Telomeres and telomerase: Their implications in human health and disease

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Part 1:
The Roles of Telomeres and Telomerase

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Telomeres cap ends of chromosomes

Telomere functions:
- Capping
- Replication
- Recombination
- End-end fusion
- Degradation
Telomeric DNA contains simple tandem repeats specified by telomerase.

- Tetrahymena: TGGGG
- Vertebrates: TTAGGG
- S. cerevisiae: (TG)1-6TG2-3
- K. lactis: ACGGATTTGATTAG
- C. albicans: ACGGATGTCTAACTT

**Telomere Structure**

- G-rich sequence

**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

- 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 GGGGT repeat tracts on minichromosomes in a ciliate were heterogeneous.
   *Blackburn and Gall, 1978*

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

3. Telomeric DNA gradually grew longer as trypanosome cells multiplied.
   *Bernards et al., 1983*
4. Yeast telomeric TG1-3 repeat DNA was added directly to the ends of Tetrahymena T2G2 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

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

WAS A NEW ENZYME AT WORK IN CELLS THAT COULD EXTEND TELOMERIC DNA?
**ASSAY FOR TELOMERE TERMINAL TRANSFERASE**

**INPUT OLIGOMER**

\[ 5' G G G G T T G G G G T T G G G T T G G G G T T 3' OH \]

Tetrahymena cell S-100

\[ Mg^{++} + dGTP + TTP \]

\[ G G G G T T G G G G T T G G G G T T g g g g g g \]

….. up to 30 \( G_4T_2 \) repeats

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Greider and Blackburn, 1985, 1987
Many tandem repeats could be added to a DNA primer by telomerase activity in the test tube

\[ ggttggttgggggttggtttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggttggggtg \]

..... up to 30 G4T2 repeats

Greider and Blackburn, 1985, 1987

Radiolabeled addition products made by telomerase

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

Greider and Blackburn, 1985, 1987
A YEAST TELOMERIC OLIGOMER PRIMES GGGGTT ADDITION

INPUT OLIGOMER

TGTTGTTGGCTGGTTGG  
Tetrahymena cell S-100 
Mg++ 
dGTP + TTP

(TG$_1$-3)$_N$ PRIMER

..... up to 30 G$_4$T$_2$ 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

GGGGTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGTGGGGTGGGGGTTTTGGGGT GG

The repeats added by telomerase started in a different place in the repeat depending on the 3' end sequence of the primer

Alignment?

Greider and Blackburn, 1985, 1987
Telomerase is a unique polymerase

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

Greider and Blackburn, 1985, 1987, 1989

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

Greider and Blackburn, 1985, 1987
WHAT DOES TELEOMERASE DO FOR CELLS?

The solution to telomere attrition
Telomerase: a telomere-synthesizing reverse transcriptase

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Plenty of telomerase

Tetrahymena thermophila

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Cells are immortal
Telomeres and Telomerase: Their Implications in Human Health and Disease, Part 1

Inactivate telomerase
Telomeres progressively shorten
Tetrahymena became "mortal"

Yu et al, Nature 1990

Telomeres are replenished by telomerase

Cells keep dividing

Telomerase maintains the ends of chromosomes

TAKE-HOME MESSAGE:
Telomeres are replenished by telomerase
Telomeres and Telomerase: Their Implications in Human Health and Disease, Part 1

Are other telomerase components up-regulated?
Is there a DNA damage response?

Survivor cells (Type II) Called ALT cells in mammals

Viability

Colony forming units/OD

Telemere Length Analysis
The Telomerase Deletion Response (TDR)

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

2. The TDR also overlaps with:
   - DNA damage response
   - “Environmental stress” cellular response
   - Change to an aerobic metabolism program
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:
Even well before senescence, catastrophic shortening of occasional telomeres

- even when bulk telomeres still LONG

Chan and Blackburn, 2003

A Protective Function for Telomerase

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

Chan and Blackburn, 2003