Telomeres

What are they?

- Ends of linear chromosomes
  - Repetitive DNA sequence: TTAGGG in vertebrates
  - Specialized proteins
  - Form a "capped" end structure

Why are telomeres important?

- Telomeres allow cells to distinguish chromosome ends from broken DNA
  - If DNA is broken there are two options after the cell cycle is stopped: Repair or Death
    - Repair can occur in two ways:
      - Homologous Recombination (HR) -- Error-free but need homologue nearby
      - Non-homologous end-joining (NHEJ) -- Error-prone but saves chromosome from degradation
- Telomeres prevent chromosome fusions by NHEJ
  - Fusion-bridge-breakage cycles leads to genomic instability which in turn can result in cell death or neoplastic transformation
- Telomeres are specialized structures that are essential for protecting chromosome ends and ensuring chromosome stability
- Telomeres also provide a mechanism for "counting" cell divisions

Telomere shortening - the end replication problem

- Telomeres shorten with each cell division (S phase)
  - The "end replication" problem:
    - DNA replication is bidirectional
    - DNA polymerases are unidirectional
    - DNA polymerases must initiate replication from a primer
  - Therefore: each round of DNA replication leaves 50-200 bp DNA unreplicated at the 3' end
- Cells with telomeres that are 10-12 kb in length (average) divide 50-60 times

    - Telomeres are 4-6 kb [5-7 kb] in length (average)
- Cellular senescence is triggeredwhen telomeres are on average 4-6 kb

How do immortal cells avoid telomere loss (i.e. solve the end replication problem)?

Telomerase = the key to replicative immortality

- Enzyme (reverse transcriptase) with protein + RNA subunits
- Adds telomeric repeats directly to 3' overhang (uses its own RNA component as a template)
    - Vertebrate telomere repeat: TTAGGG
    - Vertebrate telomerase RNA template: AATCCC
- Overcomes telomere shortening/end replication problem
    - Added back to somatic cells
        1. Prevemts telomere shortening
        2. Prevents replicative senescence
    - However cells that express telomerase still undergo cellular senescence in response to DNA damage, oncogenes, etc.
- Expressed by germ cells and early embryonic cells
- NOT expressed by most somatic cells (human)
- Expressed by certain stem cells, but highly regulated
- Expressed by 80-90% of tumor cells!
    - remaining 10-20% still need to overcome end replication problem -- do so by alternative telomere lengthening mechanism (ALT), probably recombination

Telomere Hypothesis of Aging

- Hypothesis: Telomeres shorten with age (T cells, tissue with high cell turnover)
    - Therefore, telomere shortening is a cause of aging
- **What is wrong with this hypothesis?**
    - Telomere contributes to aging ONLY IF replicative senescence contributes to aging
        - Therefore, telomerase will prevent aging and/or restore youthfulness
    - Telomerase protects against replicative, but not other forms of cellular senescence

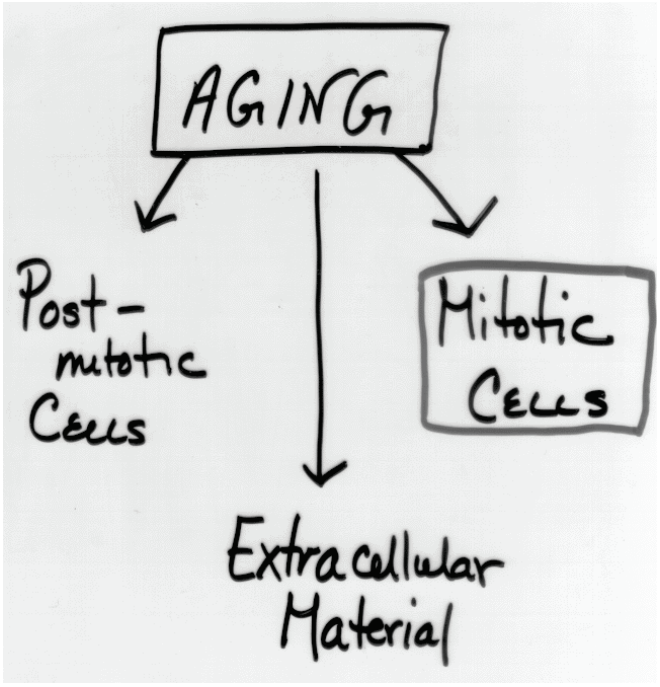
Telomerase: Useful for expanding cells for medical use

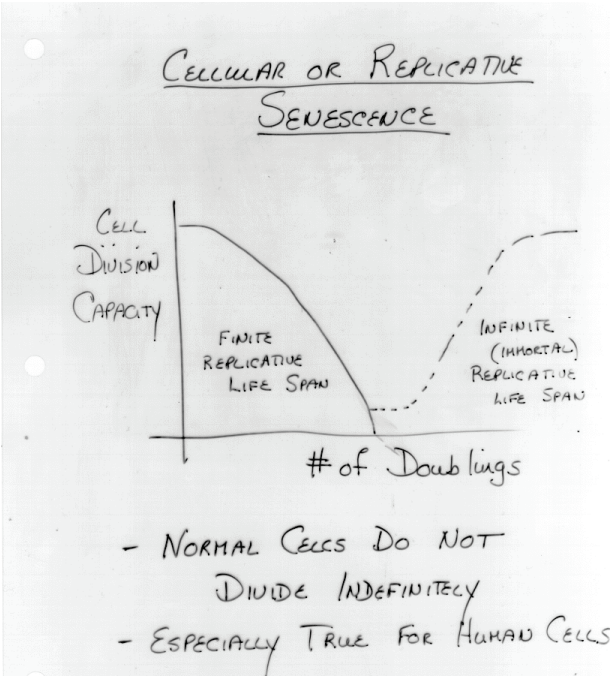
Telomerase Inhibitors: Useful for inhibiting or killing tumor cells

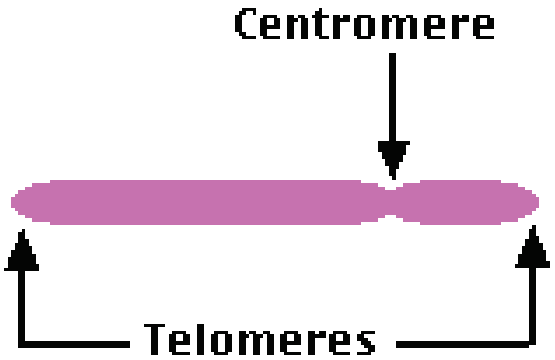
Summary

- Telomeres are essential for chromosome stability
- Telomere shortening causes replicative senescence. Other inducers of cellular senescence are telomere - independent
- Telomerase prevents telomere shortening and replicative senescence
- The telomere hypothesis of aging hinges on the cellular/replicative senescence hypothesis of aging

Dr. Campisi's diagrams from last year's lecture:







Cellular or Replicative Senescence

Exceptions:

1) Germ Line
2) Tumor Cells
3) Possibly Some Stem Cells

- Importance of Cell Senescence
- What Happens To Cells When They Senesce?
- How Do Cells Know When To Senesce?
- What Genes Control Replicative Senescence?

Importance of Replicative Senescence

- Tumor Suppression
  (Tumor cells overcome senescence in order to develop a malignant phenotype)
- Aging
  (Senescent cells accumulate with age, can destroy tissue integrity and function)

What Happens To Cells When They Senesce?

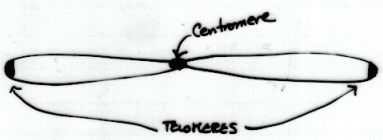
- Permanent Growth Arrest
- Resistance to Apoptosis
- Altered Function

Relation to: Tumor Suppression
Aging

How Do Cells Know When To Senesce?

Telomere Shortening

- Telomeres are the ends of linear chromosomes

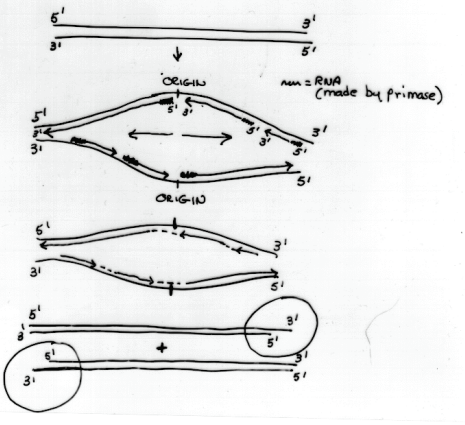


- Telomeres are composed of a repeated DNA sequence TTAGGG and specialized proteins
- Telomeres stabilize chromosomes

Telomeres Shorten With Each Cell Division

Why?

1) DNA replication is bidirectional
2) DNA polymerases move in only one direction (5'→3')
3) DNA polymerases need a RNA or DNA primer



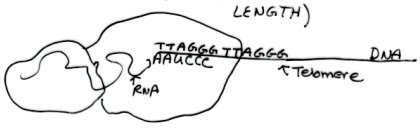
Telomere Length

Sperm, Eggs	20 kb
Fetal Cells Neonatal Cells	~ 15 kb
Adult Cells	~ 10 kb
Senescent Cells	~ 5 kb

Cells Senesce when telomeres shorten to about 5 kb (60-80 cell divisions)

What About Cells That Do Not Senesce?

Telomerase: Specialized enzyme, adds TTAGGG to chromosome ends (maintains telomere length)

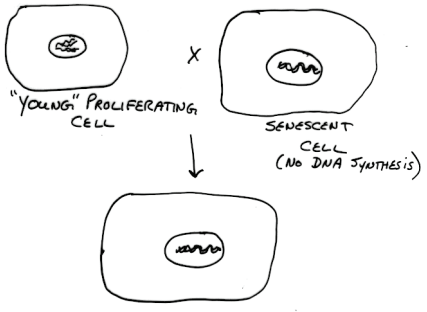


Most Cells Do Not Express Telomerase

Exceptions: Germ Cell Precursors
Tumor Cells
Some Stem Cells

What Are the Genes That Control Replicative Senescence?

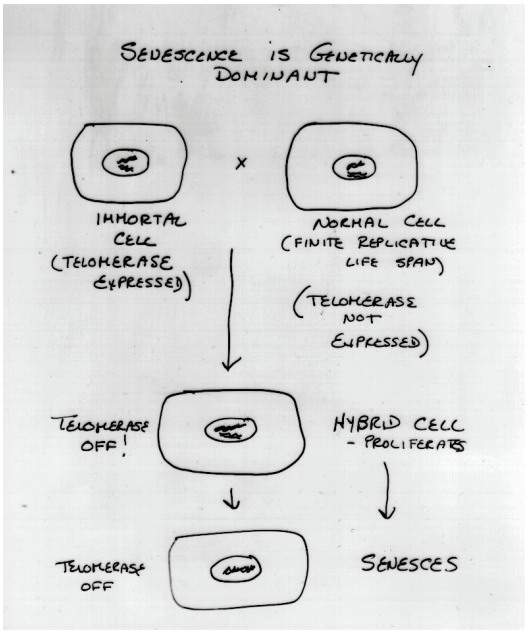
Senescence is Genetically Dominant



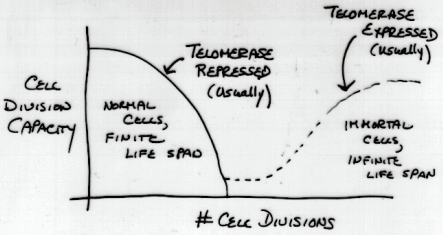
No DNA Synthesis

Senescent Cells Express Dominant Inhibitors of DNA Synthesis

Argues against damage hypothesis



- Normal Cells Express a Dominant Inhibitor of Telomerase
- Lack of Telomerase **alone** Does Not Arrest Cell Proliferation, But Does Cause Eventual Senescence



What Causes Cells to Stop Dividing?

Are There Genes That Prevent Senescence, Even in the Absence of Telomerase?

p53

Expressed by all normal cells

Binds DNA / Controls Expression of Genes Needed for Cell Proliferation and Cell Death

Level and Activity Determines Whether Cells Proliferate or Die

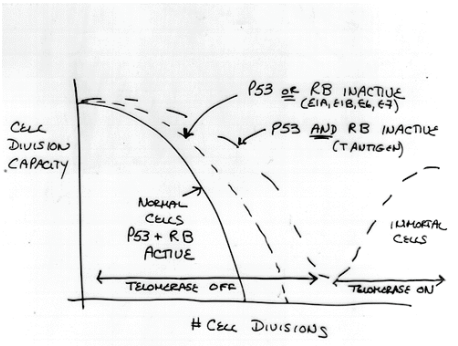
RB

EXPRESSED BY ALL NORMAL CELLS

BINDS AND CONTROLS CELLULAR TRANSCRIPTION FACTORS

ACTIVITY DETERMINES WHETHER CELLS PROLIFERATE

CAN ALSO REGULATE CELL DEATH (INDIRECT?)



GENES CRITICAL FOR CELL SENESCENCE:

TELOMERASE REPRESSOR

P53
RB > genes controlled by P53 and RB

GENETICALLY ENGINEERED MICE (p53, p16 KO)

CELLS: FAIL TO SENESCE

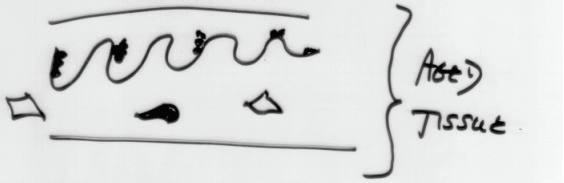
ANIMALS: TUMORS AT A YOUNG AGE

NO CELL SENESCENCE = CANCER

CELL SENESCENCE = AGING

CELL SENESCENCE & AGING

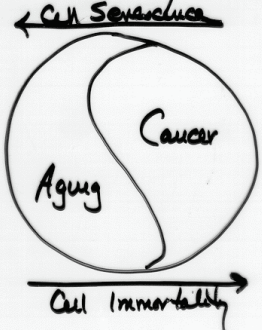
- DONOR AGE
- SPECIES LIFE SPAN
- PREMATURE AGING SYNDROMES
- SENESCENT CELLS ACCUMULATE WITH AGE IN HUMAN TISSUE (but not many!)

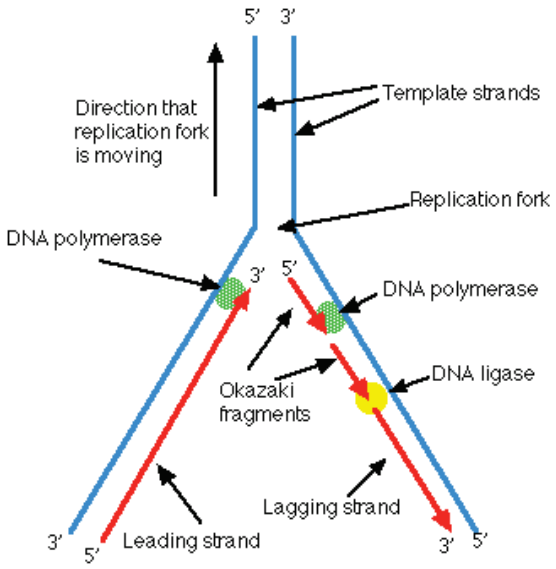


SENESCENT CELLS EXPRESS MOLECULES THAT ACT AT A DISTANCE

- Proteases
- Anti-Angiogenic Factors
- GROWTH FACTORS
- INFLAMMATORY Cytokines

SENESCENT CELLS Produce Molecules that can disrupt normal tissue function and integrity





Each eukaryotic chromosome consists of **a single molecule of DNA** associated with a variety of proteins.

The DNA molecules in eukaryotic chromosomes are linear; i.e., have two ends. (This is in contrast to such bacterial chromosomes as that in E. coli that is a closed circle, i.e. has no ends.)

The DNA molecule of a typical chromosome contains

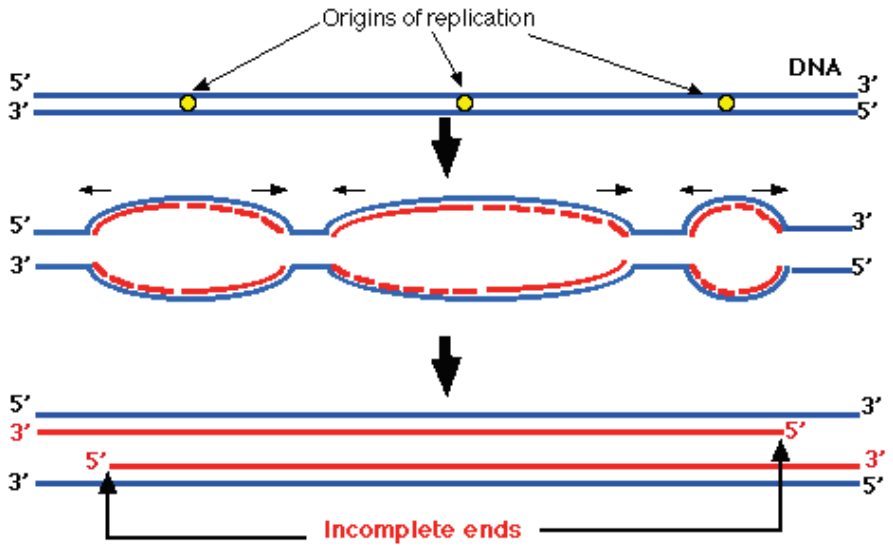
- a linear array of genes (encoding proteins and RNAs) interspersed with
- much noncoding DNA.

Included in the noncoding DNA are

- long stretches that make up the **centromere** and
- long stretches at the ends of the chromosome, the **telomeres**.

Telomeres are crucial to the life of the cell. They keep the ends of the various chromosomes in the cell from accidentally becoming attached to each other.

The telomeres of humans consist of as many as 2000 repeats of the sequence 5' **GGTTAG** 3'.



Replication of linear chromosomes presents a special problem.

DNA polymerase can only synthesize a new strand of DNA as it moves along the **template** strand in the 3' –> 5' direction. This works fine for the 3' –> 5' strand of a chromosome as the DNA polymerase can move uninterruptedly from an **origin of replication** until it meets another bubble of replication or the end of the chromosome. However, synthesis using the 5' –> 3' strand as the template has to be **discontinuous**. When the replication fork opens sufficiently, DNA polymerase can begin to synthesize a section of complementary strand — called an Okazaki fragment — working in the opposite direction. Later, a **DNA ligase** ("DNA ligase I") stitches the Okazaki fragments together.

In the figure on the right, the horizontal black arrows show the direction that the replication forks are moving. Wherever the replication fork of a strand is moving towards the 3' end, the newly-synthesized DNA (red) begins as Okazaki fragments (red dashes).

This continues until close to the end of the chromosome. Then, as the replication fork nears the end of the DNA, there is no longer enough template to continue forming Okazaki fragments. So the 5' end of each newly-synthesized strand cannot be completed. Thus each of the daughter chromosomes will have a shortened telomere.

It is estimated that human telomeres lose about 100 base pairs from their telomeric DNA at each mitosis.

This represents about 16 GGTTAG repeats. At this rate, after 125 mitotic divisions, the telomeres would be completely gone.

Is this why normal somatic cells are limited in the number of mitotic divisions before they die out?

Telomeres and Cellular Aging

Telomeres are important so their steady shrinking with each mitosis might impose a finite life span on cells. This, in fact, is the case. Normal (non-cancerous) cells do not grow indefinitely when placed in culture.

Cells removed from a newborn infant and placed in culture will go on to divide almost 100 times. Well before the end, however, their rate of mitosis declines (to less than once every two weeks). Were my cells to be cultured (I am 81 years old), they would manage only a couple of dozen mitoses before they ceased dividing and died out.

This phenomenon is called **replicative senescence** [More]. Could shrinkage of telomeres be a clock that determines the longevity of a cell lineage and thus is responsible for replicative senescence?

Evidence:

Some cells do not undergo replicative senescence:

- the cells of the germline (the germplasm);
- unicellular eukaryotes like Tetrahymena thermophila;
- stem cells, including "adult" stem cells and cancer stem cells.

It turns out that these cells are able to maintain the length of their telomeres. They do so with the aid of an enzyme **telomerase**.

Telomerase

Telomerase is an enzyme that adds telomere repeat sequences to the 3' end of DNA strands. By lengthening this strand, DNA polymerase is able to complete the synthesis of the "incomplete ends" of the opposite strand.

Telomerase:

- is a **ribonucleoprotein**.

- Its single snoRNA molecule — called **TERC** ("TElomere RNA Component") — provides an CCAAUC (in mammals) template to guide the insertion of GGTTAG.
- Its protein component — called **TERT** ("TElomere Reverse Transcriptase") — provides the catalytic action.
- Thus telomerase is a reverse transcriptase; synthesizing DNA from an RNA template.

Telomerase is generally found only in

- the cells of the germline, including embryonic stem (ES) cells;
- unicellular eukaryotes like Tetrahymena thermophila;
- some, perhaps all, "adult" stem cells (including cancer stem cells) and "progenitor" cells enabling them to proliferate.

When normal somatic cells are transformed in the laboratory with DNA expressing high levels of telomerase, they continue to divide by mitosis long after **replicative senescence** should have set in. And they do so without any further shortening of their telomeres. This remarkable demonstration (reported by Bodnar et. al. in the 16 January 1998 issue of **Science**) provides the most compelling evidence yet that telomerase and maintenance of telomere length are the key to cell immortality.

Telomerase and Cancer

The crucial feature that distinguishes a cancer from normal tissue is its ability to grow indefinitely. Most (85–90%) cancers express telomerase — at least in the population of cancer stem cells that divide uncontrollably causing the tumor to grow.

Perhaps agents that prevent the expression of the gene for telomerase — or prevent the action of the enzyme — will provide a new class of weapons in the fight against cancer. But

- if telomerase activity — however brief — is essential for all cells, we had better be careful, and
- if lack of telomerase hastens replicative senescence, it may also hasten the aging of the tissues that depend on newly-formed cells for continued health — a tradeoff that may not be worth making.

Telomerase and Transplanted Cells

One approach to gene therapy it to

- remove cells from the patient,
- transform them with the gene for the product that the patient has been unable to synthesize,
- return them to the patient.

One problem with this approach is that the cells — like all normal somatic cells — are mortal. After a series of mitotic divisions, they die out. That is the reason the children described in the link above required periodic fresh infusions of their transformed T cells.

What if their cells could be transformed not only with the **therapeutic gene** but also with an **active telomerase gene**? This should give them an unlimited life span.

But if cancer cells regain the ability to make telomerase, might not the reverse be true; that cells transformed with an active telomerase gene might become cancerous?

Perhaps not. The cells described by Bodnar et. al. in the 16 January 1998 issue of **Science** have continued to grow in culture and have been subjected to a number of tests to see if they have acquired any properties of cancer cells in culture.
The results are encouraging. While these cells continue to divide indefinitely as cancer cells do,

- They still show **contact inhibition** as normal cells do when grown in culture.
- They do not grow into **tumors** when injected into immunodeficient mice (as cancer cells do).
- They are still fussy about their diet — unable to grow on the simple media that supports cancer cells in culture.
- They still retain a normal karyotype; something that cancer cells seldom do.

However, studies with whole animals — transgenic mice that express abnormally high levels of TERT — reveal that they do suffer an elevated incidence of cancer.

Telomeres and Cloning

The now-famous sheep Dolly was cloned using a nucleus taken from an adult sheep cell that had been growing in culture. The cell donor was 6 years old, and its cells had been growing in culture for several weeks.

What about Dolly's telomeres? Analysis of telomere length in Dolly's cells reveals that they were only 80% as long as in a normal one-year-old sheep. Not surprising, since the nucleus that created Dolly had been deprived of telomerase for many generations.

Two other sheep — cloned from embryonic, not adult, cells — also had shortened telomeres although not as short as Dolly's. Perhaps the length of time the cells spent in culture before they were used accounts for this.

Does this mean that Dolly is doomed to a shortened life? She seemed healthy at first and even had babies of her own. But medical problems — probably unrelated to her telomeres — ended with her being euthanized at a relatively young age.

But her short telomeres do add another question to the debate about cloning mammals from adult cells.