Roles of telomeres and telomerase in human health and disease

Elizabeth Blackburn, Ph. D.
UCSF

Blackburn lab

Current

Carol Anderson

Josh Cheong

Beth Cimini

Francesca Gazzaniga

Kyle Lapham

Shang Li

Jue Lin

Imke Listerman

Jason Lukas

Jason Lukas

Tet Matsuguchi

Dana Smith

Brad Stohr

Tanya Williams

Lifeng Xu

Recent

Sveta Makovets

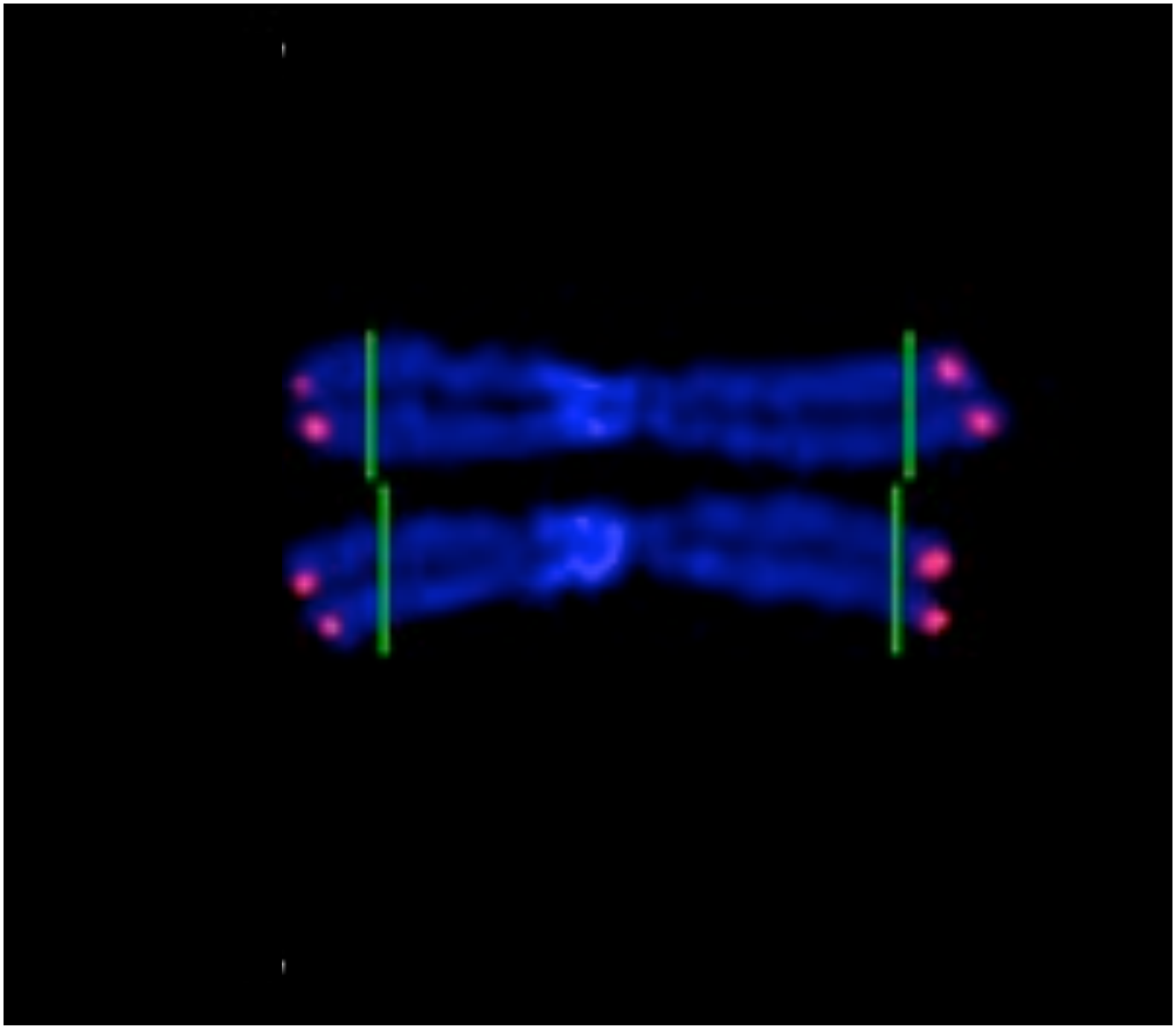
Jeff Seidel

Sherry Wang

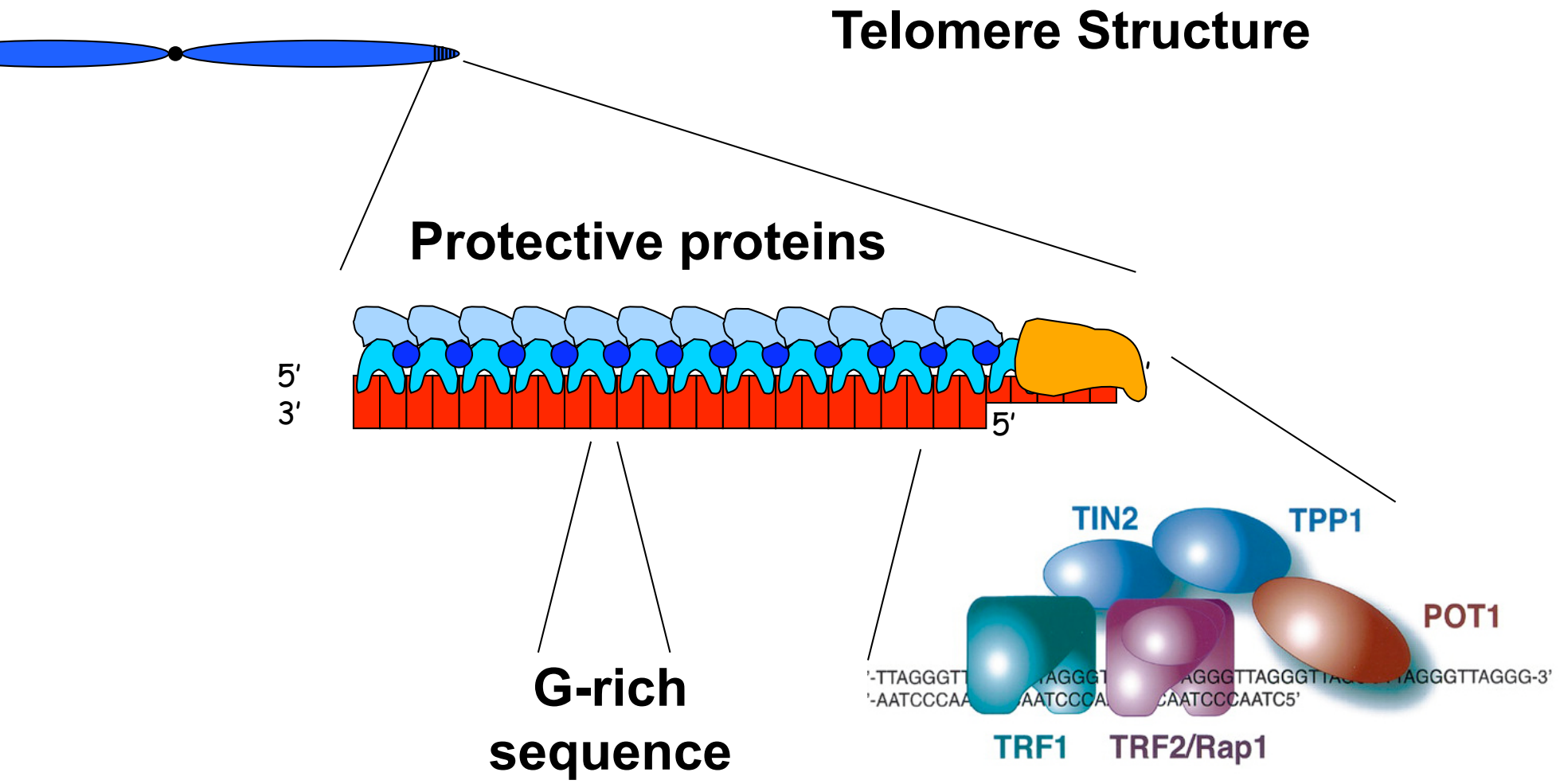
Dan Levy

Shivani Nautiyal

Telomeres cap ends of chromosomes

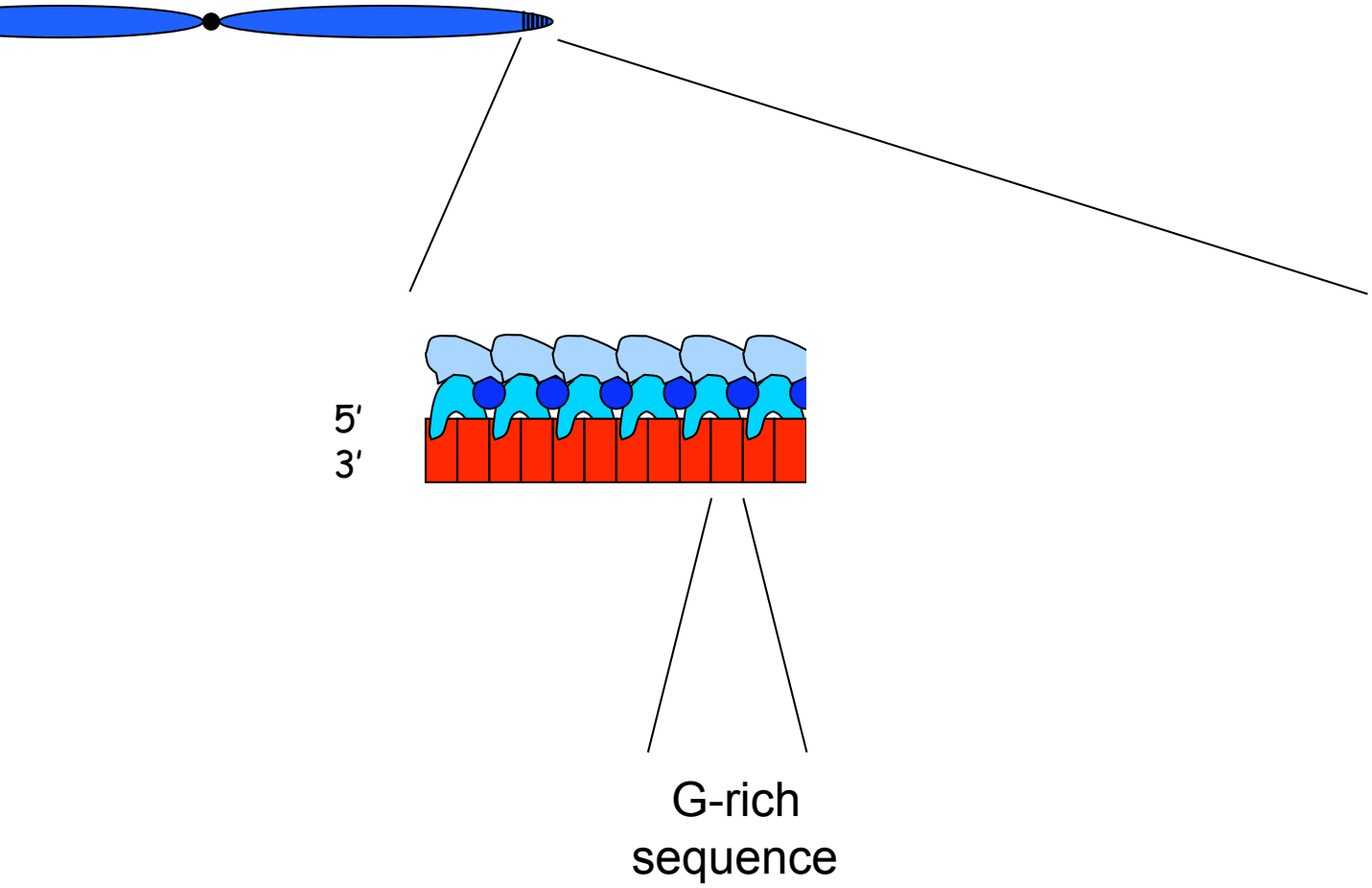


Telomeres form a protective “cap” that prevents improper repair

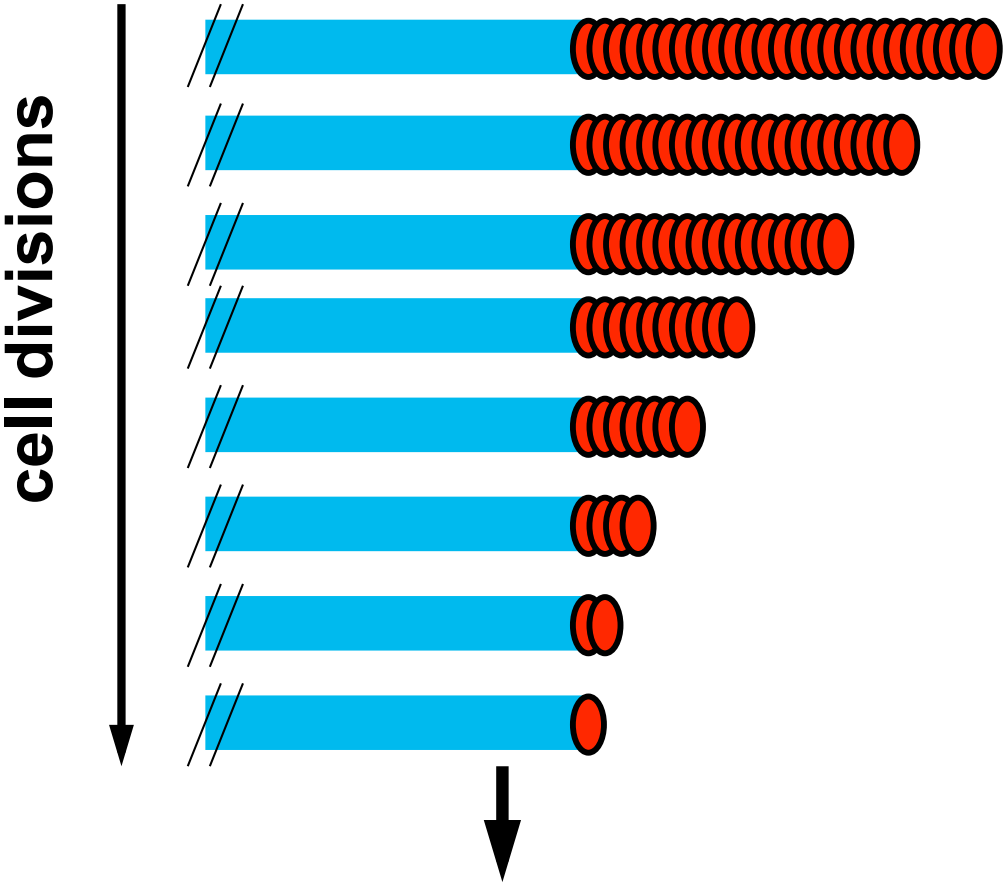


Mammalian: de Lange T. G&D, 2005

Telomere Shortening



Predicted, if DNA replication alone acts on DNA:
Loss of DNA from the chromosome end
(the DNA 'end-replication problem')

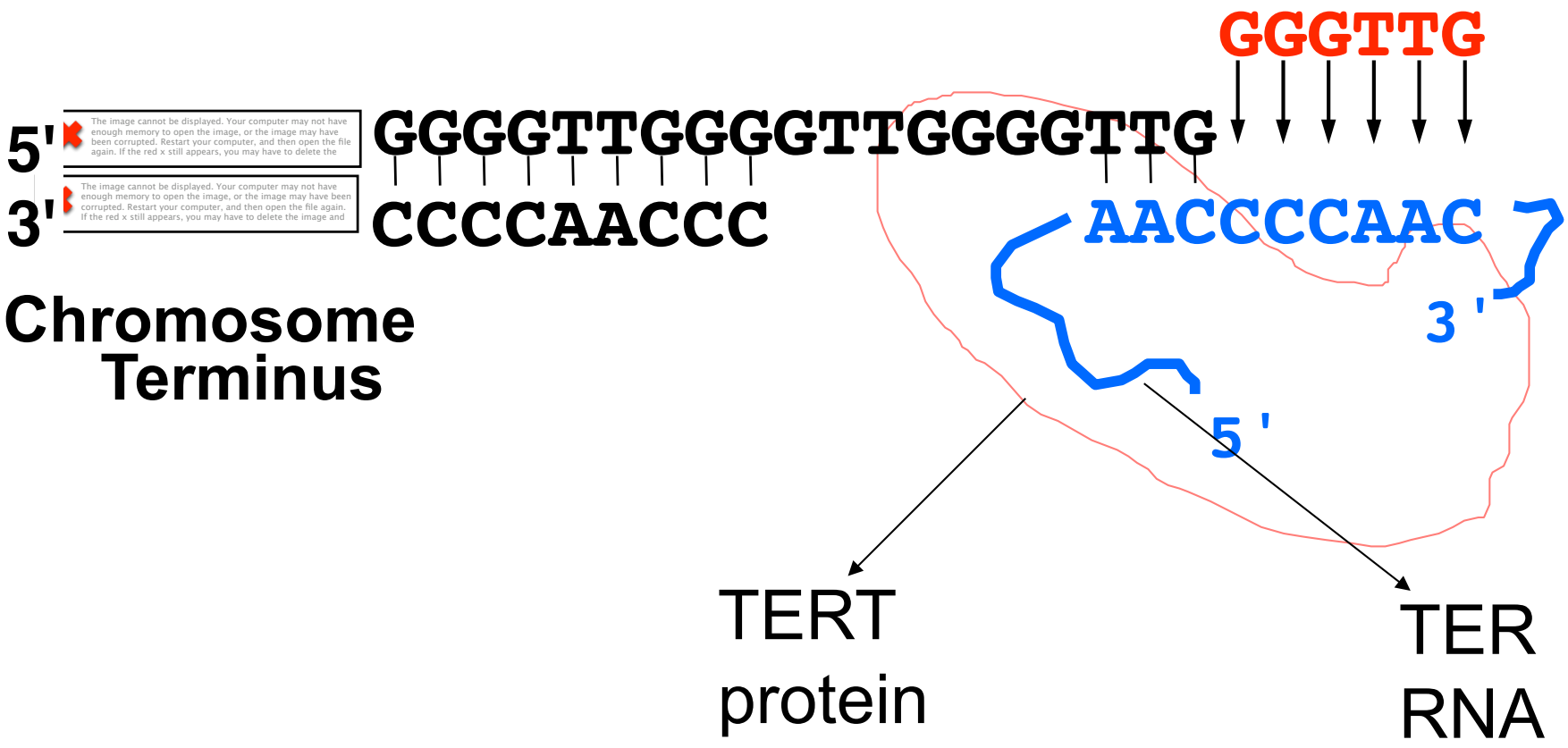


Watson, 1972, Olovnikov, 1971

WHAT DOES TELOMERASE DO?

The solution to telomere attrition

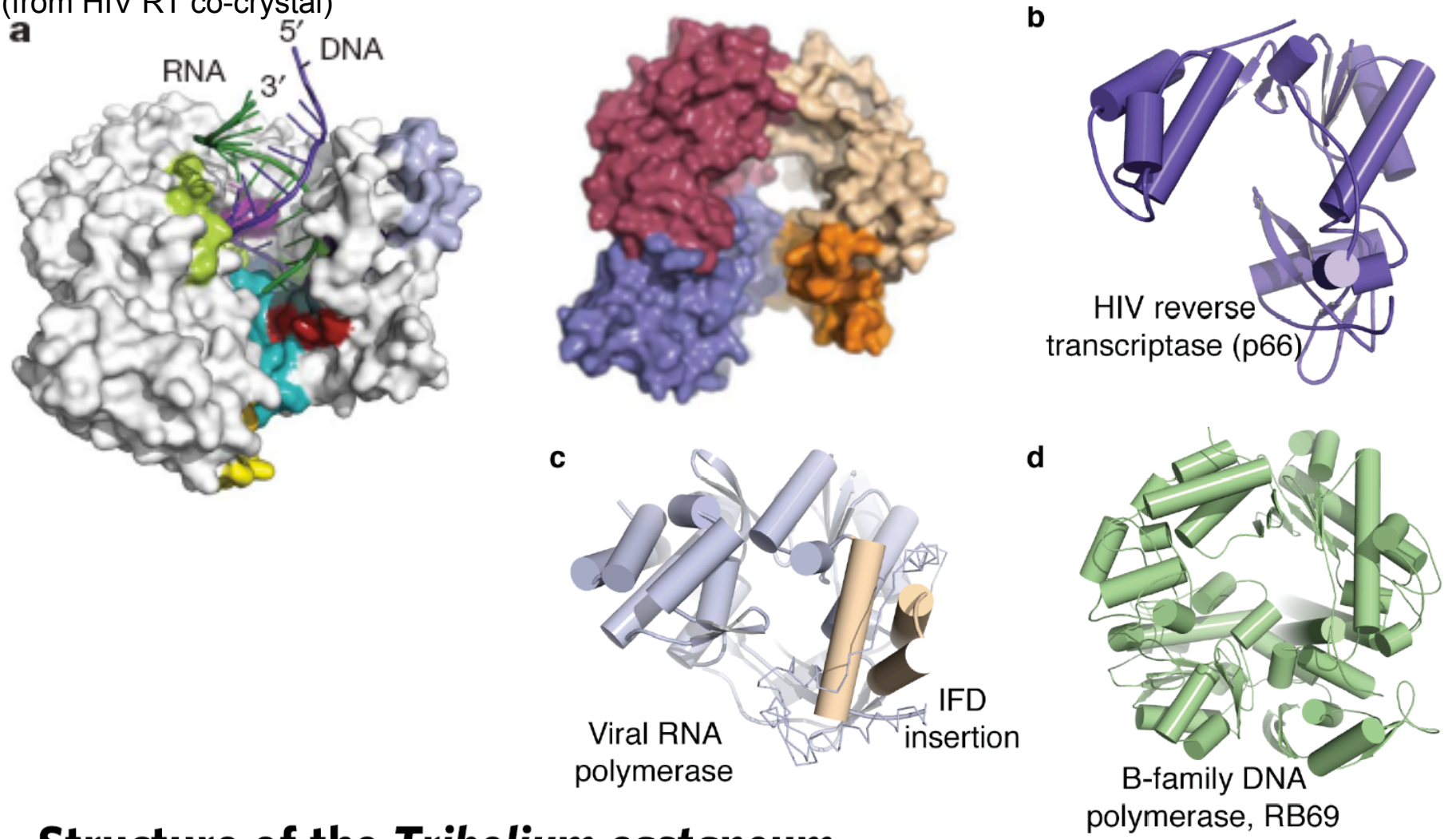
Telomerase: a telomere-synthesizing reverse transcriptase



Greider and Blackburn, 1985, 1987, 1989

TERT apo-protein

TERT
with partial RNA-DNA modeled in
(from HIV RT co-crystal)

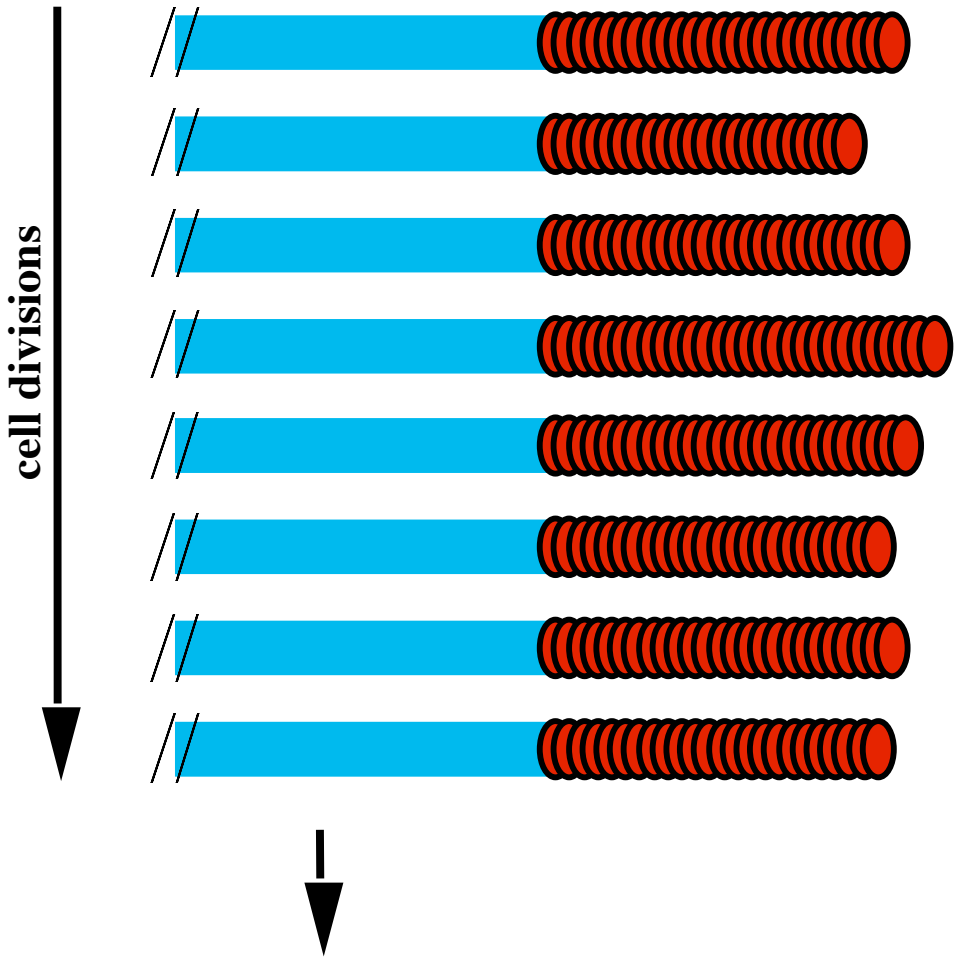


Structure of the *Tribolium castaneum* telomerase catalytic subunit TERT

Andrew J. Gillis[1], Anthony P. Schuller[1] & Emmanuel Skordalakes 2008

Telomerase RNA base sequence also affects telomerase enzymatic action

WHAT DOES TELOMERASE DO FOR CELLS?



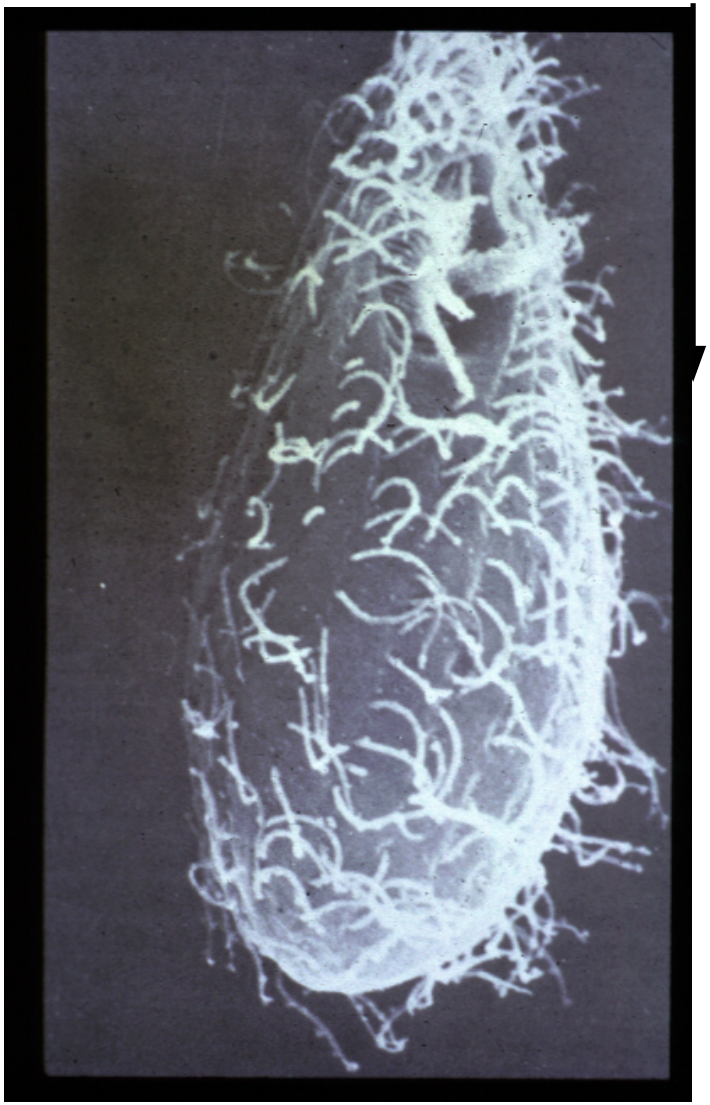
Plenty of telomerase:

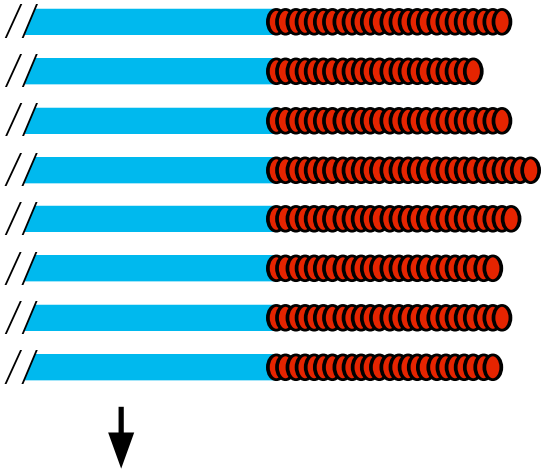
homeostasis balanced

Cells keep dividing

Tetrahymena thermophila

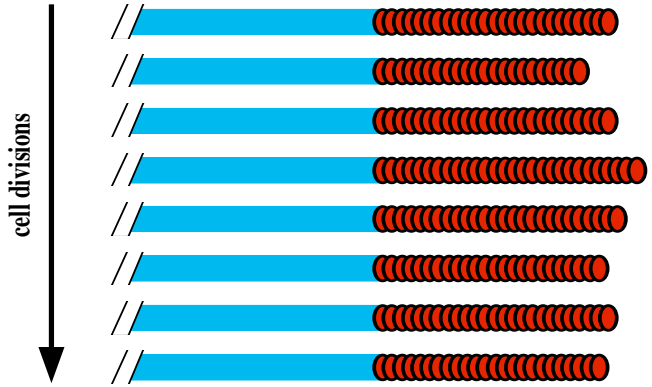
Plenty of telomerase





Telomeres replenished by telomerase

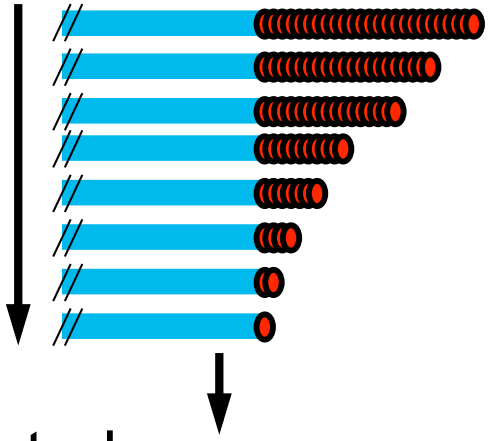
Cells are immortal



Telomeres replenished by telomerase

Cells are immortal

Inactivate telomerase



Telomeres progressively shorten

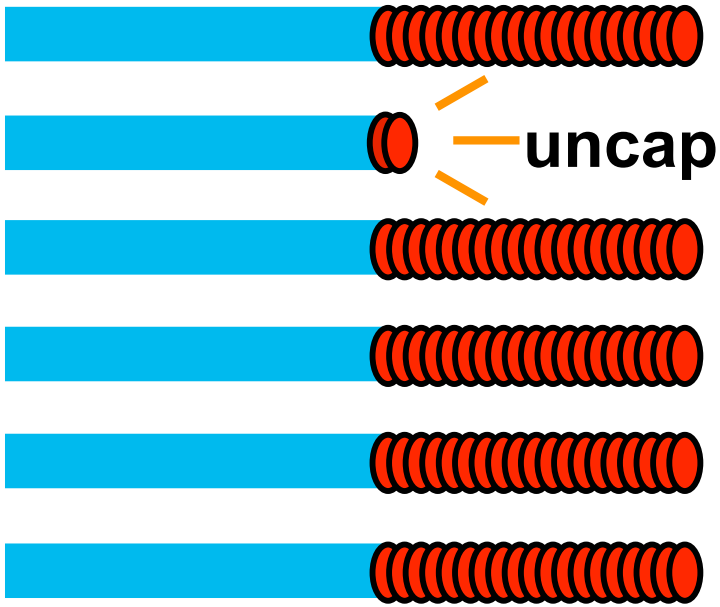
Tetrahymena ceased divisions
They became “mortal”

Yu et al, Nature 1990

WHAT ELSE DOES TELOMERASE DO?

An experiment in yeast

Remove active telomerase:
Even well before senescence, catastrophic shortening of occasional telomeres



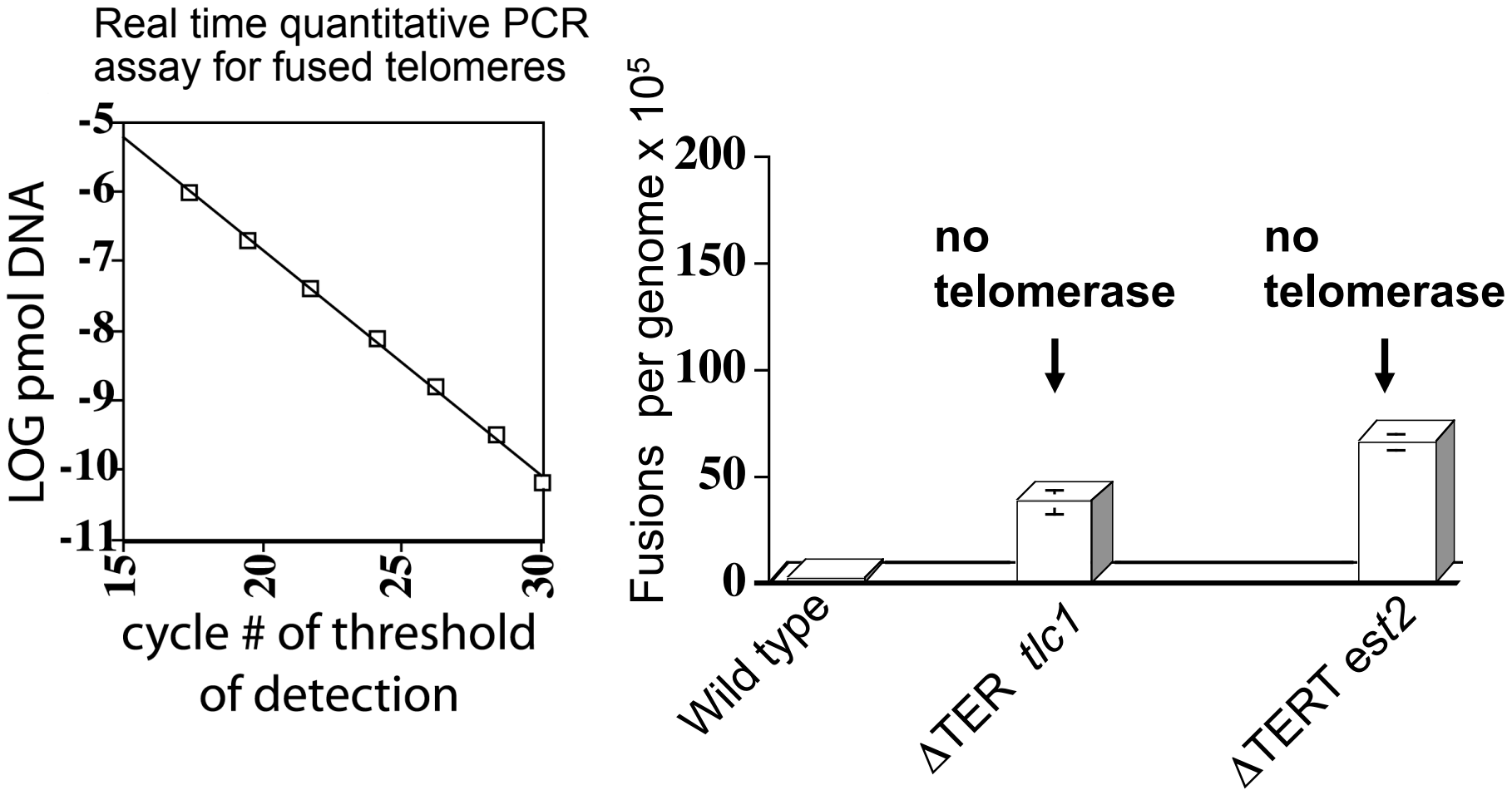
uncapping → fusions freq. 1 in 10^3

(Wild type freq. <1 in 10^5)

- even when bulk telomeres still LONG

Chan and Blackburn, 2003

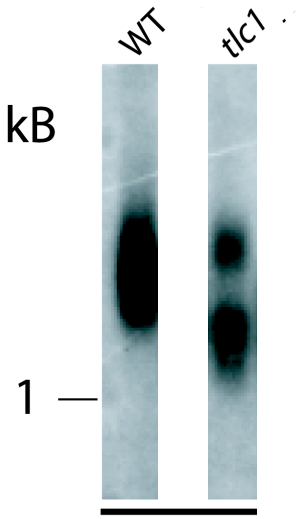
Telomerase protects telomeres from fusing to a DSB



Chan and Blackburn, 2003

Telomere-DSB fusion occurs in telomerase(-) cells, even with long telomeres.

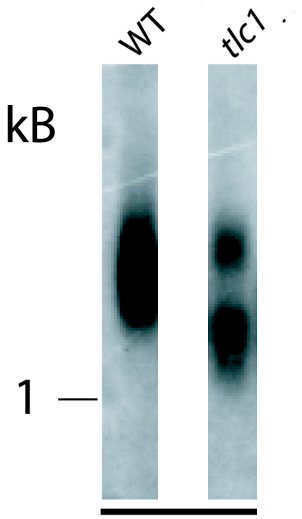
“Parent”

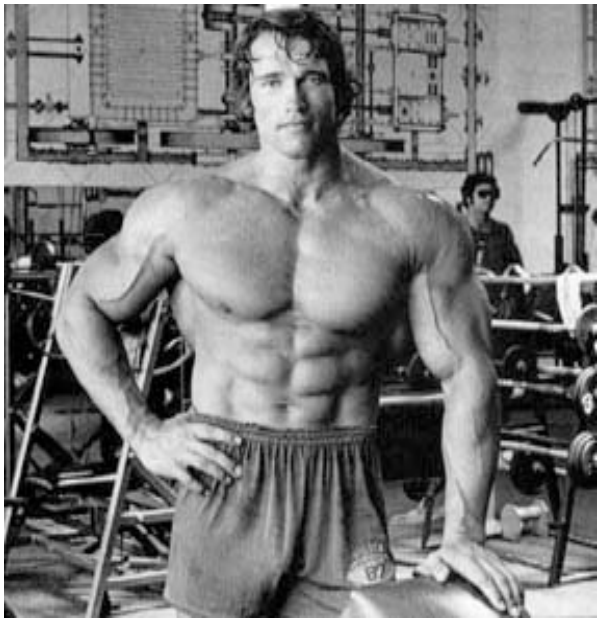


Chan and Blackburn, 2003

Telomere-DSB fusion occurs in telomerase(-) cells, even with long telomeres.

“Parent”

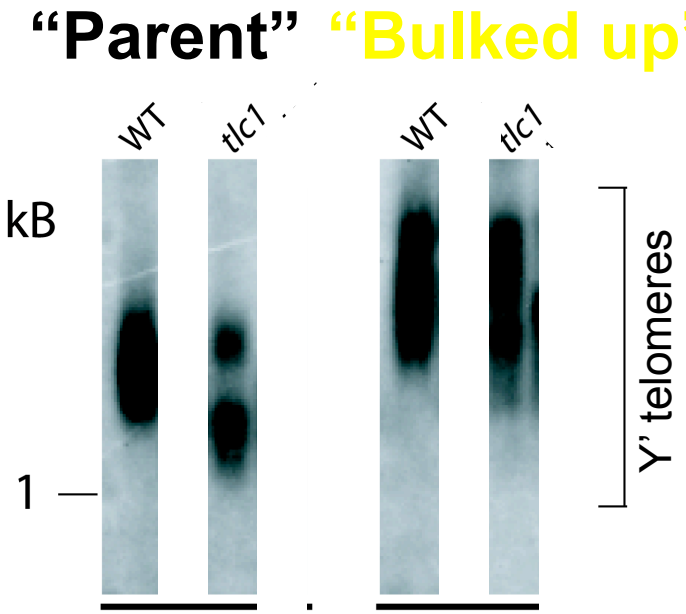


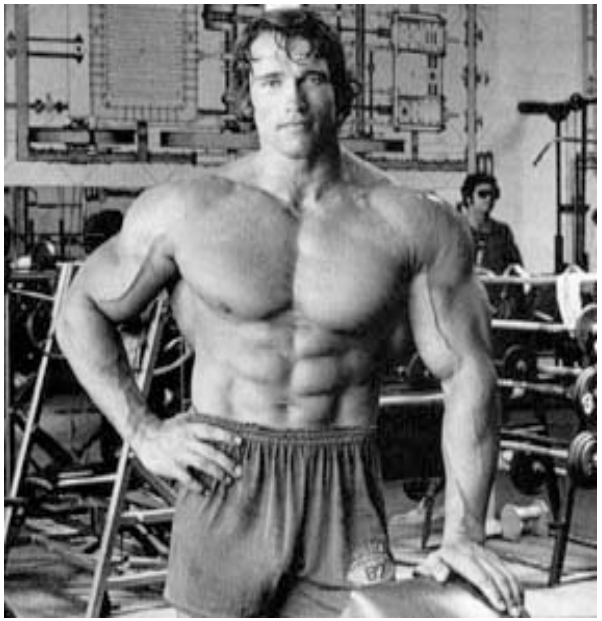


Bulked up telomeres

Chan and Blackburn, 2003

Telomere-DSB fusion occurs in telomerase(-) cells, even with long telomeres.

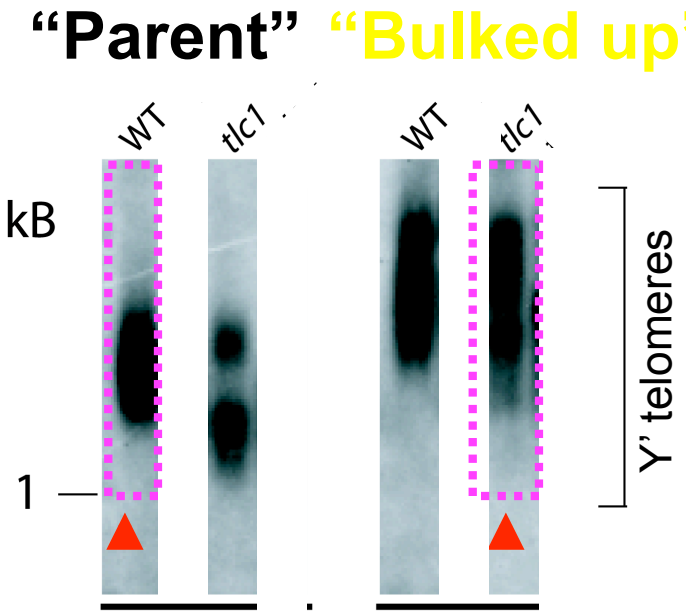




Bulked up telomeres

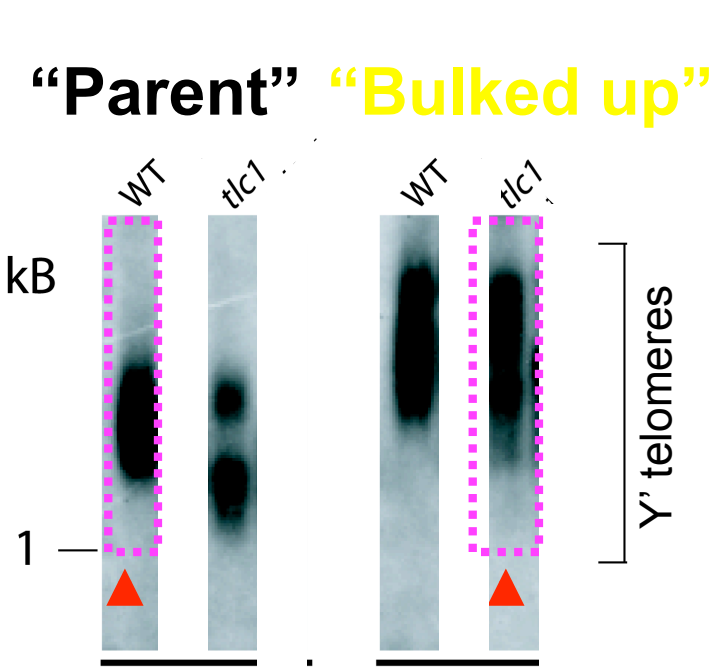
Chan and Blackburn, 2003

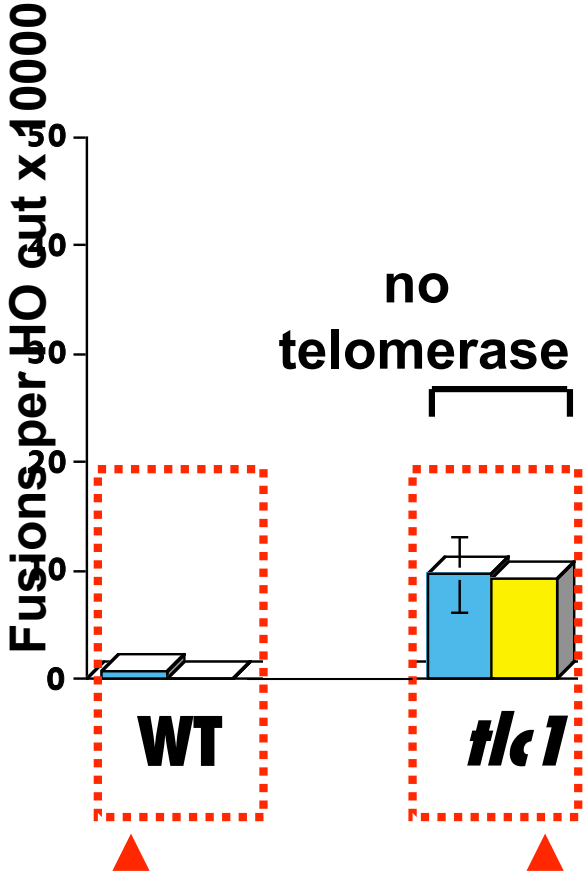
Telomere-DSB fusion occurs in telomerase(-) cells, even with long telomeres.



Chan and Blackburn, 2003

Telomere-DSB fusion occurs in telomerase(-) cells, even with long telomeres.





Chan and Blackburn, 2003

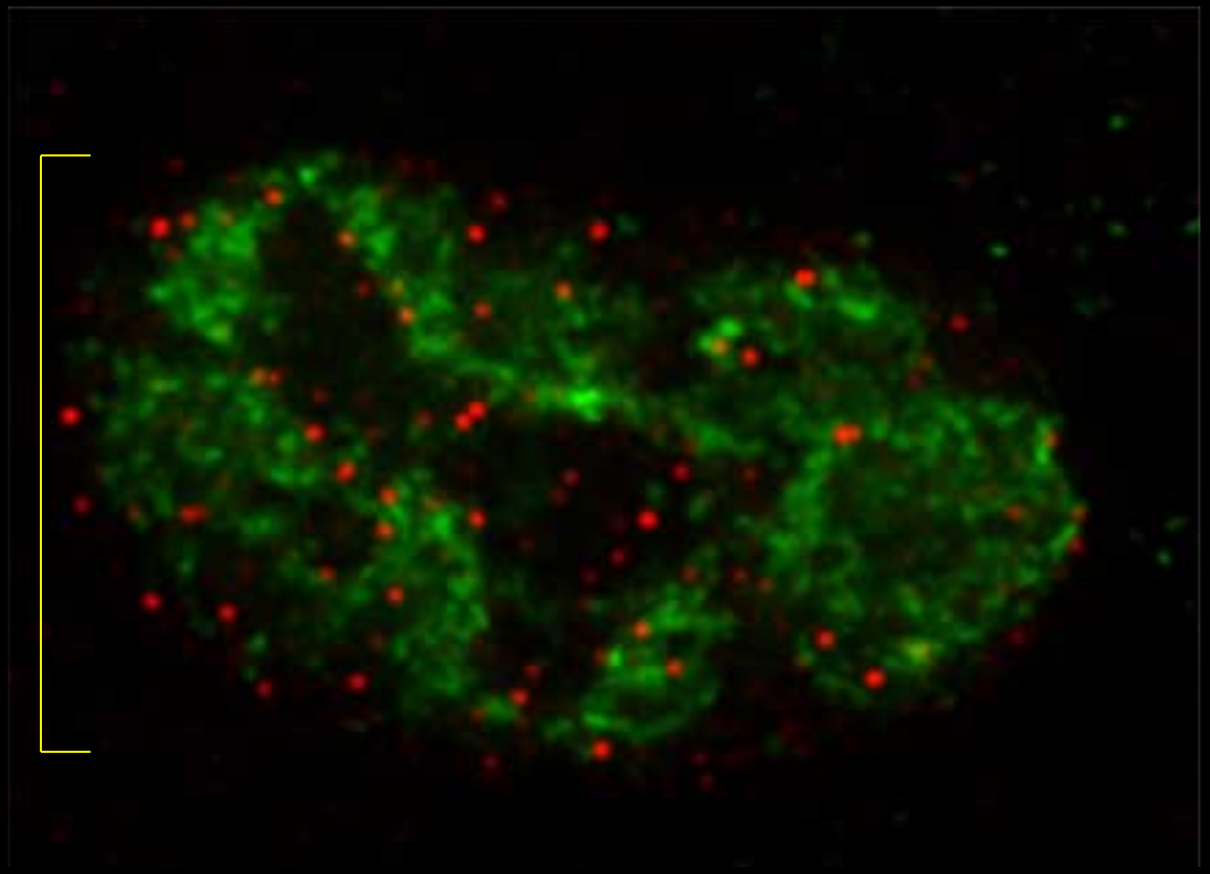
A Protective Function for Telomerase

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

Chan and Blackburn, 2003

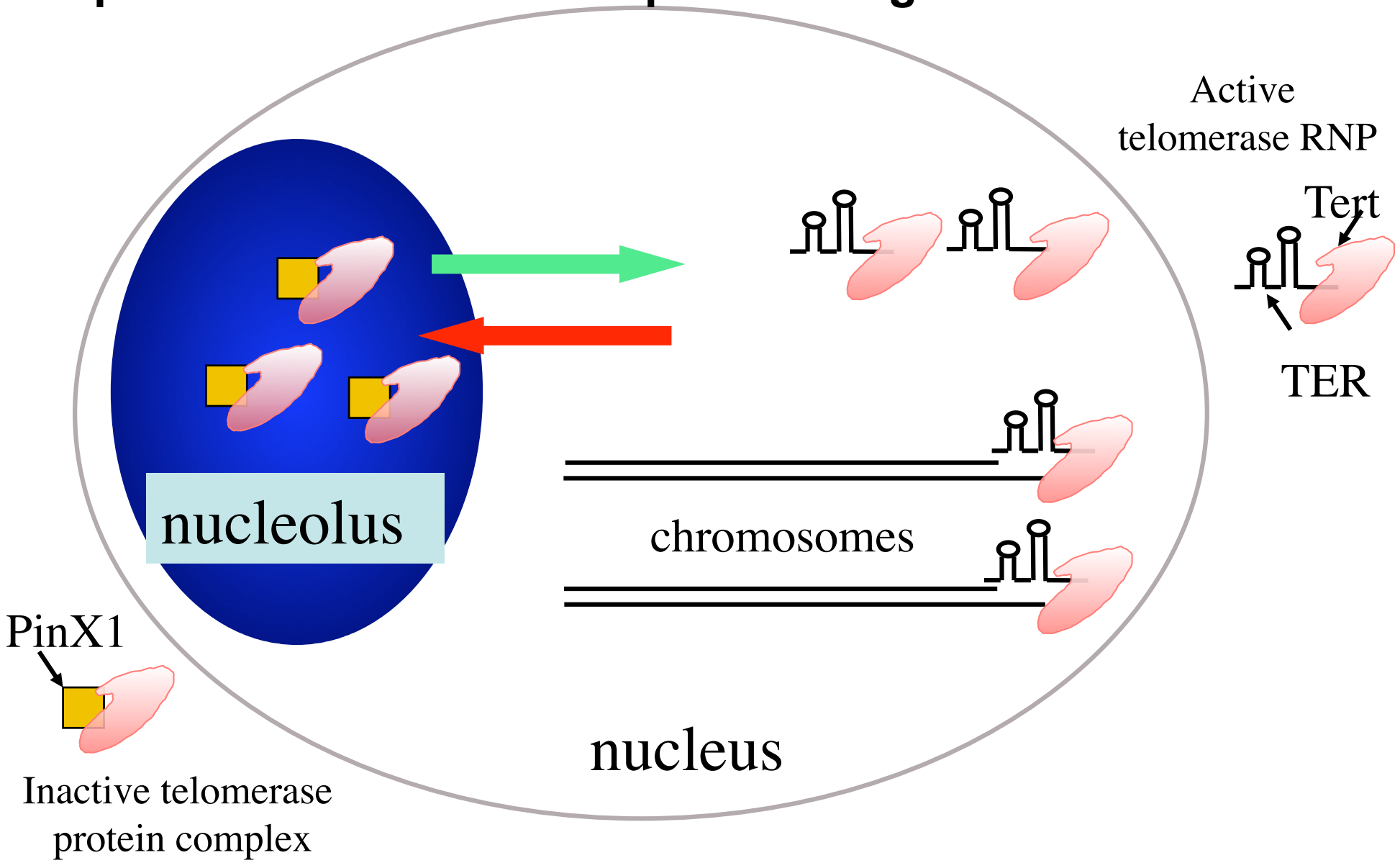
Telomerase appears to be widely distributed in the nucleus, not just at telomeres

The cancer cell nucleus



GFP-hTERT (green) and telomeres (TRF2 =red) in a representative LOX melanoma cell nucleus - deconvolution images projection

Telomerase protein Tert sequestrates with nucleolar PinX1 protein in a distinct complex lacking telomerase RNA



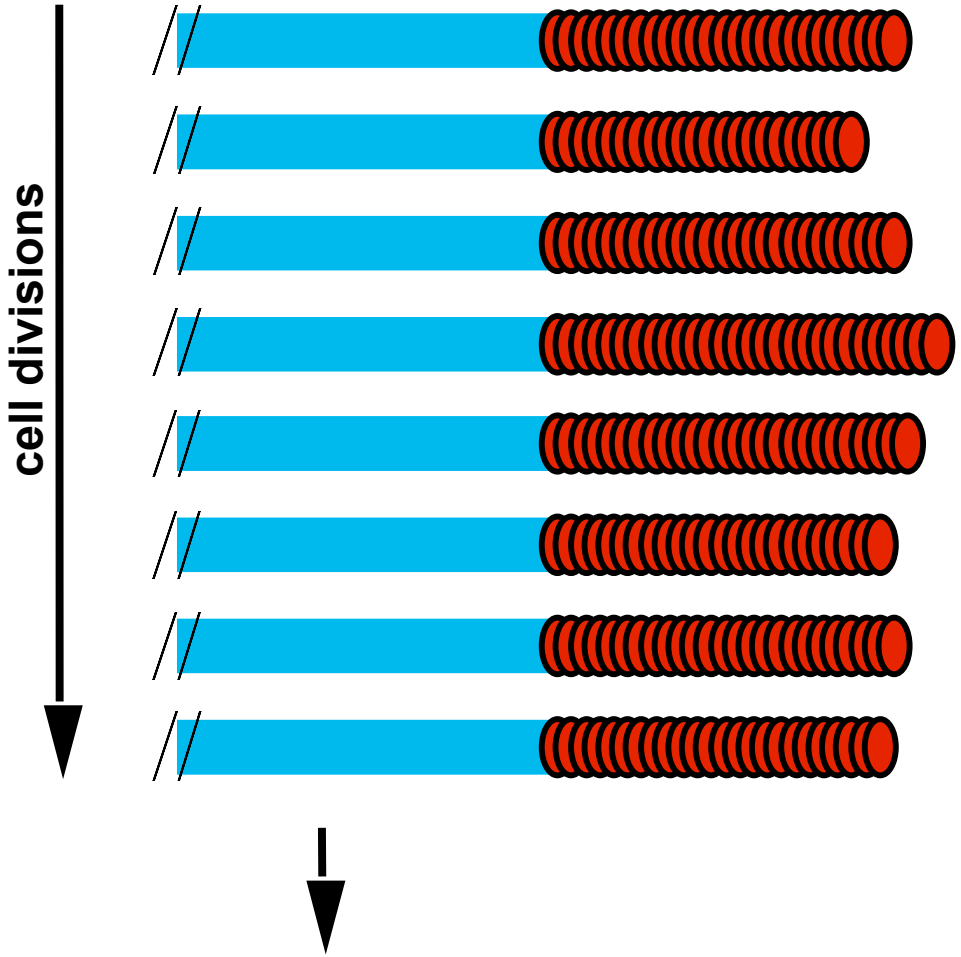
J. Lin and E. H.Blackburn, Genes and Dev. 2004

Unexpected effects of telomerase RNA depletion in cancer cells

Shang Li

Collaborating lab (UCSF)

Mo Kashani-Sabet



Telomeres replenished by telomerase

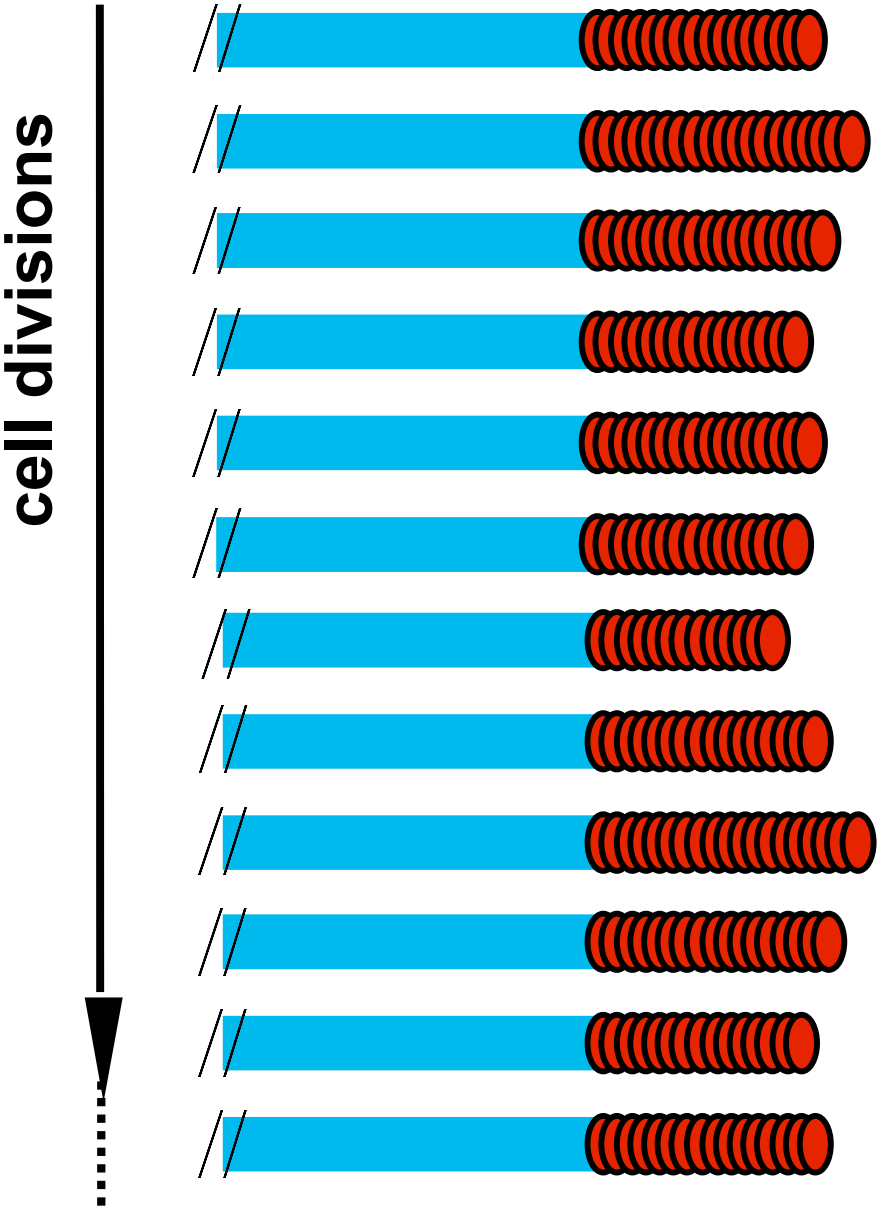
IN HUMANS

- Active: **stem cells, germ cells**

- Detectable: **many normal adult cell types (regulated activity)**

- Highly active: ~90% of human tumors

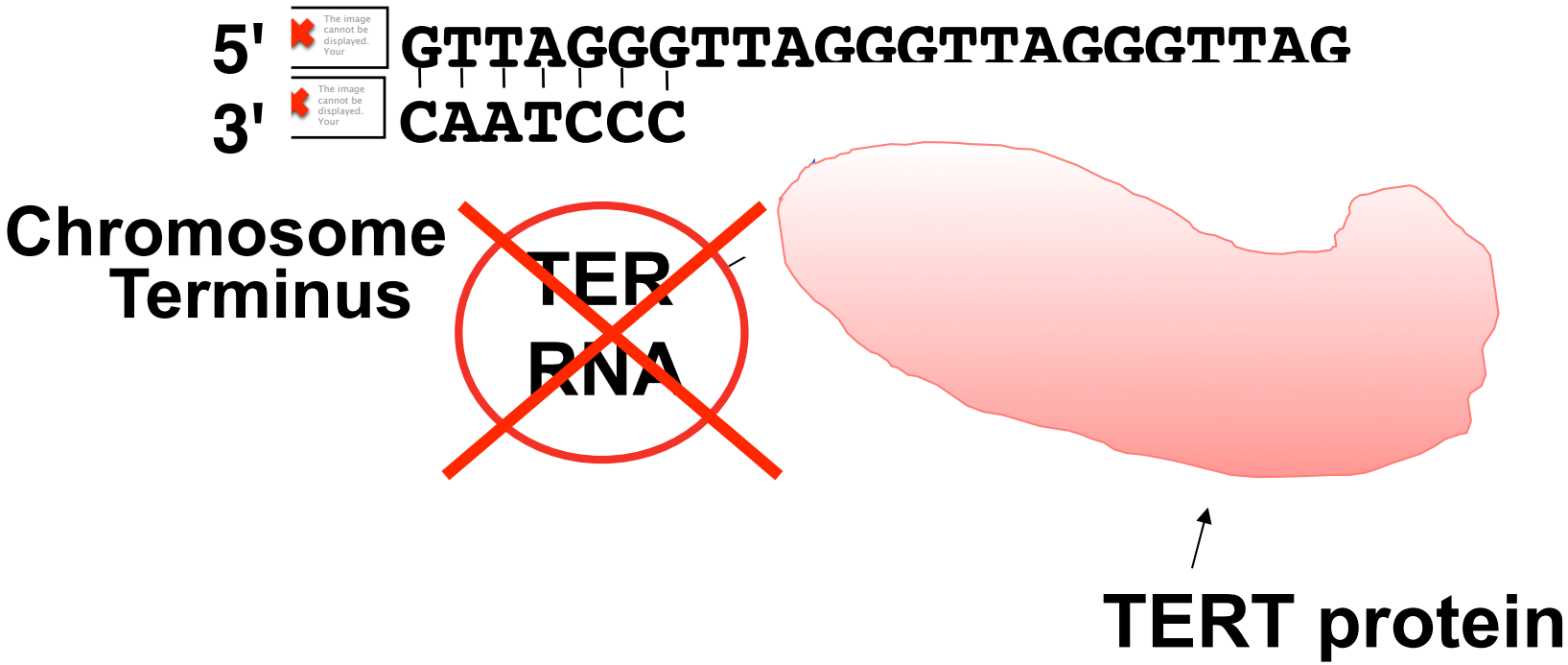
Plenty of telomerase:homeostasis balanced



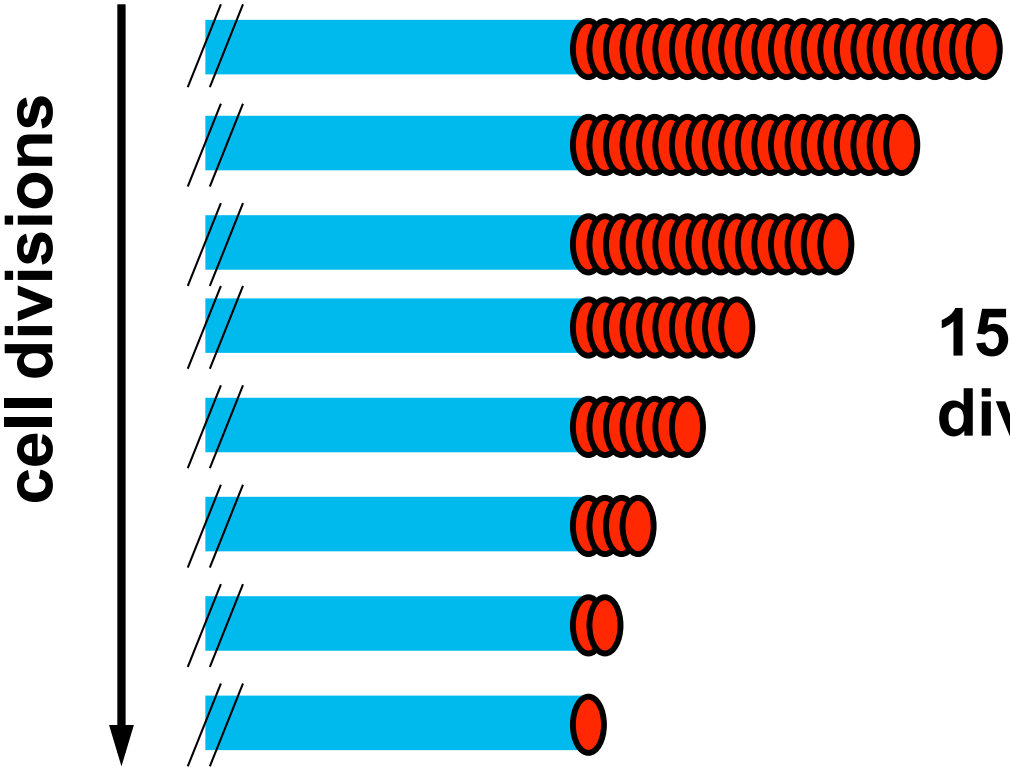
Cells keep dividing

Telomerase: a telomere-synthesizing ribonucleoprotein reverse transcriptase

Human Telomerase



OLD - Predicted, if inhibit or knock down telomerase:

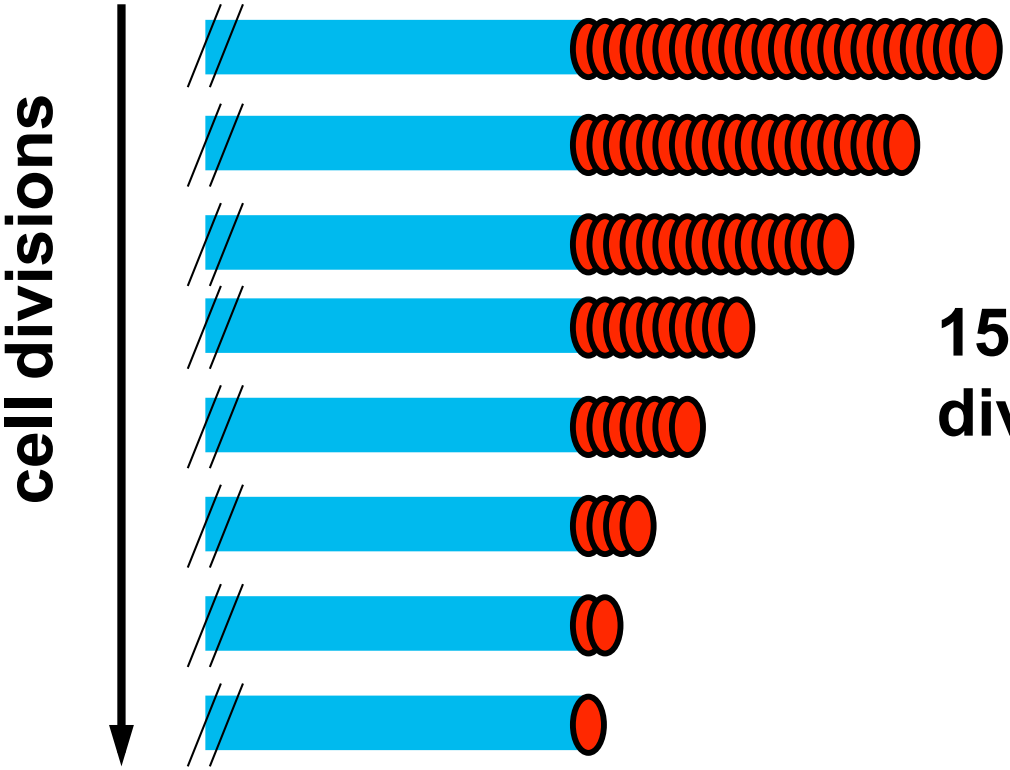


150-200 bp/cell division

- only gradual shortening of all telomeres

- DELAY before eventual senescence or apoptosis

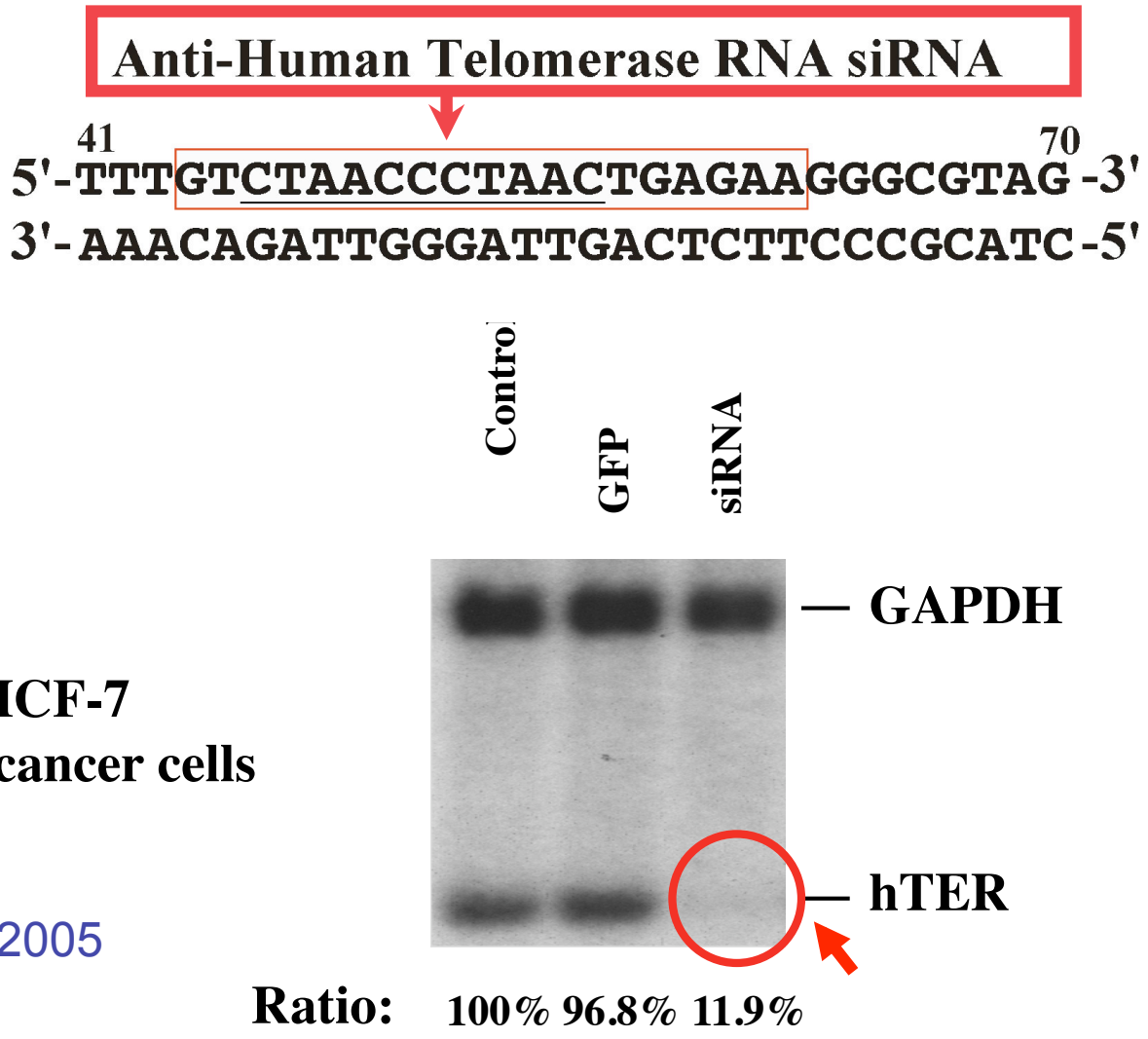
OLD - Observed, if inhibit ~~or knock-down~~ telomerase enzyme, *but keep RNP level high*:



150-200 bp/cell division

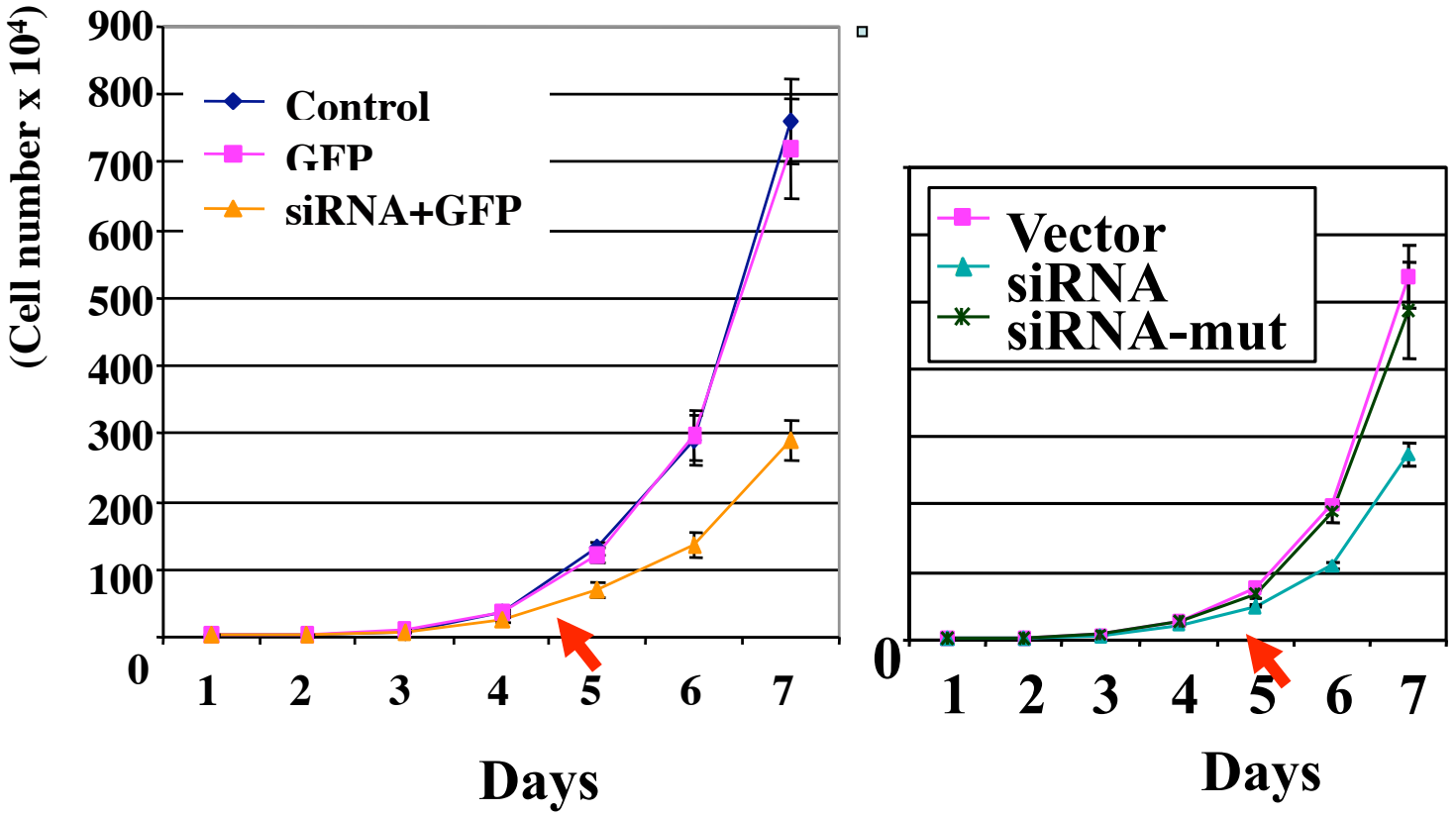
- only gradual shortening of all telomeres
- DELAY before eventual senescence or apoptosis

Knockdown of human telomerase RNA - e.g., hairpin shRNA



Li et al. 2004, 2005

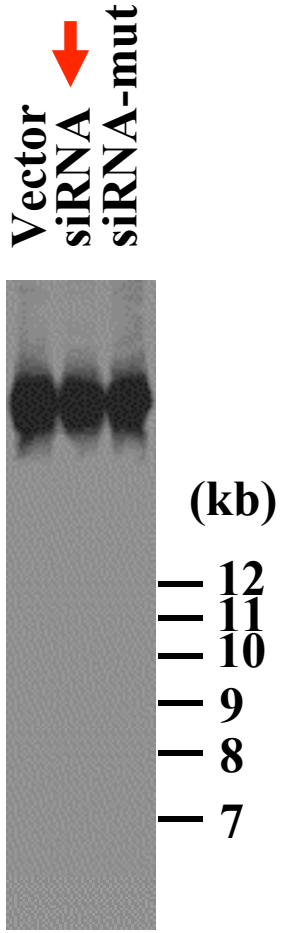
Lentiviral-expressed anti-hTER siRNA (RNAi) RAPIDLY inhibits growth of LOX melanoma cells



(bulk unselected cell populations)

Li et al 2005

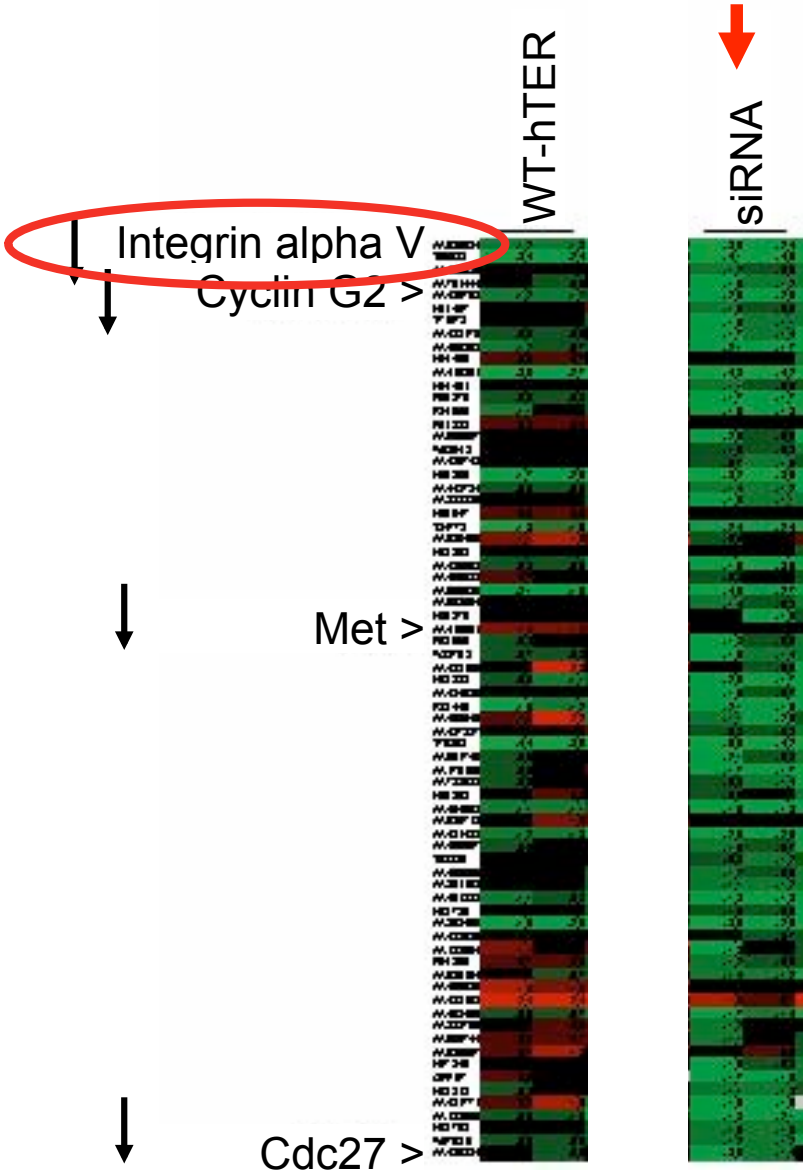
YET - Bulk telomere shortening had NOT yet been induced by the telomerase RNA knock down



Teloblot

Li et al 2005

LOX melanoma cells



Depleting telomerase RNA rapidly down-regulates a specific set of genes - includes cell cycle and tumor progression genes

HCT116 cells

Li et al 2005

Effects of telomerase knock-down on metastasis?

YES

Knock-down of telomerase
(by ribozyme or RNA-interference)
inhibits metastasis in *in vivo*
mouse melanoma models
B16 cells
LOX cells

Kashani-Sabet et al, 2004; Nosrati et al 2005; Baghari et al 2006; Li et al, unpublished

Telomerase knock-down in cancer cells

RAPIDLY inhibits cancer cell growth
p53 is not required for this

NO telomere uncapping or DNA damage response

Metastasis is reduced

Li et al, Cancer Res 2004; Li et al, 2005; Bagheri et al 2006

Telomerase knock-down in cancer cells

Metastasis is reduced
HOW?

RAPIDLY downregulates cell cycle and tumor progression genes

Glucose metabolism downregulated

Cell differentiation program induced?

Li et al, Cancer Res 2004; Li et al, 2005; Bagheri et al 2006

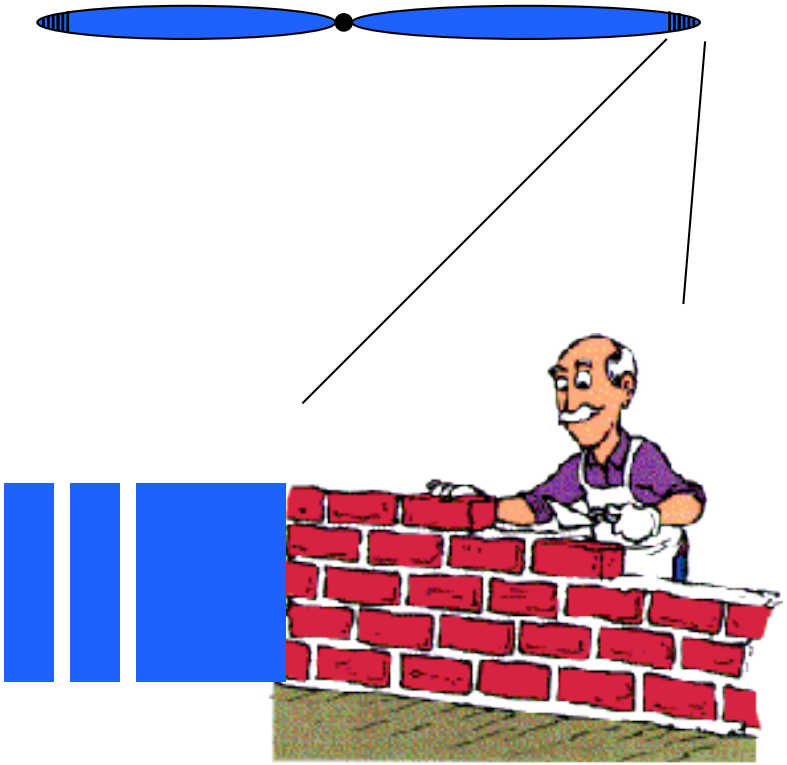
Does high telomerase promote stem cell-ness?

High telomerase levels (conditionally induced TERT subunit over-expression) in mice promoted proliferation specifically in stem cells, even in mice deleted for the telomerase RNA component gene

S. Artandi and coworkers:
Sarin et al.
Nature 436, 1048-1052 (2005)

A NEW CONCEPT FOR TELOMERASE:

Humble bricklayer



…..and also upper level manager of cell's programs?

Indra Nooyi: PepsiCo's chief executive

How do we age?

A multi-faceted process?

- increased susceptibility to diseases

-how much is

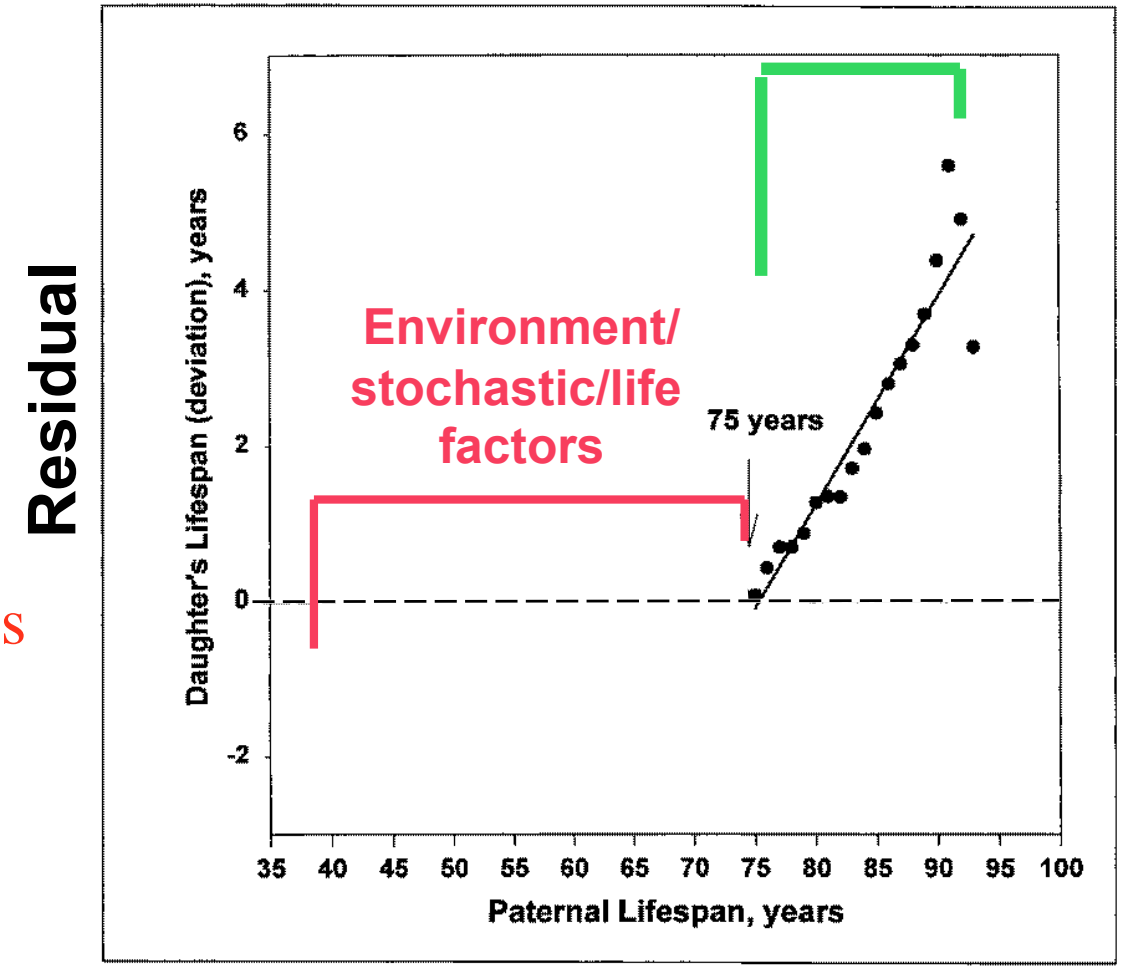
-genetic?

-environment/life factors?

Genetics contributes a lot

- **Residual zero**

  expected if inheritance is unimportant for determining life span



Residual Increasing with parental life span

expected if life span is inherited

FIG. 2. Daughter's life span (deviation from the cohort mean) as a function of paternal life span. Based on the data for 5,779 daughters from European royal and noble families born in 1800–1880 and survived by age 30. Data are smoothed by 5–year moving average.

Residual: **the** difference between each daughter's life span and the cohort mean life span

Gavrilova NS and Gavrilov LA. (2001) Journal Anti-Aging Med.

Atzmon et al Journal of the American Geriatrics Society, 2004. Vol. 52, 274

cardiovascular disease, diabetes and cancer

most deaths in the elderly.

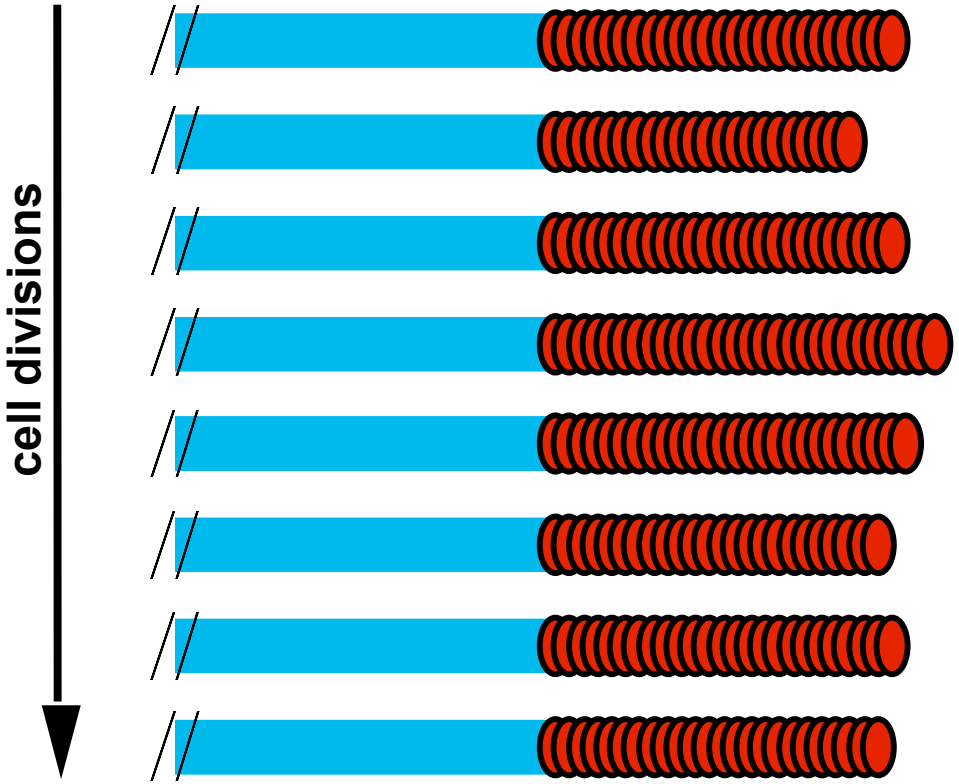
-how much is

-genetic?

-environment/life factors?

In humans

In vivo

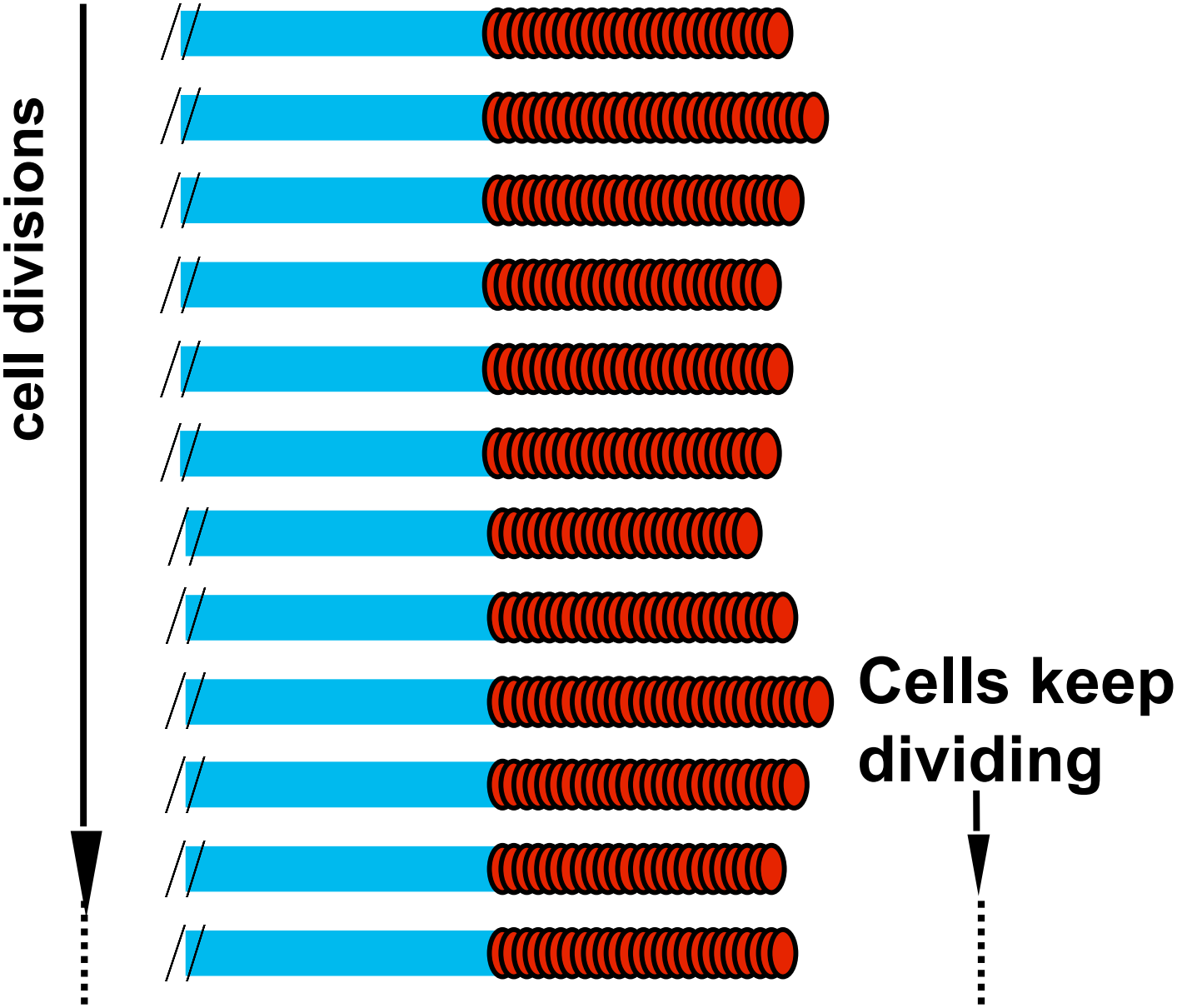


Telomeres replenished by telomerase

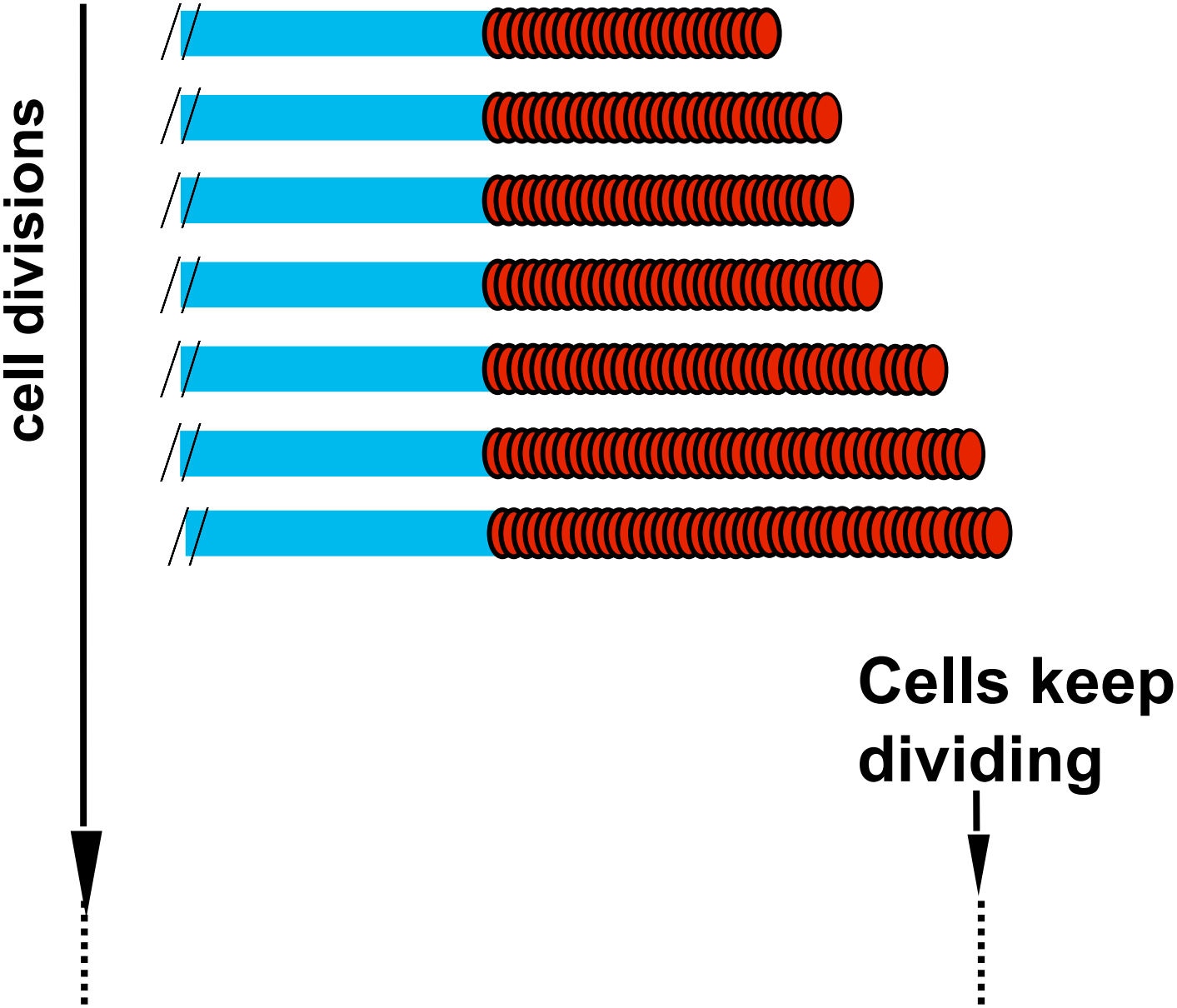
Cells keep dividing

- **Active:** stem cells, germ cells
- **Detectable:** many normal adult cell types (highly regulated)
- **Highly active:** ~90% of human tumors

Plenty of telomerase:homeostasis balanced

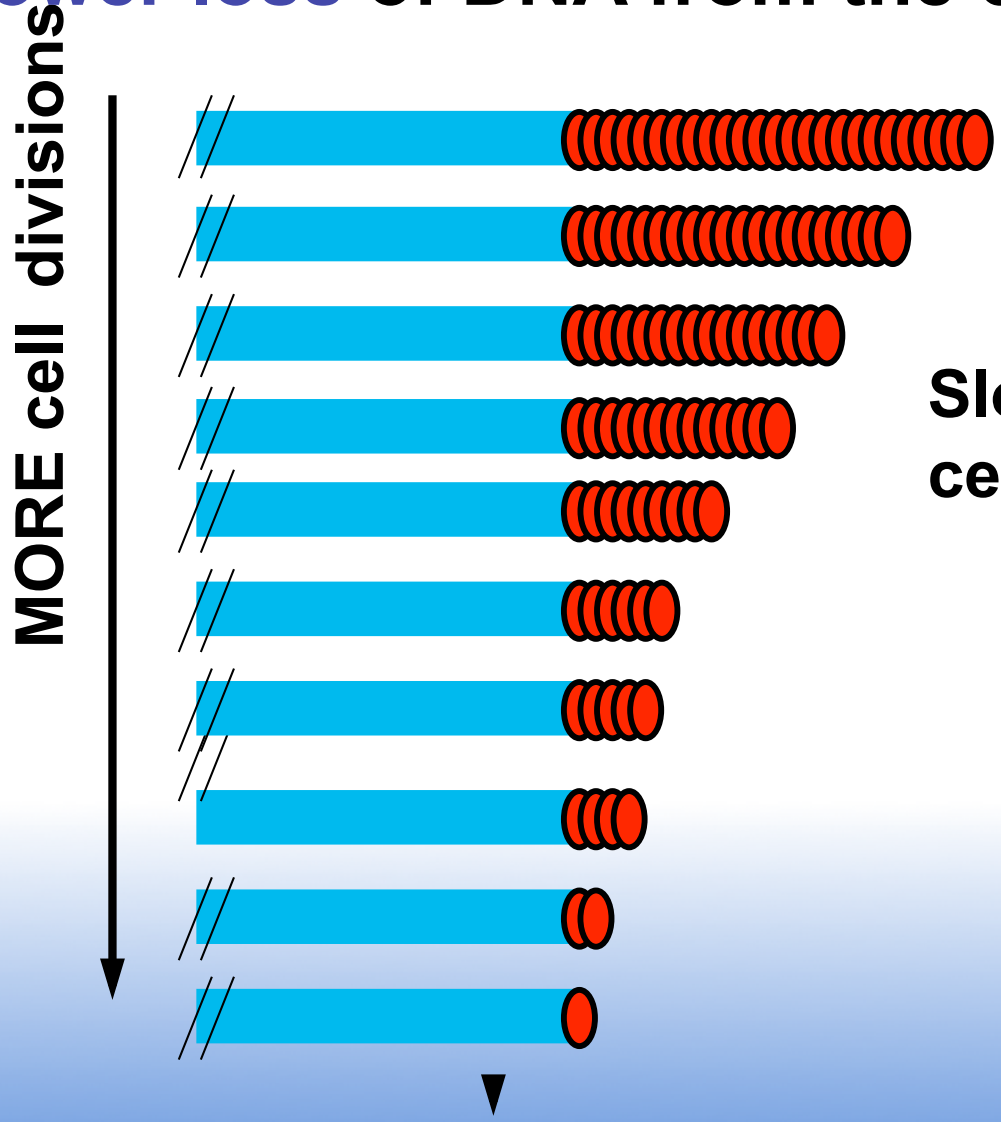


Extra telomerase:telomeres will even grow



Predicted, if some telomerase:

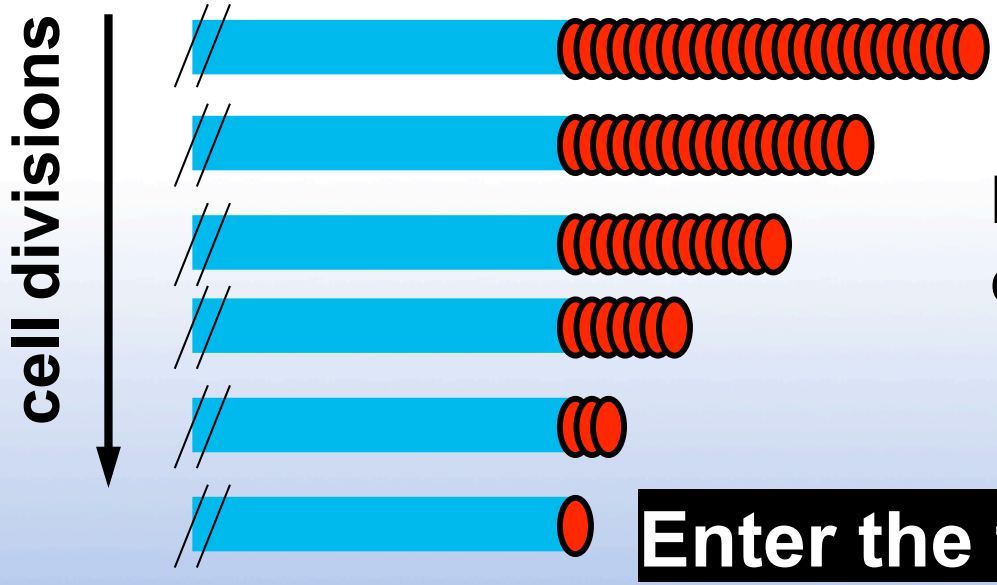
Slower loss of DNA from the chromosome end



Slower net loss/ cell division

senescence comes later

Predicted, if less telomerase:
Faster loss of DNA from the chromosome end



Faster net loss/ cell division

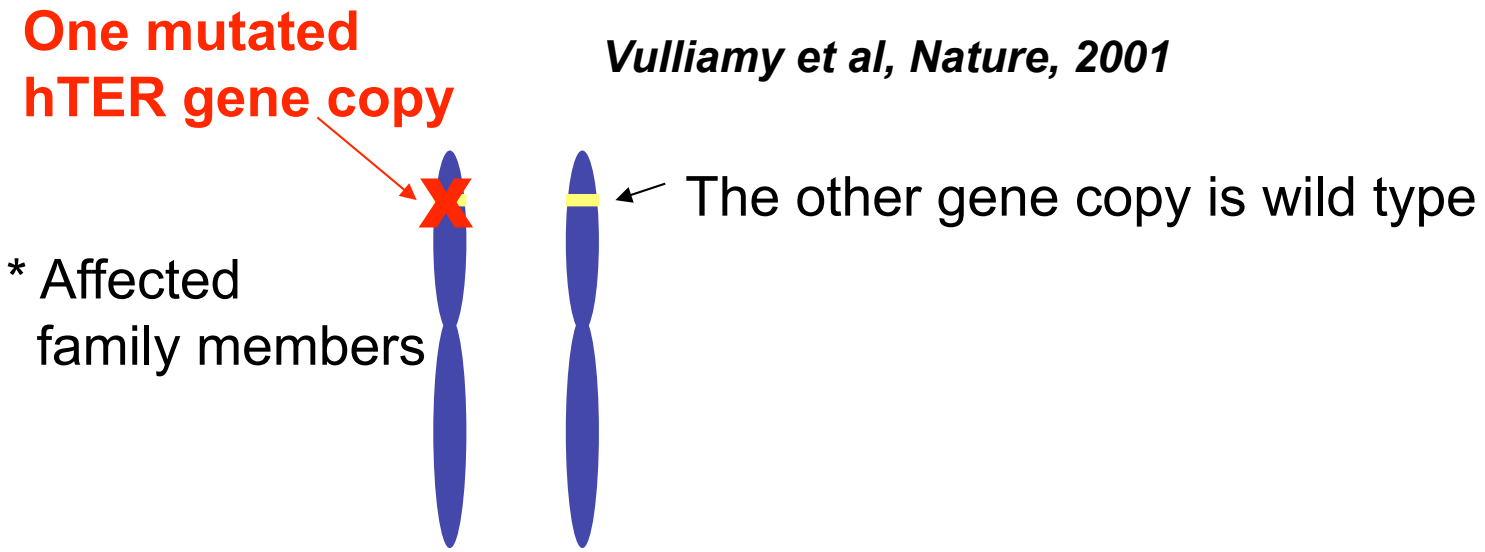
Enter the twilight zone faster…

Senescence sooner

-how much is

-genetic?

-environment/life factors?



A rare inherited condition in humans:

Premature death from progressive bone marrow failure * when one gene copy of telomerase RNA is deficient

***death in early adulthood to middle age**

- **shorter telomeres**
- **immune system becomes exhausted**
- **cancer-prone**

A full human lifespan requires **both** telomerase RNA alleles to be functional: Telomerase RNA gene product **quantity** matters

Effects *in vivo* of common variations in telomere maintenance in humans?

Reported: people aged 60 years or older with shorter blood cell telomeres have higher mortality rates

Shorter telomeres associate with:

- 3.2 fold **higher mortality** rate from heart disease
- 8.5 fold **higher mortality** rate from infectious disease
- **poorer survival** overall from aggregate of all causes

Cawthon et al, Lancet 361: 393-395, 2003

What came first?

Chronic life stress, acceleration of cellular aging, and risks of cardiovascular disease

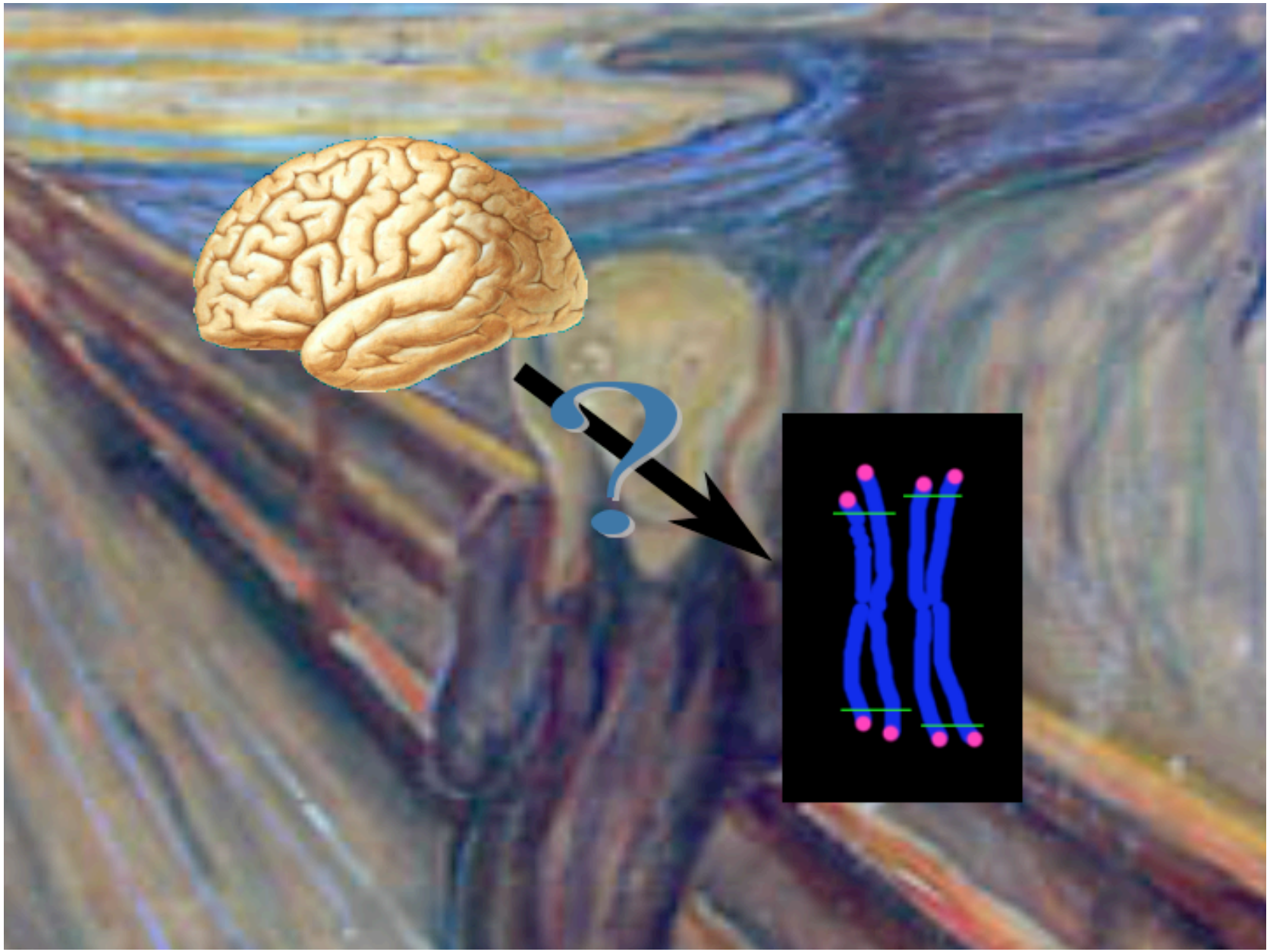
Blackburn Lab Group

- Jue Lin

Collaborators

- **Elissa Epel, UCSF**
- Richard Cawthon, U. Utah
- Nancy Adler, UCSF
- Firdaus Dhabhar, Ohio State
- Jason Morrow, Vanderbilt University
- Frank H. Wilhelm, University of Basel, Basel, Switzerland
- Owen Wolkowitz, UCSF
- Christyn Dolbier, East Carolina University, Greenville, NC
- Wendy Mendes, Harvard University

Chronic stress wears down telomeres



Study 1: Caregiver mothers and chronic stress

First Questions

Are

- level of perceived stress (both groups)
- duration of caregiving (range = 1 - 12 years)

related to markers of cell aging?

Two of the Markers of Cellular Aging

- Telomerase activity
- Telomere length

Epel et al, 2004, PNAS

Study 1: Caregiver mothers and chronic stress

Perceived stress (whole sample) was associated with

- **shorter telomeres**
- **lower telomerase activity**

	Perceived stress
Telomere length	-.31**
Telomerase activity	-.24*

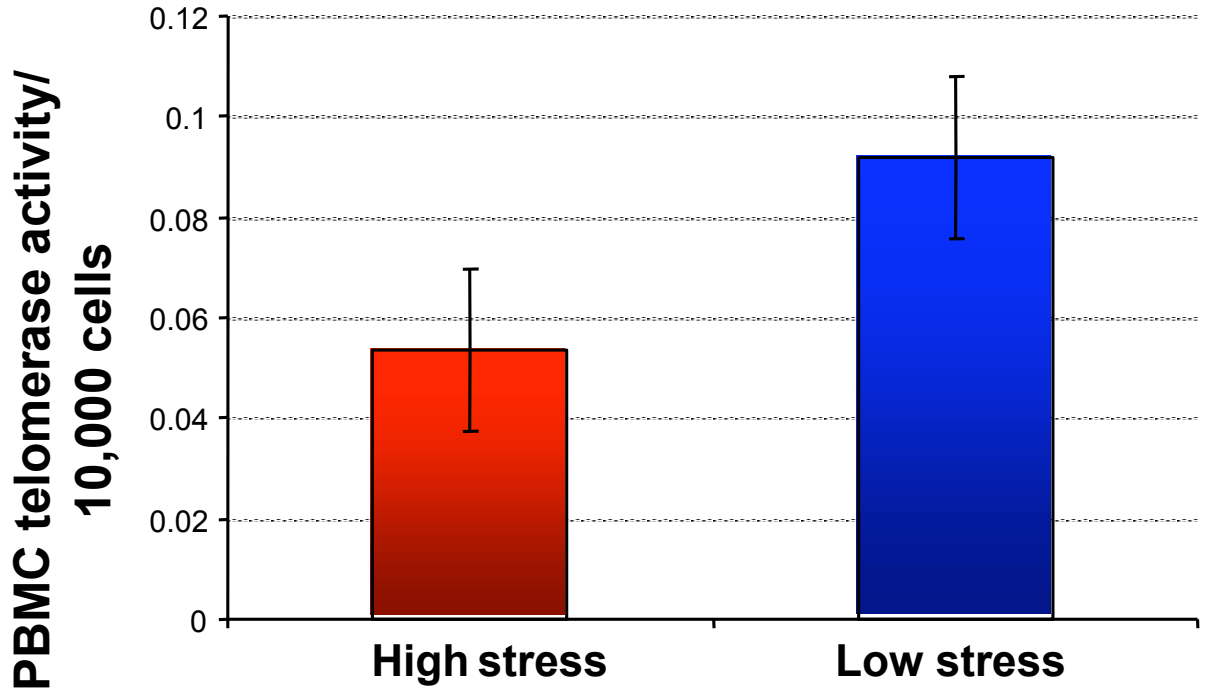
These relationships also hold after accounting for chronological age

Epel et al, 2004, PNAS

**: p< 0.01, *:P < 0.05

Study 1: Caregiver mothers and chronic stress

Telomerase activity is ~ 50% lower in the high stress group



M = .053, SE = .016 M = .092, SE = .016

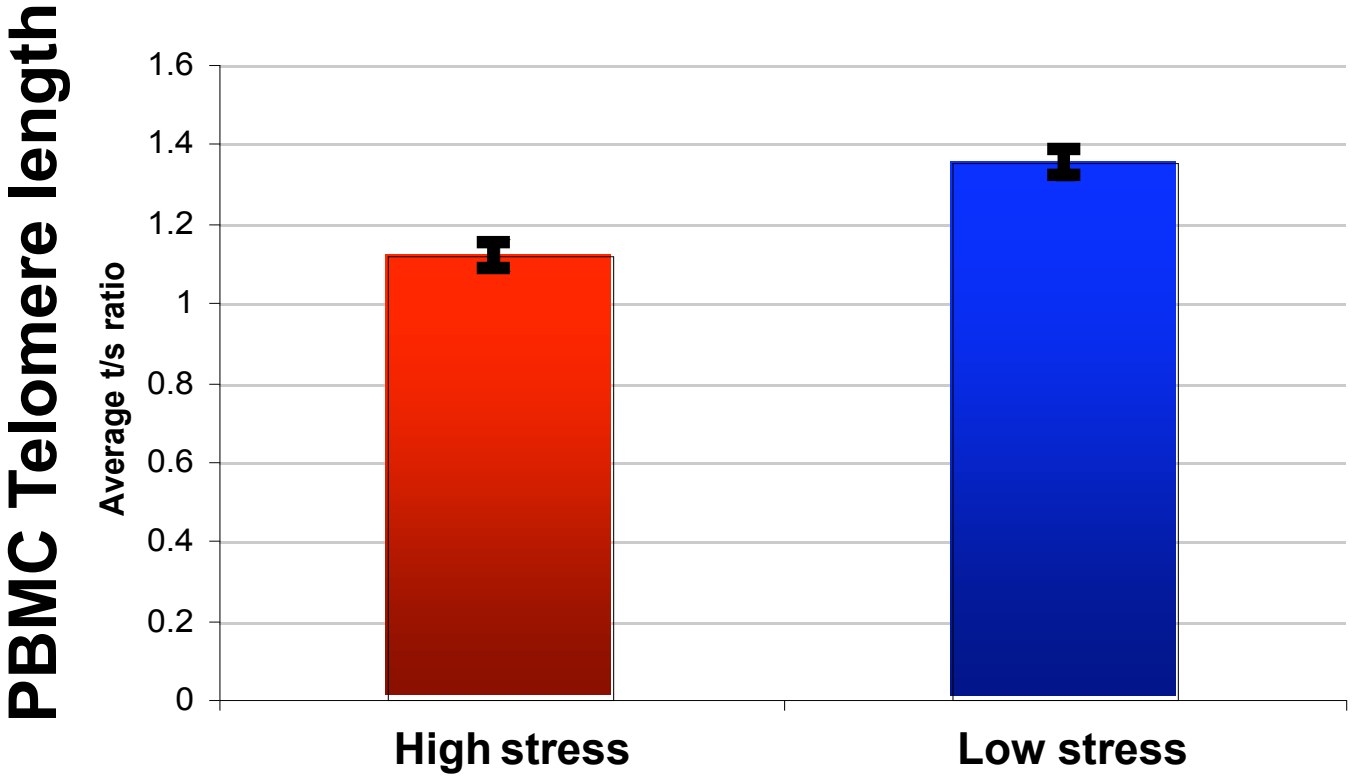
p < .045

Telomerase activity in the lowest and highest stress quartiles of the whole sample are compared.

Epel et al, 2004, PNAS

Study 1: Caregiver mothers and chronic stress

More telomere shortening in high stress group (equiv. 9 - 17 yrs of extra “aging”)

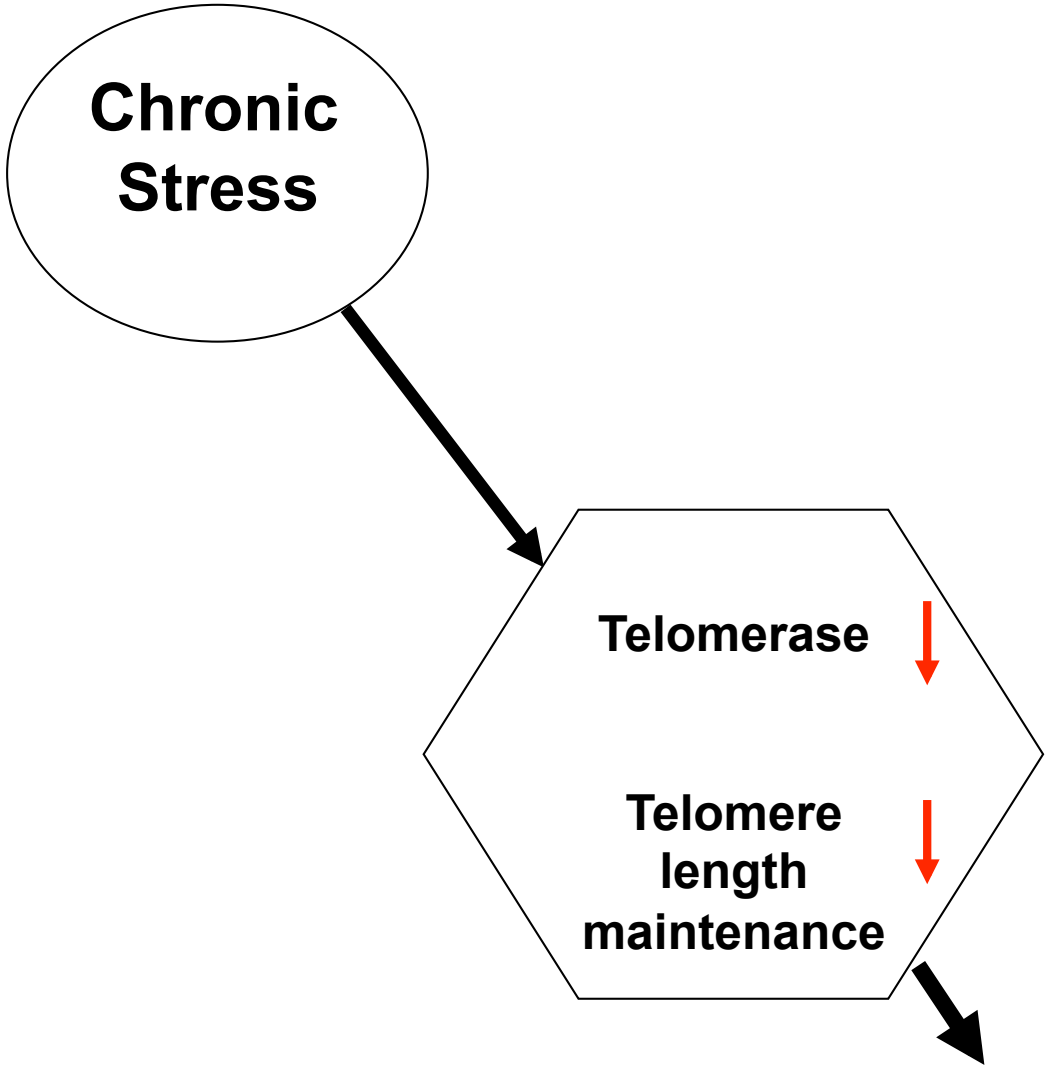


Telomeres in the lowest and highest stress quartiles of the whole sample are compared.
controlling for age and body mass index: $F(3,27) = 12.8$, $p < .001$

- **Stress perception & caregiving duration are quantifiably linked to cell aging markers**
  - **Telomerase**
  - **Telomere length**

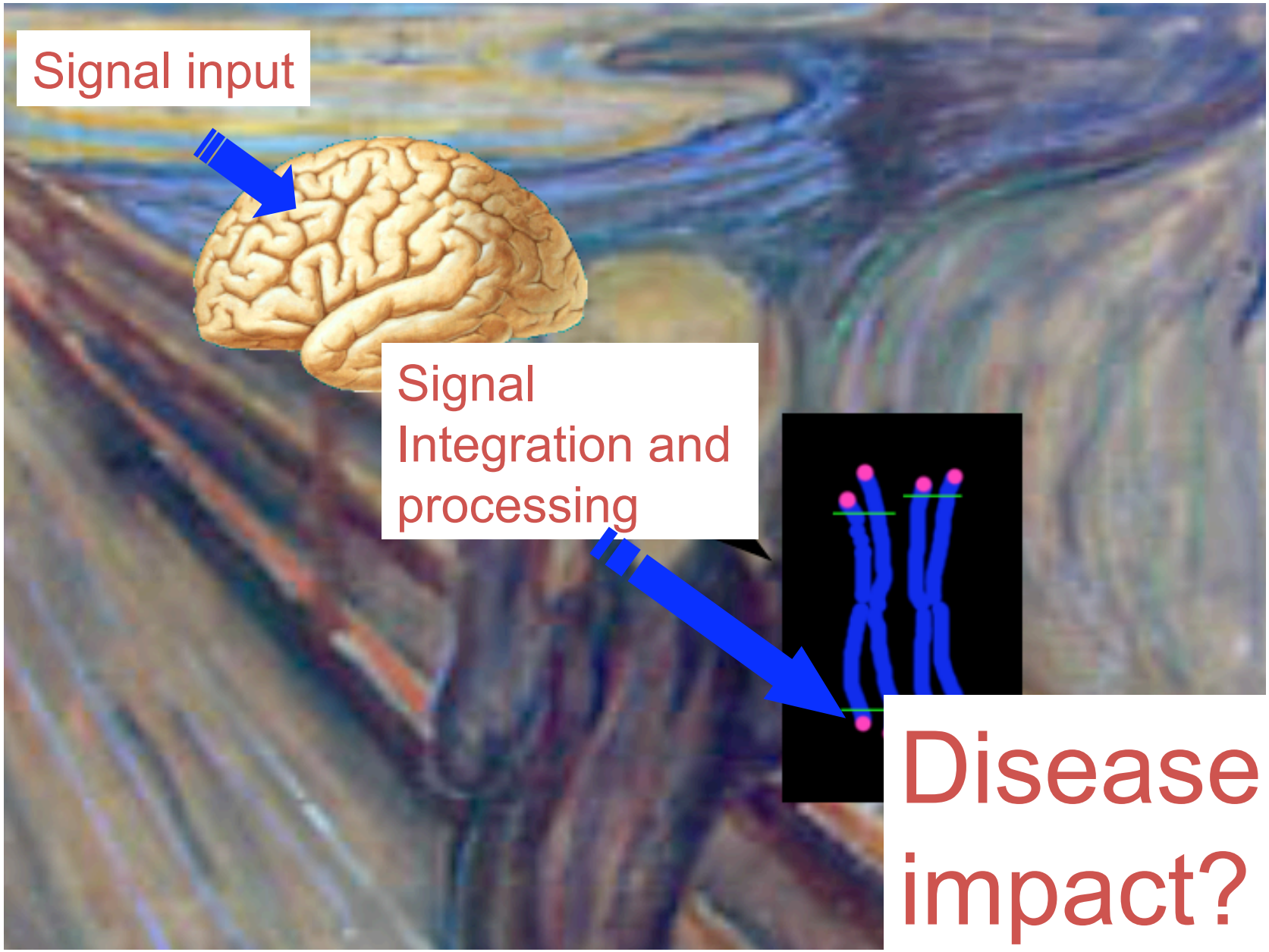
  - **Causal directions?**
  - **Possible mechanisms?**

Chronic stress - reduces telomere length maintenance

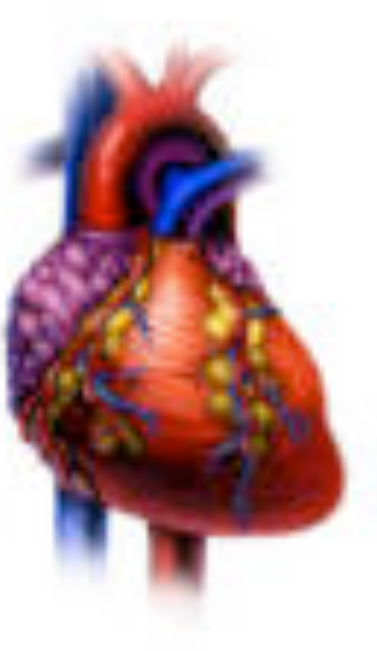


Reduces ability of cell to replenish itself

A new connection



Telomere maintenance and risk of cardiovascular disease



A link in vivo

In the largest epidemiological study of risk factors for cardiovascular disease, six prominent factors were shown to be:

smoking
poor lipid profile
high blood pressure
diabetes
abdominal obesity
psychological stress
(*Yusef et al, Lancet 2004:304).*

•psychological stress

*** Chronic stress was associated with markers of cellular aging**
•Lower telomerase activity
•Shorter telomere length

***Epel et al, 2004**

Links to telomere maintenance in many cohorts …

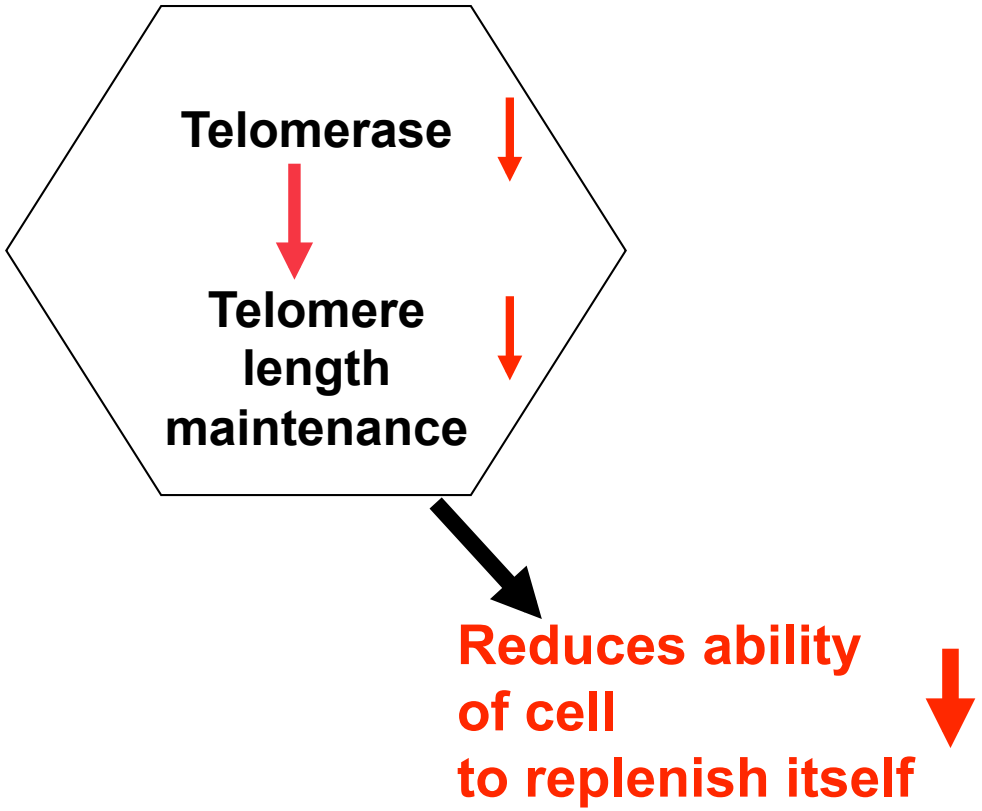
… many independent studies

(mainly with mean telomere length data so far)

Low telomere length linkage to very common disease states

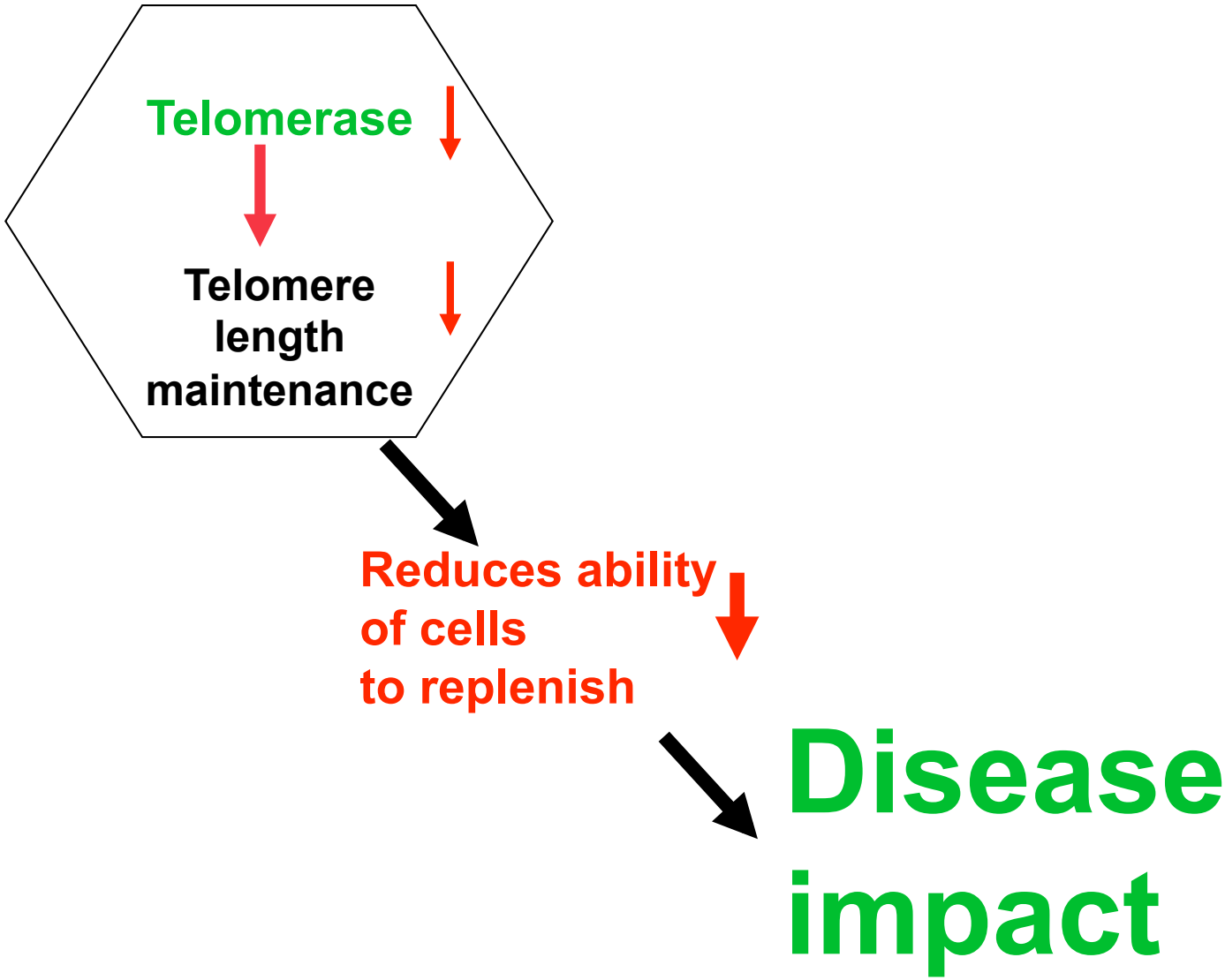
Cancer	Vulliamy, T. et al. (2001) Joshua et al., Shen et al (2007)
Cardiovascular disease (plaques, heart attacks, calcificoric aortic valve stenosis)	Brouilette, S. et al. (2003) Benetos, A. et al. (2004) Kurz, D. J. et al. (2006) Starr et al (2007) Brouilette et al (2007)
Vascular dementia	von Zglinicki, T. et al. (2000)
Degenerative conditions (osteoarthritis, osteoporosis)	Zhai, G., et al. (2006) Valdes, A. M. et al. (2007)
Diabetes	Valdes, A. M. et al. (2005) Aviv, A. et al. (2006)
General risk factors for chronic disease - obesity and insulin resistance	Gardner, J. P. et al. (2005)
Pulmonary fibrosis	Armanios, M. et al. (2007)

Telomerase - an upstream determinant of telomere length maintenance

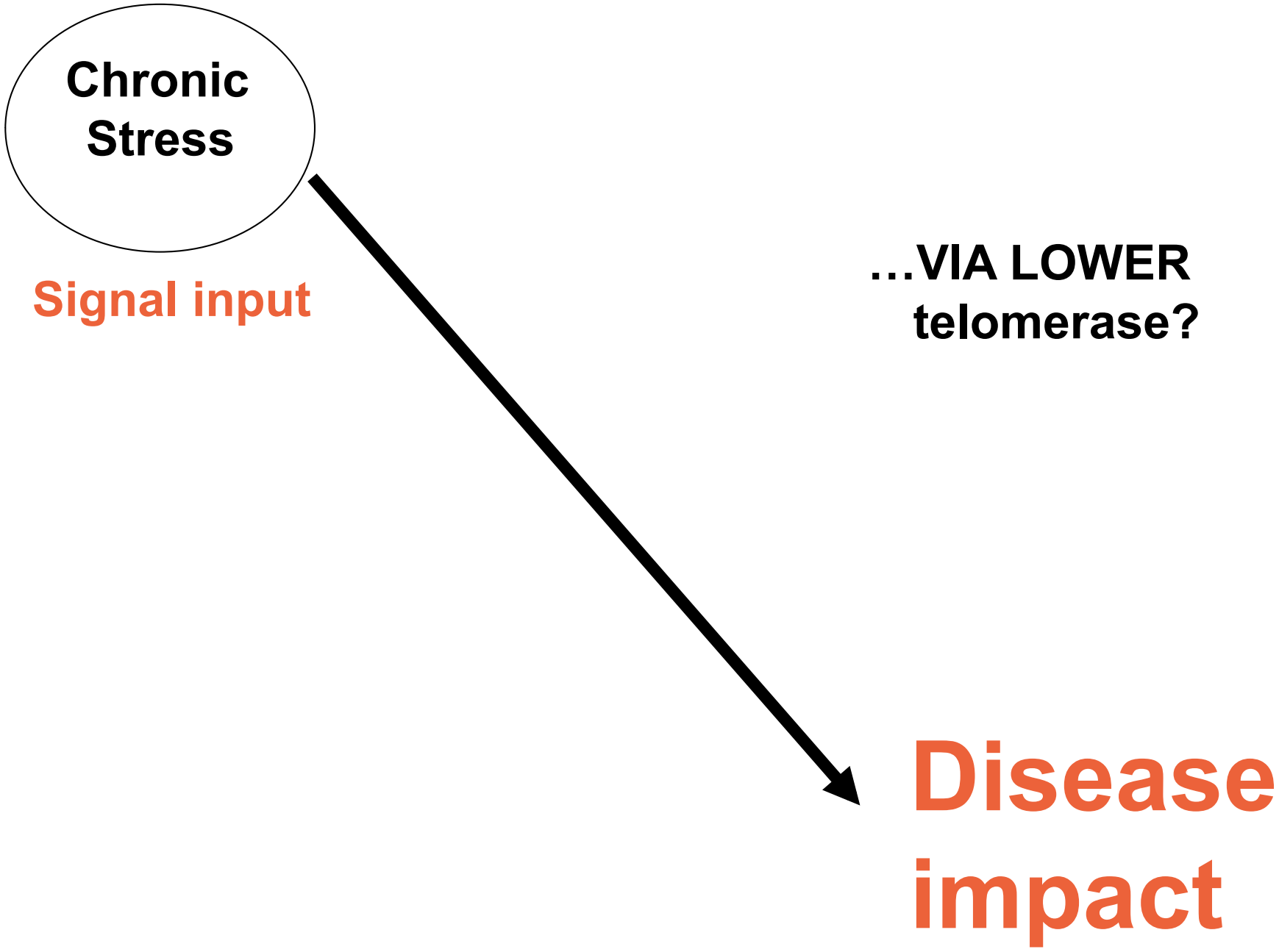


Telomerase - an upstream determinant of telomere length maintenance

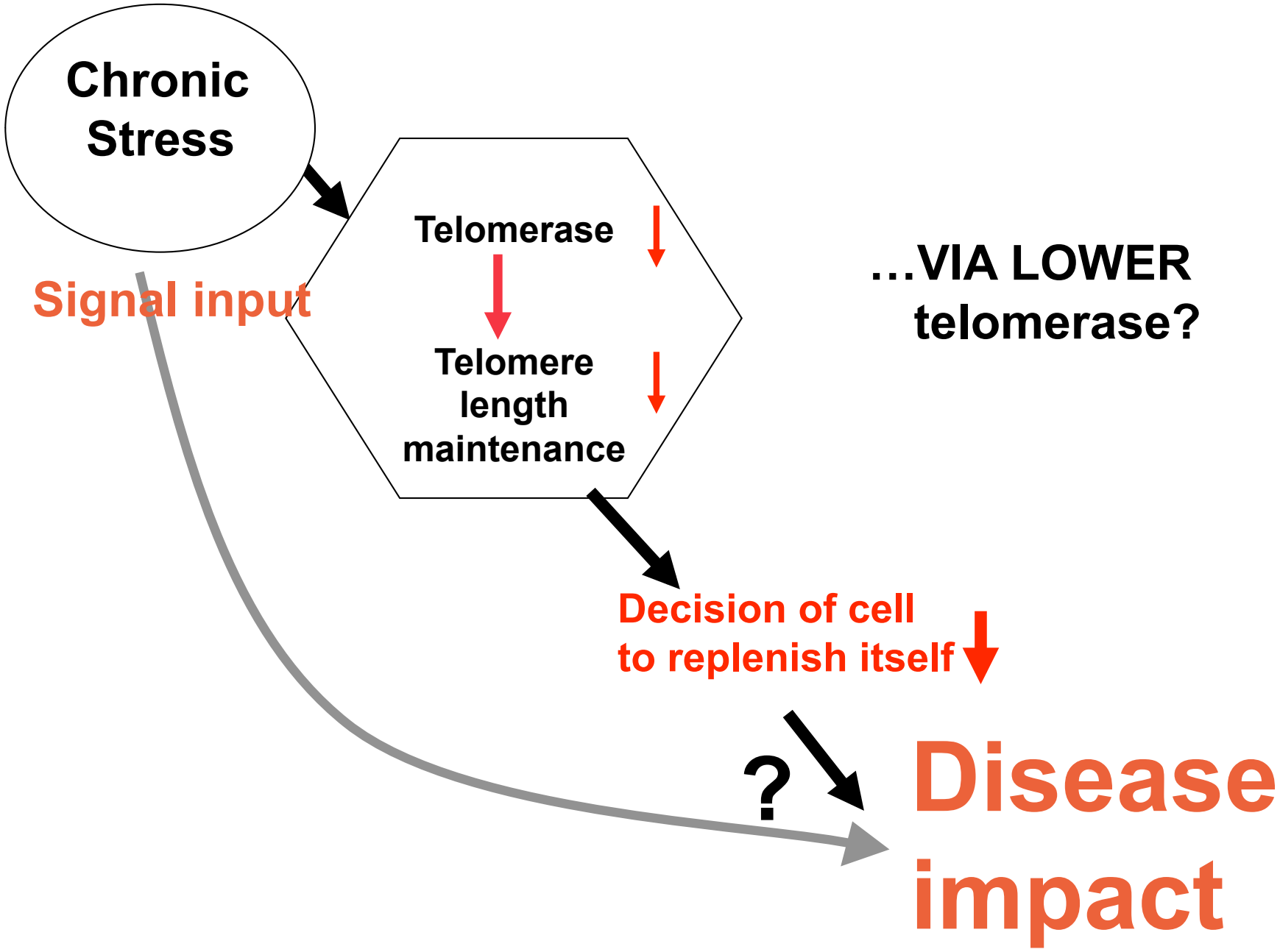
Known genetic defects in telomerase genes cause disease risk in mice and humans



Chronic stress - an upstream determinant of disease risk



Chronic stress - an upstream determinant of disease risk

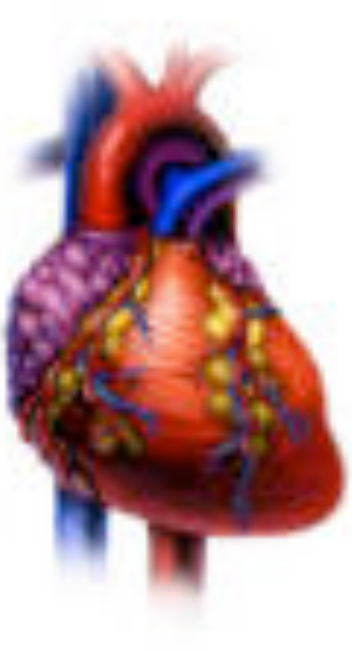


SUMMARY

1. - Forms of chronic psychological stress are associated with low telomerase and shortening of telomeres.

2. - It is emerging that low telomerase and shortening of telomeres in human cells *in vivo* are associated with (and contribute to?)
disease susceptibility and shorter life.

Telomere maintenance and risk of cardiovascular disease



A link in vivo

In the largest epidemiological study of risk factors for cardiovascular disease, six prominent factors were shown to be:

smoking	•smoking
poor lipid profile	•cholesterol/blood lipids
high blood pressure	•resting cardiovascular activity
diabetes	•fasting glucose
abdominal obesity	•adiposity
psychological stress	•psychological stress

(***Yusef et al, Lancet 2004:304).***

Stress was associated with markers of cellular aging

•Lower telomerase activity

•Telomere length

***Epel et al, 2006**