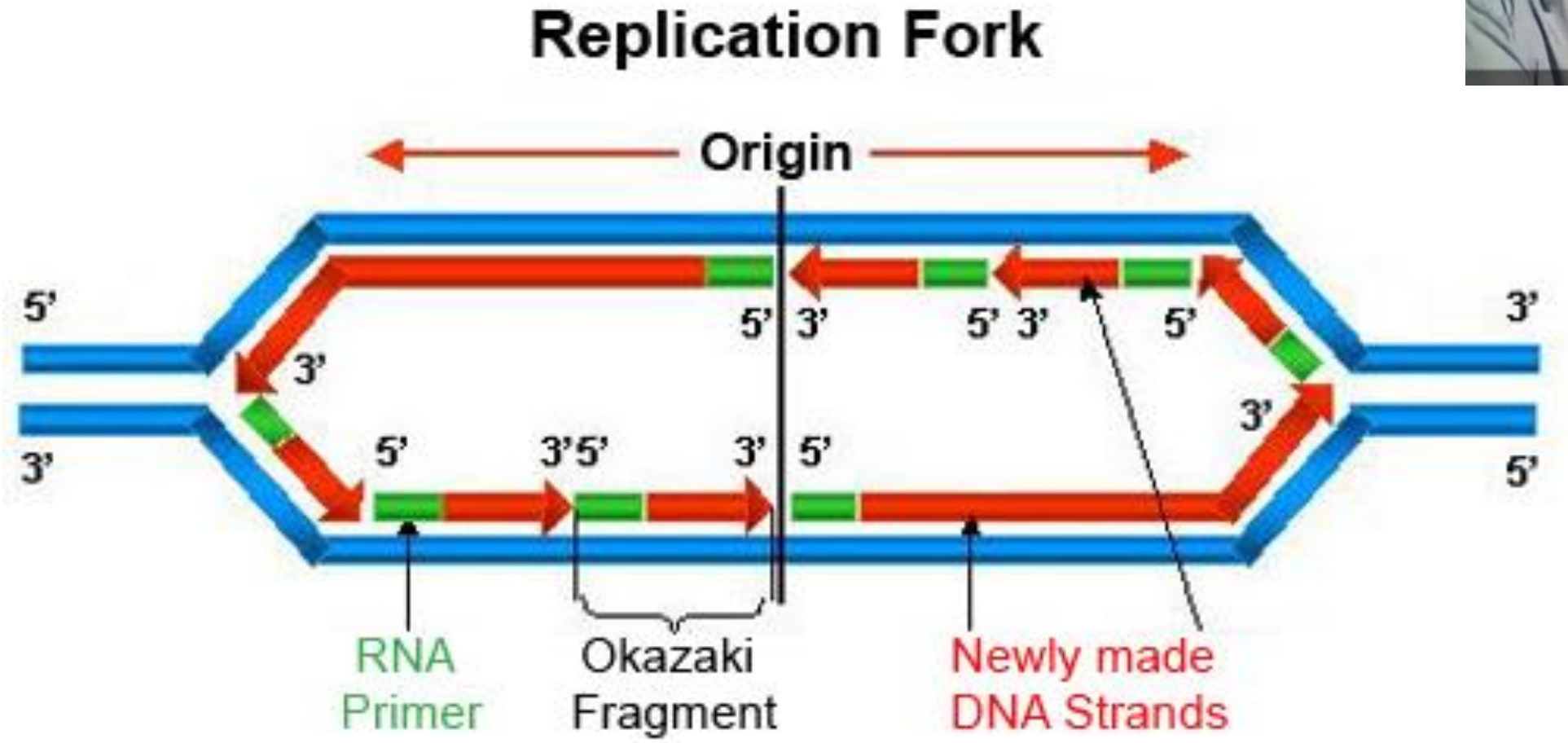
In vitro cell culture

Finite # of passages:
Hayflick Limit~50

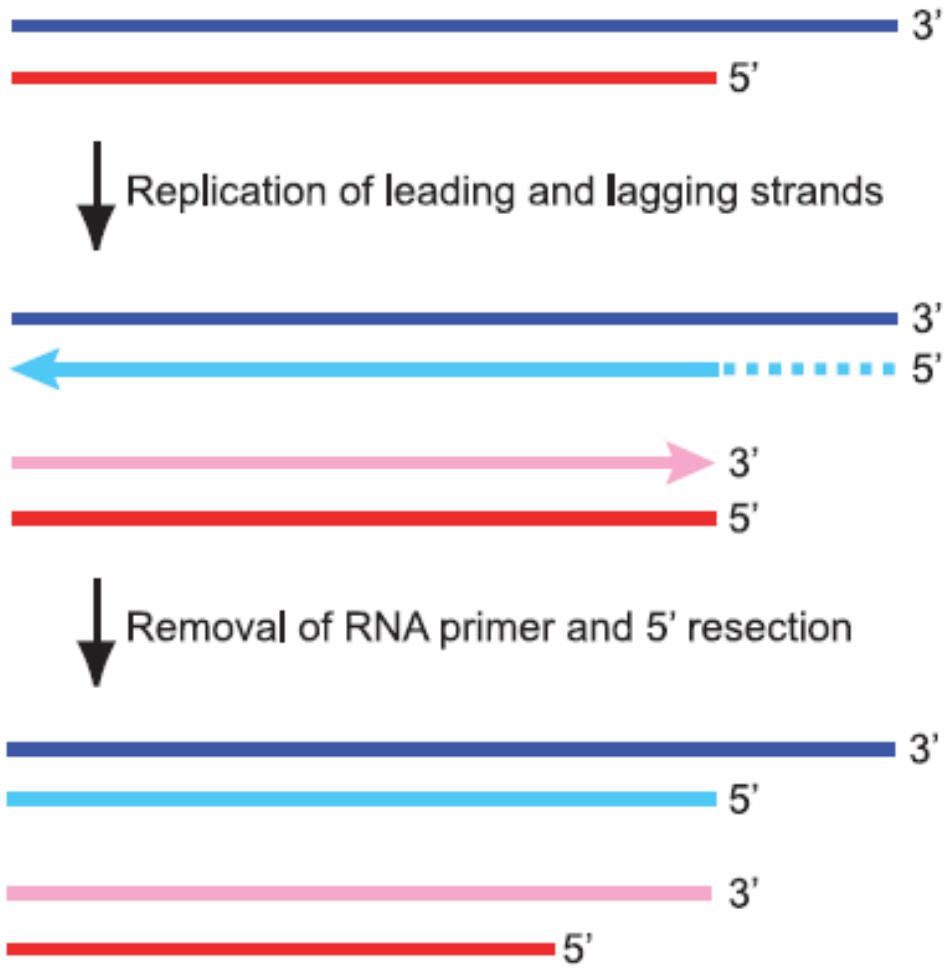




Connection between **Hayflick's limit** and knowledge of the mechanism of **DNA replication - End-replication problem** – termini of linear DNA remain unreplicated (Olovnikov, 1971)



End replication problem - *continued*



by lagging strand synthesis

by leading strand synthesis

DNA replication

Lagging strand

Leading strand

Ligation of Okazaki fragments

Lagging strand

Leading strand

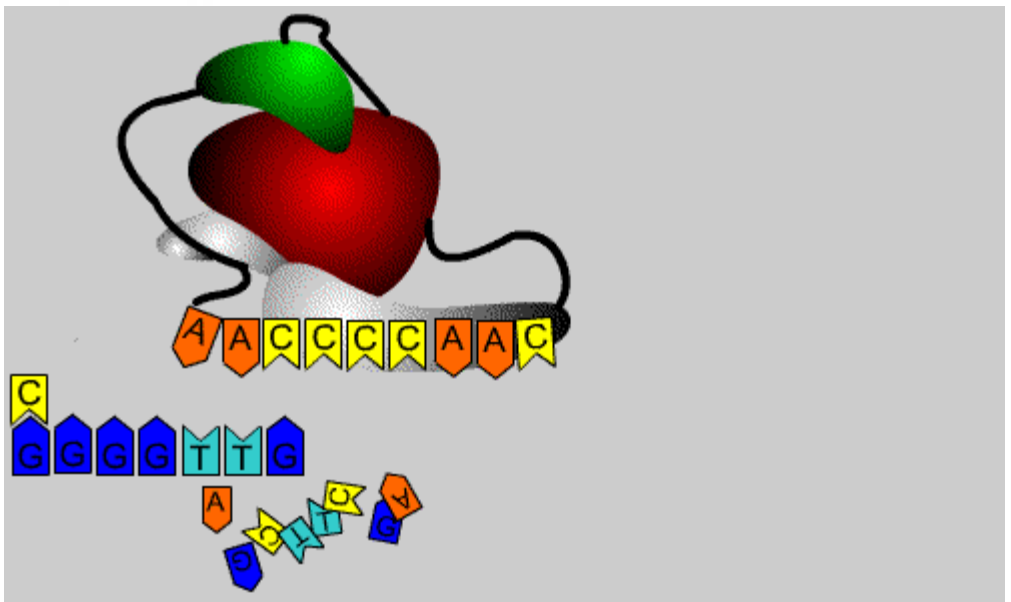
Osterhage and Friedman. 2009. JBC, 284, 16061-16065

Biology of telomeres goes molecular

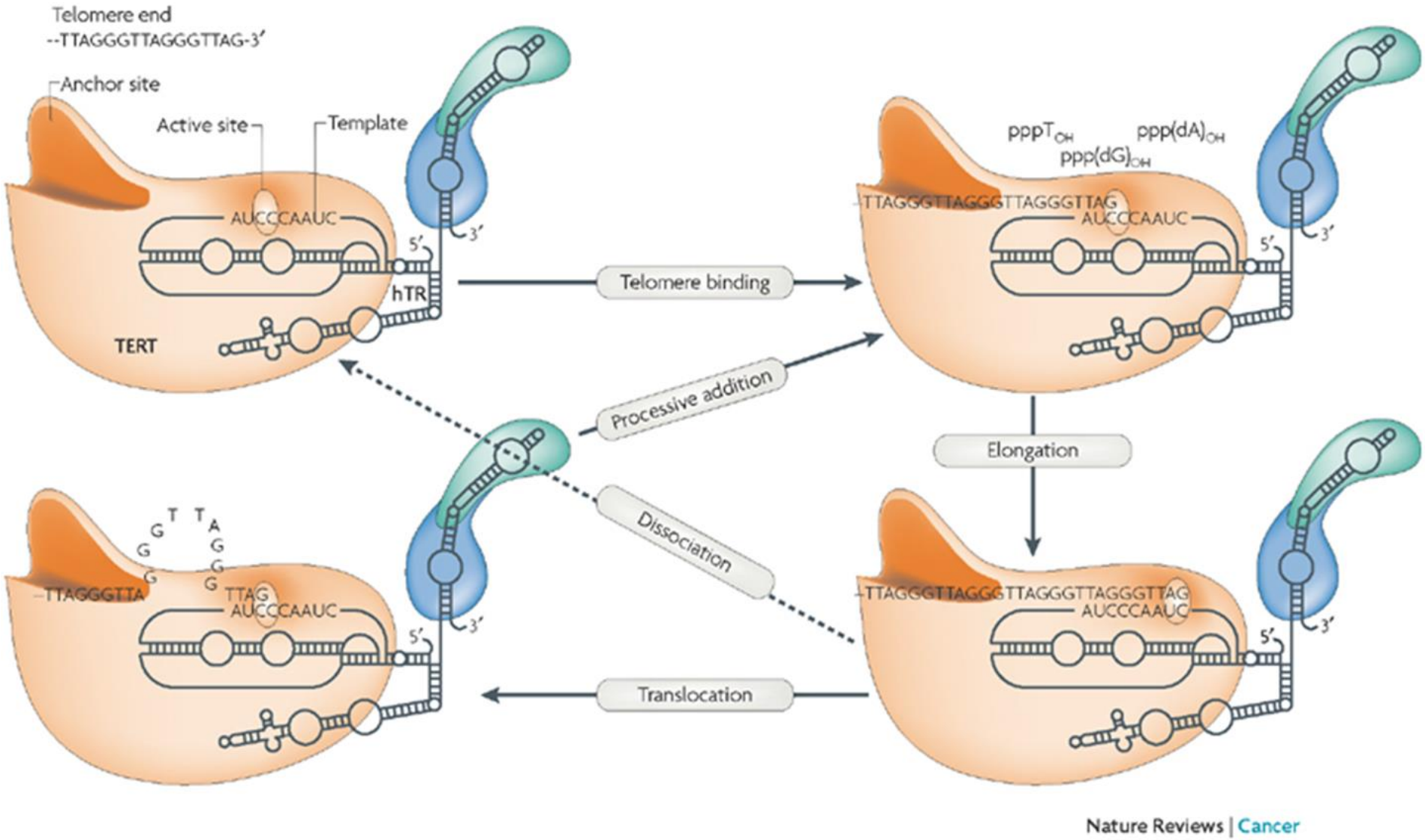
Telomere DNA of minichromosomes of protozoan *Tetrahymena* is composed of a **short tandem repeat DNA sequence - [TTGGGG]** (*E. Blackburn, J. Gall, 1978*)

Telomere synthesis is performed by an **enzyme whose activity is sensititve both to proteinase and RNase.** Telomere terminal transferase? (*C. Greider, E. Blackburn, 1985*).

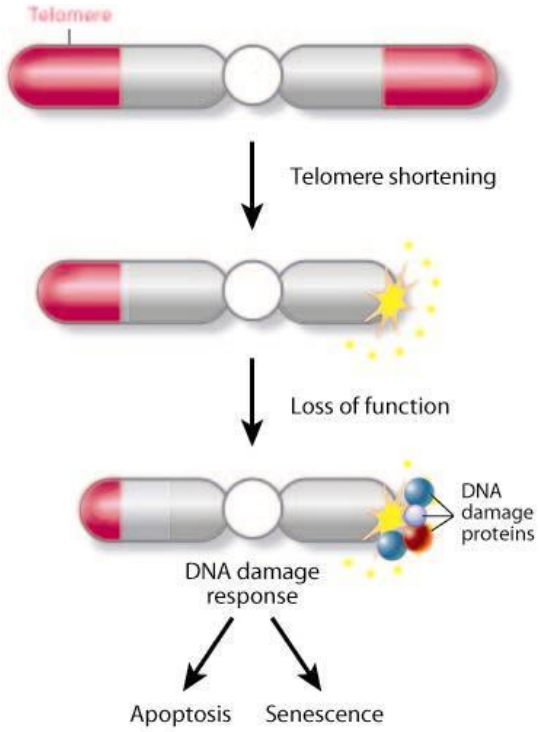
Telomere synthesis occurs as **reverse transcription, i.e.** from RNA to DNA. It is performed by **a complex of RNA and protein**, termed as **TELOMERASE** (*Greider & Blackburn, 1989*)


A A C C C C A A C
C
G G G G T T G
A

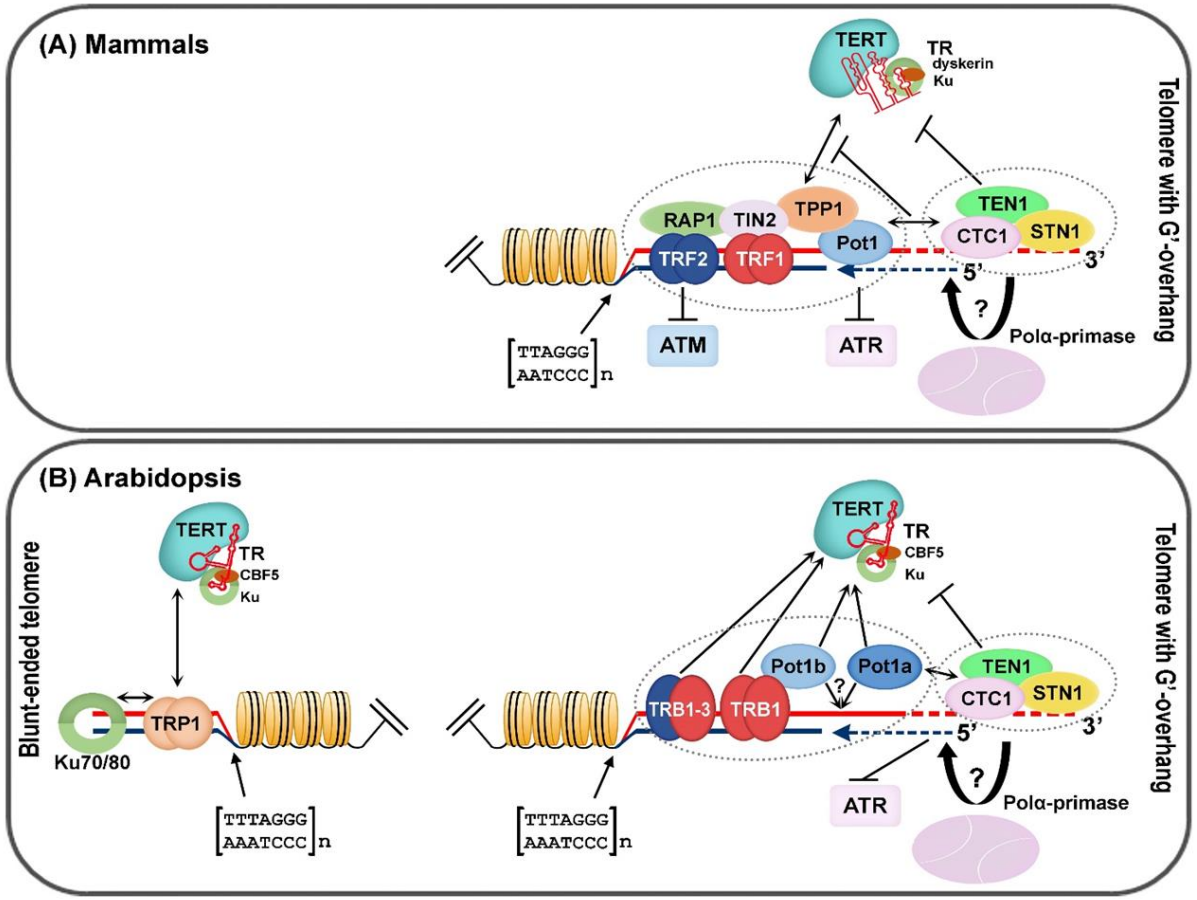
The most common way to solve the end-replication problem is telomere elongation by a specific telomere reverse transcriptase (RNA-protein complex) – **the TELOMERASE**
(C. Greider, E. Blackburn, 1985, 1989)



2. End-protection problem (Muller 1938, McClintock 1938,1941) –telomeres distinguish natural chromosome ends from unrepaired chromosome breaks. At the molecular level, specific telomere-binding proteins are responsible for this function (shelterin complex in mammals) – inhibition of DNA damage signalling and response

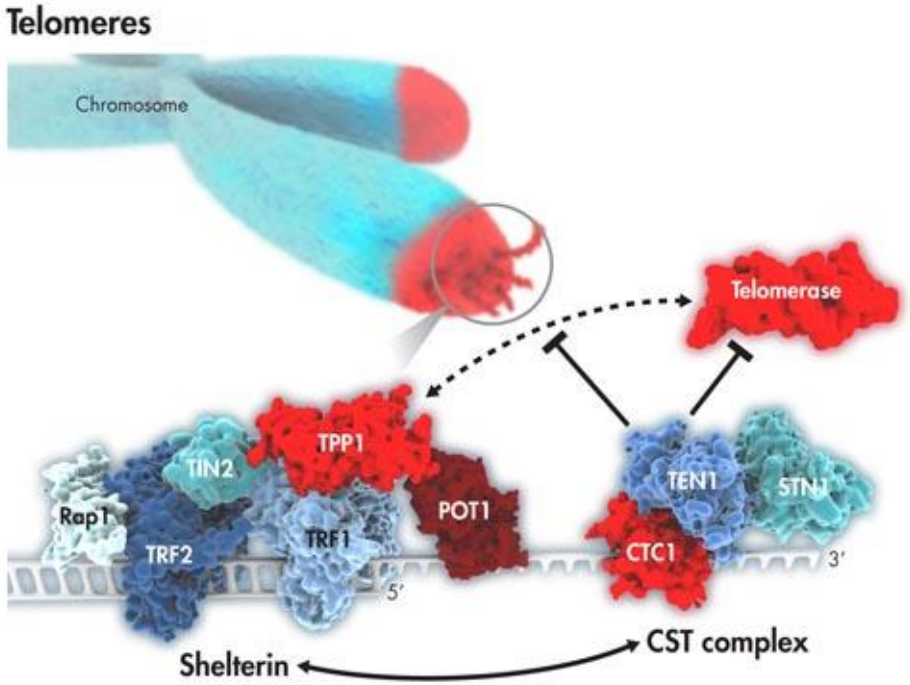


Armanios M. 2009.
Annu. Rev. Genomics Hum. Genet. 10:45–61

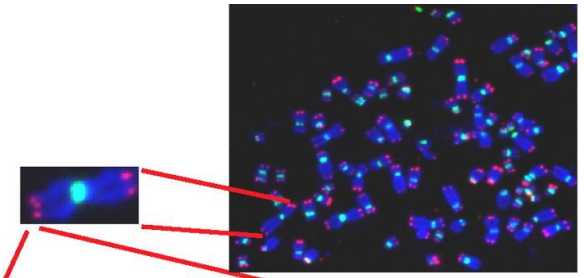


Prochazkova Schrumpfova et al., 2016

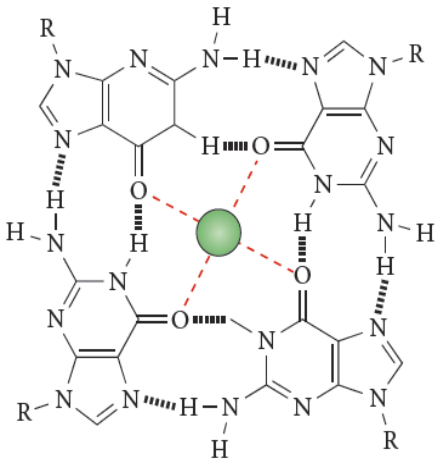
How the telomere looks like ? – a key role of proteins and their complexes (shelterin)

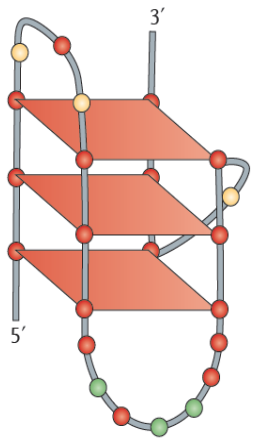


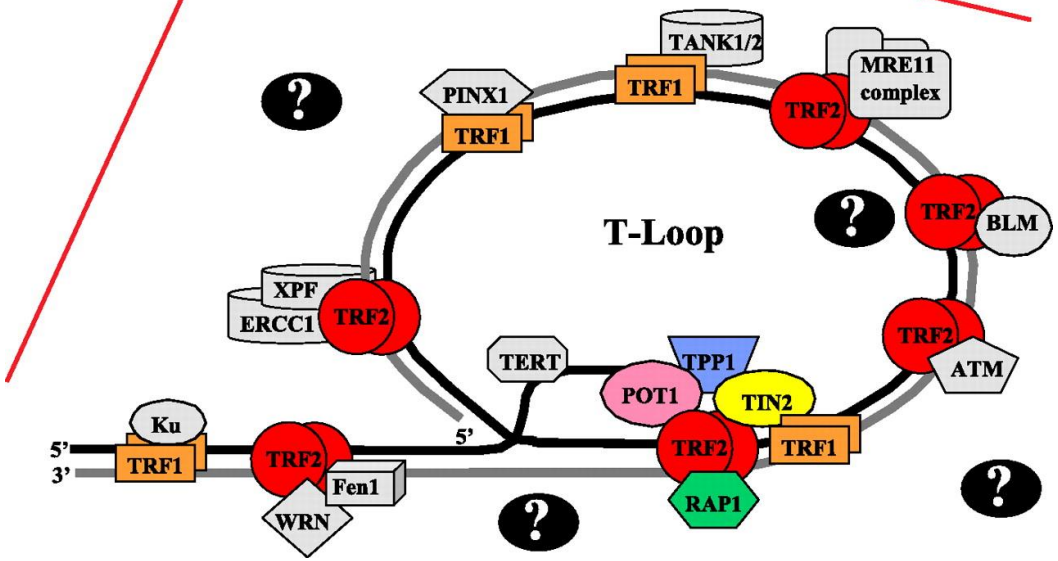
Titia de Lange, 2005



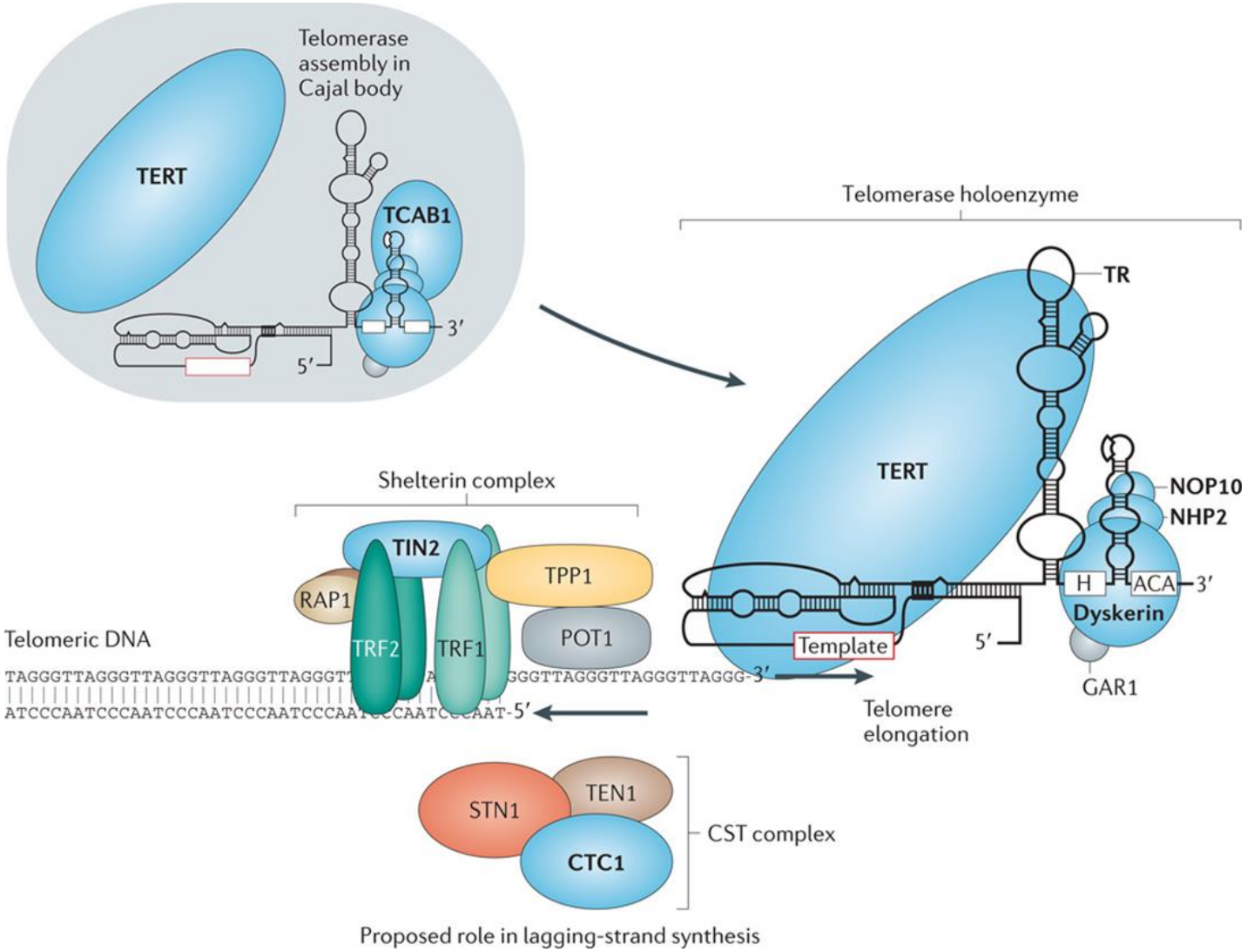
G – 4 structure of telomere DNA





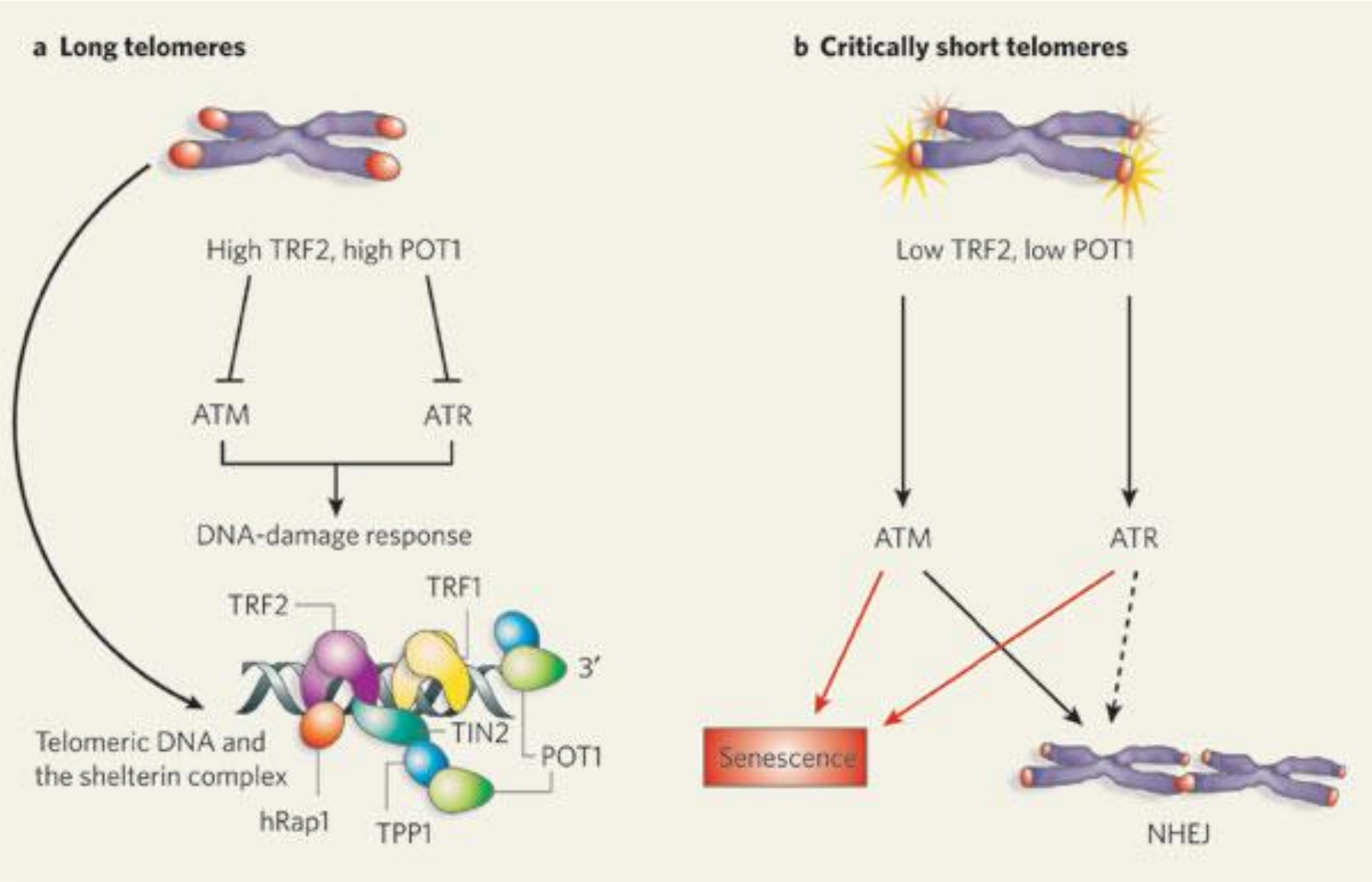


Both essential telomere functions are **interconnected,** i.e. a minimum telomere length maintenance (by telomerase) is required in dividing cells to form the end-protective telomere (shelterin) complex.



Nat Rev Genet. 2012; 13(10): 693–704.

Consequences of telomere shortening or dysfunction at the cellular level: replicative **senescence**, **apoptosis** (programmed cell death), **cancer** (when also control of genome integrity is dysfunctional - **p53, pRb, p16INK4A**



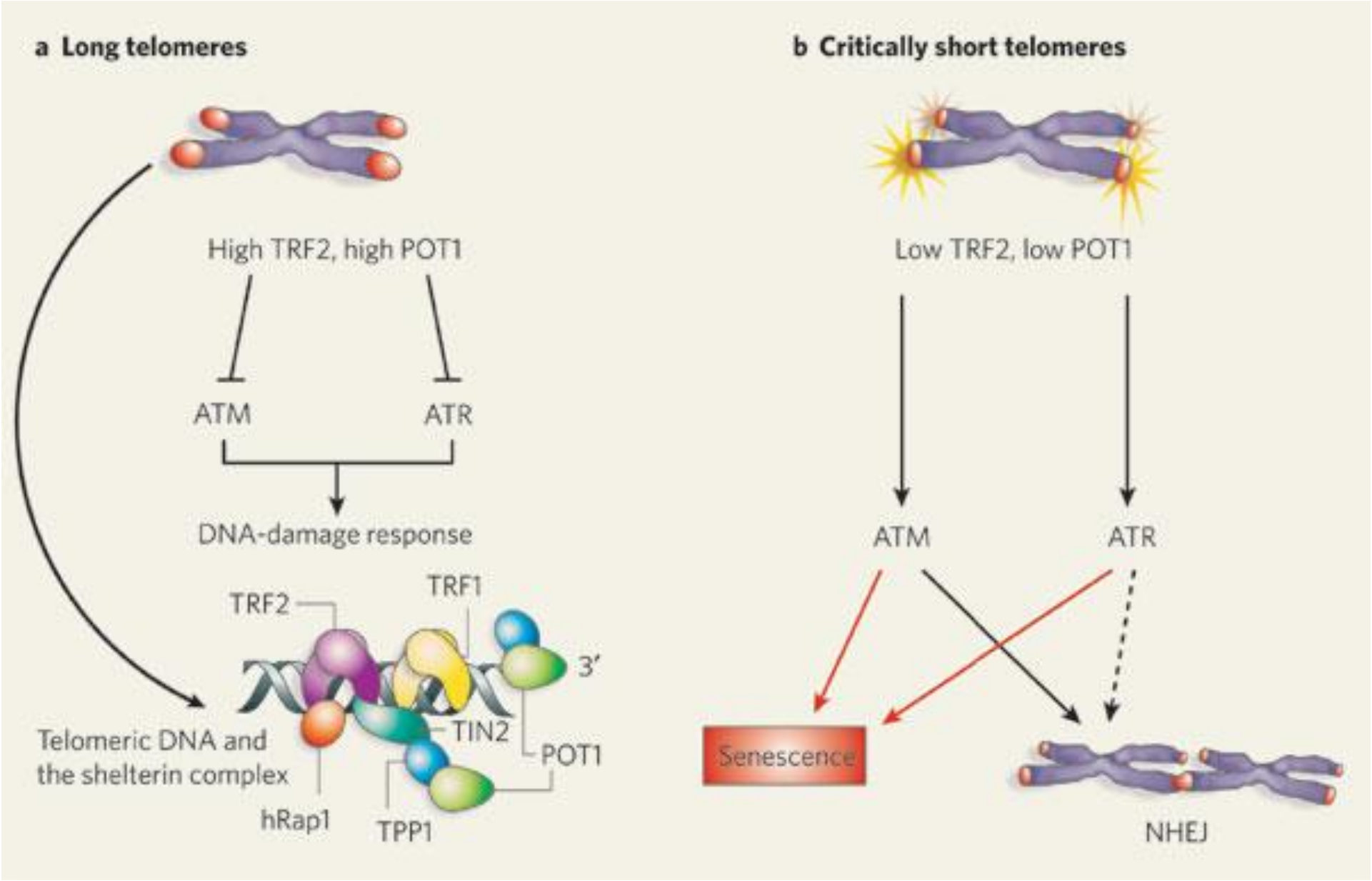
Basic knowledge on telomeres (summary of the first part)

Telomeres are **specific nucleoprotein** (chromatin) structures forming the ends of chromosomes. They protect chromosome ends from being mistaken for unrepaired chromosome breaks – 1st function: END-PROTECTION (specific telomere proteins binding to telomere DNA)

Telomeric DNAs are formed by “non-coding” DNA (usually short tandemly repeated units); therefore, their partial loss due to incomplete replication is NOT CRITICAL, even in the absence of a compensatory mechanism (telomerase, ALT)

Telomerase can elongate telomeres (*via* reverse transcription of its own RNA template), thereby resetting telomere clock (removing cell division limit – Hayflick limit) – telomerase solves the END REPLICATION problém (2nd function).

Telomeres, telomerase, aging and cancer



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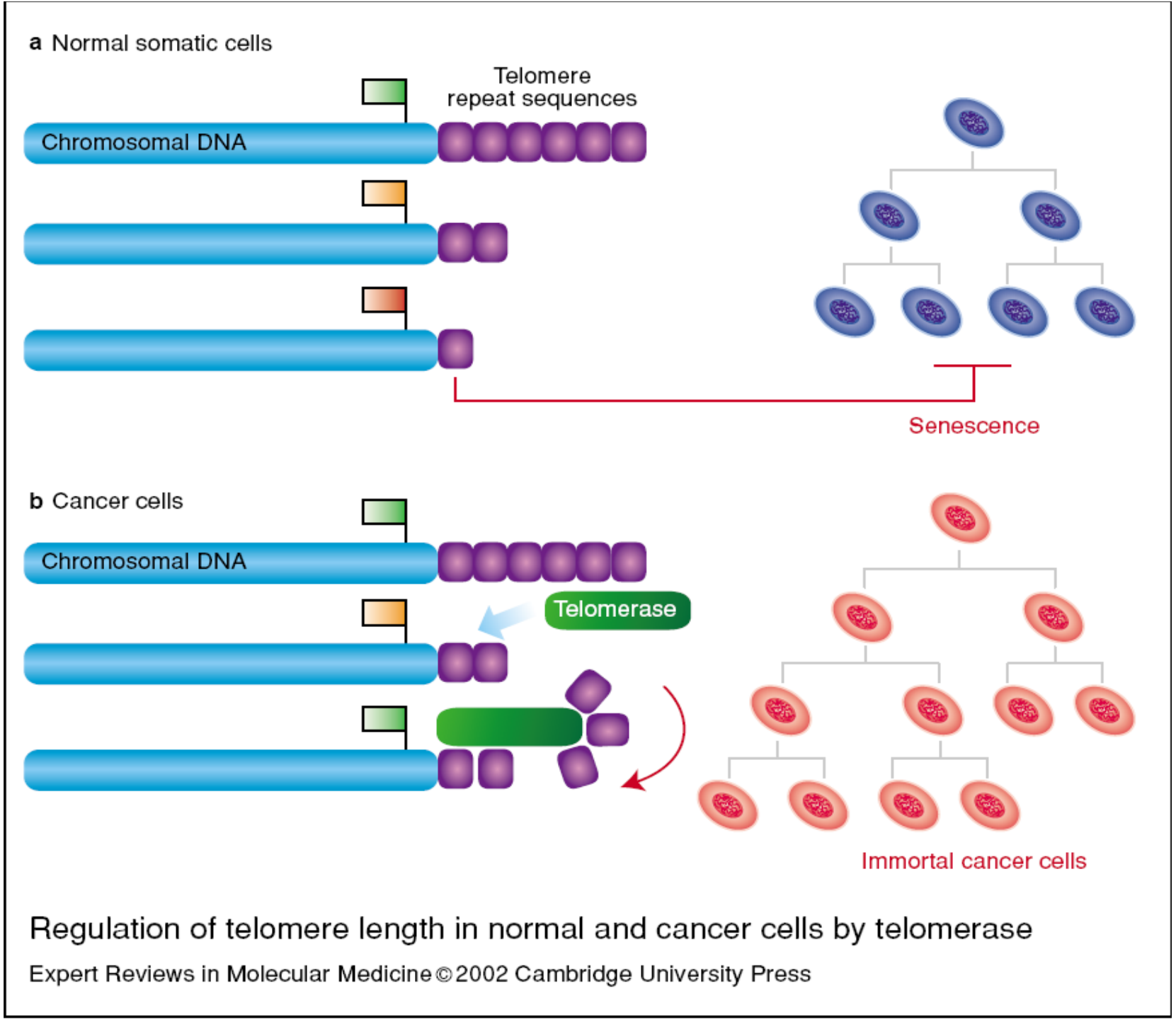
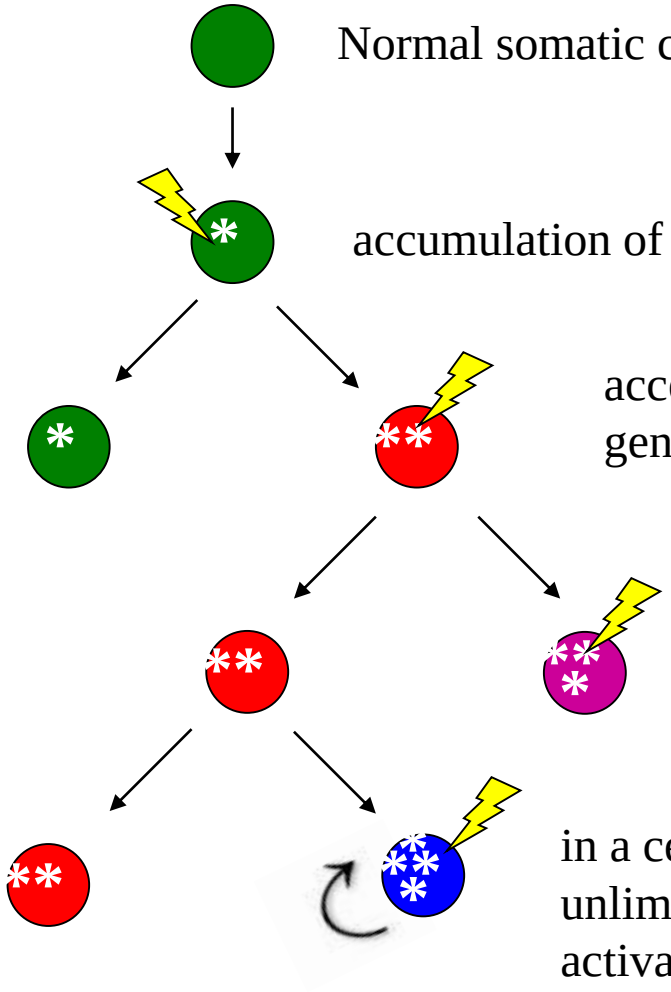


Regulation of telomere length in normal and cancer cells by telomerase

Clonal-evolution model



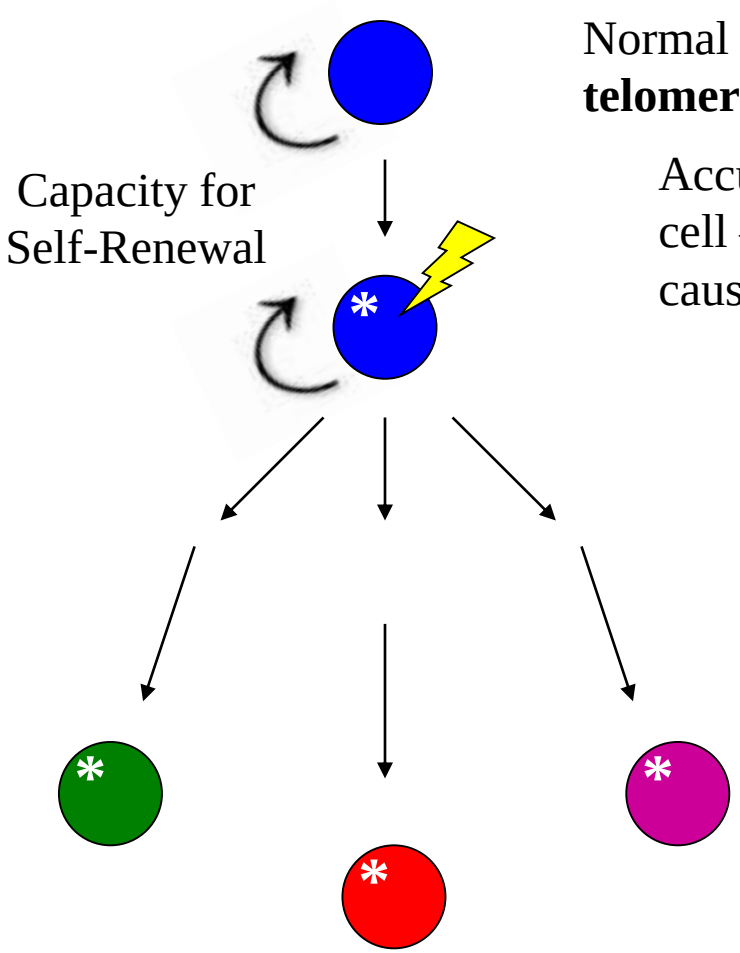
Normal somatic cell

accumulation of mutations, origin of tumor cells

accelerated proliferation of tumor cells, origin of genetically diverse clones of tumor cells

in a certain moment some of clones gains the ability of unlimited growth / self renewal (via telomerase activation, ALT activation)

Hypothesis of the origin of cancer from stem cells



Normal stem cells – gives rise to various cell types – **active telomerase**

Accumulation of several mutations – tumor stem cell – only these cells can cause metastases or cause tumors after transplantation

Majority of tumor cells does not possess the stem cell character. They may gain and accumulate other mutations, but have just limited proliferation capacity.

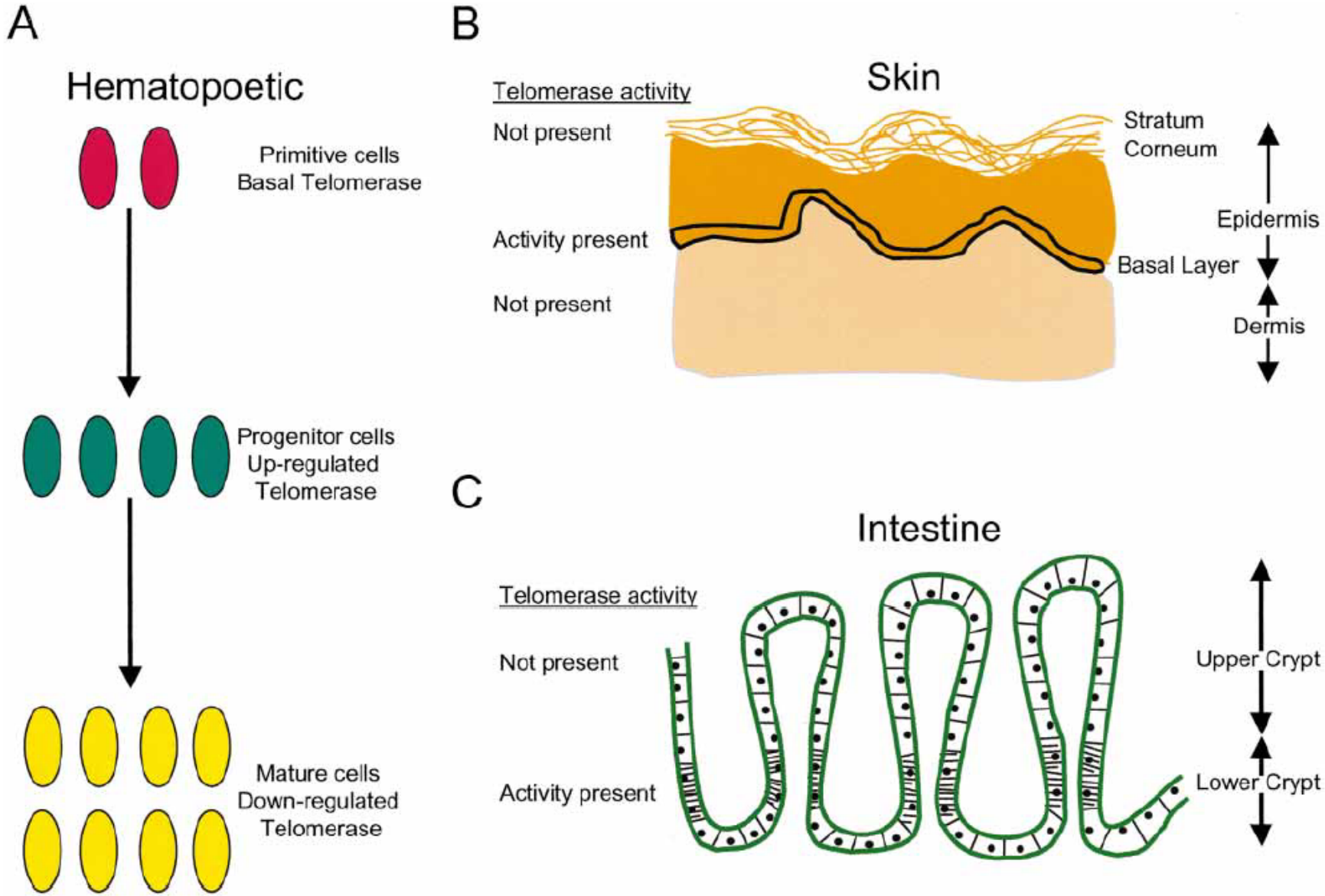
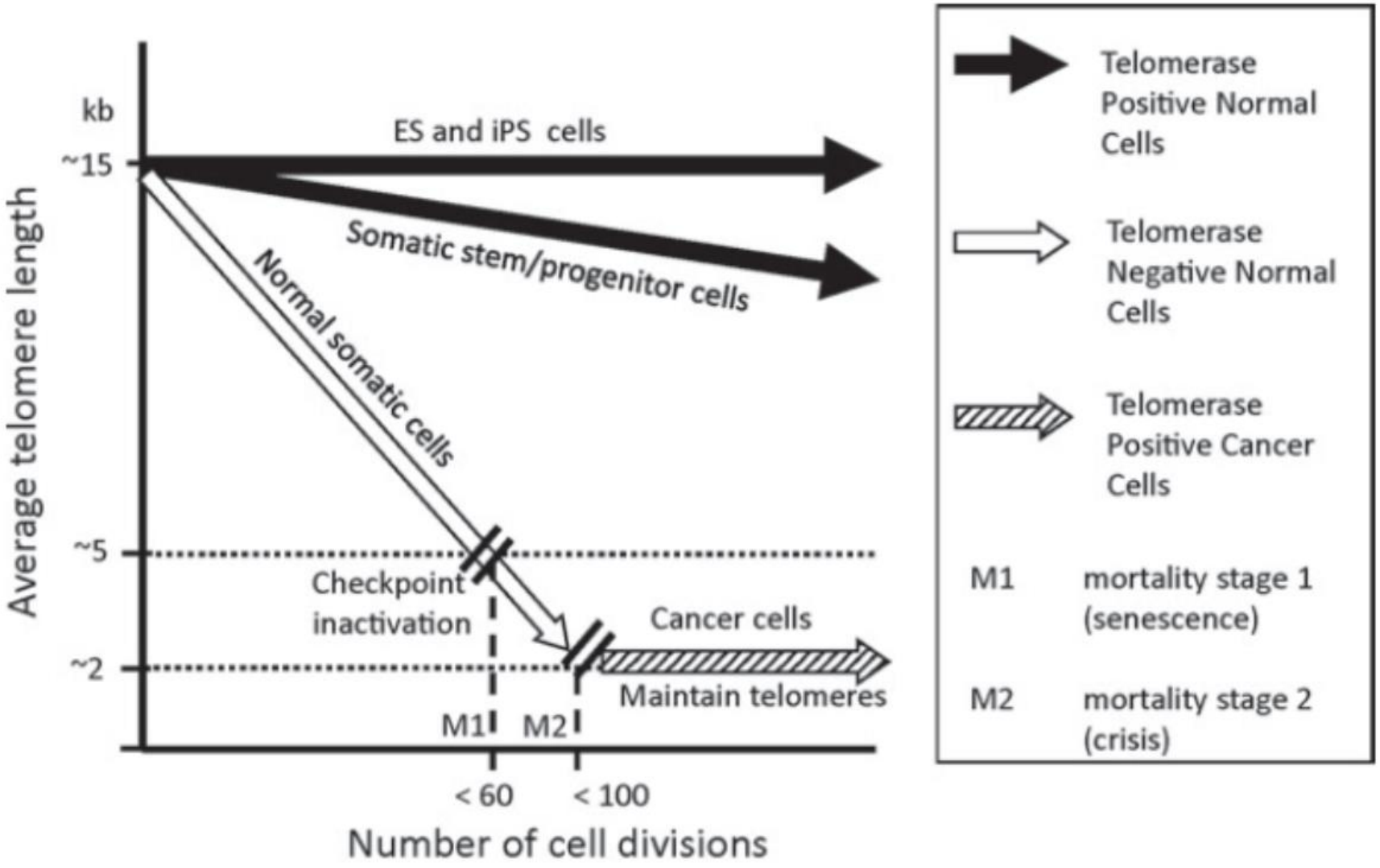


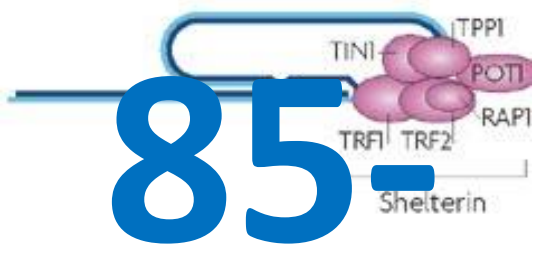
Fig. 3 Illustrative cartoon displays regions of telomerase activity in both epithelial tissues and the hematopoetic immune system in humans. (**A**) The immune system response results in telomerase upregulation coupled to proliferation, stimulated through cytokine exposure. (**B**) Basal/stem cell layer of the skin displays telomerase activity unlike the dermis or further differentiated epidermal layers. (**C**) Stem cells in the intestinal lower crypts display activity that is not present in the upper crypt regions.

Telomeres, telomerase, aging and cancer

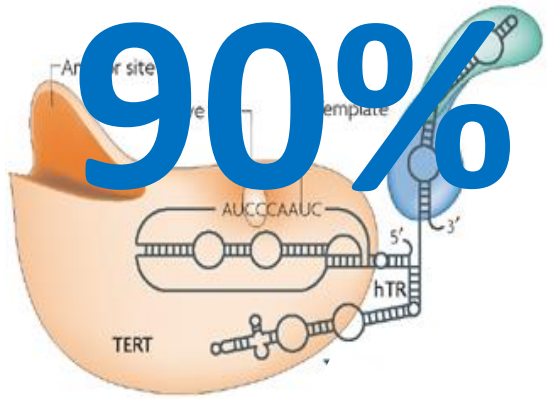


Strategies to maintain telomere lengths in cancer cells

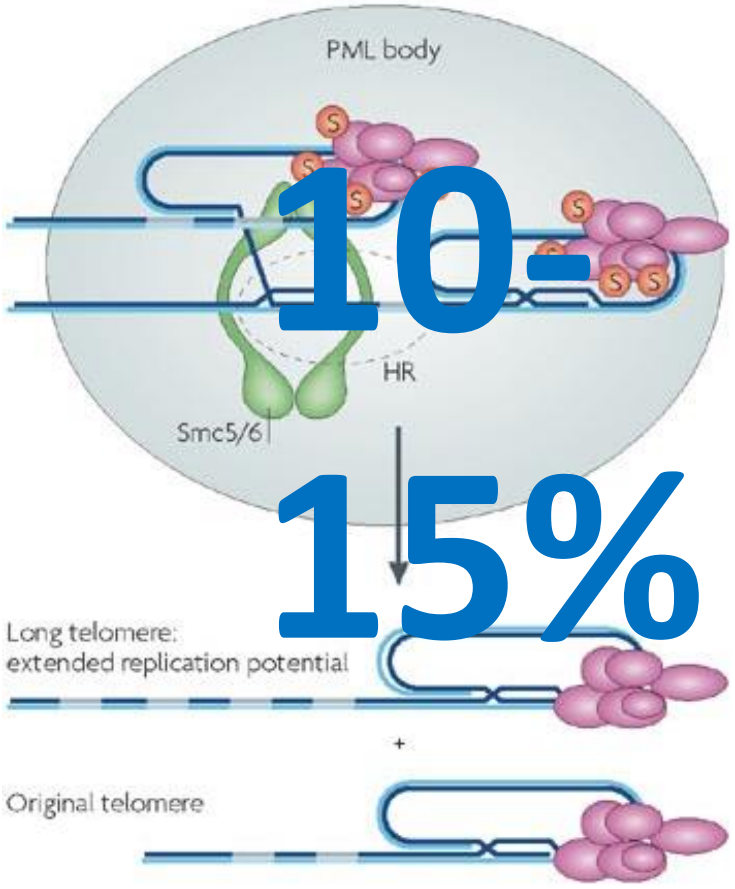
a) telomerase



85-90%

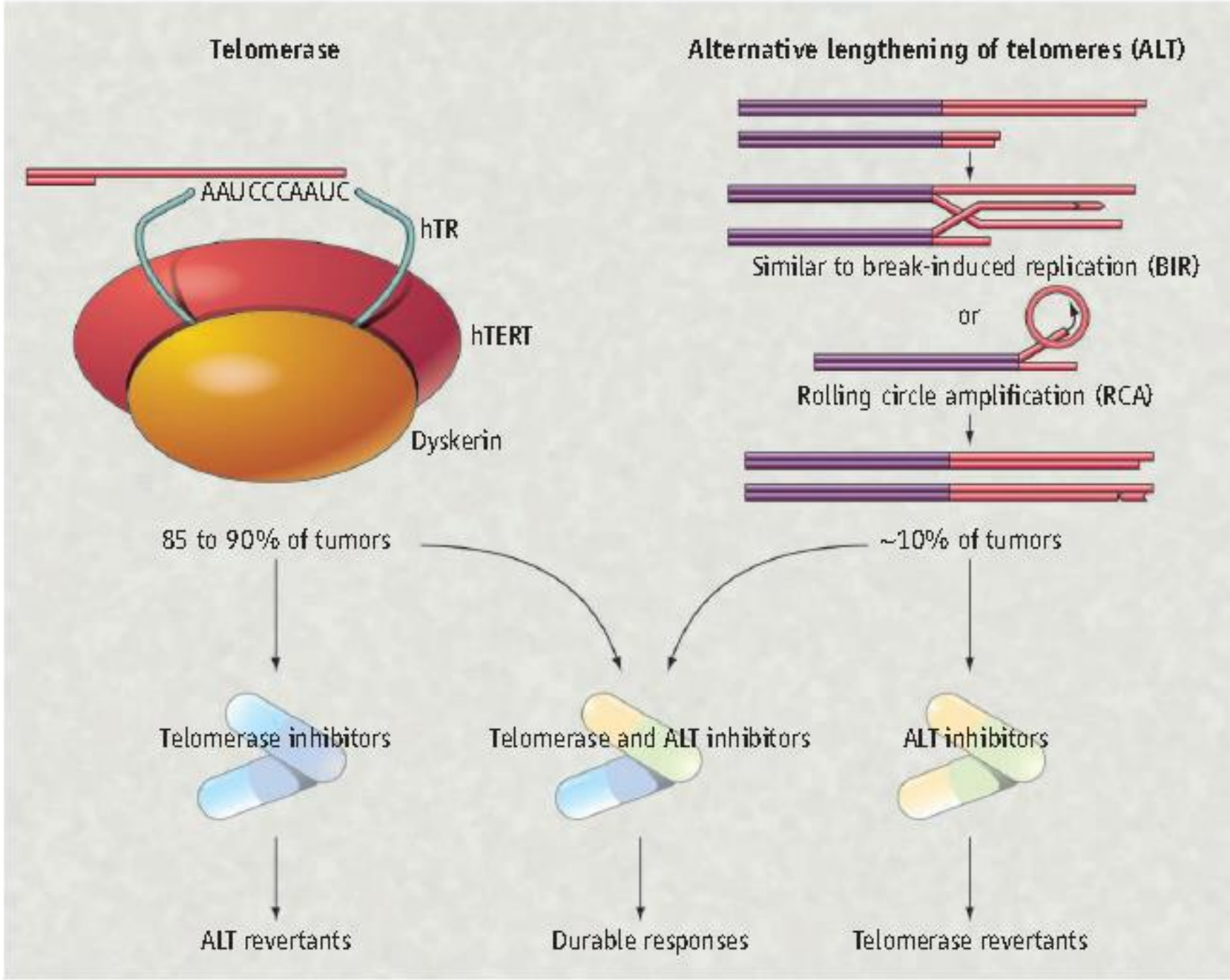


b) Alternative lengthening of telomeres (ALT)

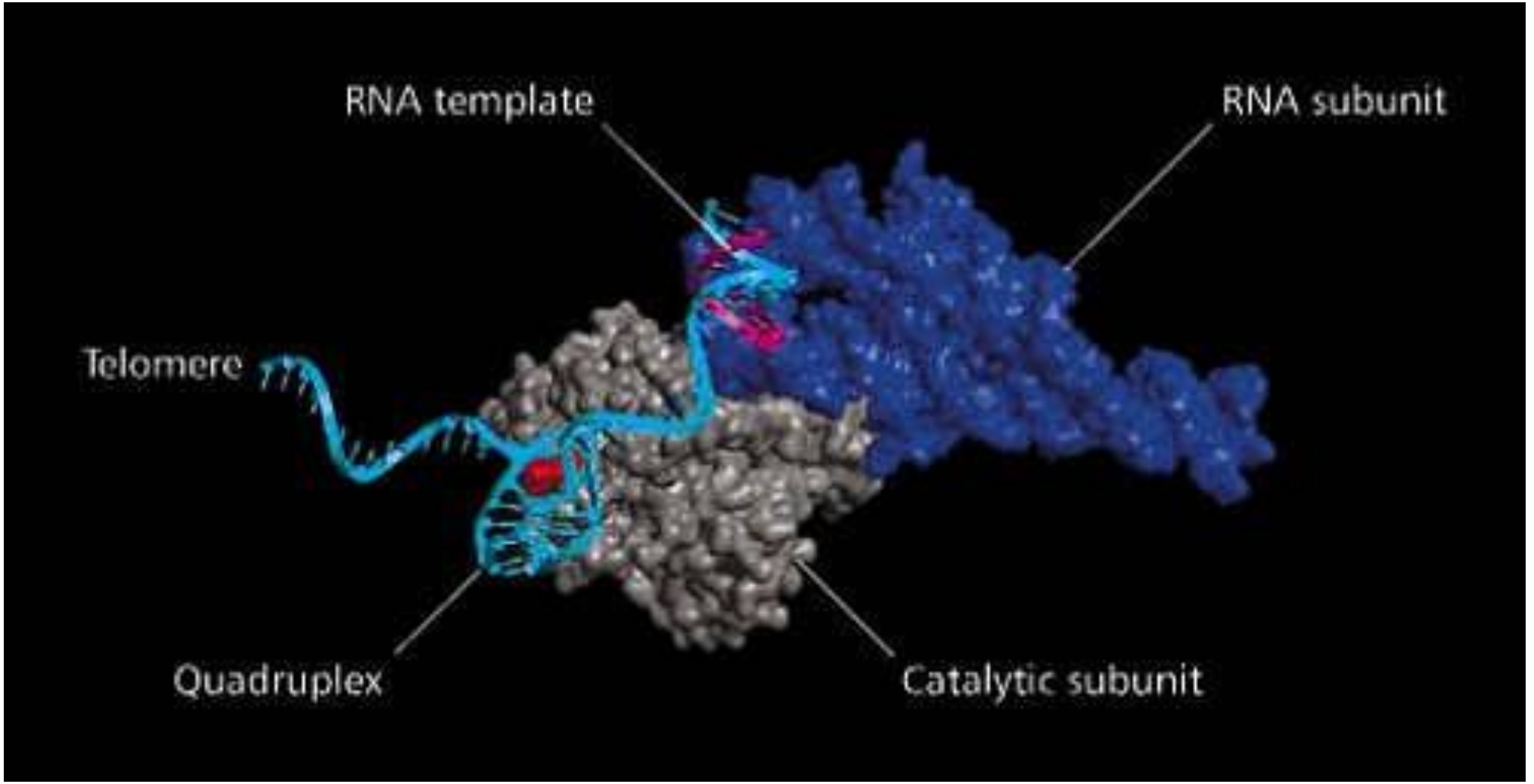


10-15%

Harley, C.B. Nat. Reviews Canc., 2008
Murray, JM and Carr, AM. Nat. Rev. Mol. Cell Biol. 2008


Telomerase
AAUCCCAAUC
hTR
hTERT
Dyskerin
Alternative lengthening of telomeres (ALT)
Similar to break-induced replication (BIR)
or
Rolling circle amplification (RCA)
85 to 90% of tumors
~10% of tumors
Telomerase inhibitors
Telomerase and ALT inhibitors
ALT inhibitors
ALT revertants
Durable responses
Telomerase revertants

Schematic picture of telomere-telomerase complex with potential target sites for inhibition



Why the telomerase represents a suitable target for anticancer therapy?

Universal

- 85-90% of all tumors is telomerase-positive

Critically important

- Telomerase activity/telomere maintenance is essential for the malignant character of cells

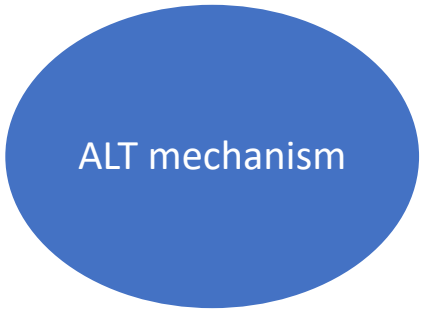
Specific

- Most of normal cells lacks telomerase activity or the activity is very low, in tumor cells the activity is increased

Which problems can be expected

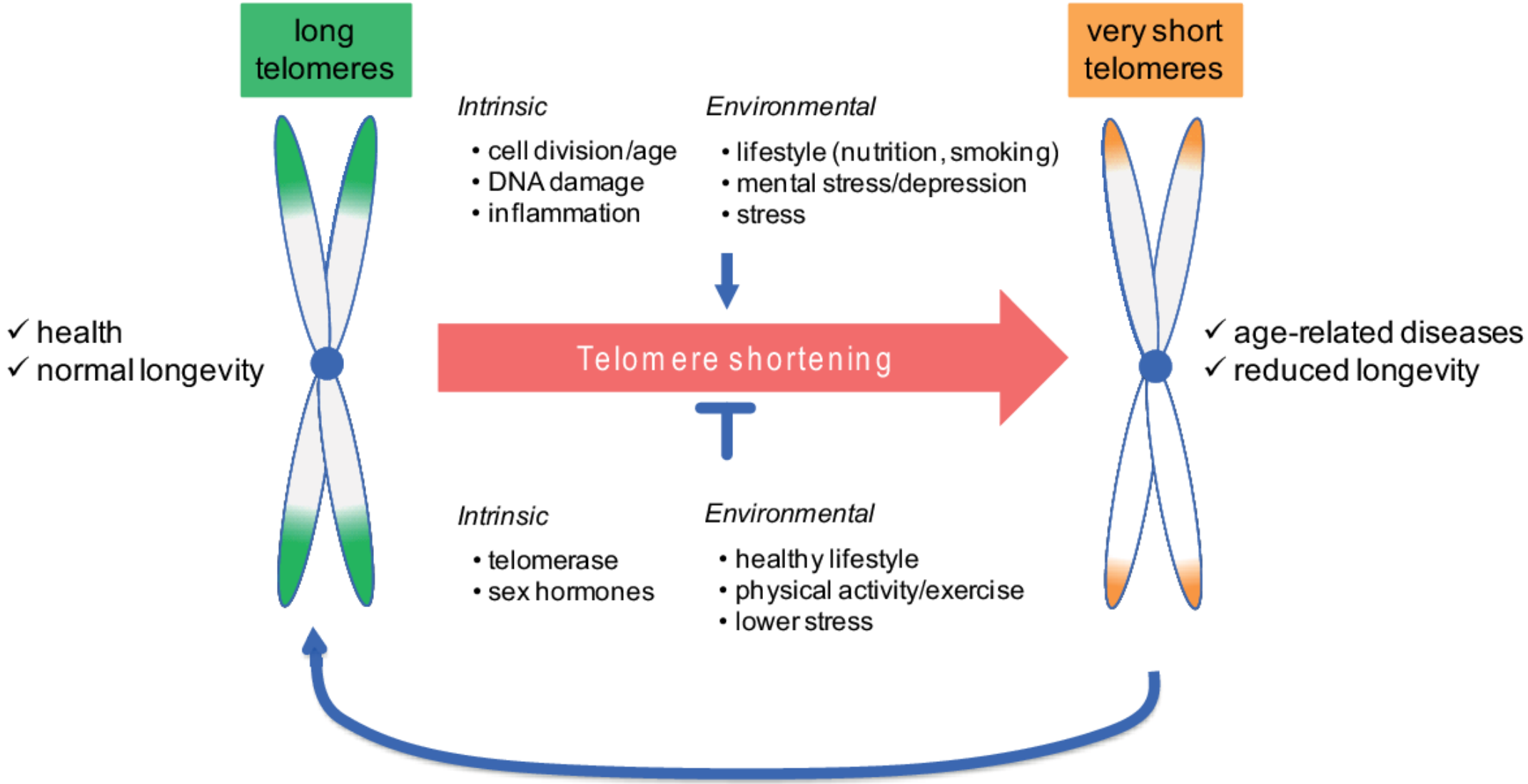
- The lag period between the administration of an inhibitor and the moment when telomeres shorten to the critical lengths which did not allow for cell proliferation

- Telomerase inhibitors may induce clones of tumor cells which will be resistant to the drug (via reactivating of telomerase or ALT)



- Alternative (recombination-based) mechanism is active in 10-15% of tumors)

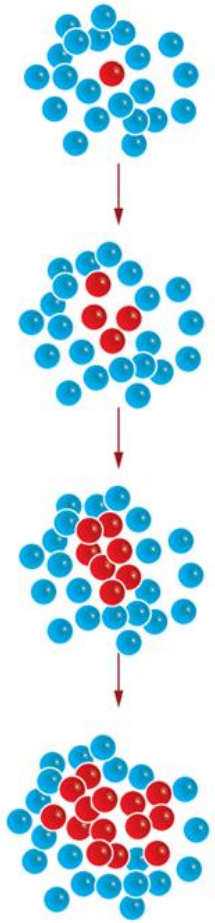
Telomeres and aging



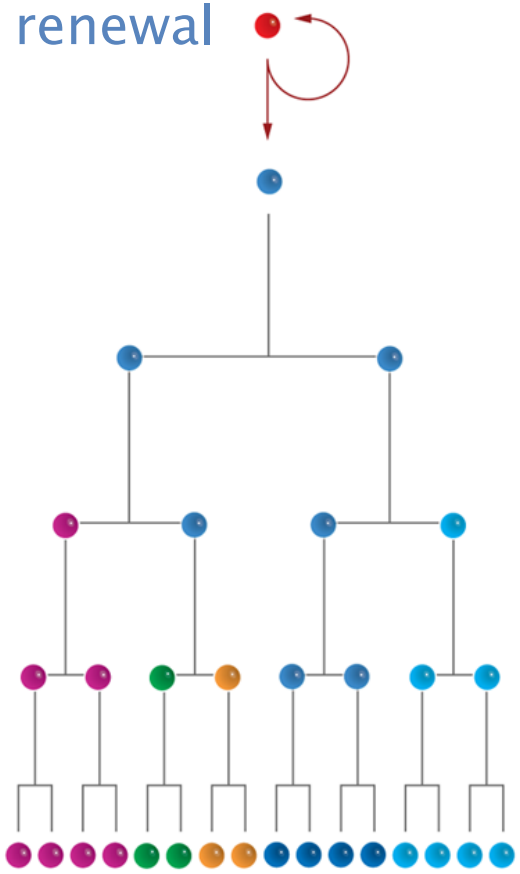
TERT gene therapy (AAV vectors)
TERT activating molecules (i.e., sex hormones, drugs, etc.)
TERT mRNA

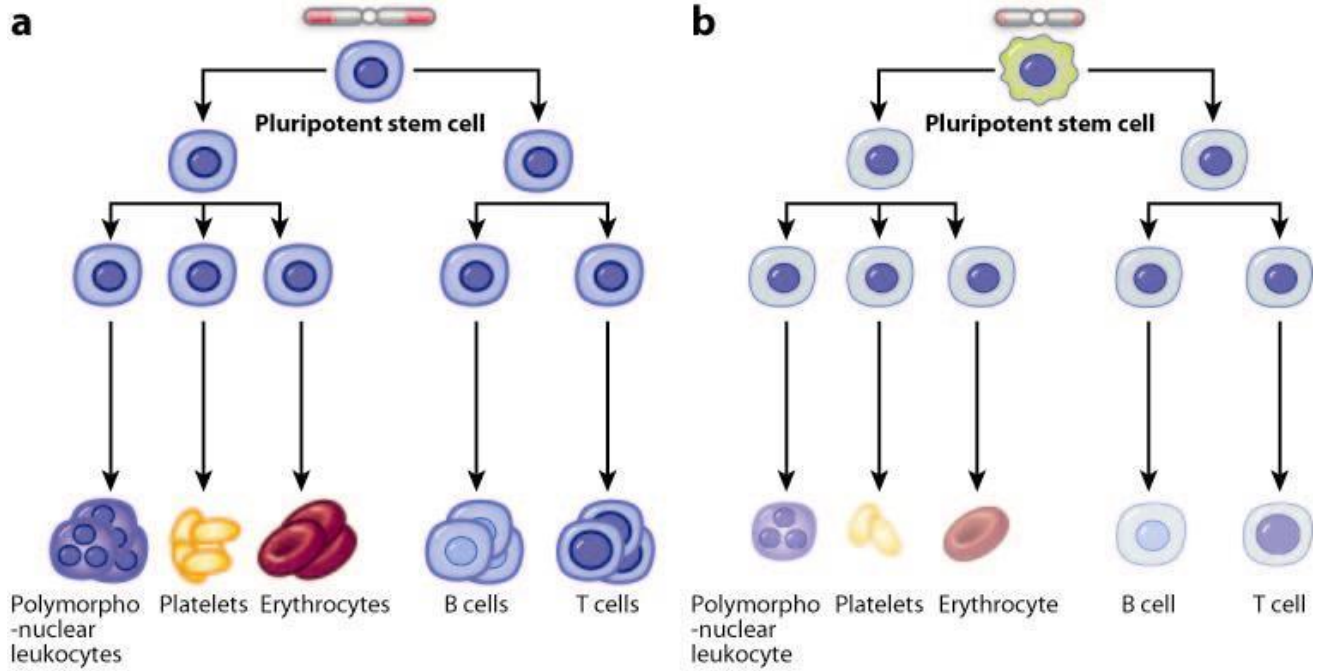
Short telomeres limit cell growth → telomere syndromes

Cancer



Tissue renewal





Armanios M. 2009.
Annu. Rev. Genomics Hum. Genet. 10:45–61

Short telomeres lead to stem cell failure in the bone marrow. (*a*) Normal hematopoiesis is hierarchical and relies on the intact capacity of a pleuripotent stem cell to self-renew and differentiate. When telomeres are short (*b*), stem cell function is impaired and the impairment leads to a progressive decline in the production of mature blood lineages in aplastic anemia.

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. (b) The shelterin complex is composed of six specialized proteins that bind telomeric DNA (Figure 2b is adapted from 60). Mutations in the shelterin component TINF2 explain a subset of severe cases of dyskeratosis congenita. The mechanism by which TINF2 heterozygous mutations lead to telomere shortening is not known.

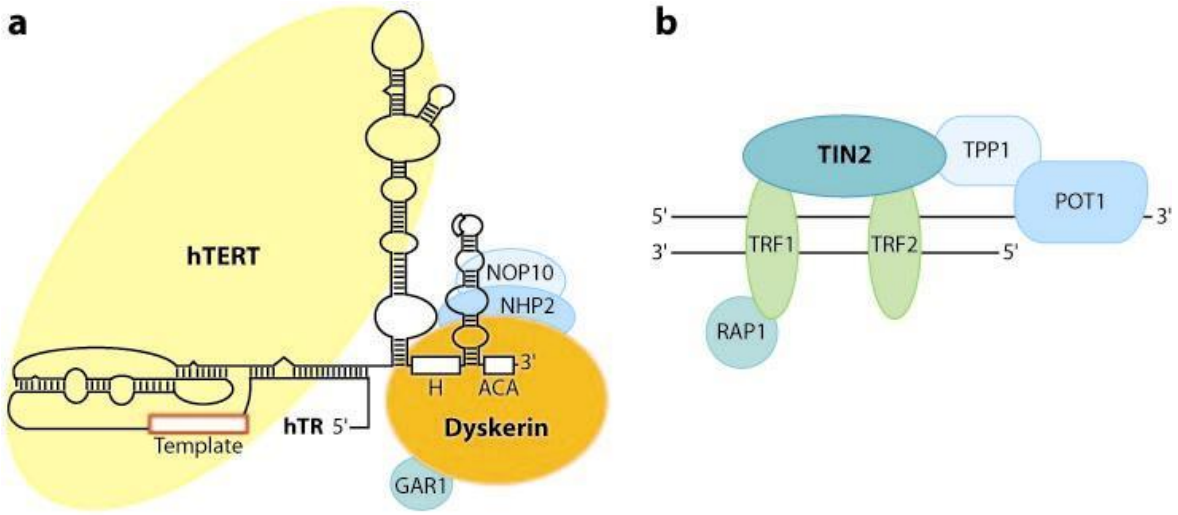


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Table 1 Mutations in telomerase and telomere genes lead to a broad clinical spectrum of syndromes of telomere shortening

Gene Name	Diagnosis	Typical age of onset in years
hTR and *hTERT*	Sporadic IPF 1–3% Familial IPF[a] 8–15% Sporadic and familial aplastic anemia ~3–5% Autosomal dominant DC[b]	Broad range 5–77
DKC1	X-linked DC Hoyeraal-Hreiderasson	Less than 30 Less than 5
TINF2	Sporadic DC Autosomal dominant DC Hoyeraal-Hreiderasson	Less than 10 - Less than 5
NOP10	Autosomal recessive DC	-
NHP2	Autosomal recessive DC	-

[a]IPF refers to idiopathic pulmonary fibrosis.
[b]DC refers to dyskeratosis congenita.

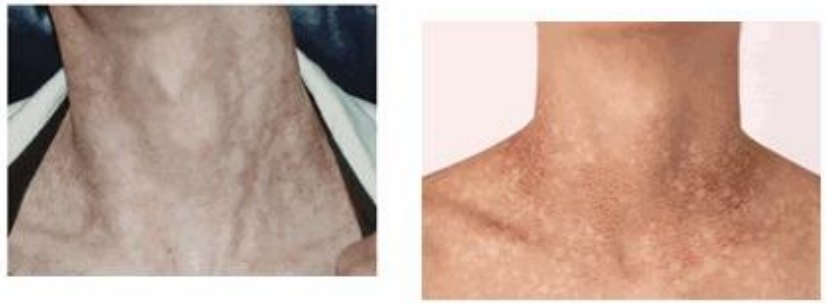
Examples of the **dyskeratosis congenita diagnostic triad**

A.Skin pigmentation

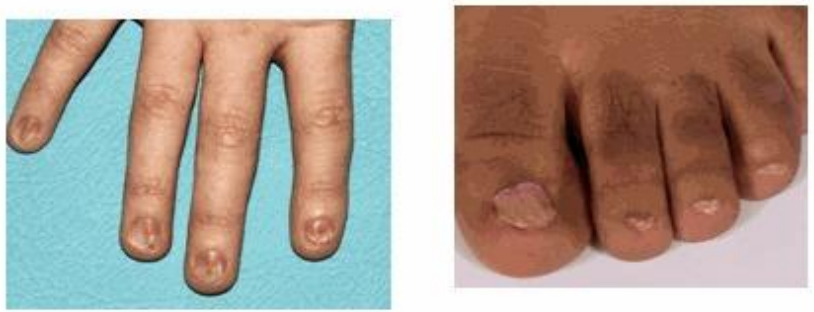
B.Dysplastic fingernails and toenails

C.Oral leukoplakia

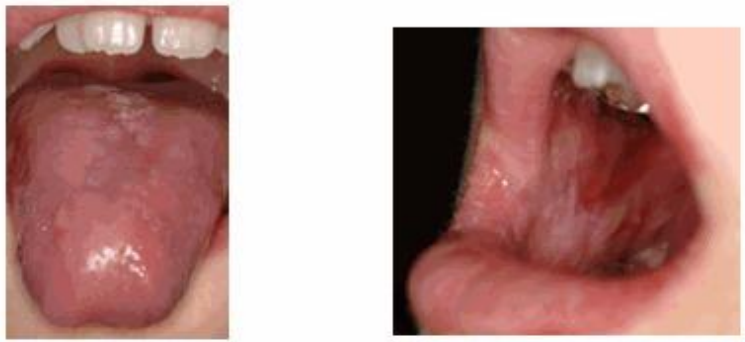
A.



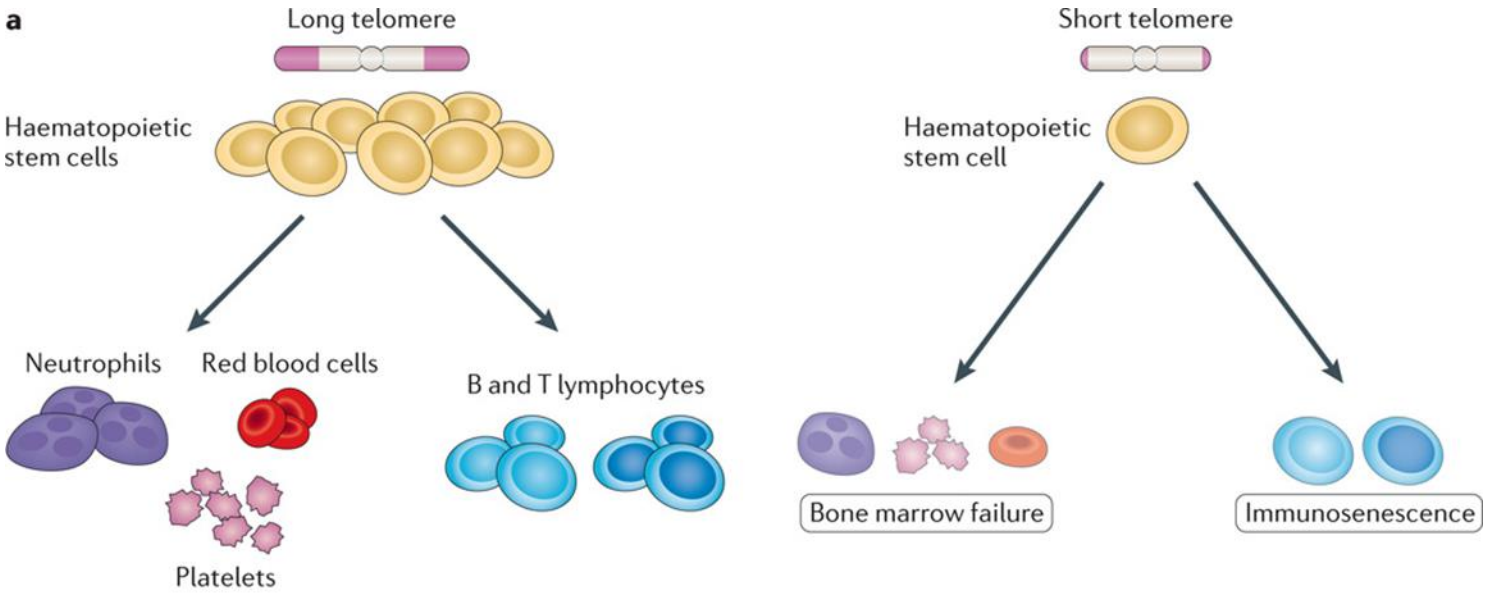
B.



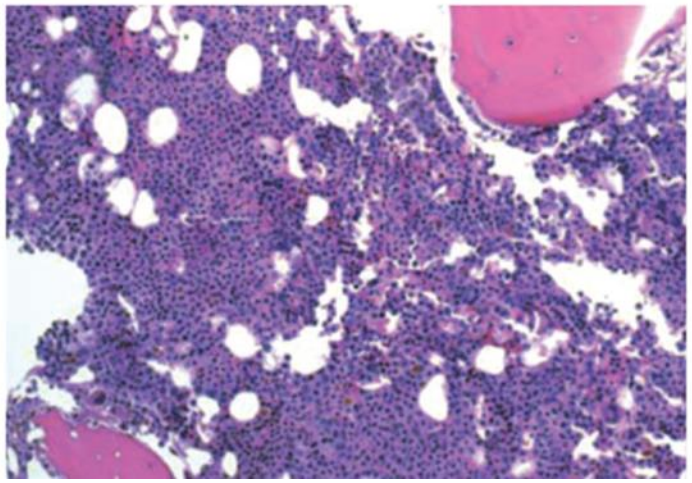
C.



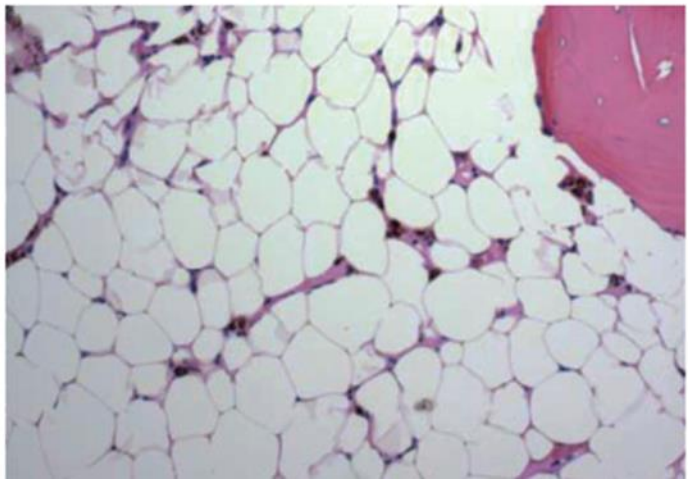
Short telomeres cause haematopoietic stem cell failure



b Normal bone marrow

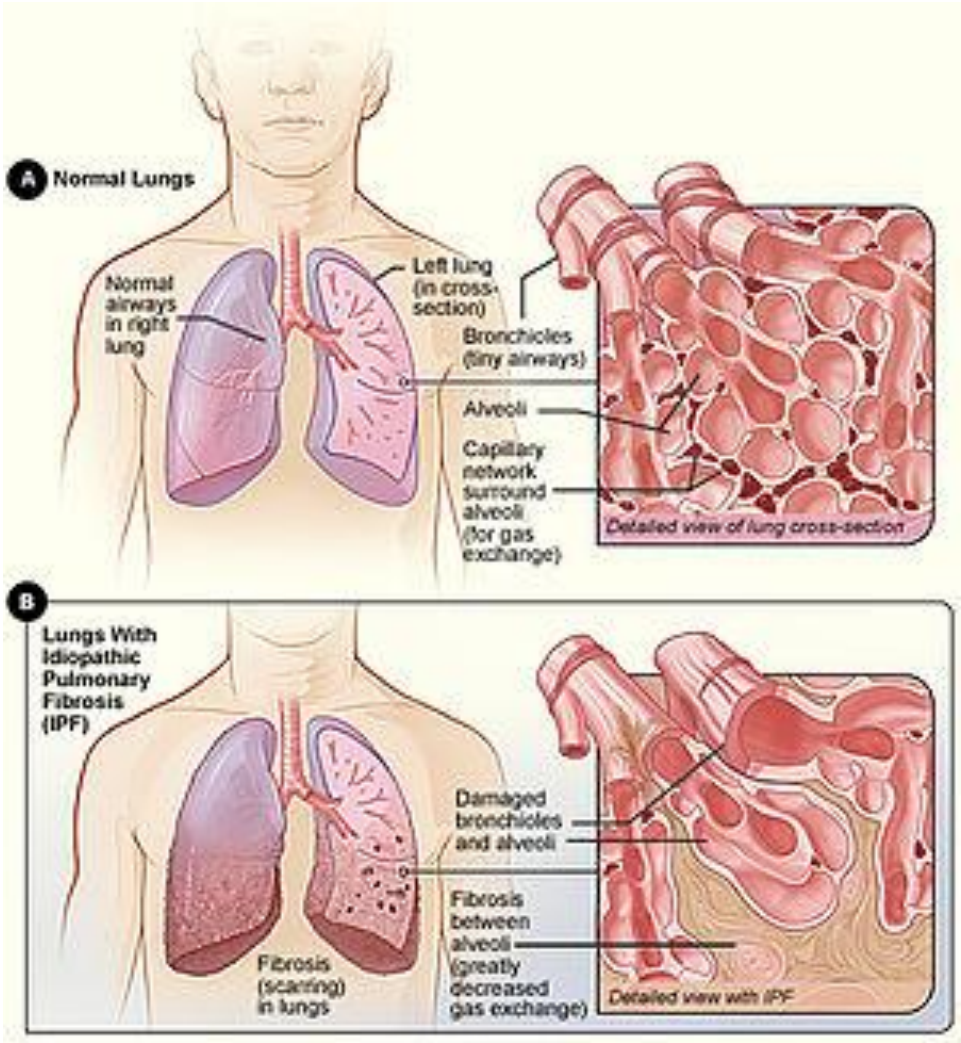


Aplastic anaemia



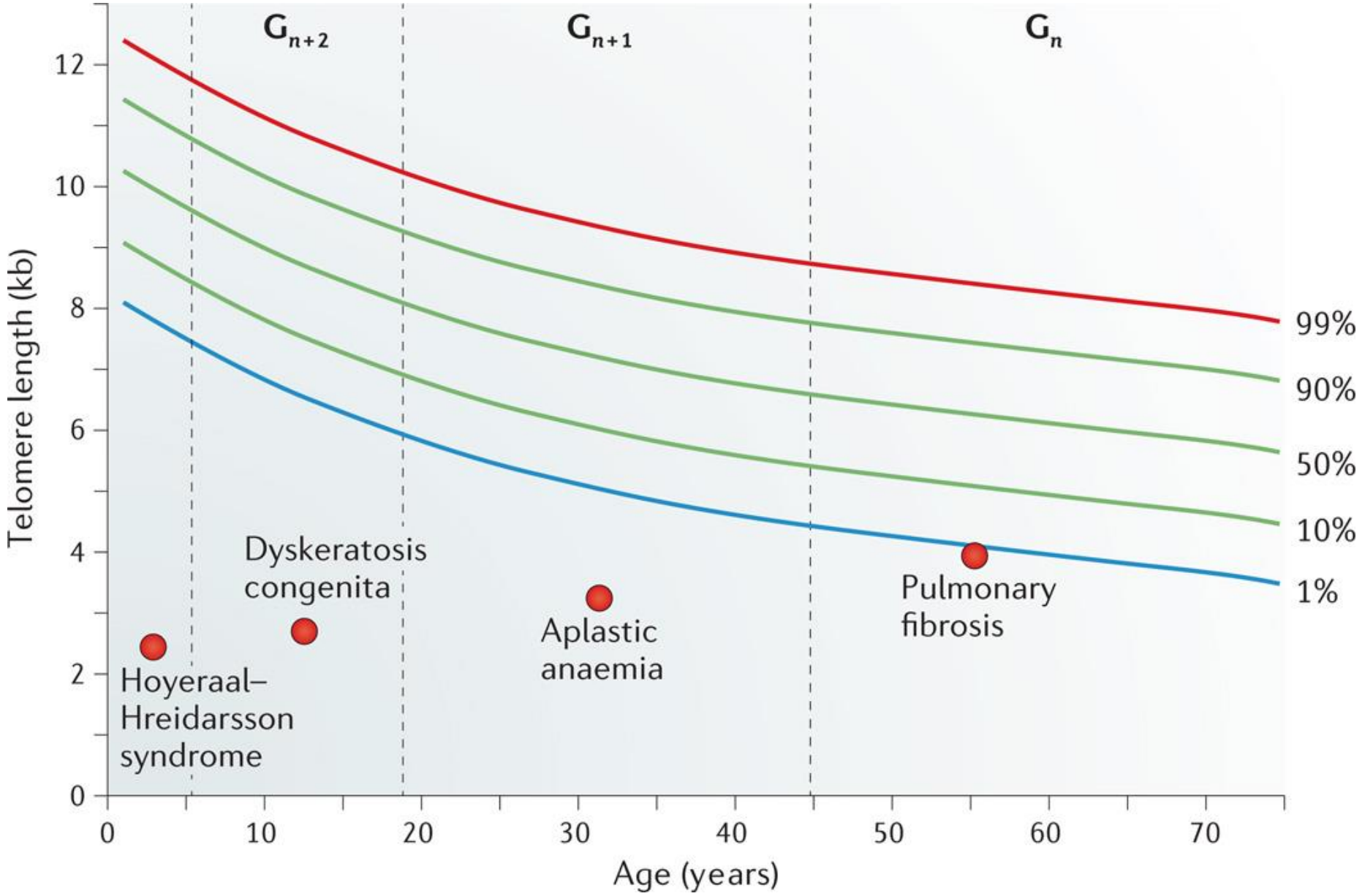
Idiopathic pulmonary fibrosis (IPF) is a specific form of plumonary fibrosis with unknown cause.
Pulmonary fibrosis involves fibrotic lesion and scarring of the lung. The build up of excess scar tissue in the lungs results in reduced lung volume. The symptoms that typify the disease are chronic cough and shortness of breath. Some familiar types of IPF are caused by mutations in the genes that encode TR and TERT

AA and IPF are commonly listed as telomere disease syndromes in parallel to DC. However, the symtoms of AA and IPF occur also in DC.



Age-dependent manifestations of telomere syndromes

Nat Rev Genet. 2012 Oct; 13(10): 693–704.

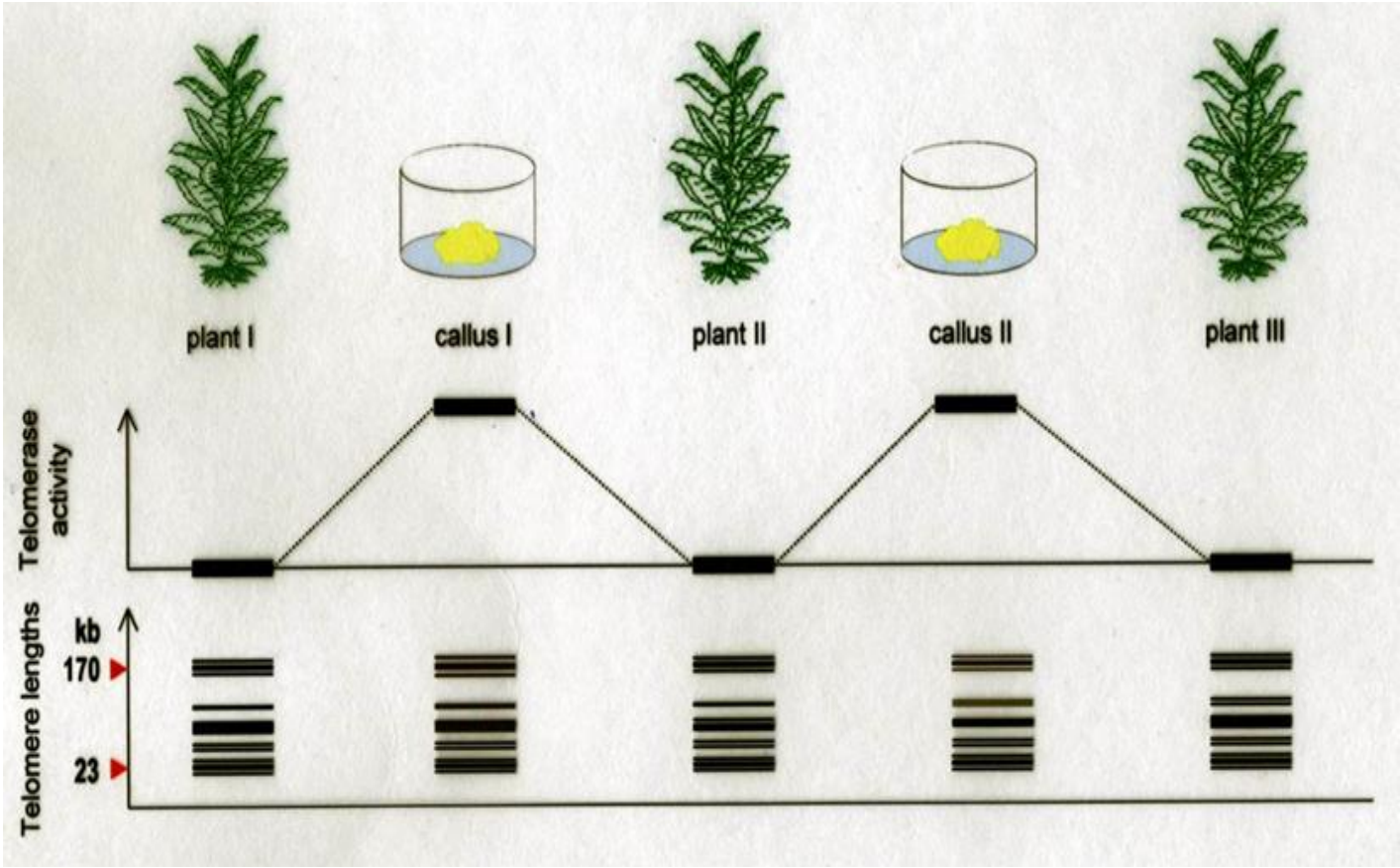


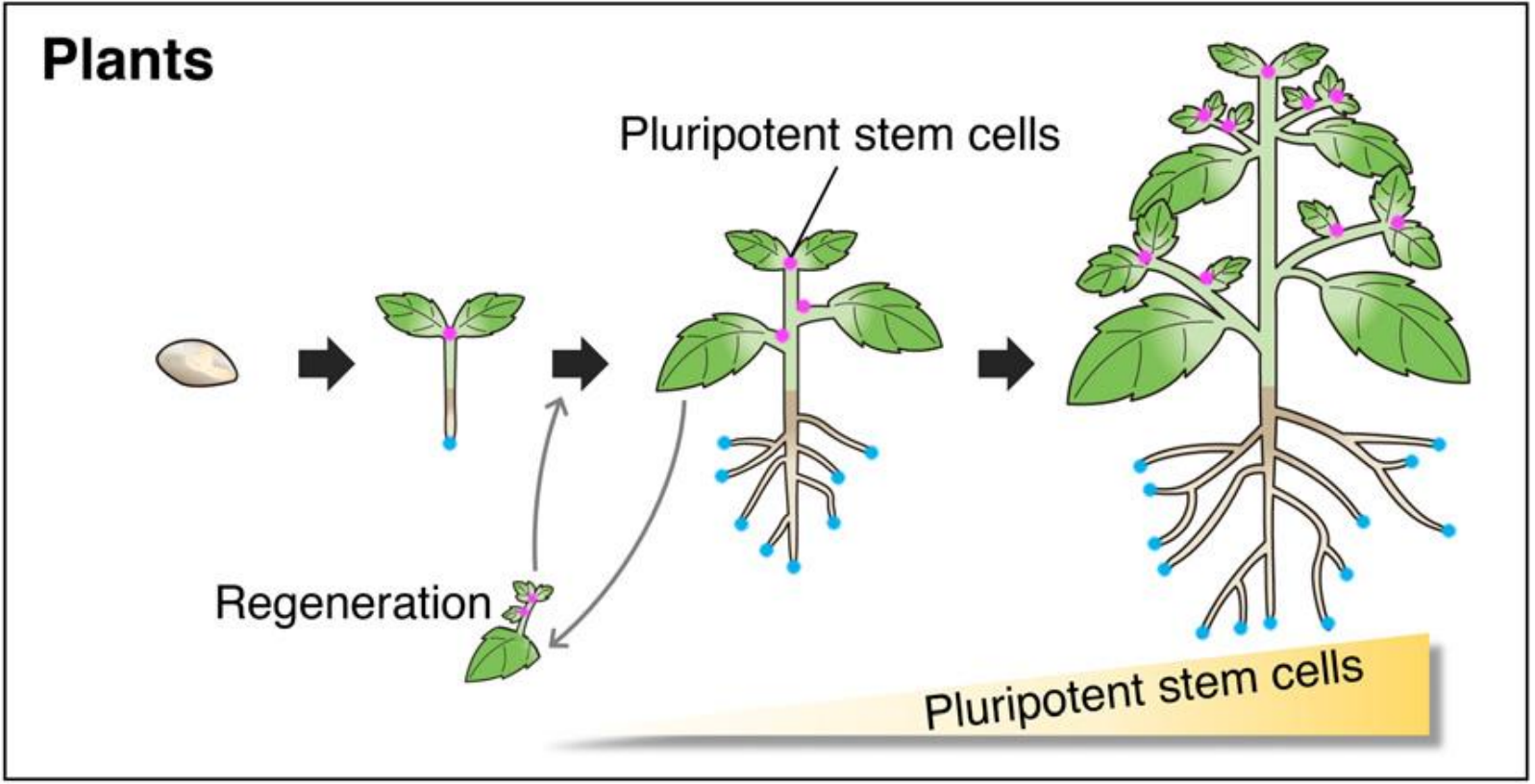
Telomeres and telomerases in plants – what we can learn from our sessile companions

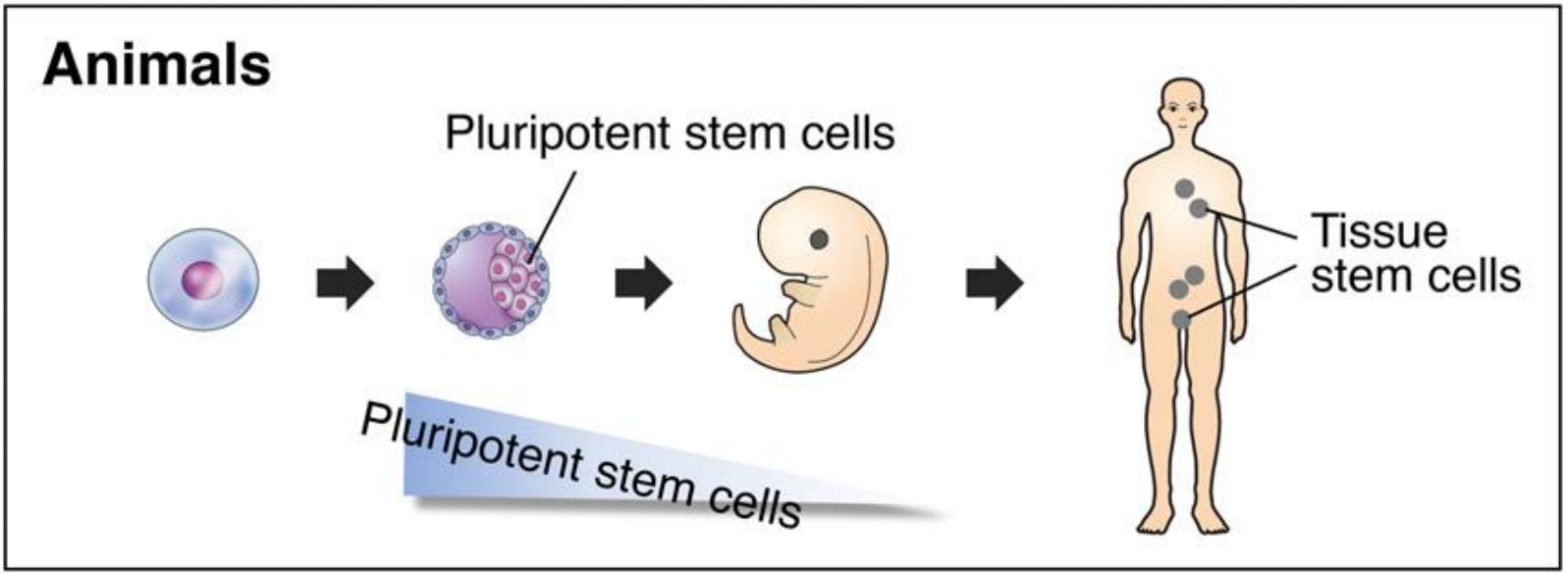
Plant development - a modular development- is substantially different from that of e.g., human. Also the sessile lifestyle of plants is challenging – they cannot escape from unfavourable conditions. Instead, they have highly developed mechanisms of adaptation or tolerance to different types of stress, as well as a **high regeneration capacity**.

Plant telomeres remain stable during ontogenesis – **no developmental telomere shortening** in plants! (*Fajkus et al., MGG 1998, Říha, Fajkus et al., Plant Cell 1998*)
Telomerase remains active in plant meristem cells (which produce all cell types of the plant body).
Even a differentiated plant cell (without telomerase activity) can re-activate telomerase upon stimulation of regeneration and cell division.

Telomerase regulation is reversible in plants - We can only envy them!







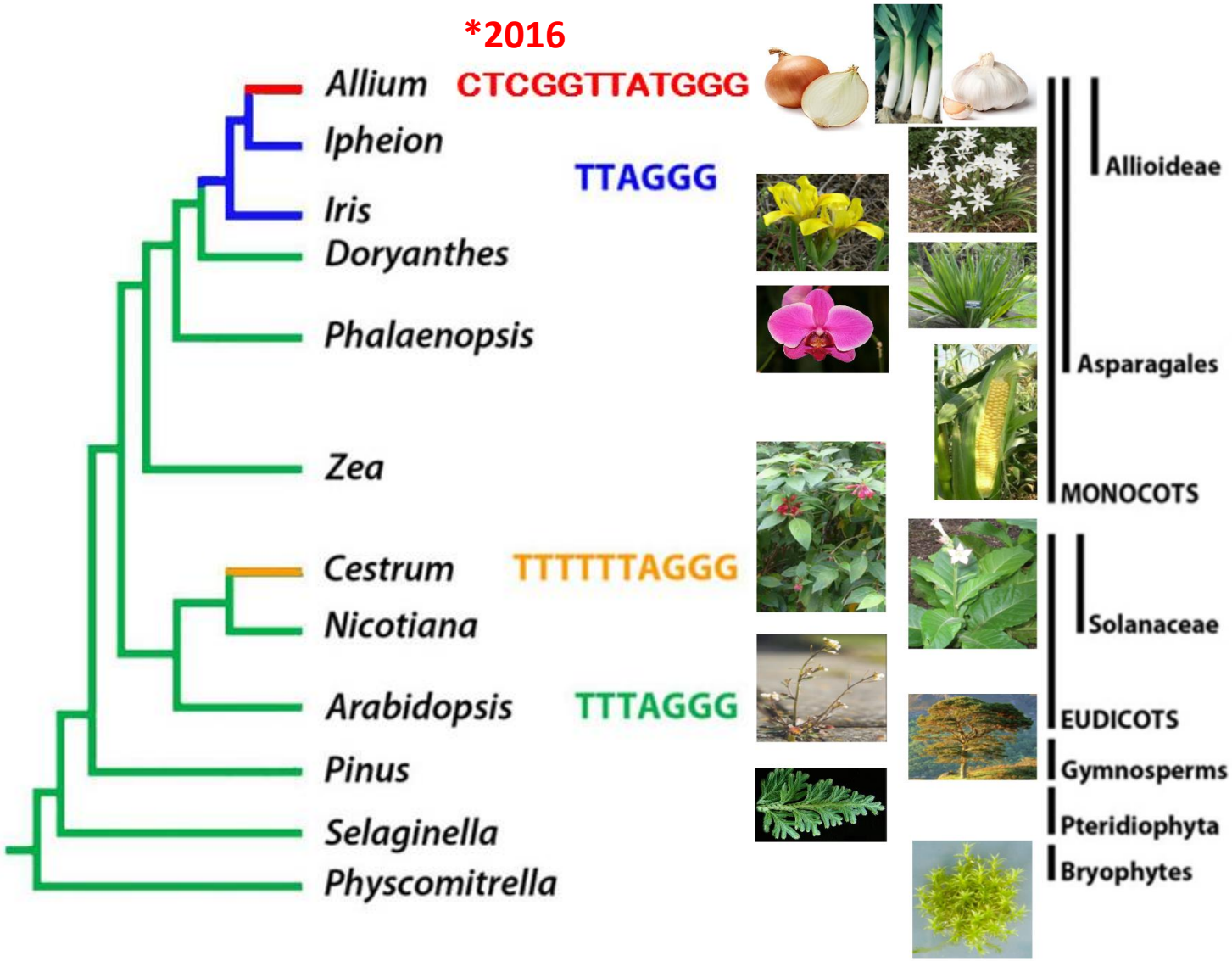
Stem cells in plants and animals.
Stem cells in the apical and axillary meristems in shoots maintain pluripotency, and **their population continuously increases** in number during development (pink). Root stem cells are unipotent, but different types are cooperatively involved in root development (blue).

In animals, pluripotent stem cells disappear soon after early embryogenesis, and, in the adult body, tissue (adult or somatic) stem cells differentiate into specific cell types and maintain tissue homeostasis.

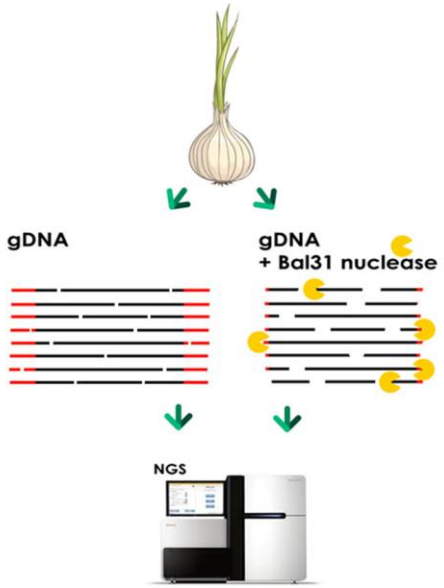
Postembryonic silencing of telomerase in most somatic cells of human and animals – probably an evolutionary protective mechanism against cancer.

Umeda et al., 2021

Evolutionary changes in plant telomere DNA repeats



Comparative NGS of of HMW and BAL31-digested DNA: a method to identify telomere repeats (even in large genomes full of repeats)



Vratislav Peška

the plant journal
SEB Society for Experimental Biology
The Plant Journal (2016) 85, 337–347
doi: 10.1111/tpj.13115

Allium telomeres unmasked: the unusual telomeric sequence $(CTCGGTTATGGG)_n$ is synthesized by telomerase

Petr Fajkus[1,2†], Vratislav Peška[2,†,*], Zdeňka Sitová[1], Jana Fulnečková[2], Martina Dvořáčková[1,2], Roman Gogela[1], Eva Sýkorová[2], Jan Hapala[1] and Jiří Fajkus[1,3,*]

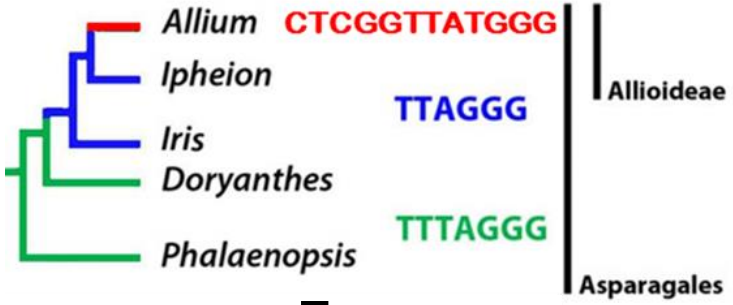
Petr Fajkus

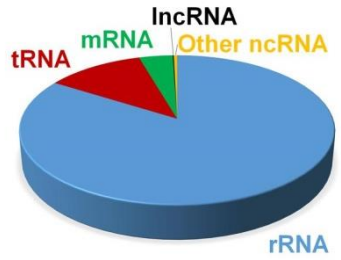
the plant journal
SEB Society for Experimental Biology
The Plant Journal (2015) 82, 644–654
doi: 10.1111/tpj.12839

Characterisation of an unusual telomere motif $(TTTTTTAGGG)_n$ in the plant *Cestrum elegans* (Solanaceae), a species with a large genome

Vratislav Peška[1,2], Petr Fajkus[1,2], Miloslava Fojtová[1,2], Martina Dvořáčková[1,2], Jan Hapala[2], Vojtěch Dvořáček[1], Pavla Polanská[2], Andrew R. Leitch[3], Eva Sýkorová[1,2] and Jiří Fajkus[1,2,*]

Characterisation of plant telomerase RNAs





Palazzo and Lee, 2015

rRNA depletion+RNAseq

RNA-seq of total RNA from **telomerase active** tissues from ***Allium*** and some other **Asparagales** species with **vertebrate/plant** type telomeres

Transcriptomes *de novo* assembly

Blast search for common RNAs with the expected template motif

Extra-long telomere repeats in *Allium* limit the number of candidate TRs !

Species	genome size (1C pg)	NGS raw reads	TRINITY	telomere repeat (length)	candidates with template region	shared sequences in all *Allium* datasets
A. angulosum	15,1	151011864	366609	CTCGGTTATGGG (12)	24	1
A. cepa	16,75	64518048	210240	CTCGGTTATGGG (12)	5	2
A. ericetorum	?	155932784	529407	CTCGGTTATGGG (12)	22	2
A. fistulosum	15,53	127291690	386217	CTCGGTTATGGG (12)	25	3
A. nutans	22,63	105013146	380366	CTCGGTTATGGG (12)	28	7
A. ursinum	30,17	145956874	441843	CTCGGTTATGGG (12)	80	1
C. elegans	9,76	95463938	338591	TTTTTTAGGG (10)	2339	not defined
S. peruviana	?	66865026	476296	TTAGGG (6)	60903	not defined
T. violacea	19,83	101341874	430607	TTAGGG (6)	65997	not defined

Petr Fajkus

Vratislav Peška

Research into the nature of the evolutionary telomere changes has led us to discover telomerase RNA subunits across the whole land plant phylogeny (*Fajkus P. et al, 2019*)

1. TRs in land plants are **monophyletic**
2. They are transcribed with **RNA Pol III** (in contrast to yeasts an vertebrates – RNA Pol II)
3. TR genes are under a control of type-3 promoter (USE+TATA)

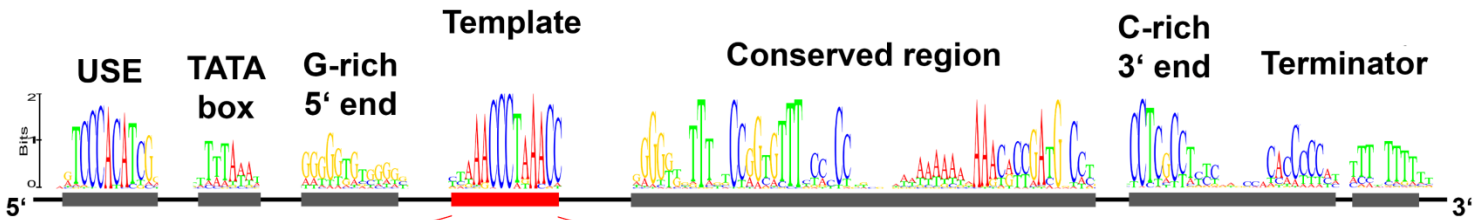
9842–9856 Nucleic Acids Research, 2019, Vol. 47, No. 18 Published online 8 August 2019
doi: 10.1093/nar/gkz695

Telomerase RNAs in land plants

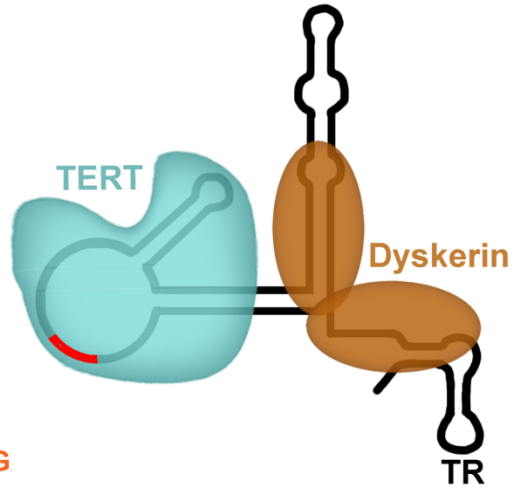
Petr Fajkus[1,2,†], Vratislav Peška[1,†], Michal Závodník[2,3], Miloslava Fojtová[1,2,3], Jana Fulnečková[1,2], Šimon Dobias[1,2], Agata Kilar[2,3], Martina Dvořáčková[3], Dagmar Zachová[3], Ivona Nečasová[2,3], Jason Sims[4], Eva Sýkorová[1,*] and Jiří Fajkus[1,2,3,*]

Telomerase RNAs in land plants

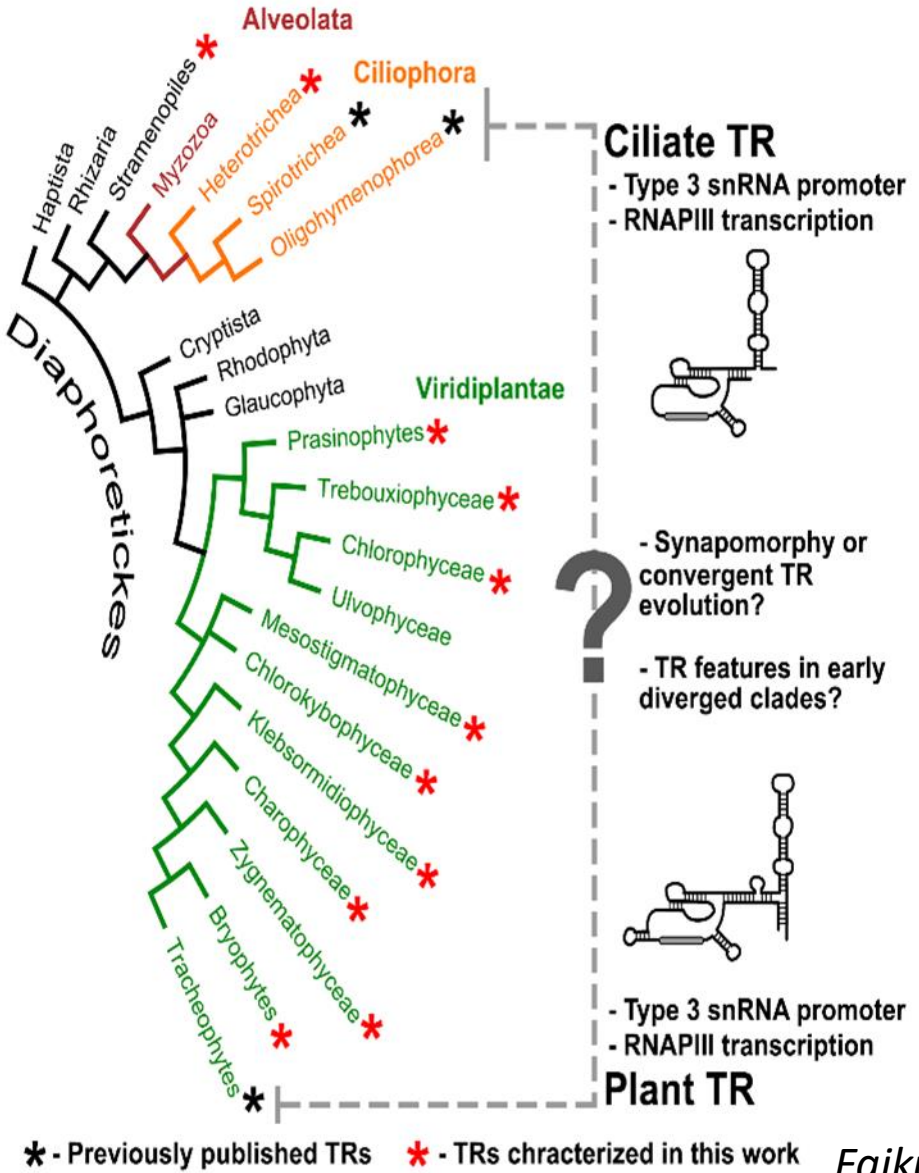
1988 - Identification of the first higher eukaryotic telomere DNA (in Arabidopsis*)*
2019 - Identification of telomerase RNA subunits across land plants

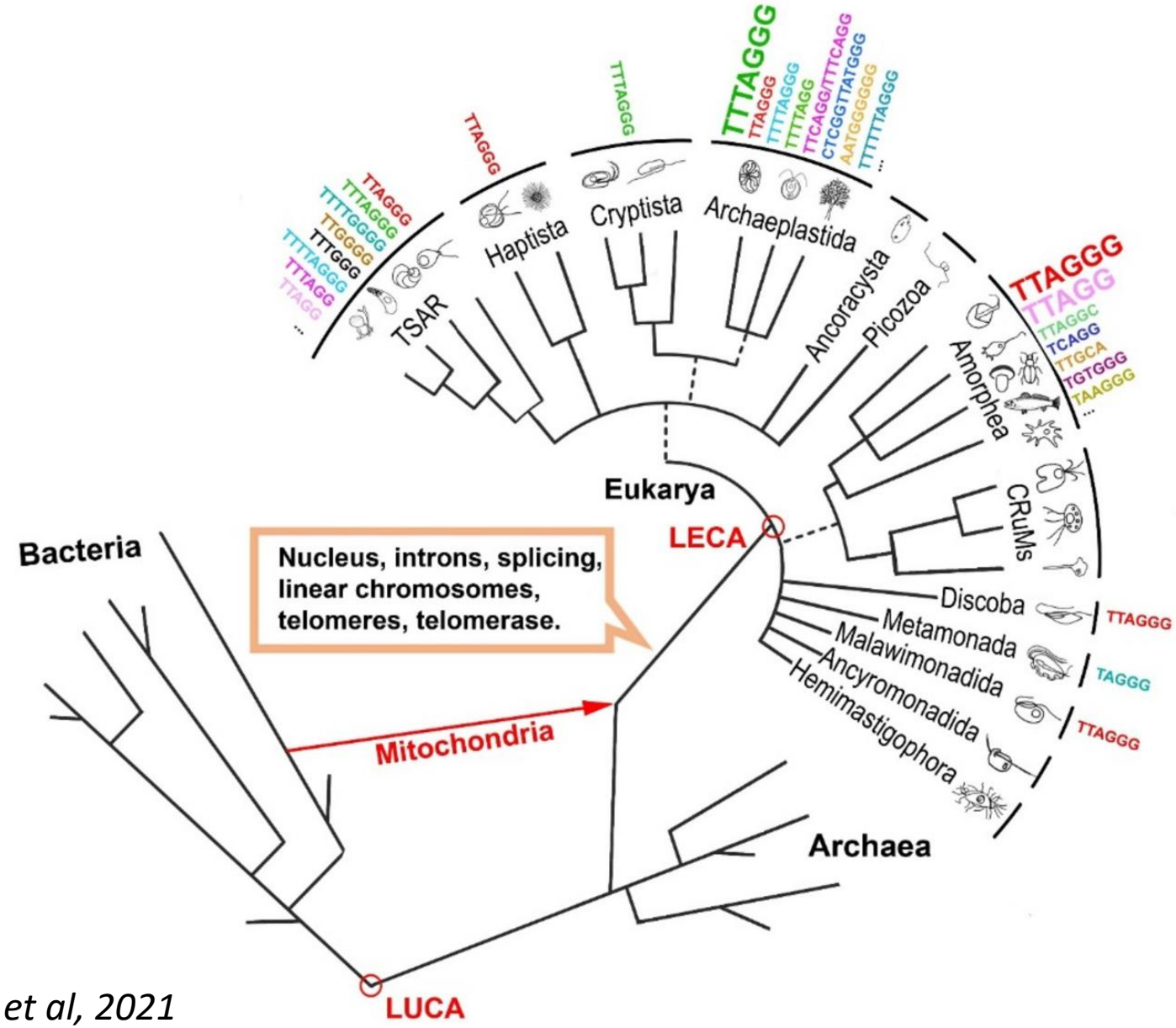


Specimen	TR Template domain (5‘ - 3‘)	Telomeric motif
i) Asparagales		
Allium cepa	AGAACCGAGCCCATAACCGATTG	CTCGGTTATGGG
Allium ursinum	TGAACCGAGCCCATAACCGATTG	
Tulbaghia violacea	CAAAGTTTACCCTAACCCTTCAT	
Scilla peruviana	TACAATACACCCTAACCCTACCT	TTAGGG
Asparagus officinalis	TCTTCGTTACCCTAACCCGTTTG	
Agave tequilana	ATGATTAAACCCTAACCCGTTGA	
Dendrobium catenatum	GTATTGAAACCCTAAACAATCCT	TTTAGGG
ii) *Cestrum* (Solanales)		
Cestrum elegas	CTGTAAAAACCCTAAAAACTGGC	TTTTTTAGGG
Petunia integrifolia	TATGTTCAACCCTAAACCACTTC	
Nicotiana sylvestris	ATTGTTAAACCCTAAACCACTTC	TTTAGGG
Solanum lycopersicum	ATGTCCAAACCCTAAACCGCTTC	
iii) *Genlisea* (Lamiales)		
Genlisea hispidula	ATCTCTCGAACCTGAACCACACC	TTCAGG/TTTCAGG
Genlisea nigrocaulis	TAATCGAAACCCTAAACCATACC	TTTAGGG
Genlisea aurea	CAATTGAAACCCTAAACCACATG	



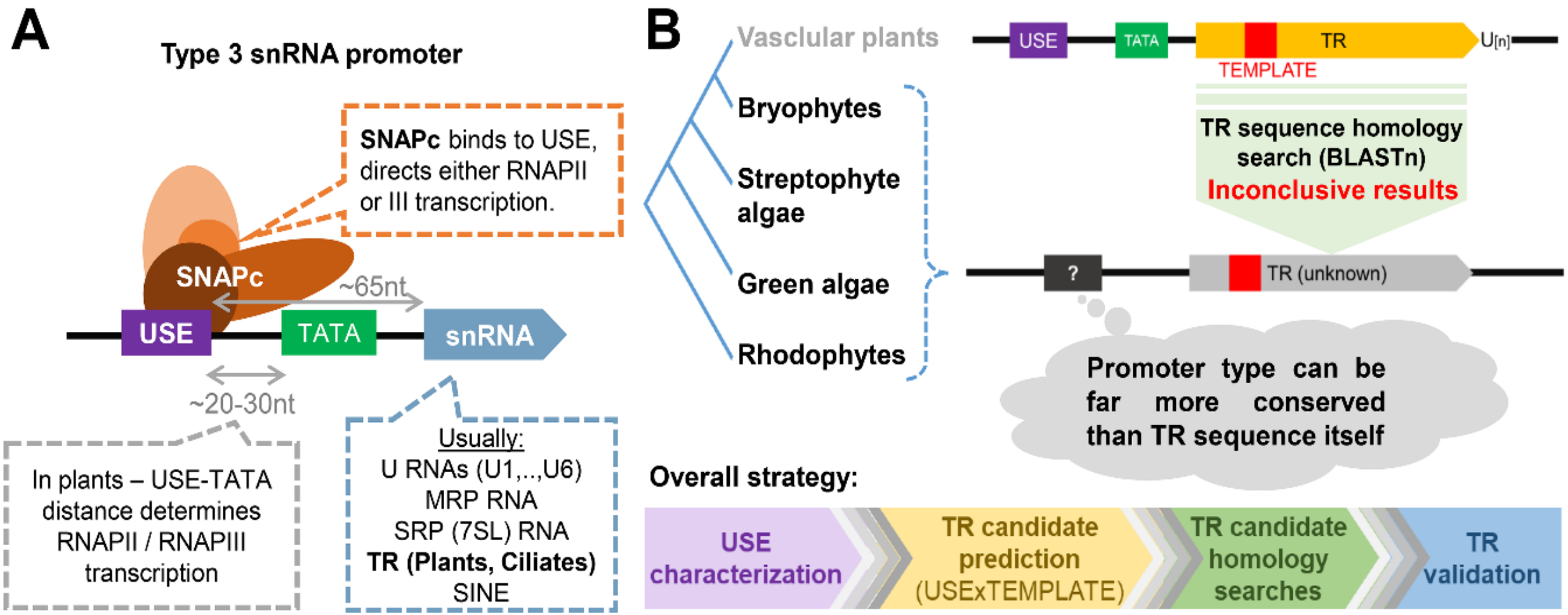
…as well as to telomerase RNA identification across the phylogenetic megagroup of Diaphoretickes (thus mapping the telomerase RNA evolution over a bilion year years ago…)





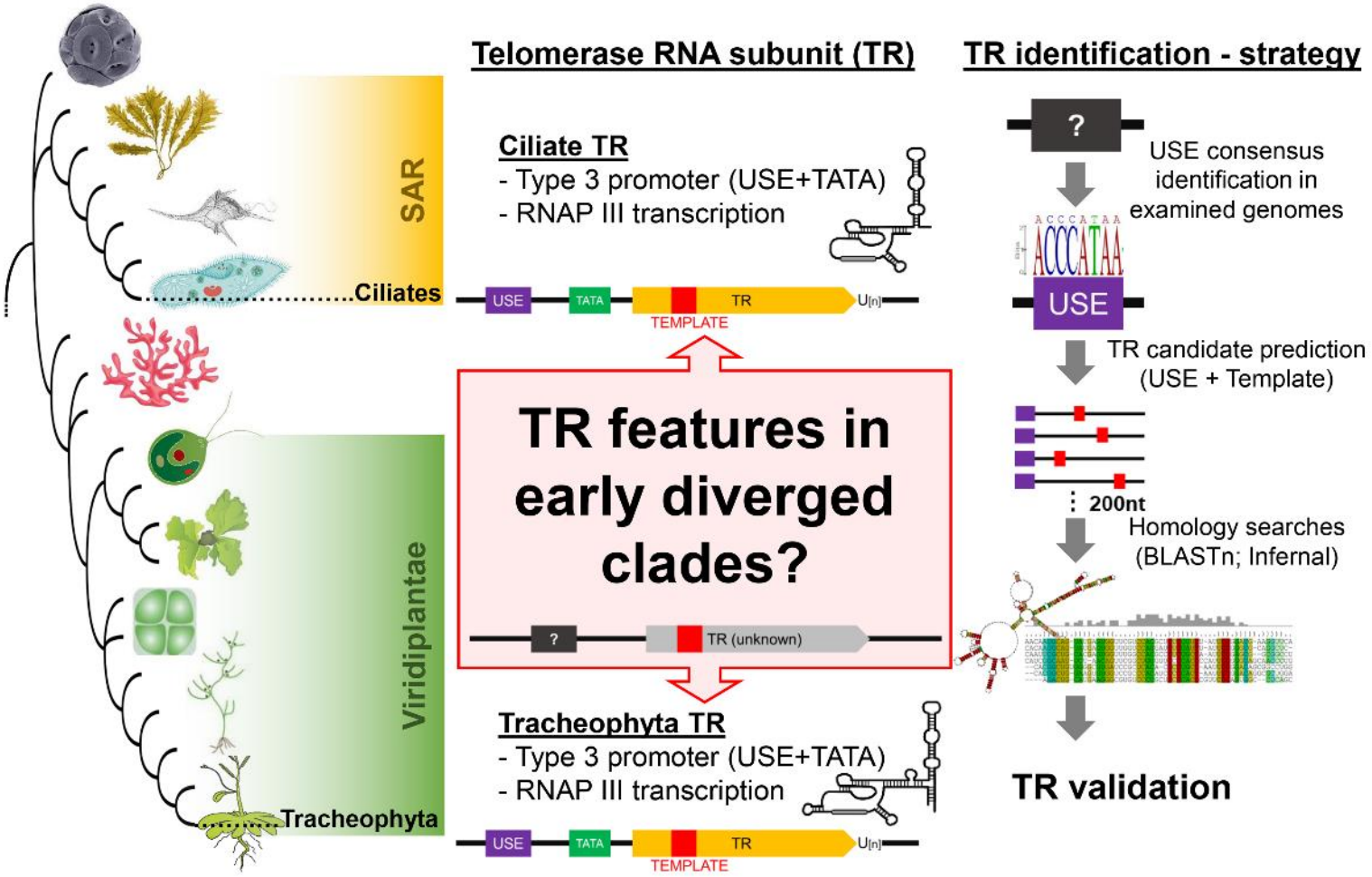
Fajkus P. et al, 2021

Evolution of TRs in a broader phylogenetic context: travelling back through time, from the branches deeper to the roots of the tree of life… But HOW to overcome the barrier of TR diversity? (*J. Chen - **TER**ribly difficult….*)



Summary

- Based on conserved type 3 promoter and template, we predicted TR canidates
- We characterise TRs in early diverging Viridiplantae taxa, as well as in ciliates and other Diaphoretickes lineages
- TRs were validated experimentally and show conservation of core TR structural domains
- These results shed light on the evolution of a key eukaryotic non-coding RNA **across more than a billion years**.



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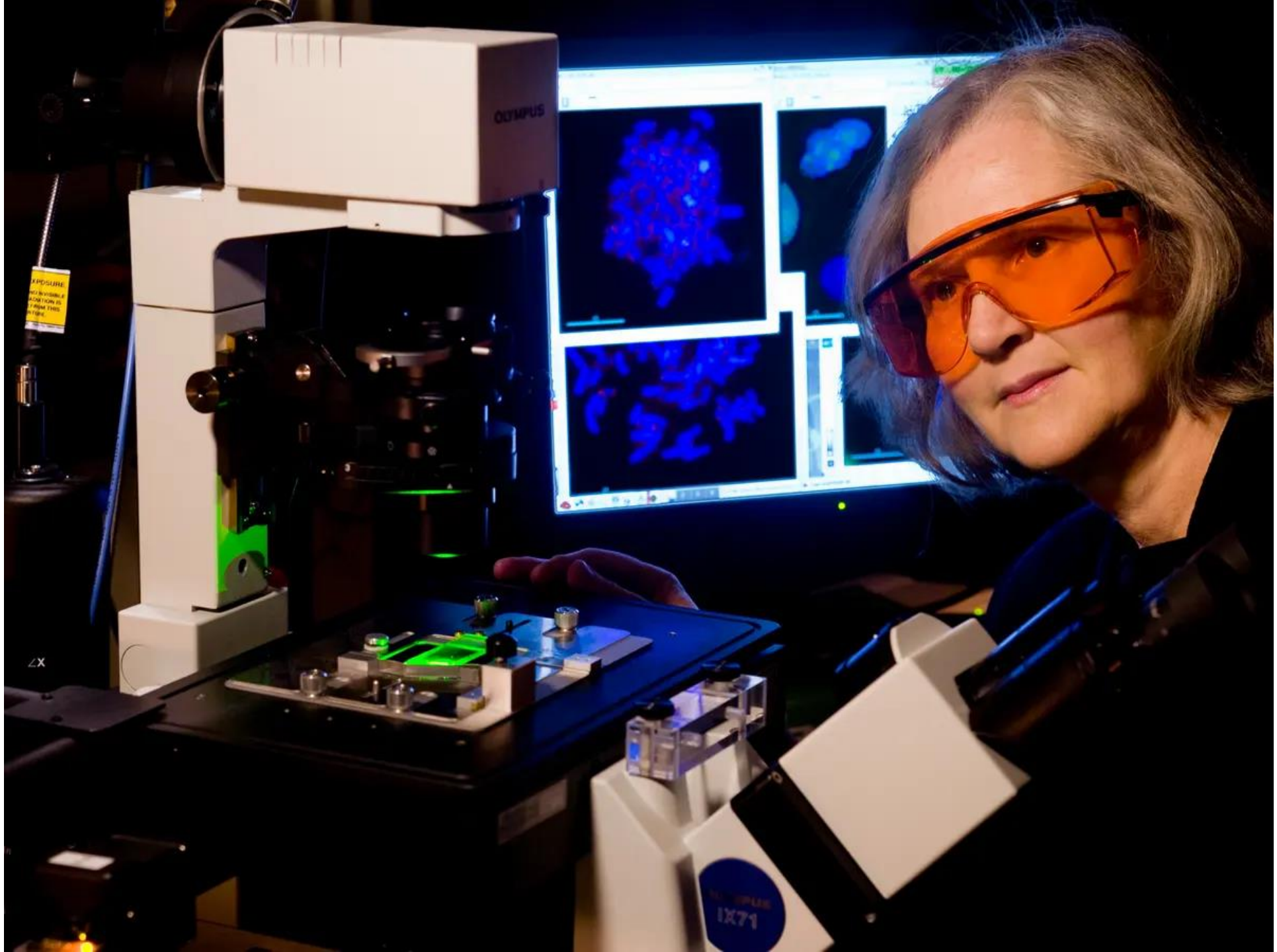
https://doi.org/10.1093/nar/gkab545

Evolution of plant telomerase RNAs: farther to the past, deeper to the roots

Petr Fajkus[1,2,*,†], Agata Kilar[2,3,†], Andrew D.L. Nelson[4], Marcela Holá[5], Vratislav Peška[1], Ivana Goffová[2,3], Miloslava Fojtová[2,3], Dagmar Zachová[2], Jana Fulnečková[1] and Jiří Fajkus[1,2,3,*]

Thank you very much for your attention and possible questions!


OLYMPUS
IX71