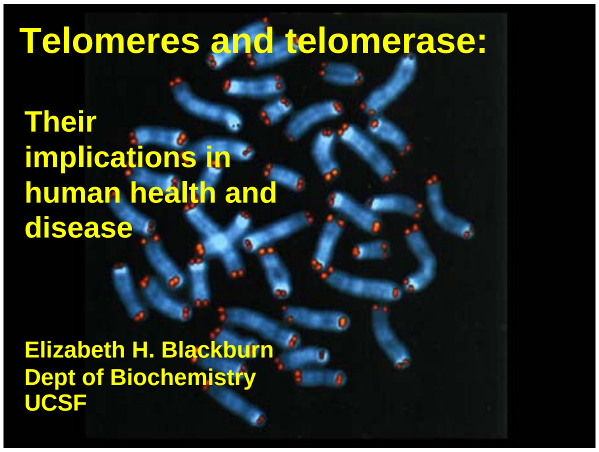
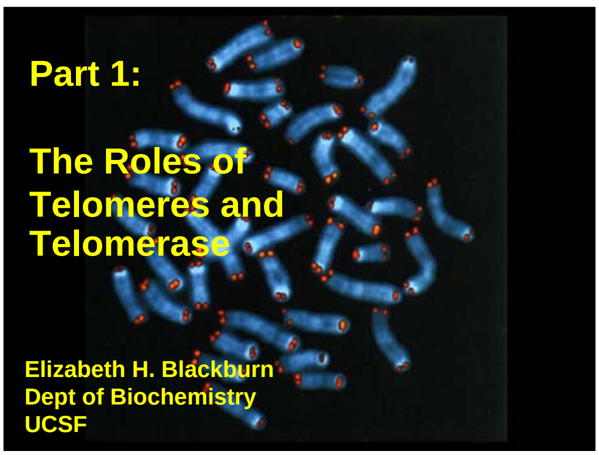
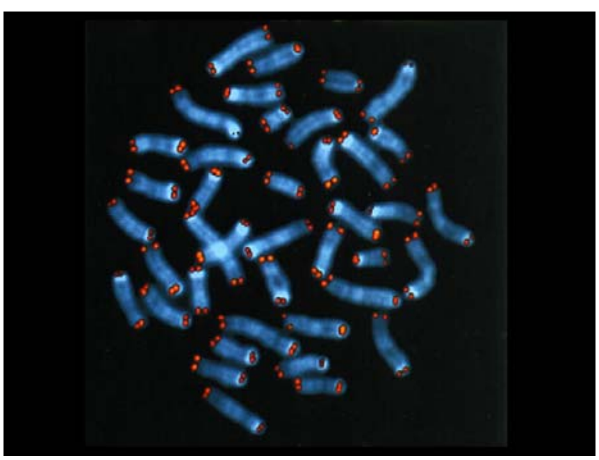
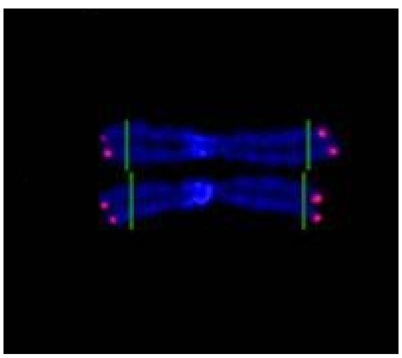
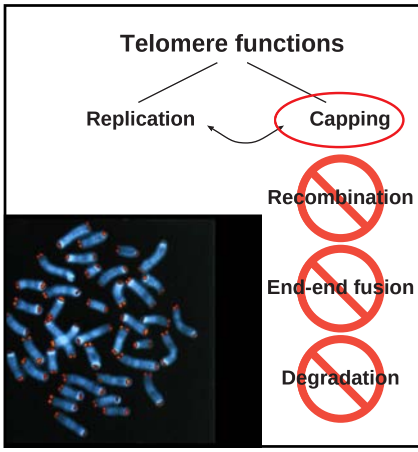

Telomeres and telomerase:
Their implications in human health and disease
Elizabeth H. Blackburn
Dept of Biochemistry
UCSF


Part 1:
The Roles of Telomeres and Telomerase
Elizabeth H. Blackburn
Dept of Biochemistry
UCSF



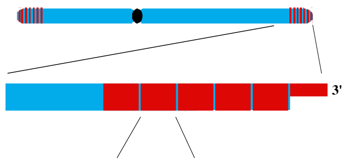
**Telomeres cap ends of chromosomes**





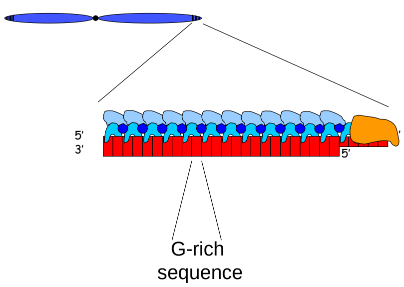
**Telomeric DNA contains simple tande**

specified by telomerase

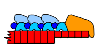


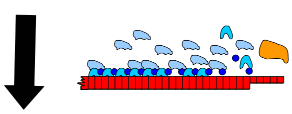

| | |
|---|---|
| Tetrahymena | TTGGGG |
| Vertebrates | TTAGGG |
| S. cerevisiae | $(TG)_{1-6}TG_{2-3}$ |
| K. lactis | ACGGATTTGATTAGG |
| C. albicans | ACGGATGTCTAACTT |

## Telomere Structure

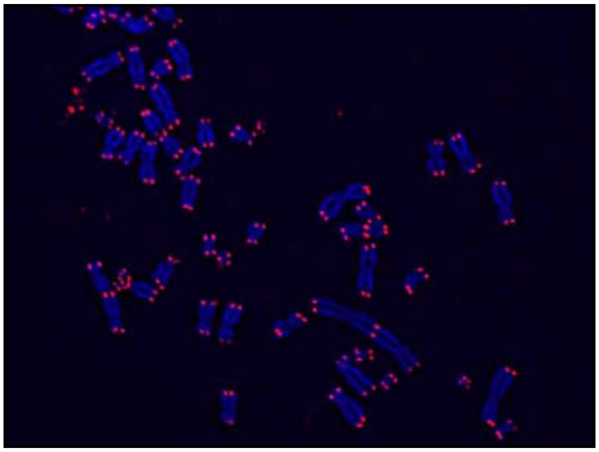


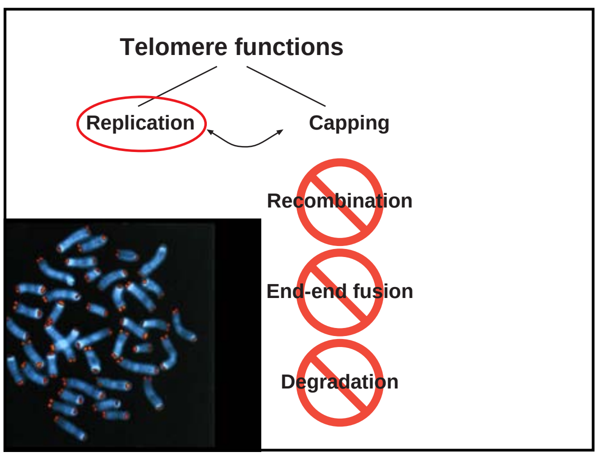

## Telomere Dysfunction

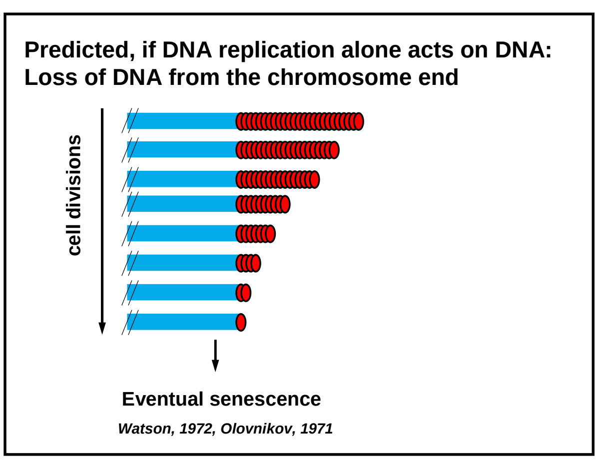




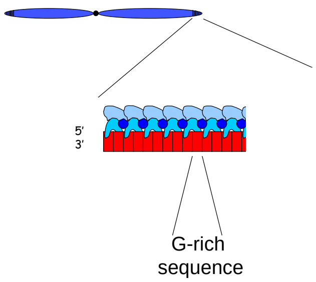
**Limits cell renewal capability**
**Can lead to genomic instability**




Telomere functions
Replication
Capping
Recombination
End-end fusion
Degradation


Predicted, if DNA replication alone acts on DNA:
Loss of DNA from the chromosome end
cell divisions
Eventual senescence
Watson, 1972, Olovnikov, 1971

**Telomere Structure**




**WHAT IS**

**TELOMERASE?**

**RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION**

RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION

1. Telomeric GGGGTT repeat tracts on minichromosomes in a ciliate were heterogeneous.
Blackburn and Gall, 1978

RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION

2. Telomeric GGGGTT repeat tract DNA was found added to various sequences in ciliate mini-chromosomes as a result of new telomeres forming on chromosomes, during development of the somatic nucleus.
Blackburn et al, 1982

RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION

3. Telomeric DNA gradually grew longer as trypanosome cells multiplied.
Bernards et al, 1983

RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION

4. Yeast telomeric TG1-3 repeat DNA was added directly to the ends of Tetrahymena $T_2G_2$ repeat telomeres maintained in yeast.

Szostak and Blackburn 1982; Shampay, Szostak and Blackburn 1984

RESULTS WITH TELOMERIC DNA THAT COULD NOT BE READILY EXPLAINED BY CURRENT MODELS FOR DNA REPLICATION

AND…….

5. Barbara McClintock had noted a maize mutant stock that had lost the normal capacity for broken maize chromosome ends to heal early on plant development.

B. McClintock, personal comm. 1983

**WAS A NEW ENZYME AT WORK IN CELLS THAT COULD EXTEND TELOMERIC DNA?**

***Tetrahymena thermophila***

**Carol Greider, UC Berkeley ca.1985**

**ASSAY FOR TELOMERE TERMINAL TRANSFERASE**

INPUT OLIGOMER

5' G G G G T T G G G G T T G G G G T T 3' OH

Tetrahymena cell S-100
Mg++
dGTP + TTP

↓

G G G G T T G G G G T T G G G G T T g g g g t t g g g

….. up to
30 $G_4T_2$ repeats

Greider and Blackburn, 1985, 1987

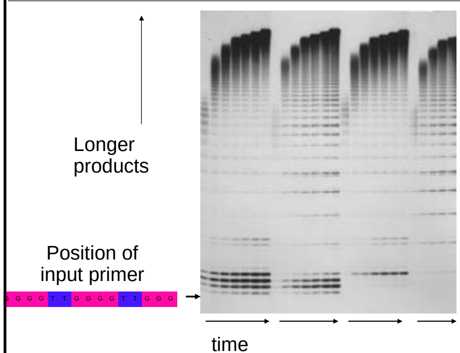
**Many tandem repeats could be added to a DNA primer by telomerase activity in the test tube**

g g t t g g g g t t g g g g t t g g g g t t g g g g t t g g

….. up to
30 $G_4T_2$ repeats

Greider and Blackburn, 1985, 1987

**Radiolabeled addition products made by telomerase**




Greider and Blackburn, 1985, 1987, M.Lee, unpub.

**Telomerase preferred, for a primer, the DNA strand corresponding to the sequence at the very 3' end of chromosomal DNA**

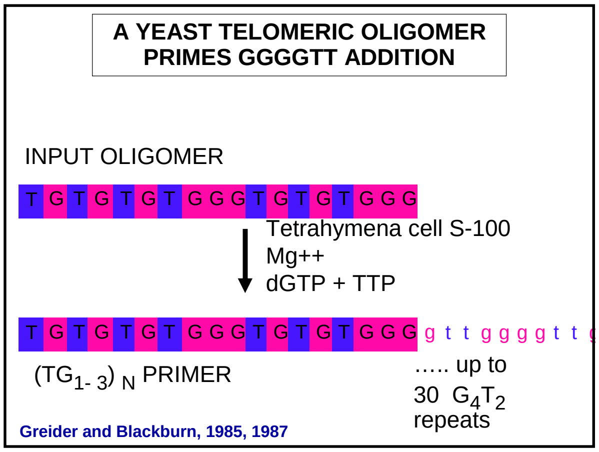
T T G G G G T T G G G G T T G G G G t t g g g g t t g g

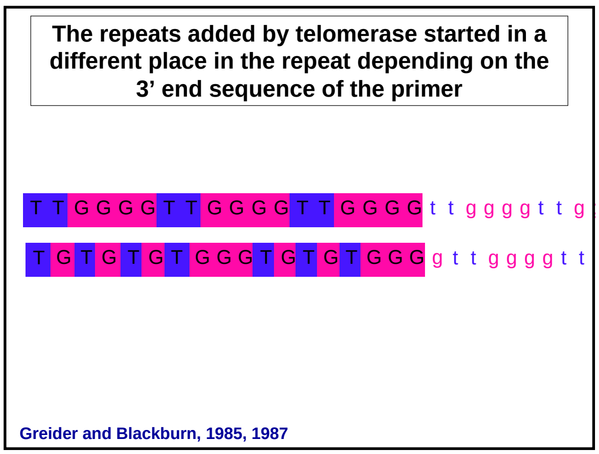
G G G G T T G G G G T T G G G G T T g g g g t t g g

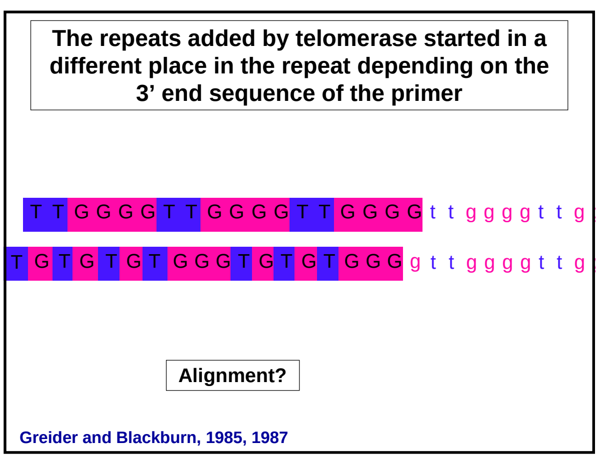
C C C C A A C C C C A A C C C C A A X

A A C C C C A A C C C C A A C C C C X

Greider and Blackburn, 1985, 1987


A YEAST TELOMERIC OLIGOMER PRIMES GGGGTT ADDITION
INPUT OLIGOMER
T G T G T G T G G G T G T G T G G G
Tetrahymena cell S-100
Mg++
dGTP + TTP
T G T G T G T G G G T G T G T G G G g t t g g g g t t
(TG1- 3) N PRIMER
….. up to 30 G4T2 repeats
Greider and Blackburn, 1985, 1987


The repeats added by telomerase started in a different place in the repeat depending on the 3' end sequence of the primer
T T G G G G T T G G G G T T G G G G t t g g g g t t g
T G T G T G T G G G T G T G T G G G g t t g g g g t t
Greider and Blackburn, 1985, 1987

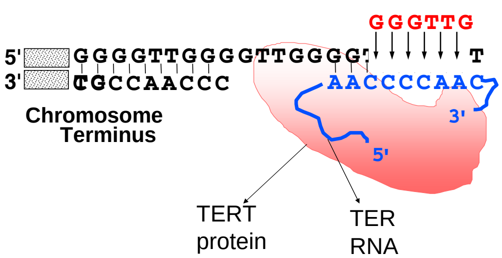

The repeats added by telomerase started in a different place in the repeat depending on the 3' end sequence of the primer
T T G G G G T T G G G G T T G G G G t t g g g g t t g
T G T G T G T G G G T G T G T G G G g t t g g g g t t g
Alignment?
Greider and Blackburn, 1985, 1987

**Tests for alignment of the primer 3' end on a potential template**


**Alignment**

Greider and Blackburn, 1985, 1987

**Telomerase:**
**a telomere-synthesizing reverse transcriptase**




Greider and Blackburn, 1985, 1987, 1989

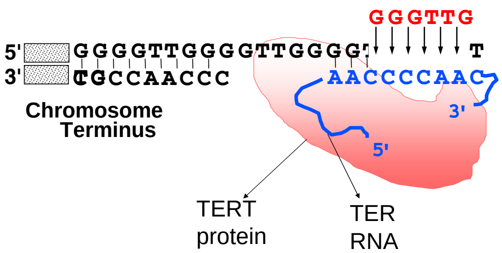
## Telomerase is a unique polymerase

- **A cellular reverse transcriptase.**
- **Contains an intrinsic RNA component.**
- **Specializes in synthesizing multiple short repeats.**

## WHAT DOES TELOMERASE DO FOR CELLS?

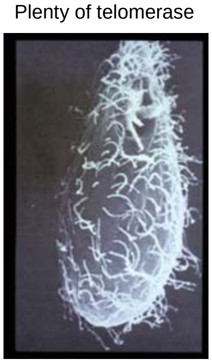
**The solution to telomere attrition**

**Telomerase: a telomere-synthesizing reverse transcriptase**



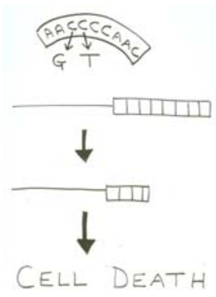

Greider and Blackburn, 1985, 1987, 1989

Plenty of telomerase



Cells are immortal

***Tetrahymena thermophila***




Inactivate telomerase

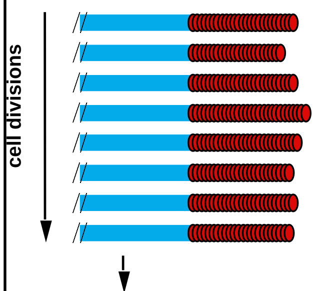
Telomeres progressively shorten

Tetrahymena became “mortal”

Yu et al, Nature 1990

## Telomerase maintains the ends of chromosomes

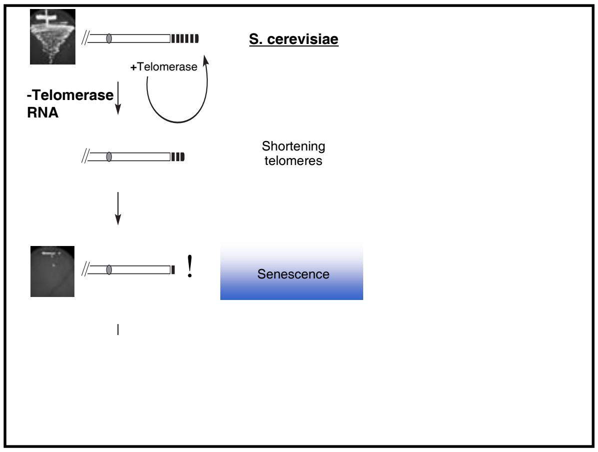
Telomeres replenished by telomerase

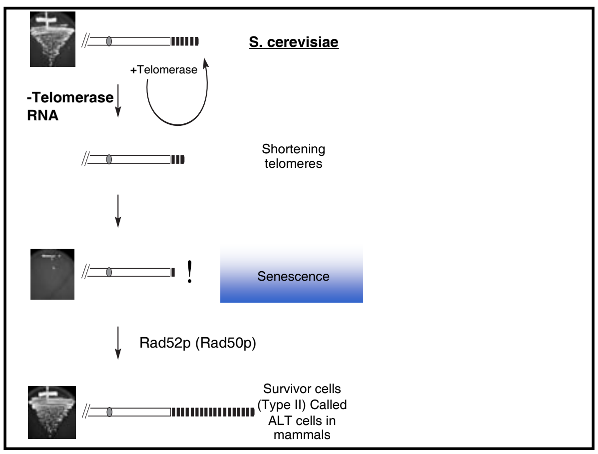


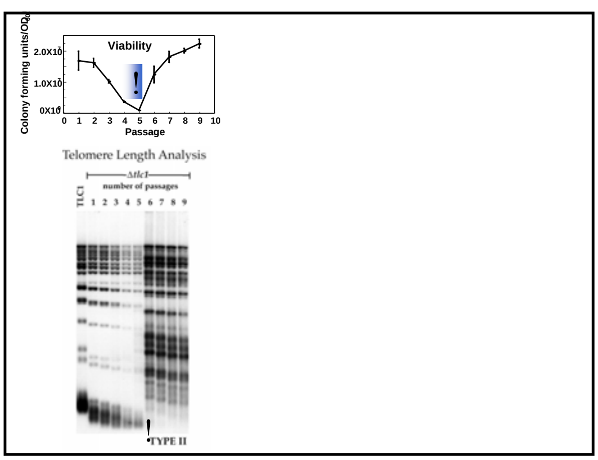

Cells keep dividing

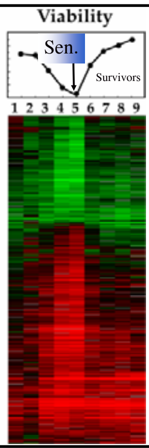
TAKE-HOME MESSAGE:

**Telomeres are replenished by telomerase**


S. cerevisiae
+Telomerase
-Telomerase RNA
Shortening telomeres
Senescence


S. cerevisiae
+Telomerase
-Telomerase RNA
Shortening telomeres
Senescence
Rad52p (Rad50p)
Survivor cells (Type II) Called ALT cells in mammals


Viability
Colony forming units/OD
Passage
Telomere Length Analysis
number of passages
TYPE II




Passage # after telomerase deletion

Patterns of Gene Expression Induced by Δ*tlc1*

Nautiyal et al, PNAS 2001

## The Telomerase Deletion Response (TDR)

1. A set of genes is uniquely upregulated in response to deletion of telomerase RNA - the "telomerase deletion signature".

Nautiyal et al, PNAS 2001

## The Telomerase Deletion Response (TDR)

CONTINUED…

2. The TDR also overlaps with:
- DNA damage response
- "Environmental stress" cellular response
- Change to an aerobic metabolism program

Nautiyal et al, PNAS 2001

**The Telomerase Deletion Response (TDR)**

CONTINUED…

3. Yeast growing without telomerase are *different*:

in ∆tlc1 survivors, transcriptional profile is

- distinct from wild type
- indicative of a cellular stress response

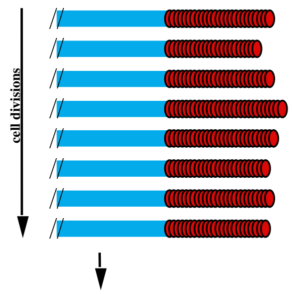
Nautiyal et al, PNAS 2001

TAKE-HOME MESSAGE:

Yeast lacking telomerase using recombination to maintain telomeres grow well but are under cellular stress

Nautiyal et al, PNAS 2001

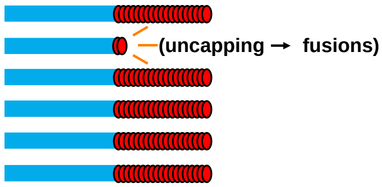
**Plenty of telomerase: homeostasis balanced**




**Cells keep dividing**

### An experiment in yeast

**Remove active telomerase:**
**<u>Even well before</u> senescence, catastrophic shortening of occasional telomeres**




**- even when bulk telomeres still LONG**

Chan and Blackburn, 2003

### A Protective Function for Telomerase I

Telomerase protects even lengthened telomeres from catastrophic shortening and fusing to a double-stranded break

Chan and Blackburn, 2003