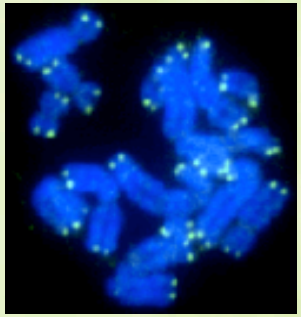
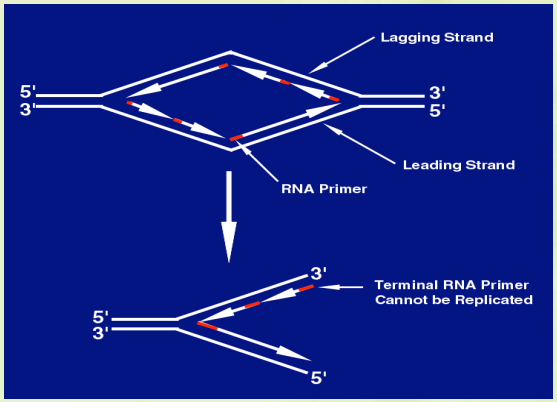
Theories of aging: telomeres and senescence



Reading: Handbook of Aging, Ch 9

A&S300-003 Jim Lund

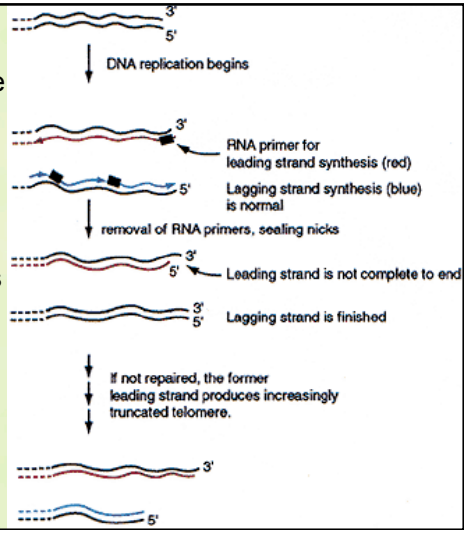
Chromosome End Replication Problem



DNA replication and telomere shortening♪

The chromosome **End Replication** Problem

DNA polymerases add bases 5' -> 3' and require a primer template



Consequences of the end replication problem

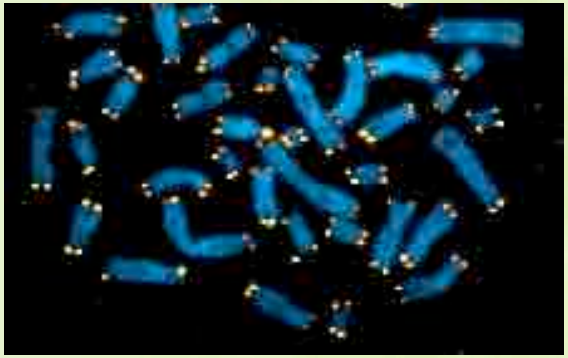
- One strand replicates to the end
- The other strand has a 8 - 12 bp gap at the 5' end.
- Each chromosome in a cell that divides repeatedly will progressively shorten.
- This will lead eventually to chromosomes shorting until genes are lost from the ends.
- Described by Olovnikow,1973.

Telomeres/telomerase maintain chromosome ends

What are telomeres?

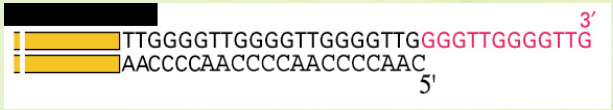
- Telomeres are…
  - Repetitive DNA sequences at the ends of all human chromosomes
  - They contain thousands of repeats of the six-nucleotide sequence, TTAGGG
  - In humans there are 46 chromosomes and thus 92 telomeres (one at each end)

Chromosome Ends are specialized structures called Telomeres



Blue = DNA White = Telomere protein (TERT)

Telomeres



Repeated G rich sequence on one strand in humans: $(TTAGGG)_n$

Repeats can be several thousand basepairs long. In humans, telomeric repeats average 5-15 kilobases.

Telomere specific proteins, eg. TRF1 & TRF2 bind to the repeat sequence and protect the ends.

Telomere functions

- Telomeres protect chromosome end from DNA repair pathways, repair leads to chromosomal fusions.
- Maintain length of chromosomes.
- Telomeres associate with the nuclear membrane and maintain nuclear organization.

Telomerase

- Telomerase is a ribonucleoprotein enzyme complex (a cellular reverse transcriptase).
  - TERT - RNA directed DNA polymerase.
  - TERC - RNA template.
- It stabilizes telomere length by adding hexameric (TTAGGG) repeats onto the telomeric ends of the chromosomes, thus compensating for the erosion of telomeres that occurs in its absence.

Telomerase is composed of both RNA and protein

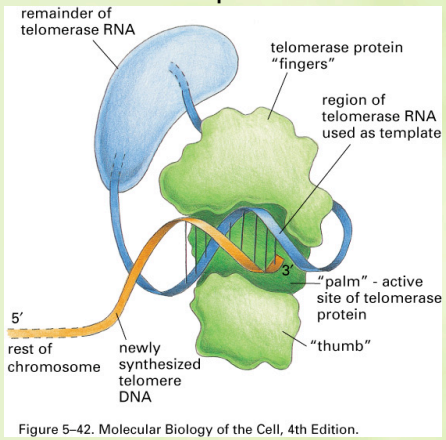


Figure 5–42. Molecular Biology of the Cell, 4th Edition.

How Does Telomerase Work?

- Telomerase works by adding back telomeric DNA to the ends of chromosomes, thus compensating for the loss of telomeres that normally occurs as cells divide.
- Most normal cells do not have this enzyme and thus they lose telomeres with each division.

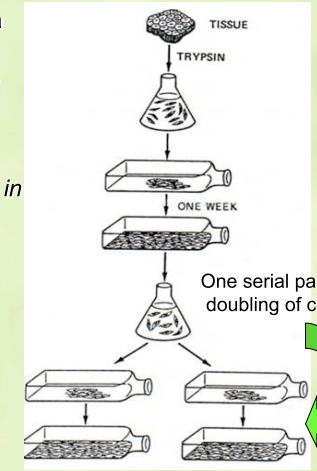
The telomere theory of aging

- Potentially immortal cells (germ cells, cancer cells) maintain telomerase activity
  - Can divide indefinitely.
- Cells with a limited replicative lifespan.
  - Should have no telomerase activity.
  - Progressively shortening telomeres.
  - Cell division serves as a mitotic clock for replicative senescence.
- Provides a mechanistic explanation for the Hayflick limit.

Hayflick limit: cells are only capable of a limited number of population doublings in culture.

Here's what is meant by the term doubling *in vitro*.

Term is used to describe replication going on in culture dishes.



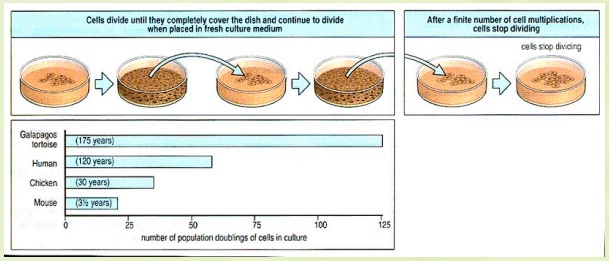
Cell proliferation potential greater in long-lived species

Organism + L.S:
-mouse about 3 years
-human about 100
-Galapagos tortoise about 150

Hayflick Limit:
-doublings about 20
-doublings about 40-60
-doublings about 140

Species	Maximum life span (years)	Maximum doubling number
Galapagos tortoise	175	125
Man	110	60
Horse	46	82
Chicken	30	35
Cat	28	92
Kangaroo	16	46
Mink	10	34
Mouse	4	28

Population doublings

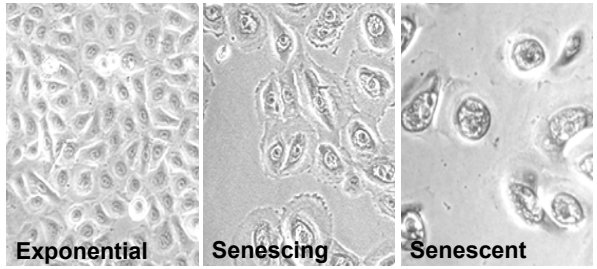


Cell proliferation potential lower from older donors

•Cells from older donors have "used up" some of doublings

Fetal Lung		Adult Lung		
Strain	Number of population doublings	Strain	Number of population doublings	Age of donor
WI-1	51	WI-1000	29	87
WI-3	35	WI-1001	18	80
WI-11	57	WI-1002	21	69
WI-16	44	WI-1003	24	67
WI-18	53	WI-1004	22	61
WI-19	50	WI-1005	16	58
WI-23	55	WI-1006	14	58
WI-24	39	WI-1007	20	26
WI-25	41			
WI-26	50			
WI-27	41			
WI-38	48			
WI-44	63			
Average range	48 (35–63)		20 (14–29)	

Senescence of keratinocytes



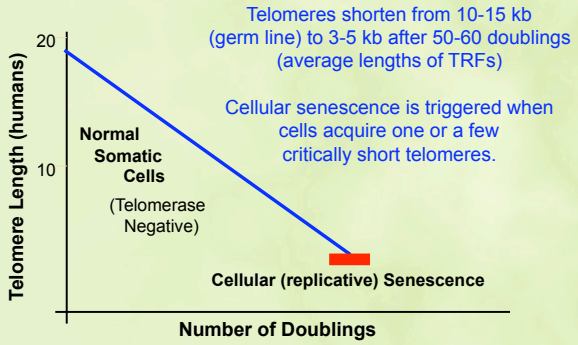
Telomerase Activity

- In humans, telomerase is active in germ cells, in vitro immortalized cells, the vast majority of cancer cells and, possibly, in some stem cells.
- High telomerase activity exists in germ cells, stem cells, epidermal skin cells, follicular hair cells, and cancer cells.
- **Inactive in most cells**: somatic cells, differentiated cells, post-mitotic cells.

Cellular senescence

- Once the telomere shrinks to a certain extent, the cell stops dividing.
  - ~4kb in human cells triggers end to cell division.
- This leads to other changes called cellular senescence:
  - Cell morphology changes.
  - Gene expression changes.

Telomere also provide a means for "counting" cell division: telomeres shorten with each cycle



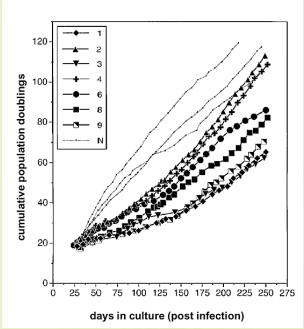
Yeast replicative lifespan regulated by telomere length

- Telomerase mutants have a short lifespan.
  - When telomeres shorten to a critical point, yeast cells stop dividing.
- Overexpression of telomerase:
  - Longer telomeres.
  - Increased replicative lifespan.
- Subtelomeric gene expression is supressed.
  - Shortening of telomeres relieves the supression.

Telomeres in mice

- Lab strains of mice have very long telomeres.
  - 30-40kb telomeres.
  - Therefore, short telomeres aren't the cause of senescence in mice!
- *Tert* knock-out mice:
  - Normal for four generations as their telomeres shorten,
  - Premature aging phenotypes present in the 5th generation.

Werner's cellular phenotype reversed by telomerase expression



Dermal fibroblasts transformed with TERT (telomerase) continue dividing, Werner's cells typically stop dividing at 20 population doublings.