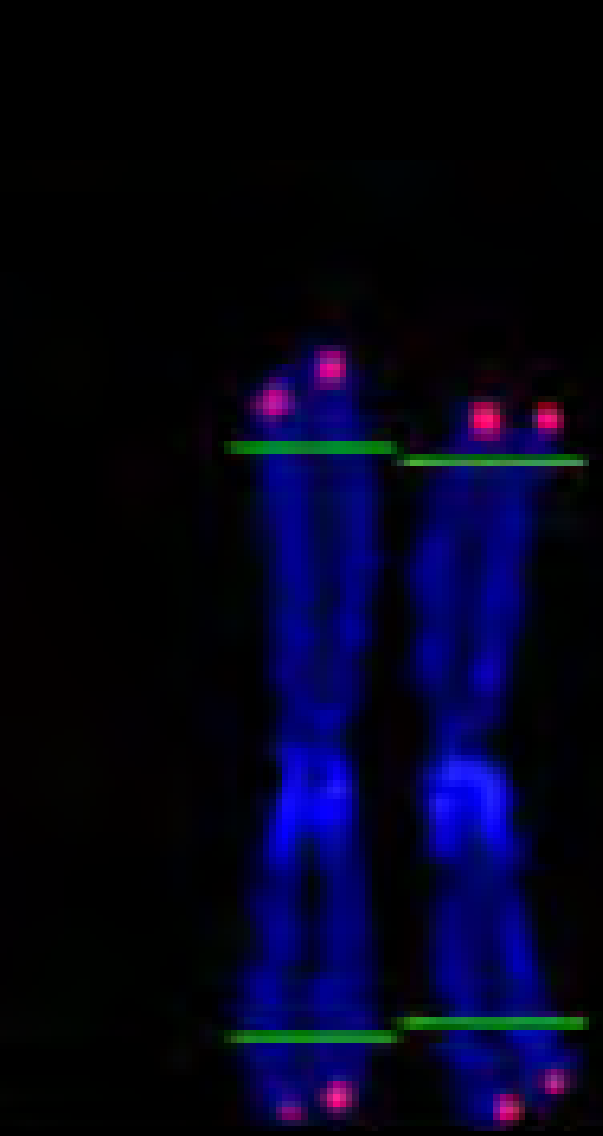


Telomeres and Telomerase: The Means to the End

Elizabeth H. Blackburn

Nobel Lecture 2009

Karolinska Institutet

Maize breeding

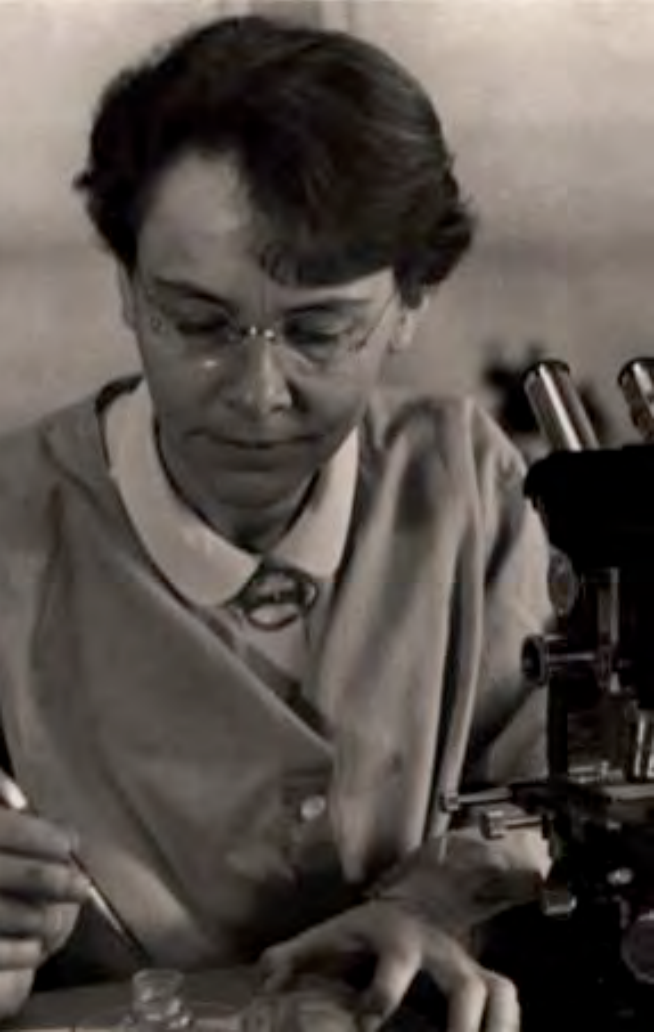
Ancient….

Mayan corn god

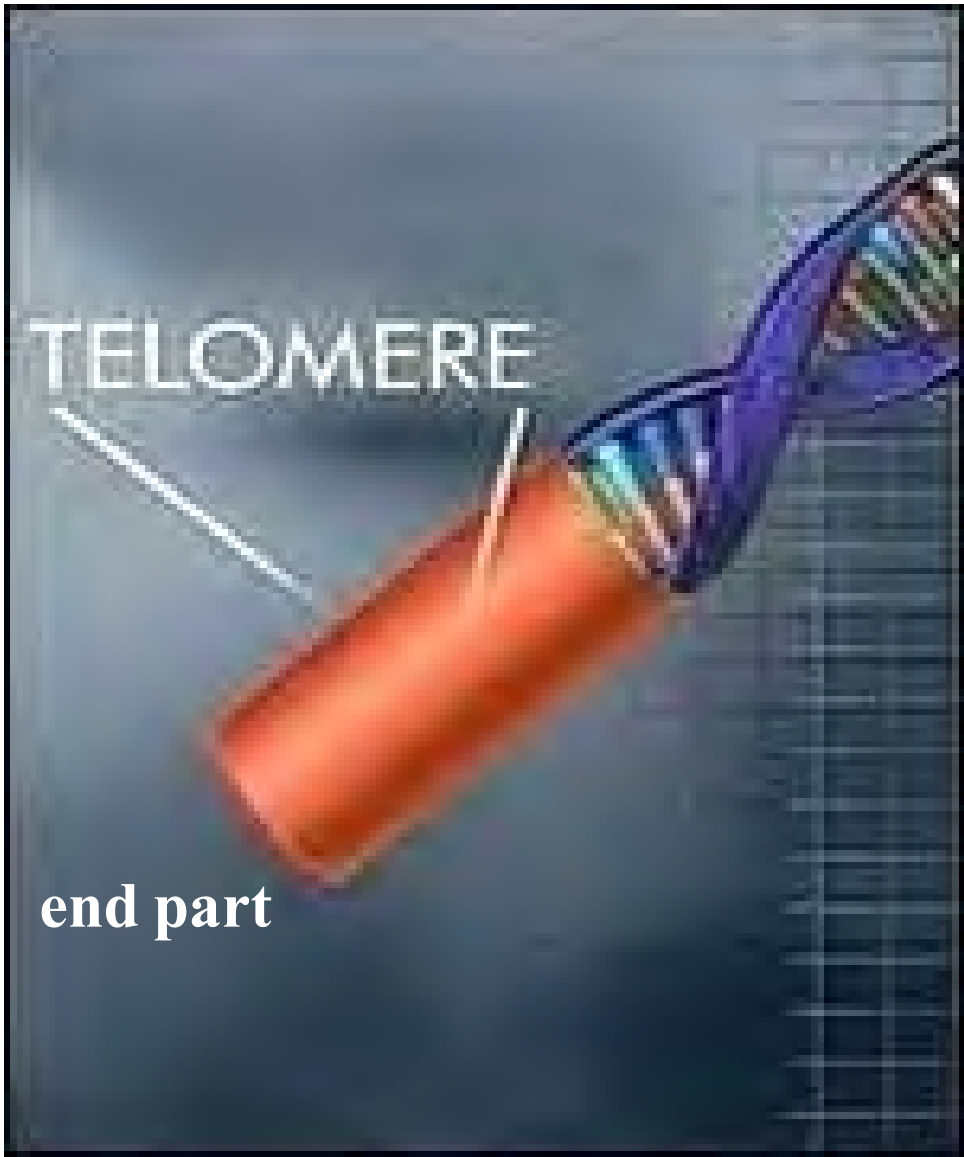
…. modern

- cytogenetics
- chromosome discoveries

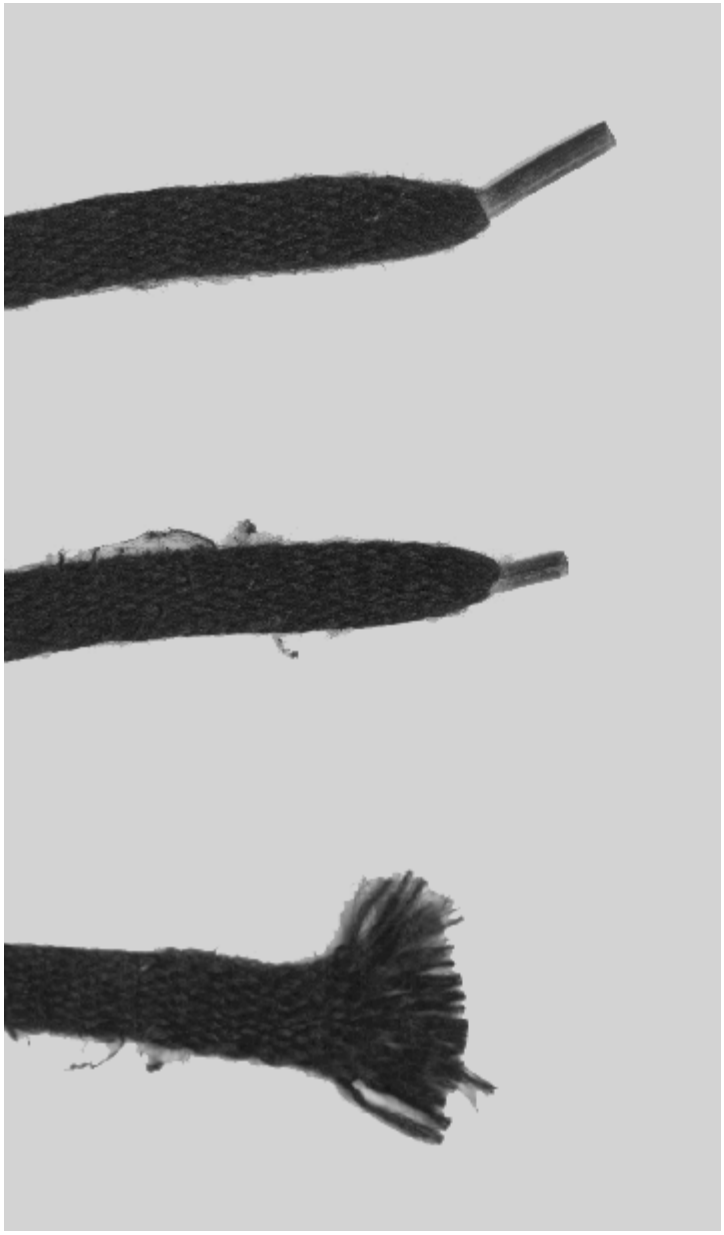
Barbara McClintock



Cold Spring Harbor, 1947

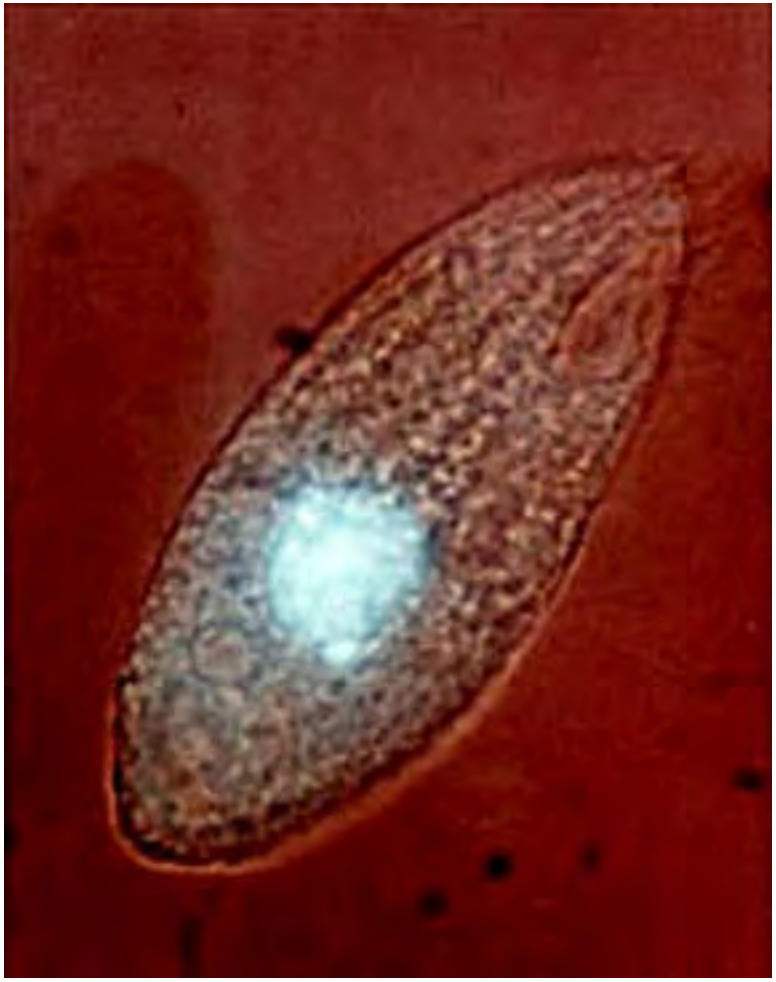


Telo-mere
(tel'uh mer or te lō mēr)
- named by Hermann Muller



***Common Shoelace*, 10x.**

ag·let (ag'lit) *n.* A tag or sheath, as of plastic, on the end of a lace, cord, or ribbon to facilitate its passing through eyelet holes.



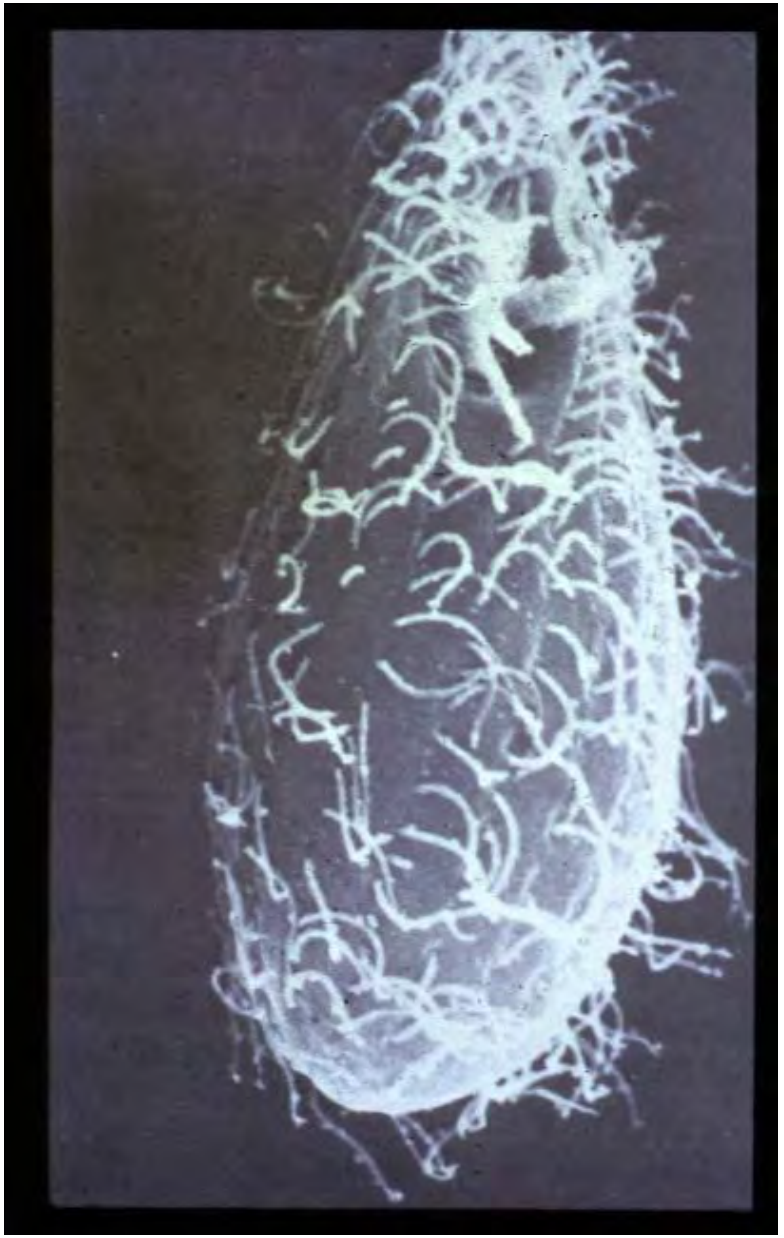
Pond scum
(a.k.a. *Tetrahymena thermophila*)

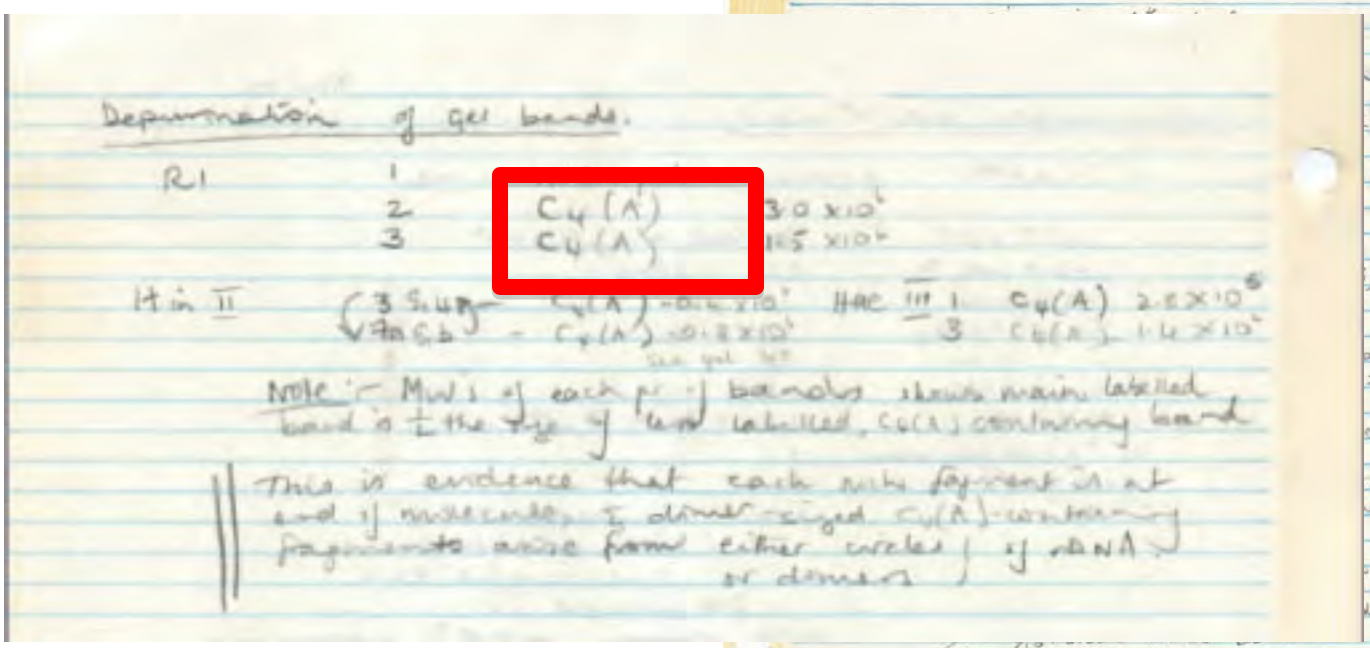
Joe Gall

Photographed in Prague, 1999, demonstrating an optical principle by which a partial solar eclipse can be viewed.

Tetrahymena thermophila

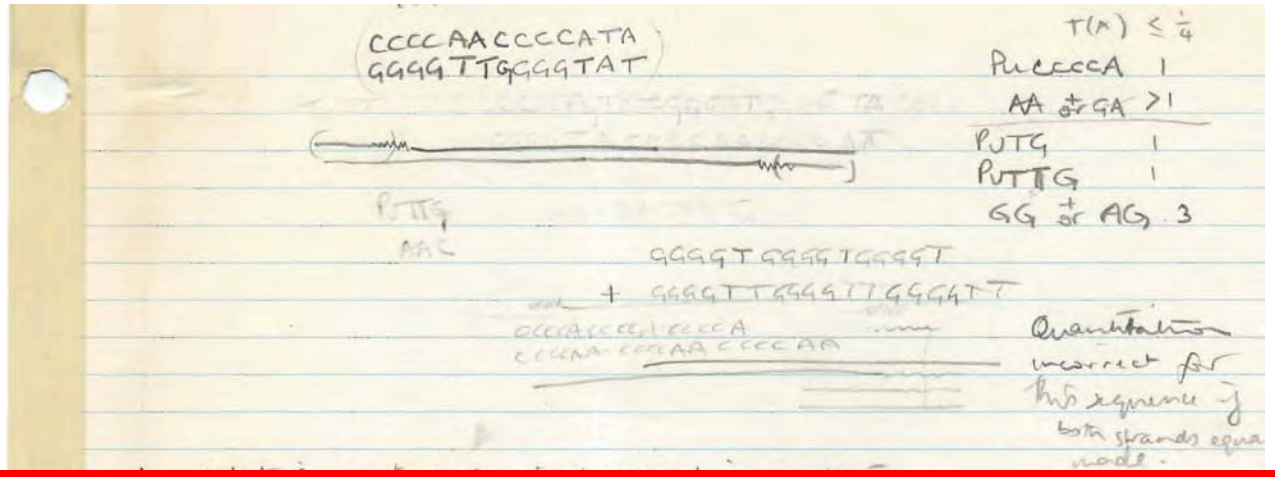
- Contains abundant very short linear chromosomes





Beginning to piece together the first telomeric DNA sequence…

… and data that the telomeric DNA repeat unit was tandemly repeated



7. Calculating no. of ³H AMP's incorporated per molecule of DNA → about 20-30 molecules per molecule of DNA (MW 13.6 x 10⁶). No evidence that the DNA is saturated or 100% substituted at this pt.

3. With long incubations, get high rel amt of C_4 at room temp + pancr. C_4 also at 0° ∴ high salt.

4. V. short incubations - low C_4 rel to other simple deps. C_4 rel amt increases ∴ time (20'-90' at 4°).

5. C_4 found almost exclusively in small Rl frag 1.5 x 10⁶ MW
Bam 2.2 x 10⁶
one Hae frag 1.4 x 10⁶
in a heterogeneous Hin II frag band ≈ 5 x 10⁵ X Bethes est. 4 x 10⁵
≈ 750 bprs.

6. If random incorporation, ∴ high amt of pancr, find ~1% of A* incorporated is in the sequence PuCCCCA. This → ~650 bases (for 10-base repeat), or MW

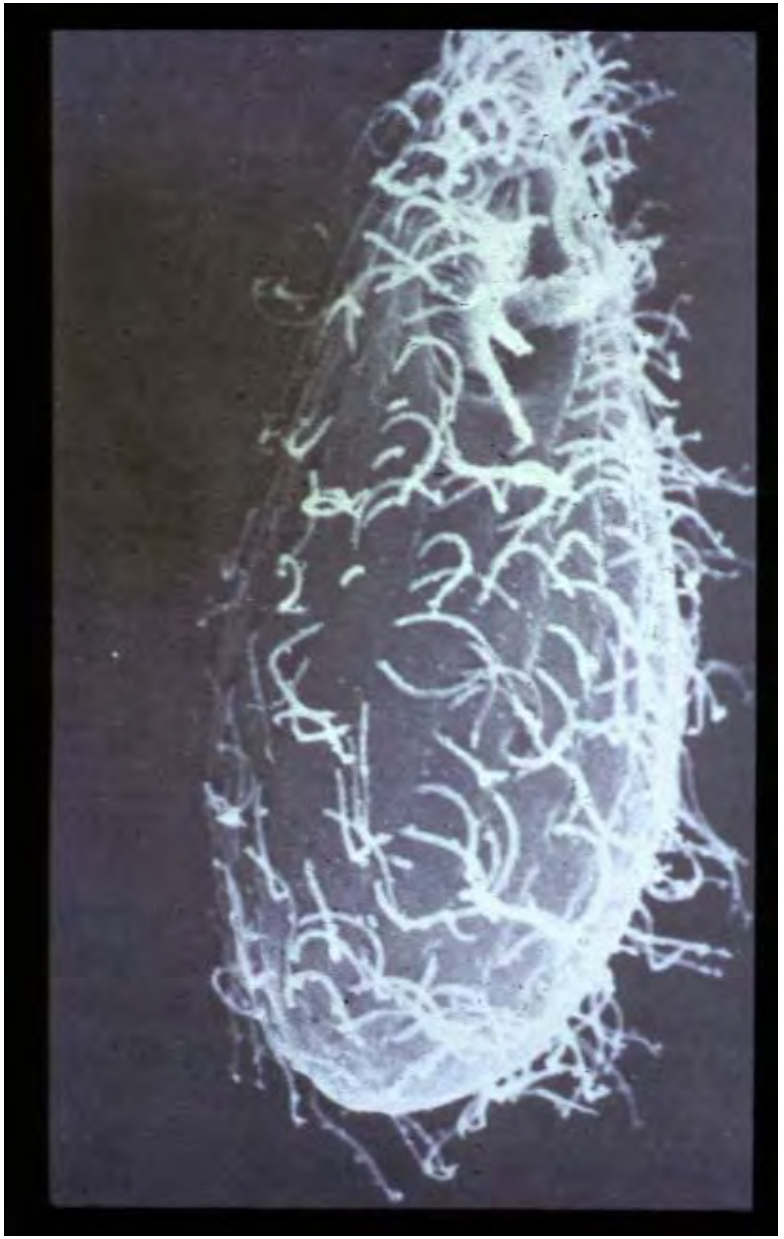
7. Calculating no. of ³H AMP's incorporated per molecule of DNA → about 20-30 molecules per molecule of DNA (MW 13.6 x 10⁶). No evidence that the DNA is saturated or 100% substituted at this pt.

Tetrahymena thermophila

- Contains abundant very short linear chromosomes

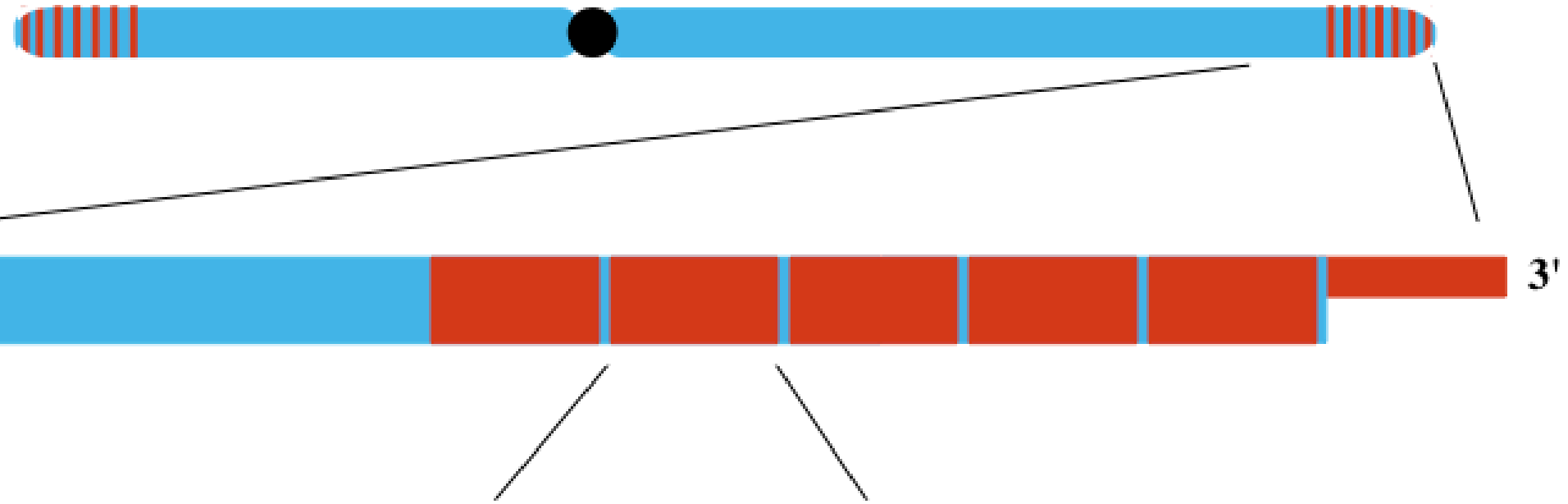
-They end in TTGGGG repeats.

Blackburn and Gall, 1978



Telomeric DNA contains simple tandem repeats

Chromosome with centromere



Tetrahymena	**TTGGGG**	Blackburn and Gall, 1978
S. cerevisiae	**$(TG)_{1-6}TG_{2-3}$**	Szostak and Blackburn 1982; Shampay, Szostak and Blackburn 1984

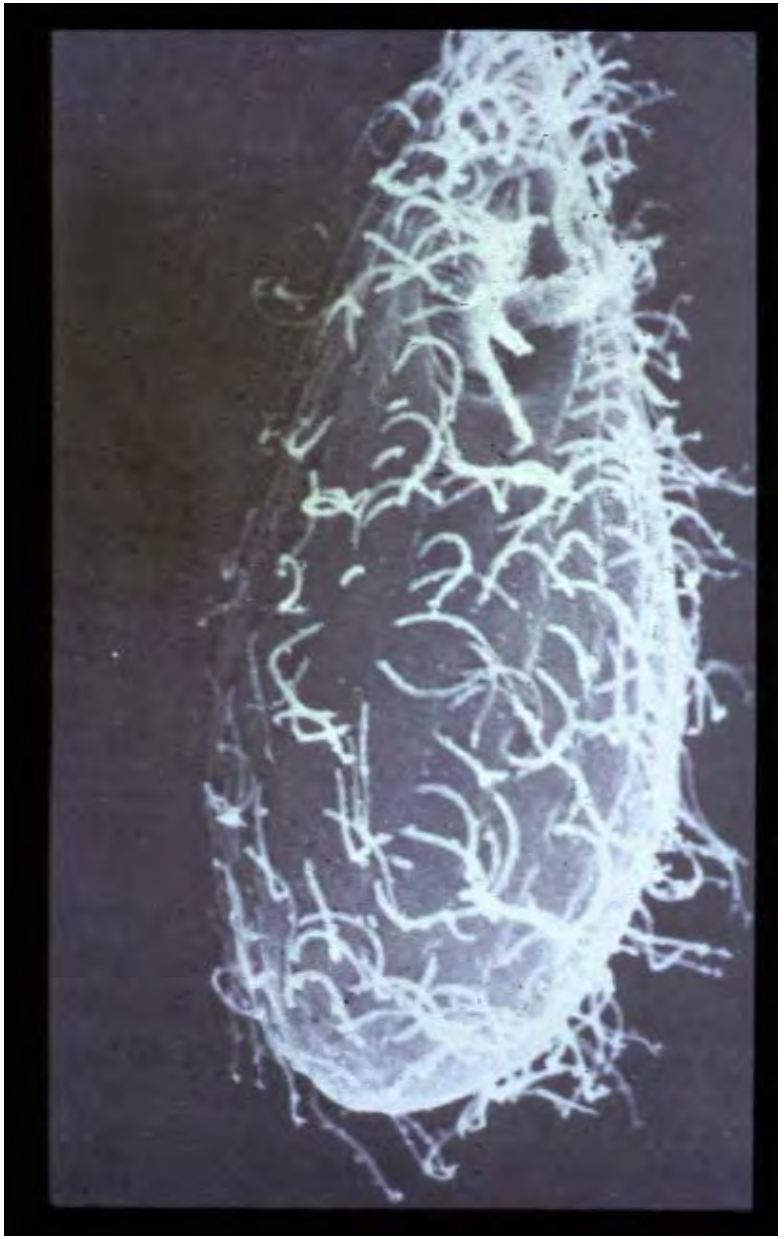
Tetrahymena thermophila

- Contains abundant very short linear chromosomes

-They end in TTGGGG repeats.

Blackburn and Gall, 1978

- How did the repeats get there?



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

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

•Telomeric GGGGTT 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

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

•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 THEN-CURRENT MODELS FOR DNA REPLICATION

•AND…….

•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?

DISCOVERY OF TELOMERASE

SYNTHETIC TELOMERE IN TEST TUBE

5’ G G G G T T G G G G T T G G G G T T 3’ OH

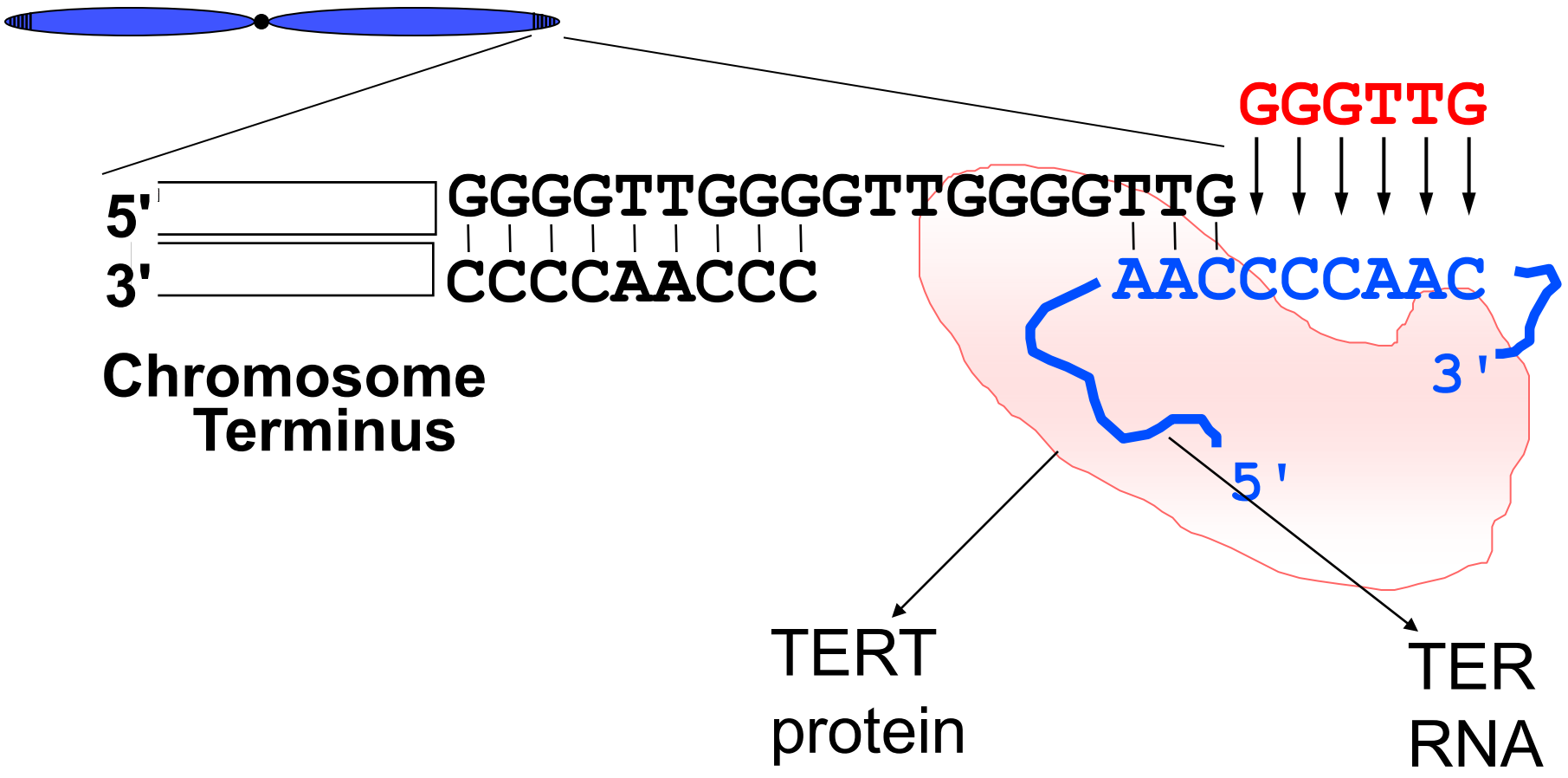
Tetrahymena cell extract
Mg++
dGTP + TTP

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

Greider and Blackburn, 1985

The solution to telomere attrition

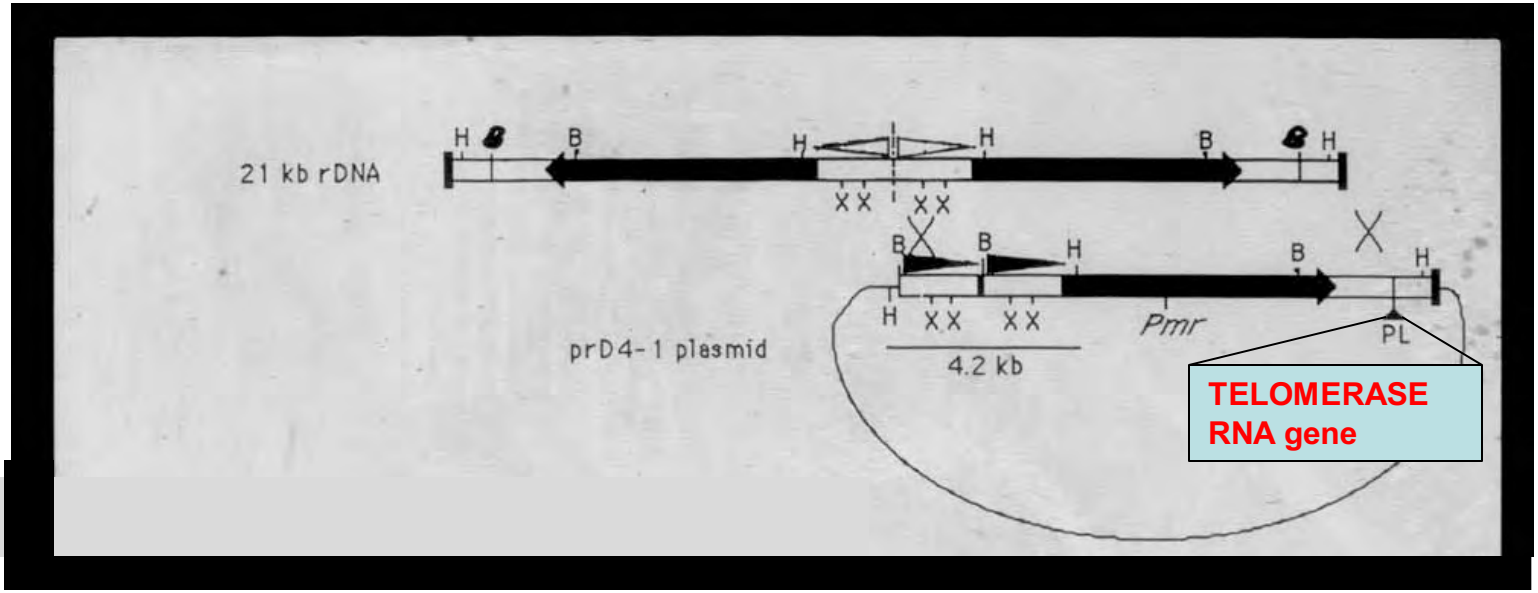
Telomerase:



Greider and Blackburn, 1985, 1987, 1989

Blackburn lab members and friends at UC Berkeley 1986

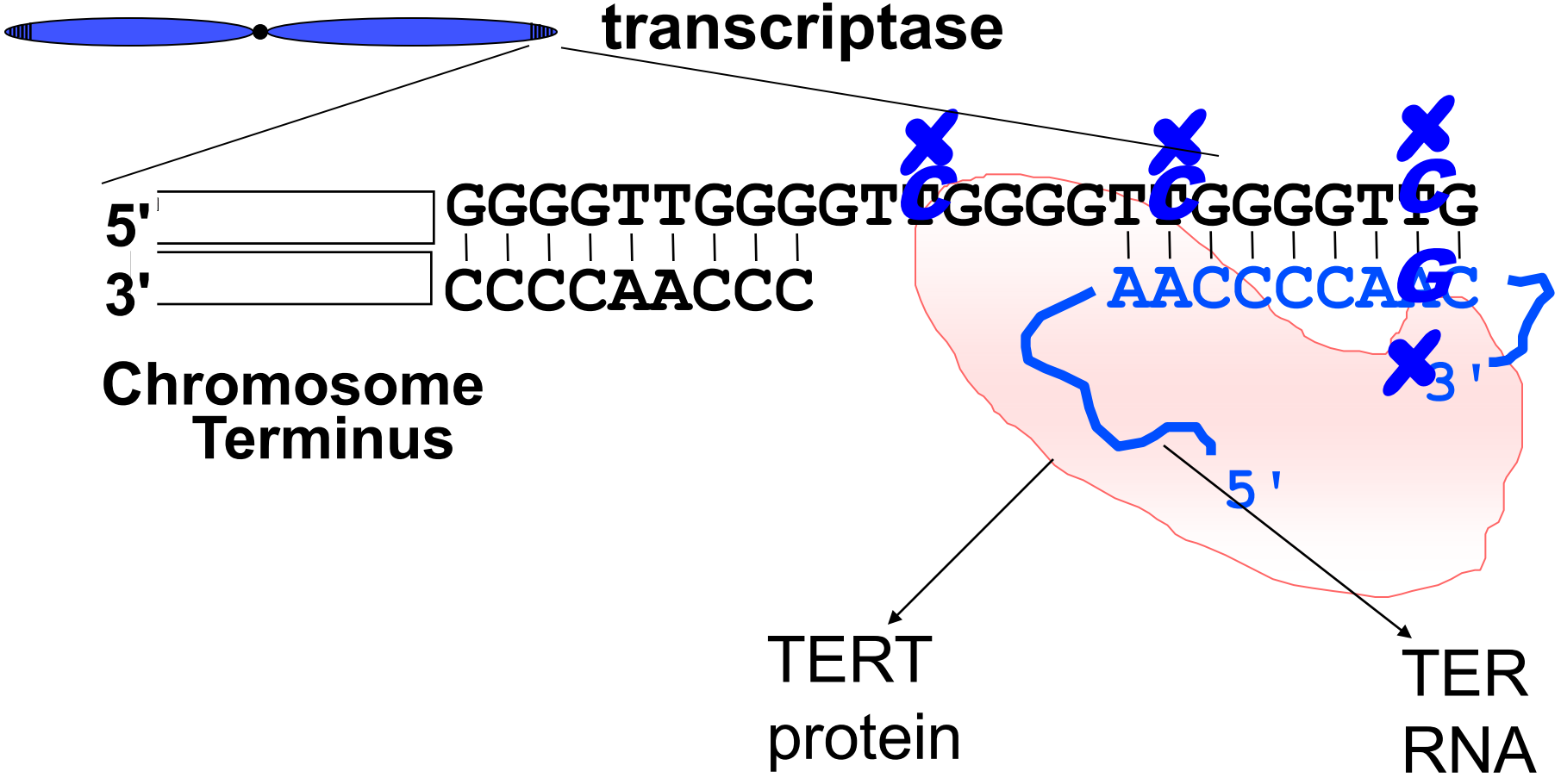
A vector based on Tetrahymena rDNA replication properties



Yu et al, 1990

The solution to telomere attrition

Telomerase: a telomere-synthesizing reverse transcriptase



Yu et al, Nature 1990

How do **Tetrahymena** cells respond when telomerase is nonfunctional?

Guo-Liang Yu

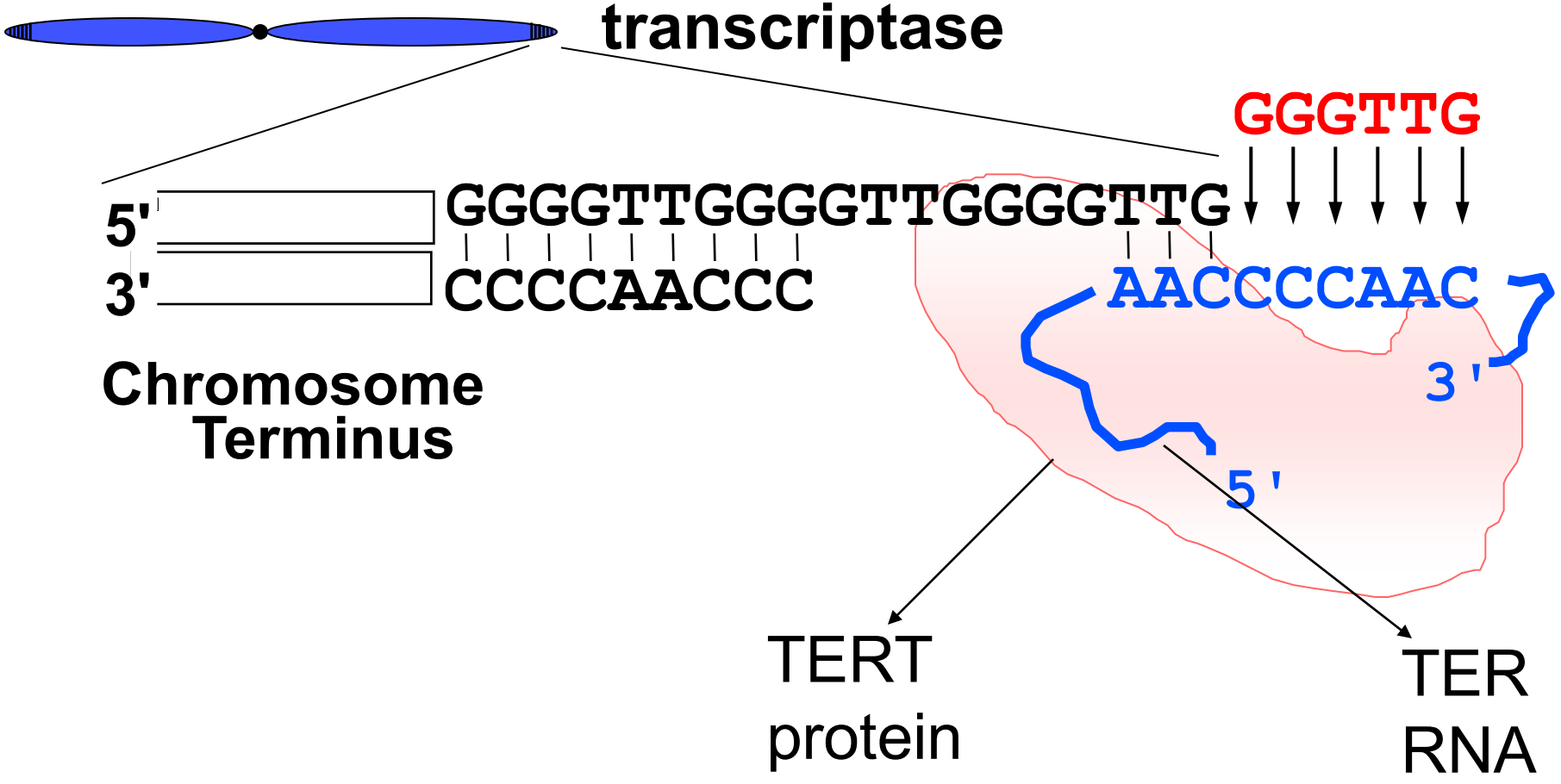
John Bradley

Laura Attardi

Yu et al, Nature 1990

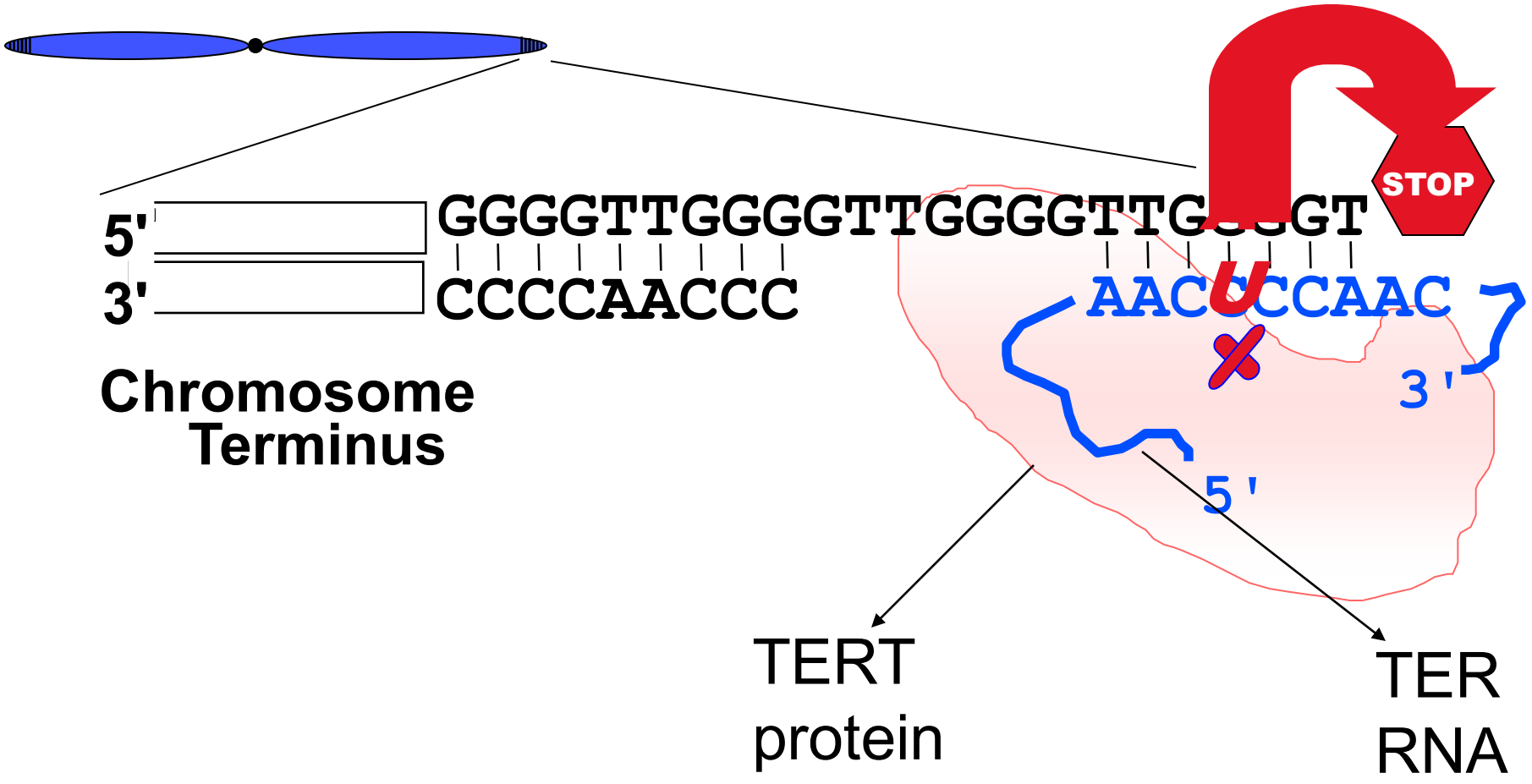
The solution to telomere attrition

Telomerase: a telomere-synthesizing reverse transcriptase



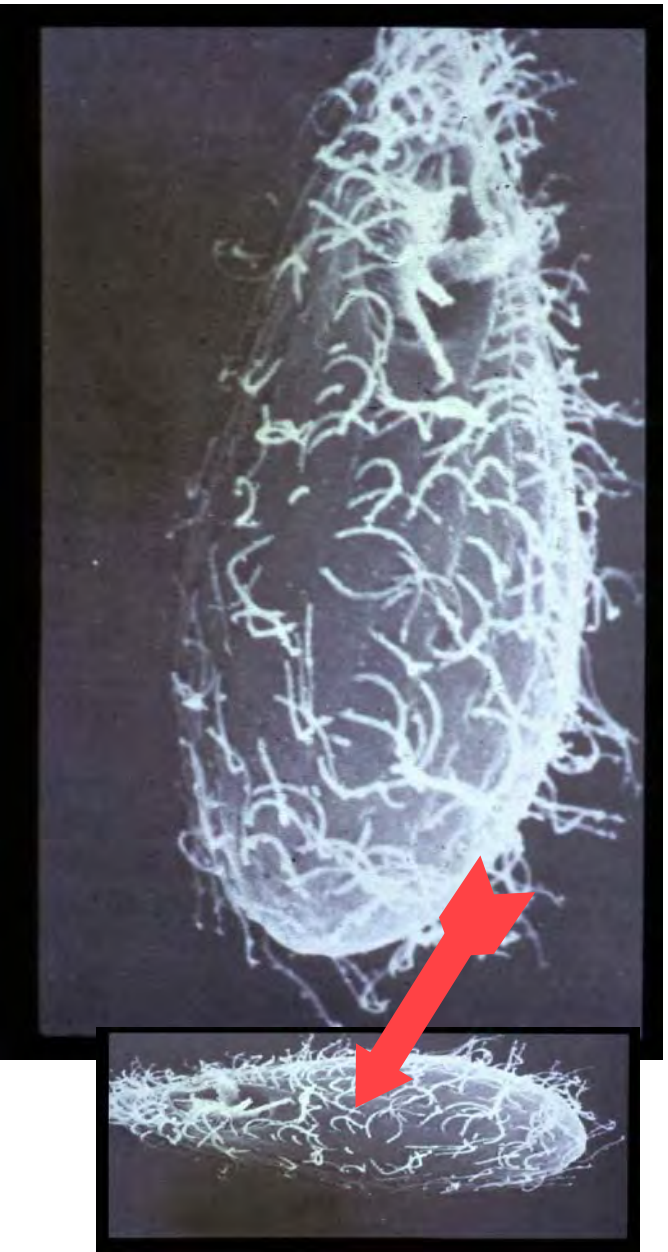
Greider and Blackburn, 1985, 1987, 1989

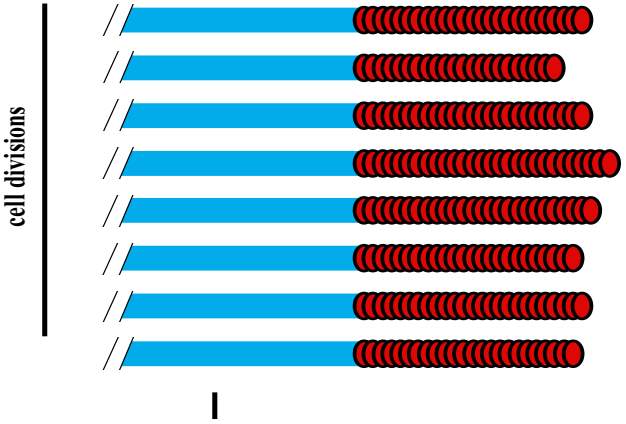
A telomerase RNA mutant unable to copy the template



Yu et al, 1990; Gilley et al, 1996

Tetrahymena thermophila

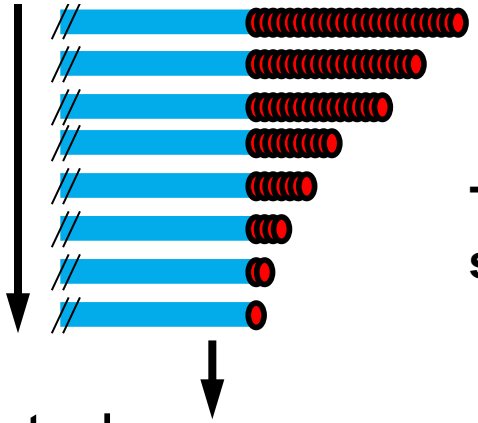




Telomeres replenished by telomerase

Cells are immortal

Plenty of telomerase



Genetically kill telomerase

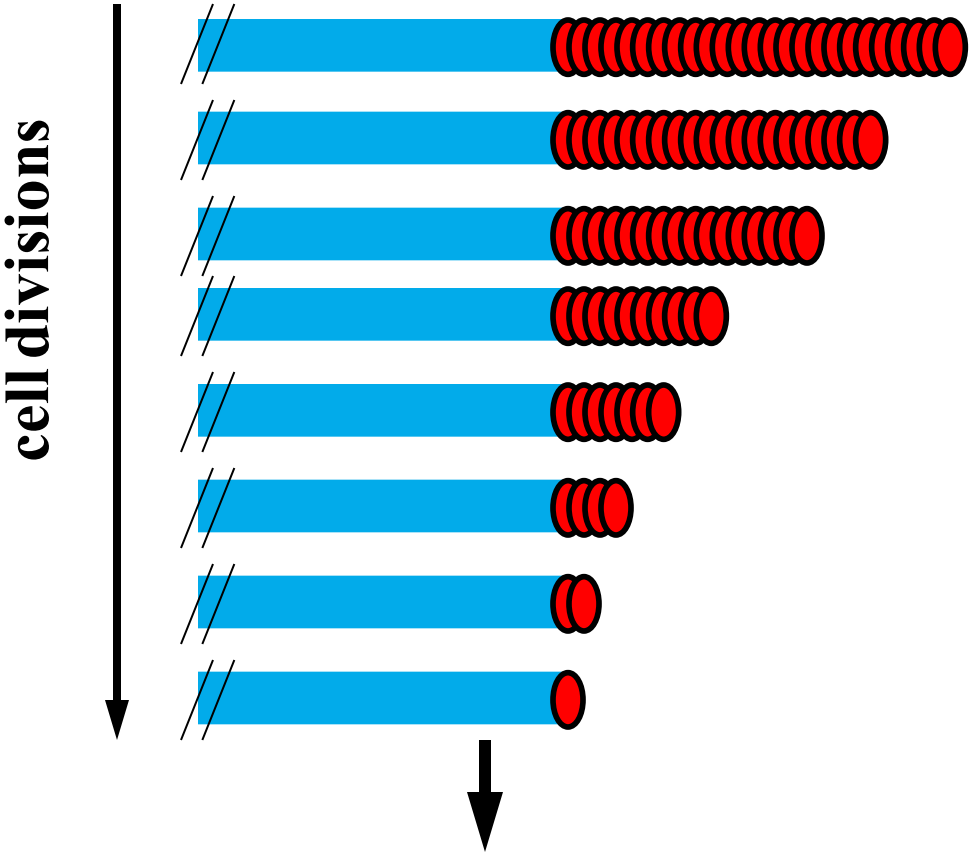
Telomeres progressively shorten

Tetrahymena ceased divisions

They become "mortal"

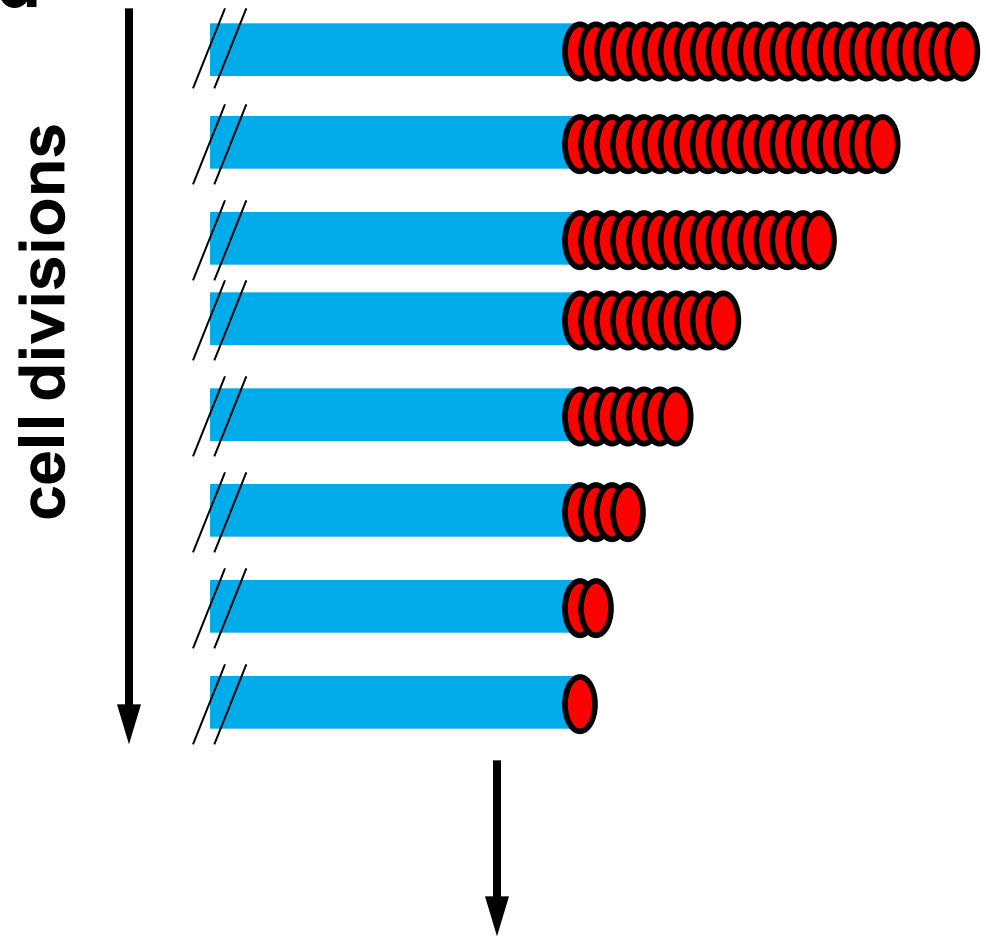
Yu et al, Nature 1990

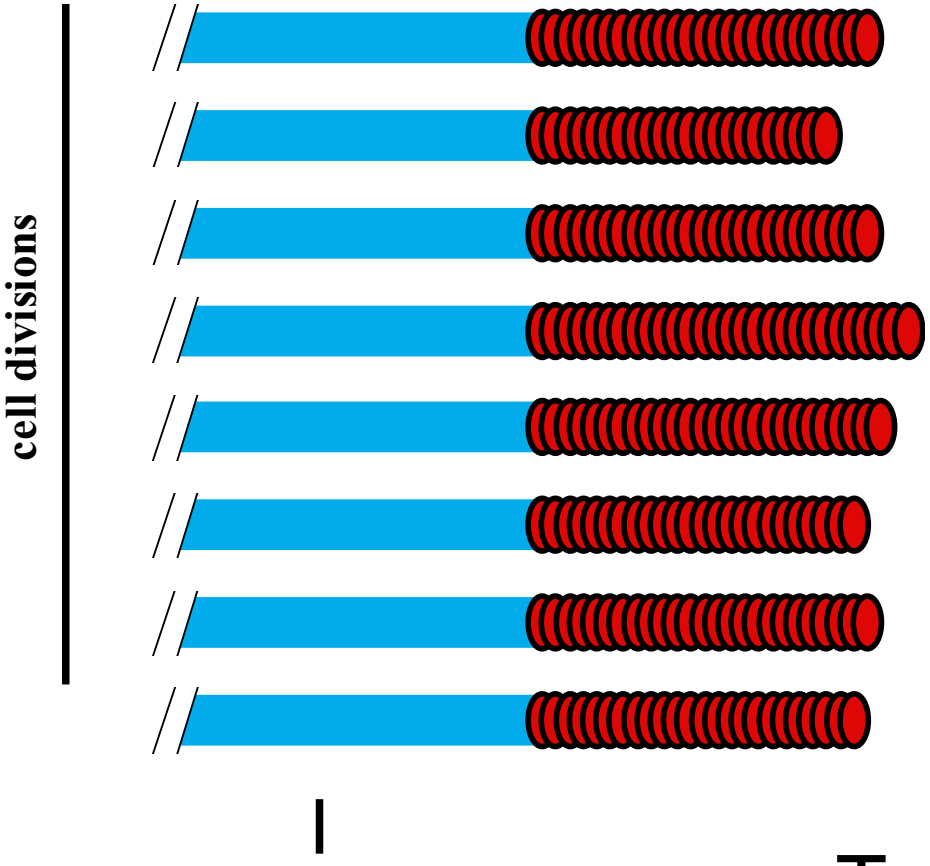
Predicted, if DNA replication alone acts on DNA: Loss of DNA from the chromosome end (the DNA 'end-replication problem')



Watson, 1972, Olovnikov, 1971

Lack of functional telomerase: Progressive loss of DNA from the chromosome end





Telomeres replenished by telomerase

Tetrahymena thermophila

Immortal

Inactivate telomerase

“Mortal”

Yu et al, Nature 1990

How did
Tetrahymena
cells
respond when telomerase is forced to make the wrong DNA sequence?

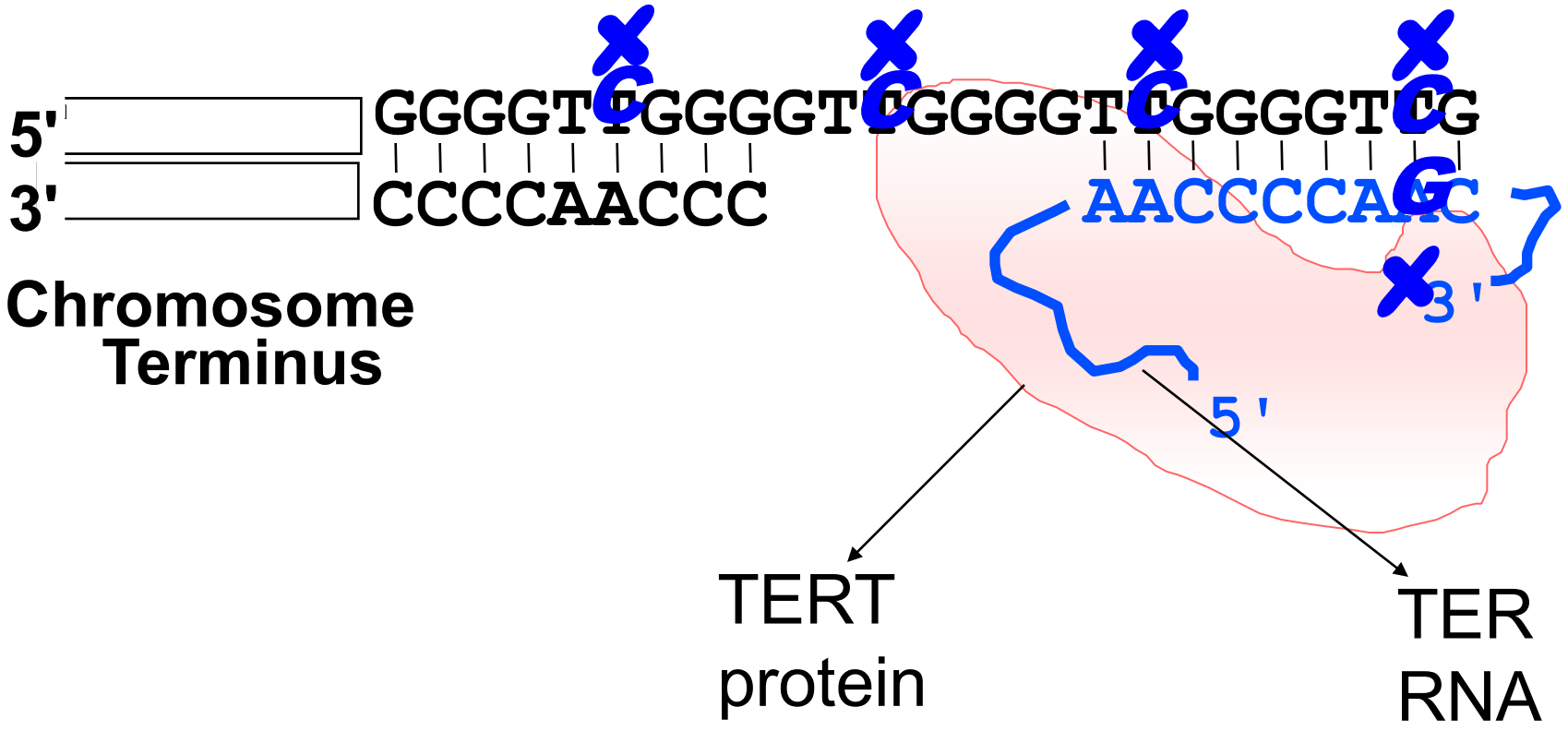
Guo-Liang Yu

John Bradley

Laura Attardi

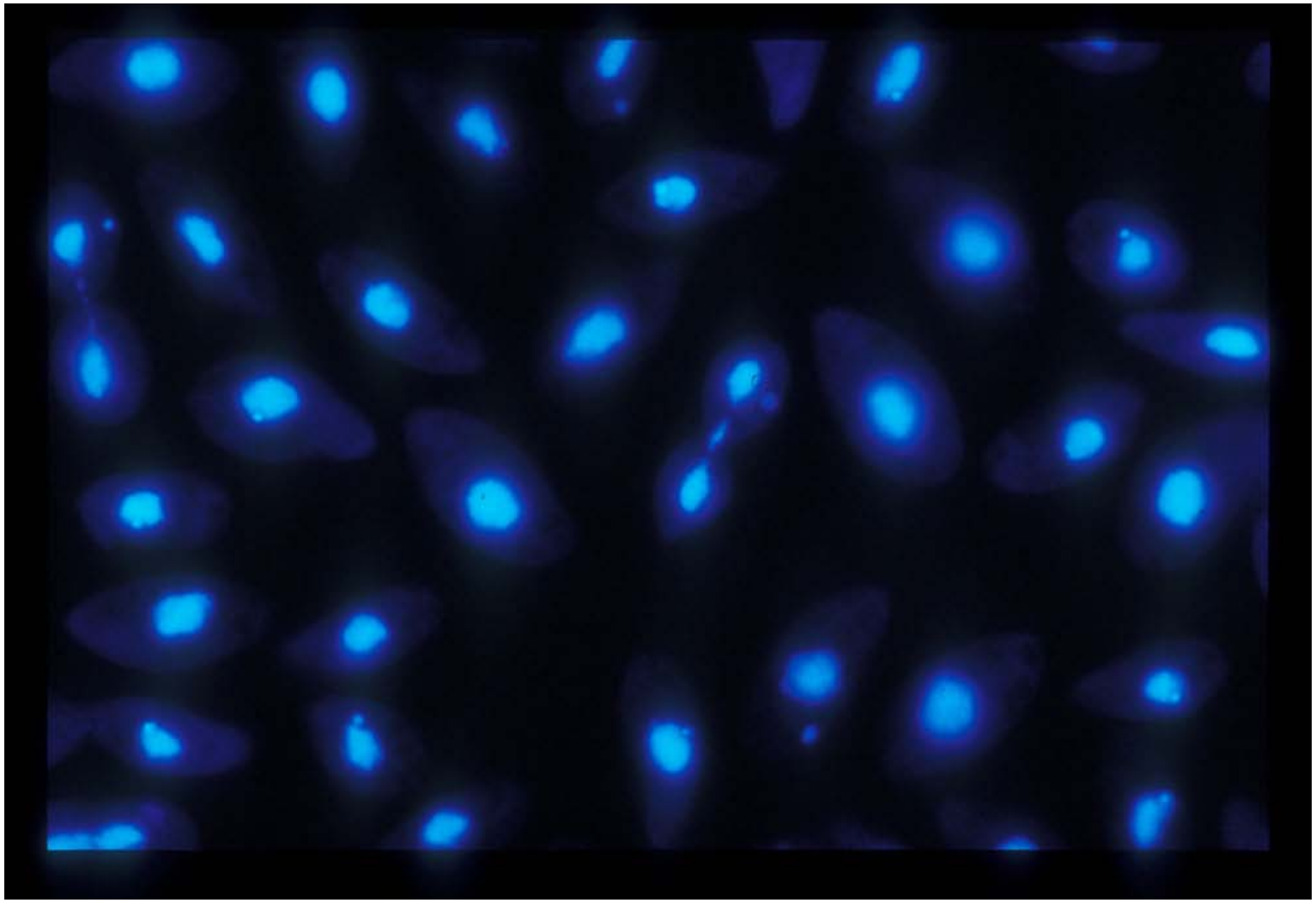
Yu et al, Nature 1990

Telomerase: a telomere-synthesizing reverse transcriptase: the sequence matters



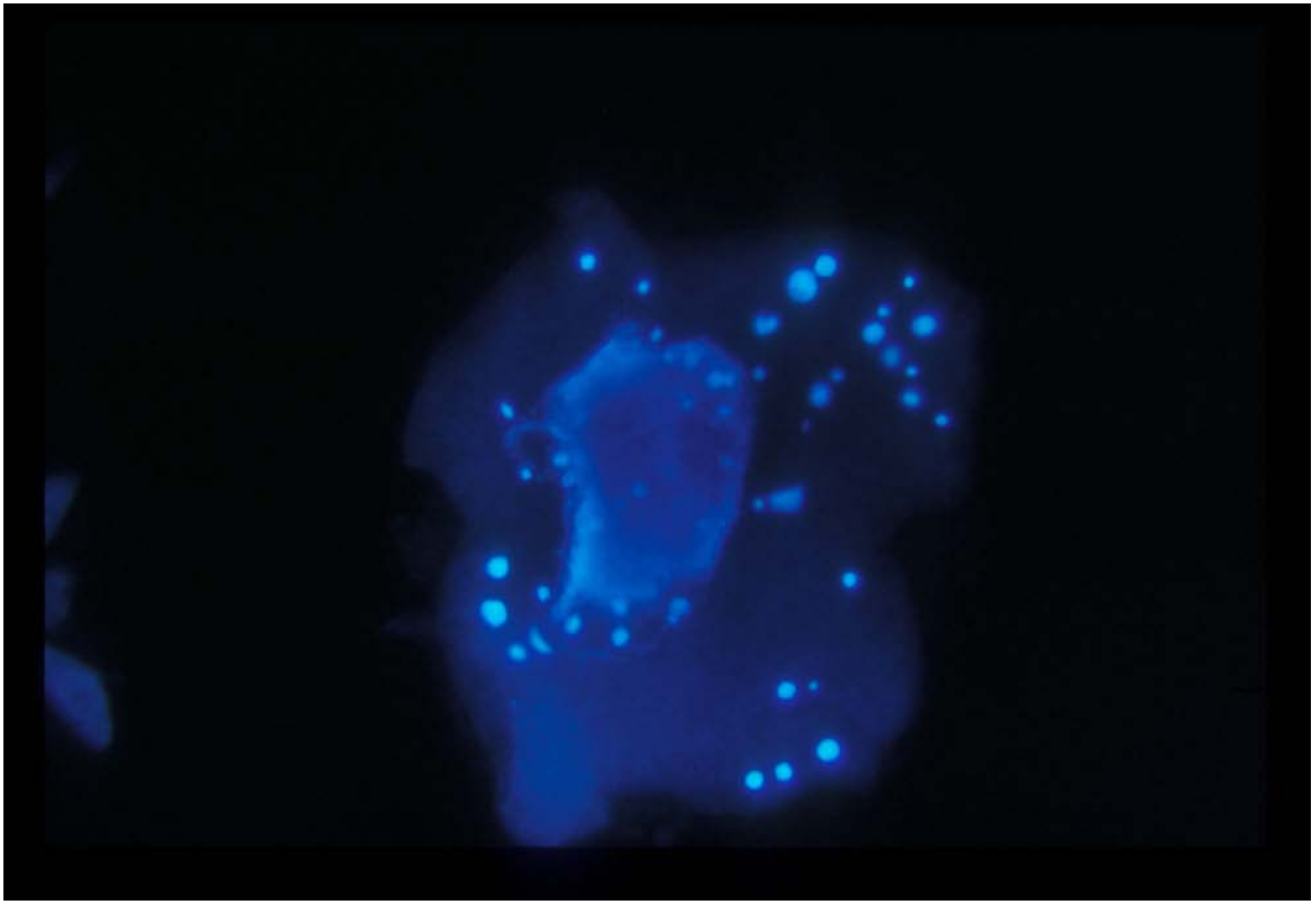
Ye at al, 1990; John Bradley unpubl.

Tetrahymena thermophila **WILD TYPE**



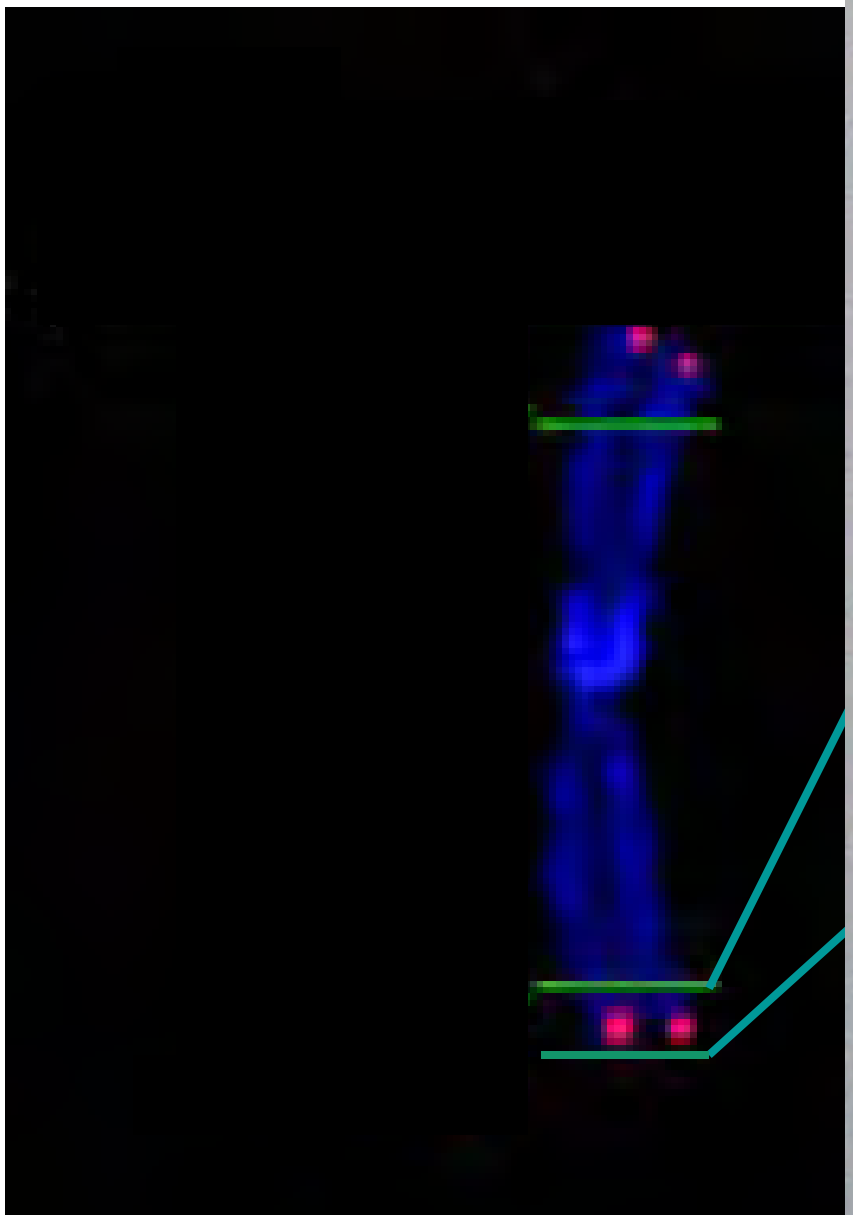
Tetrahymena thermophila

mutant-sequence telomeres

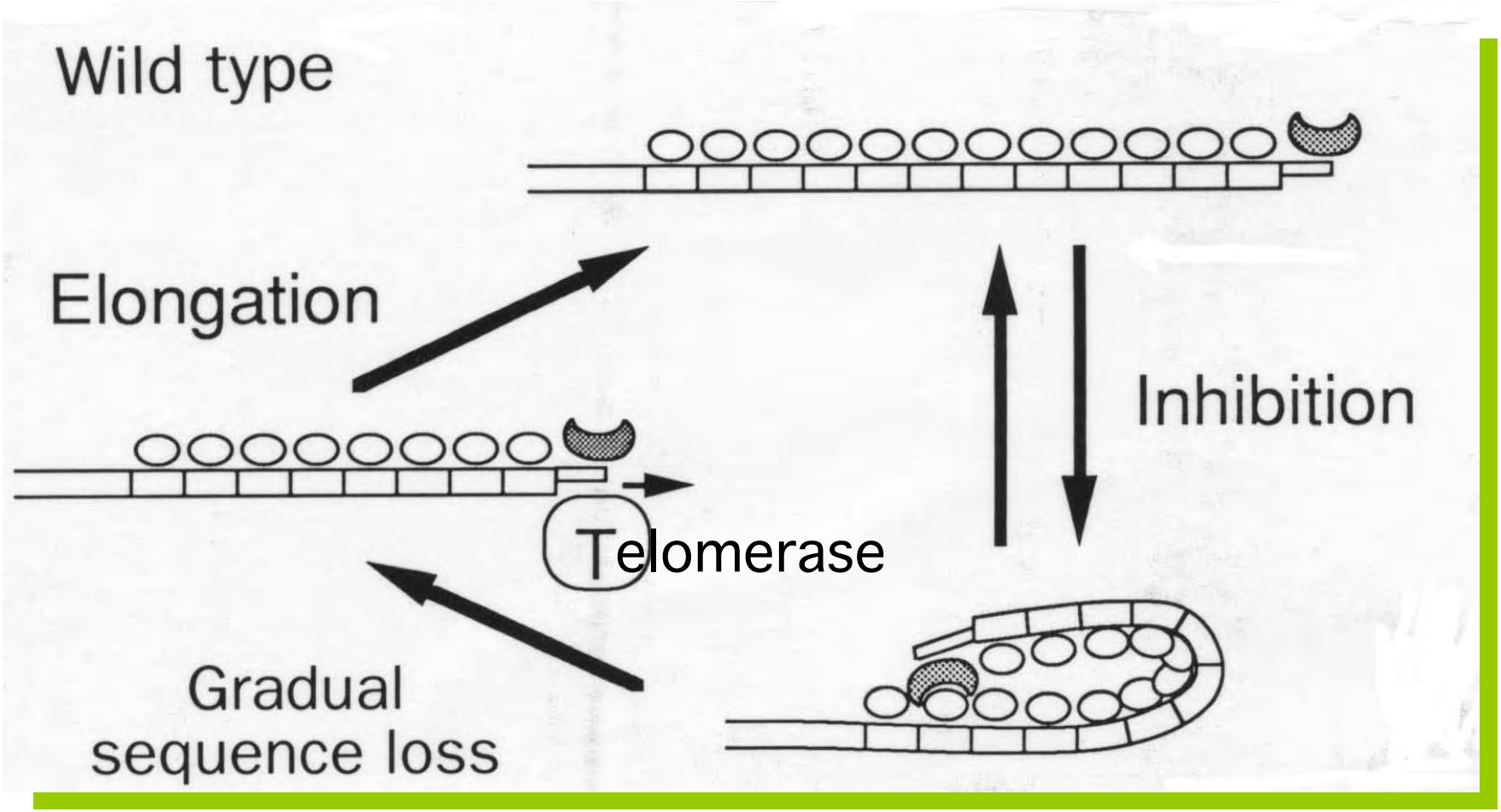


Cells rapidly lost viability!

Telomeres cap ends of chromosomes



Telomere dynamics: a homeostatic system



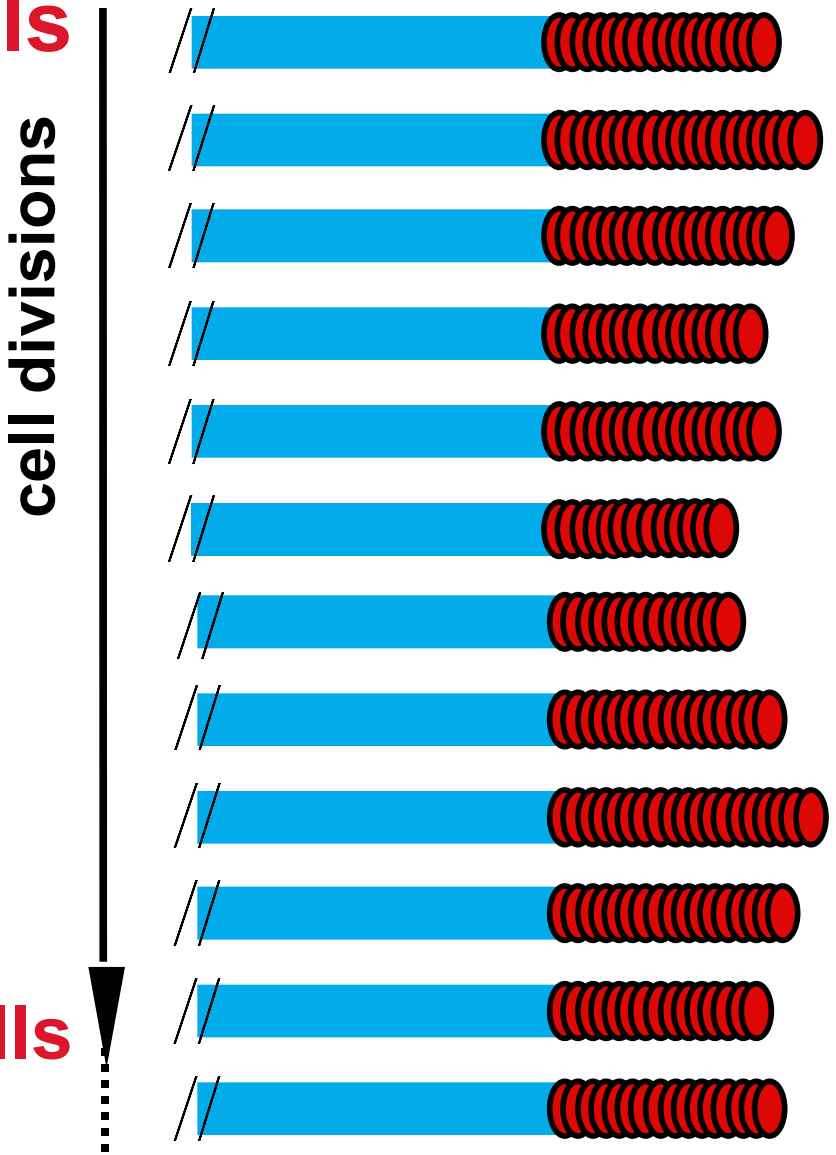
McEachern and Blackburn. Nature, 1995.

In humans?

In **cancer cells**

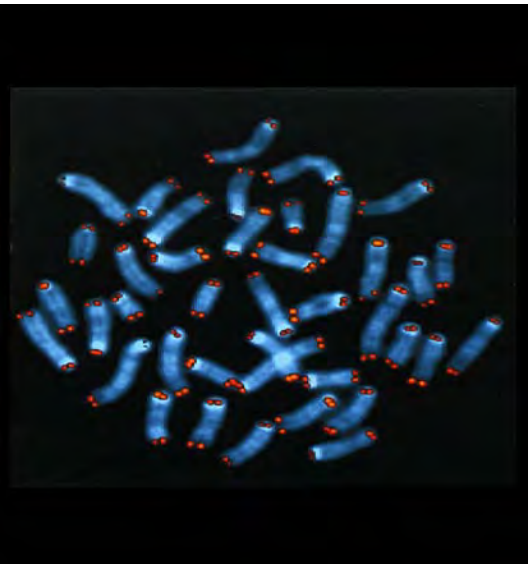
HIGH telomerase characterizes malignant human cancer cells

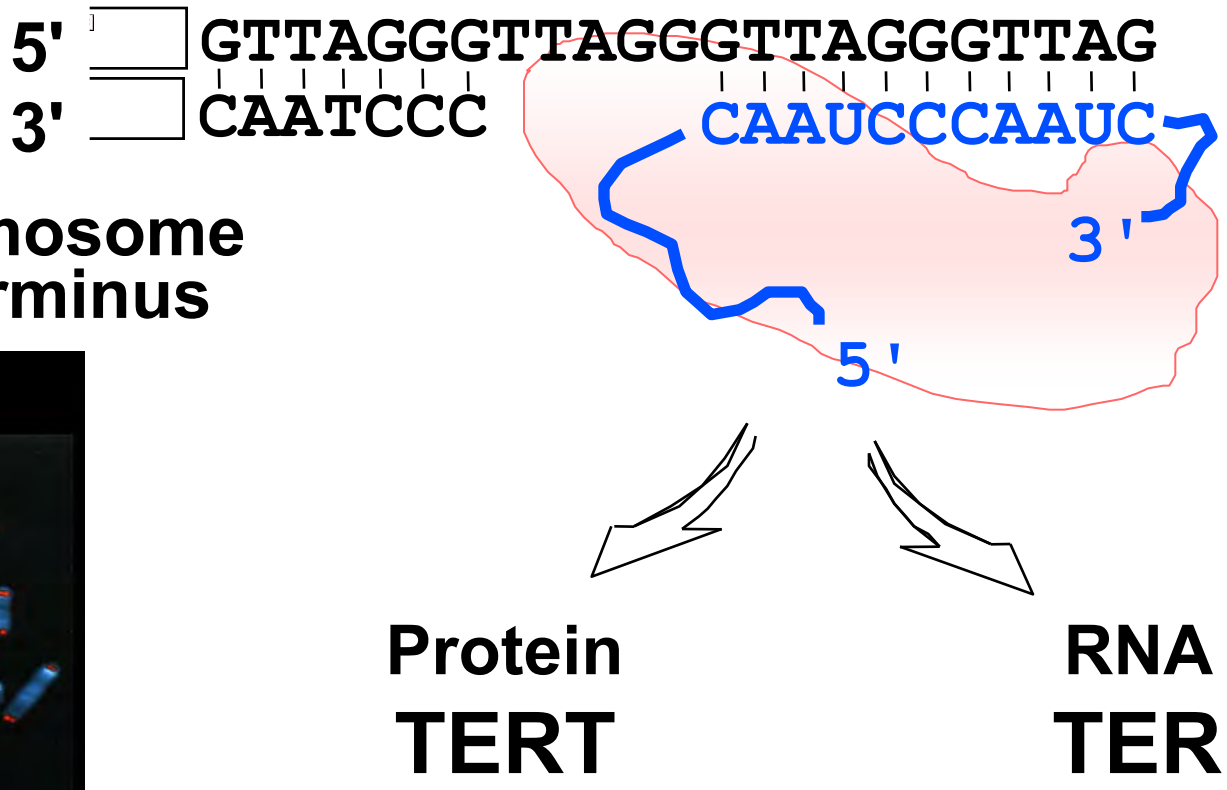
HIGH telomerase characterizes malignant human cancer cells



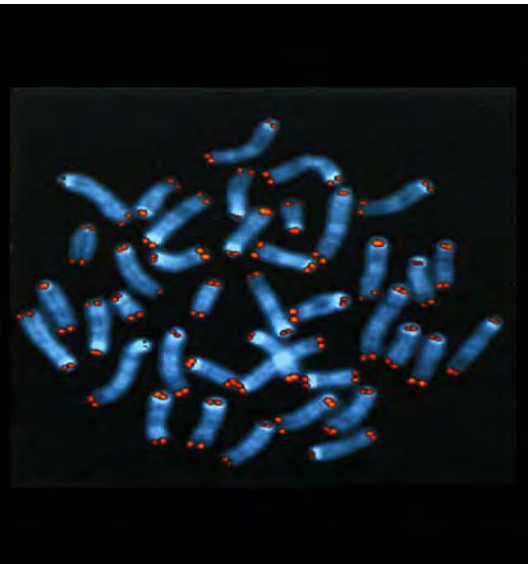
Exploiting the high telomerase of cancer cells to make toxic telomeric DNA

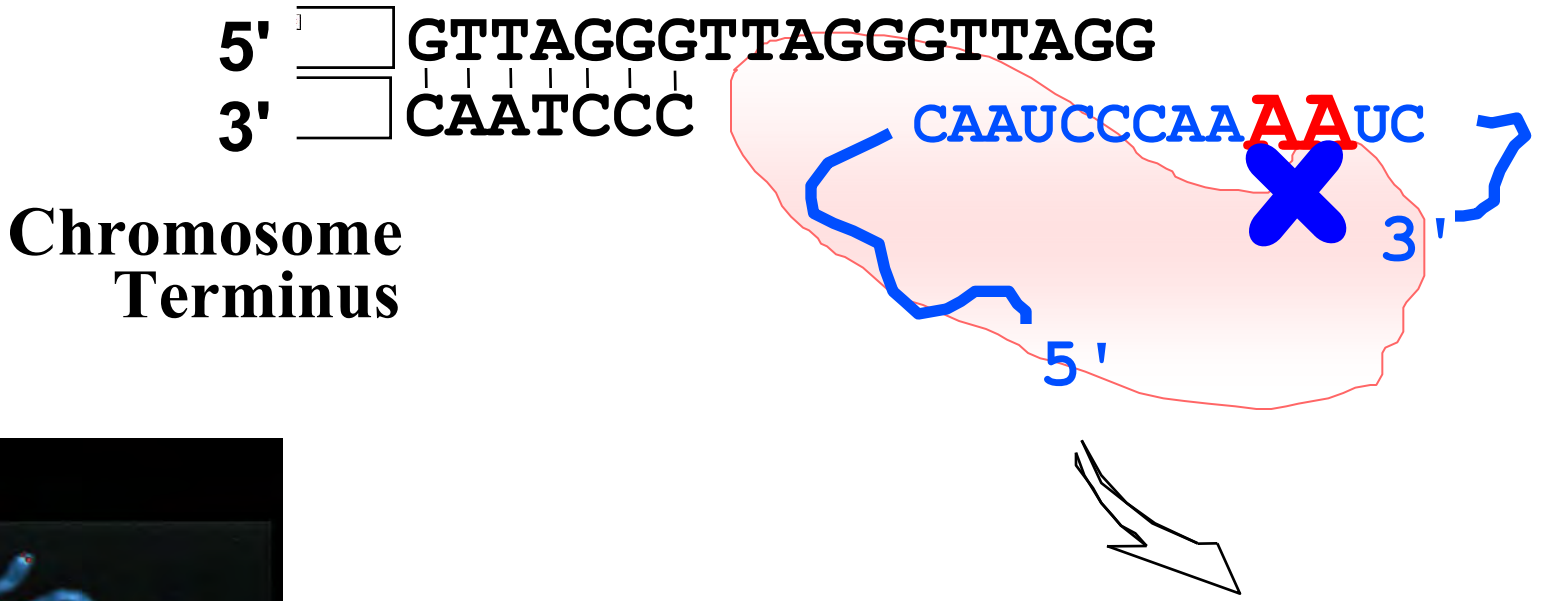
Human Telomerase



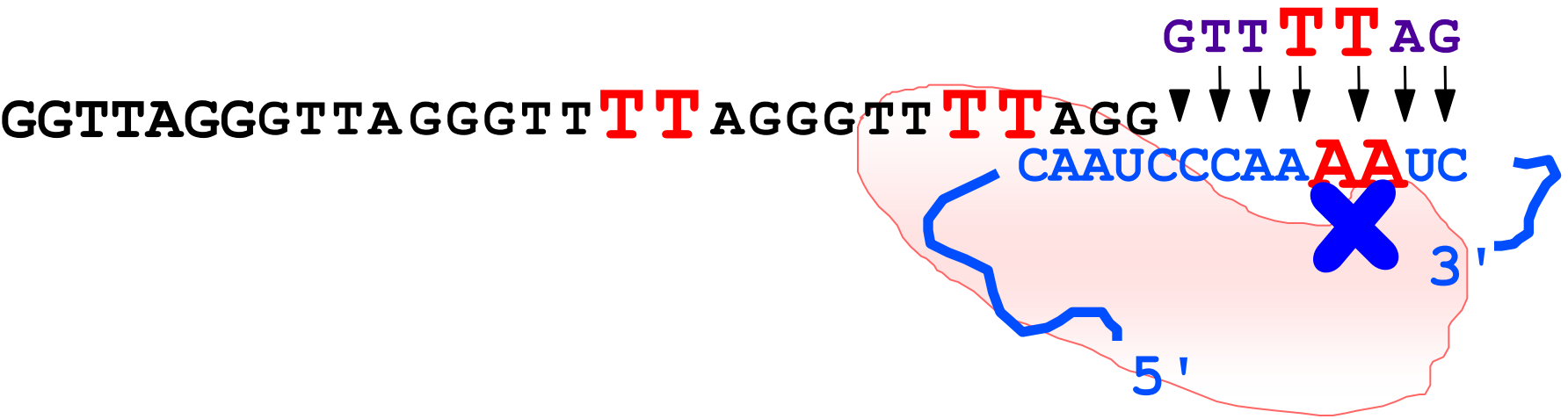


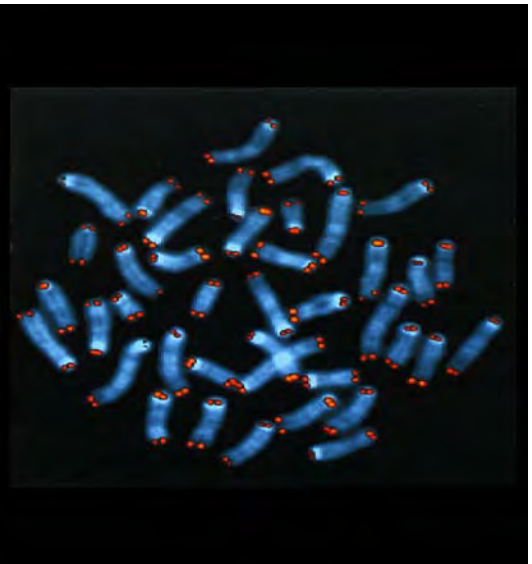
Exploiting the high telomerase of cancer cells to make toxic telomeric DNA





Exploiting the high telomerase of cancer cells to make toxic telomeric DNA





RNA

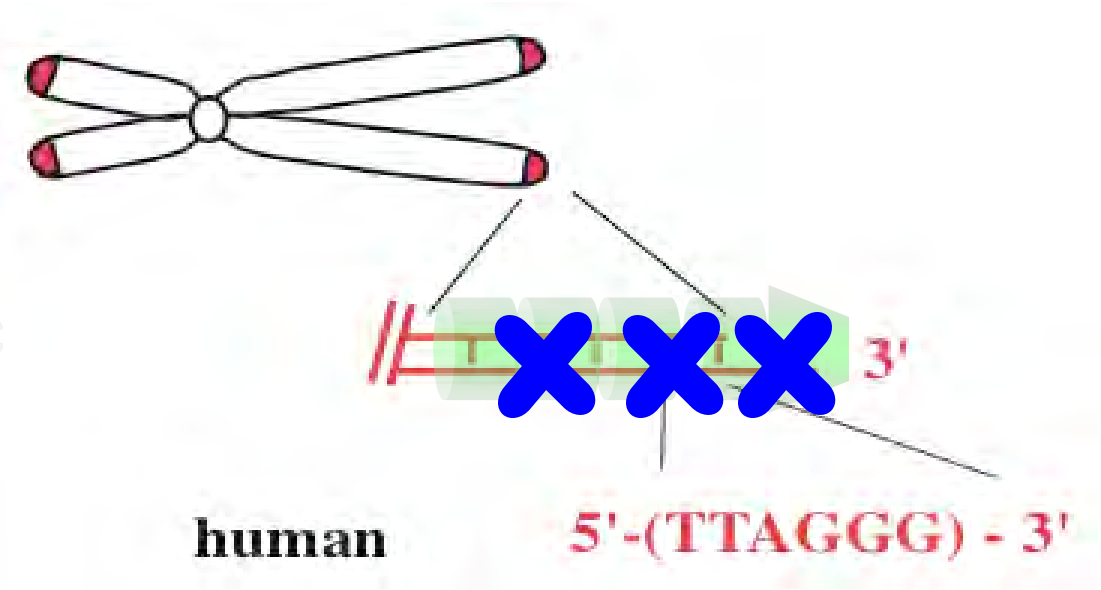
TER

Mutant-template

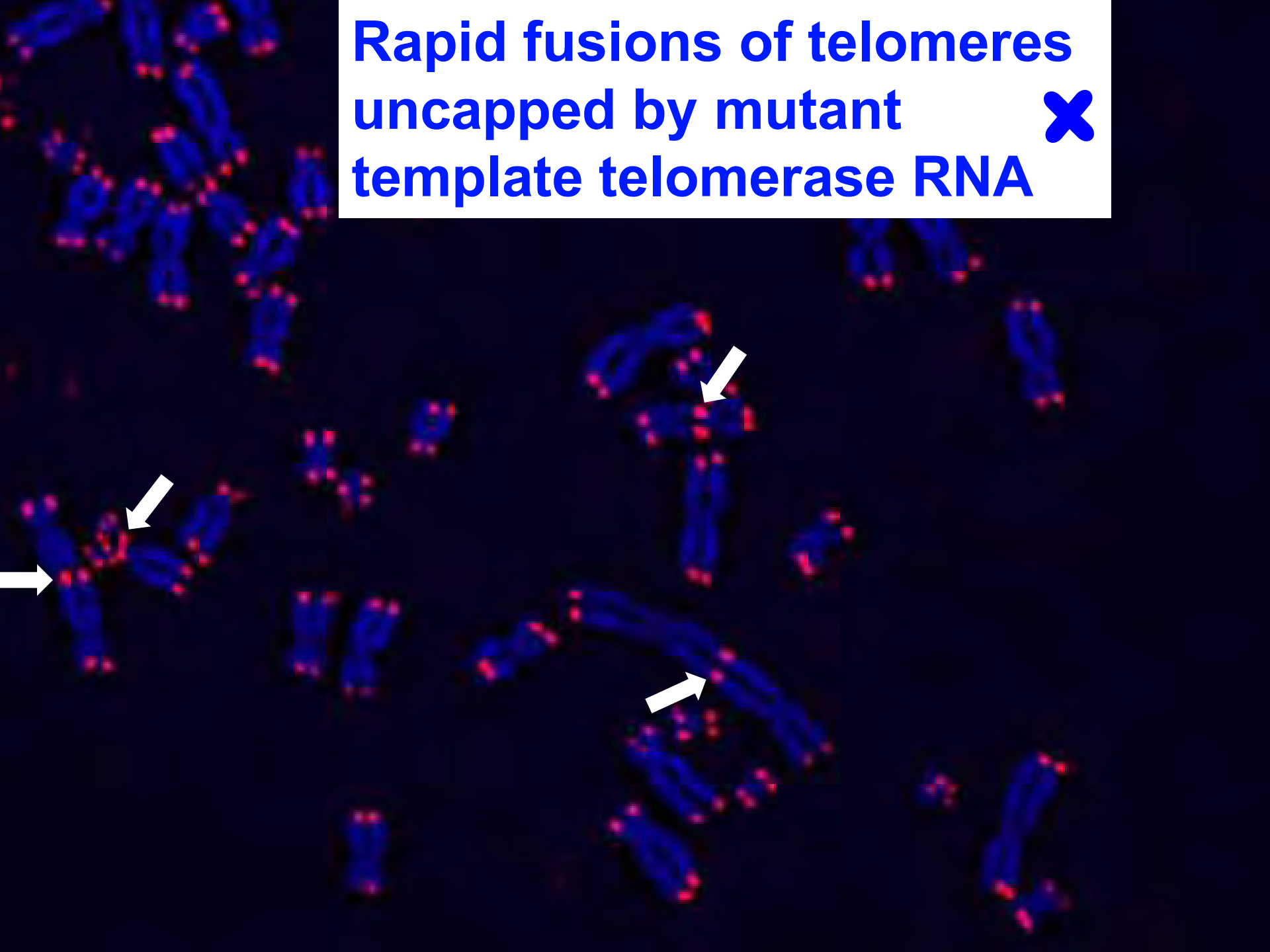
Kim et al., 2001

Exploiting the high telomerase of cancer cells to make toxic telomeric DNA

Telomeric Complex

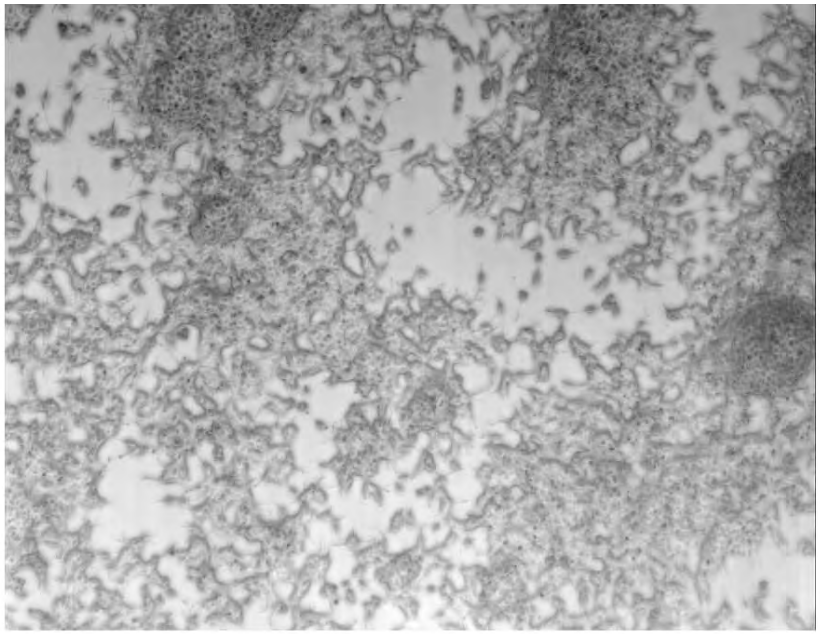


- Telomeric DNA
- Telomeric sequence-specific binding proteins

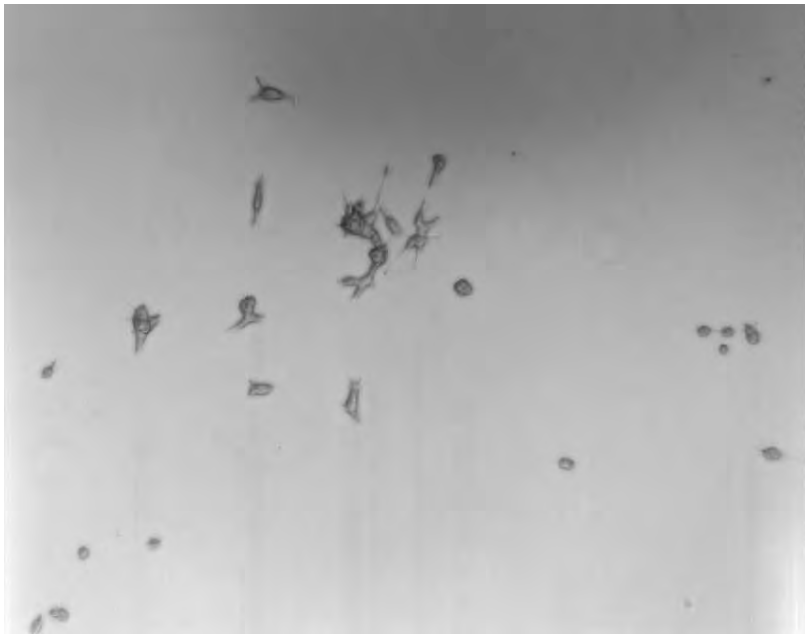

Rapid fusions of telomeres
uncapped by mutant
template telomerase RNA

Mutant telomerase RNA in human bladder cancer (LNCaP) cells

- note cell death



WT



mutant-template telomerase

What have we learned from forcing telomerase misfunction in human cancer cells?

Altering the telomeric DNA sequence

- Rapid fusions cause genomic disaster
- Independent of:
    - p53, pRb (all mutant sequences tested)
    - ATM or NHEJ (for certain mutant sequences)

Li et al, 2004; Brad Stohr, Lifeng Xu

Mutant Telomerase

We are turning the high telomerase activity of tumor cells back onto the cells to cause cell death.

Current: *in vivo* delivery to treat tumors in model systems.

In **normal** cells
in humans?

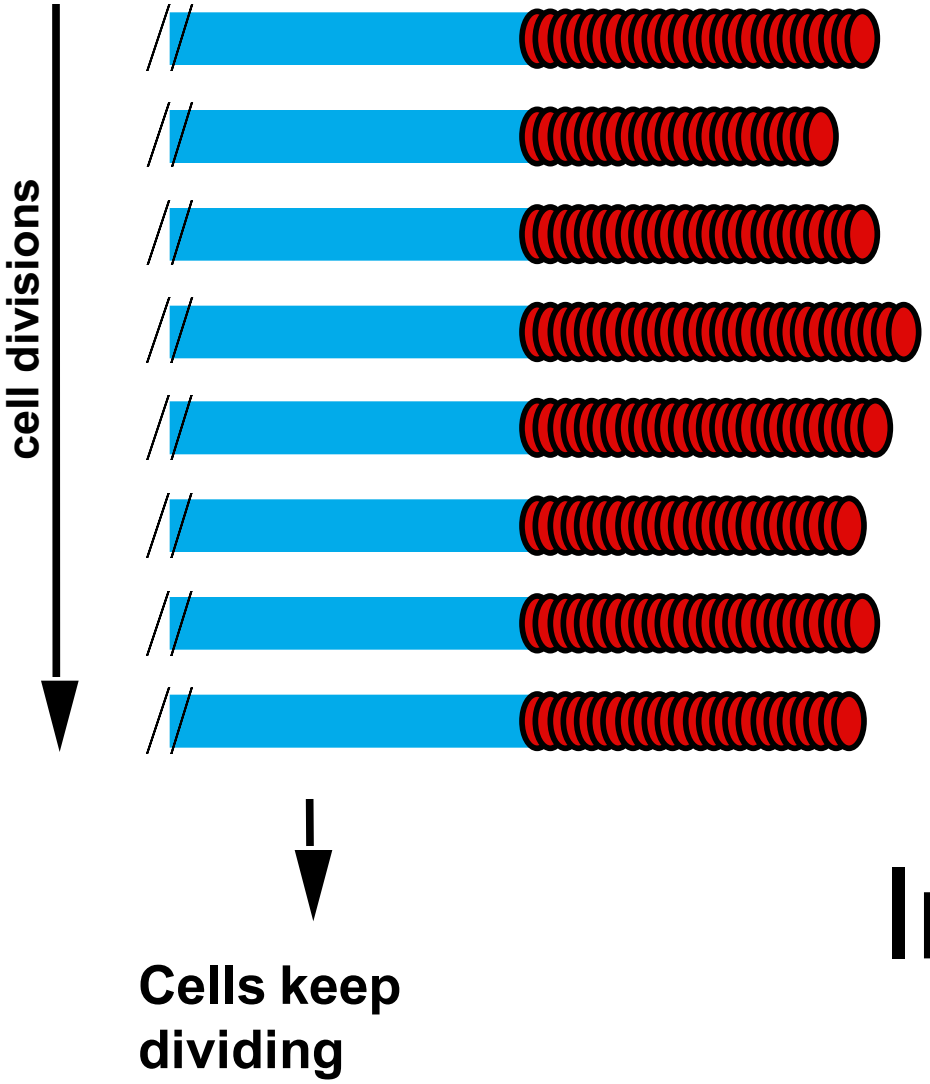
Tetrahymena thermophila

Immortal

Inactivate telomerase

“Mortal”

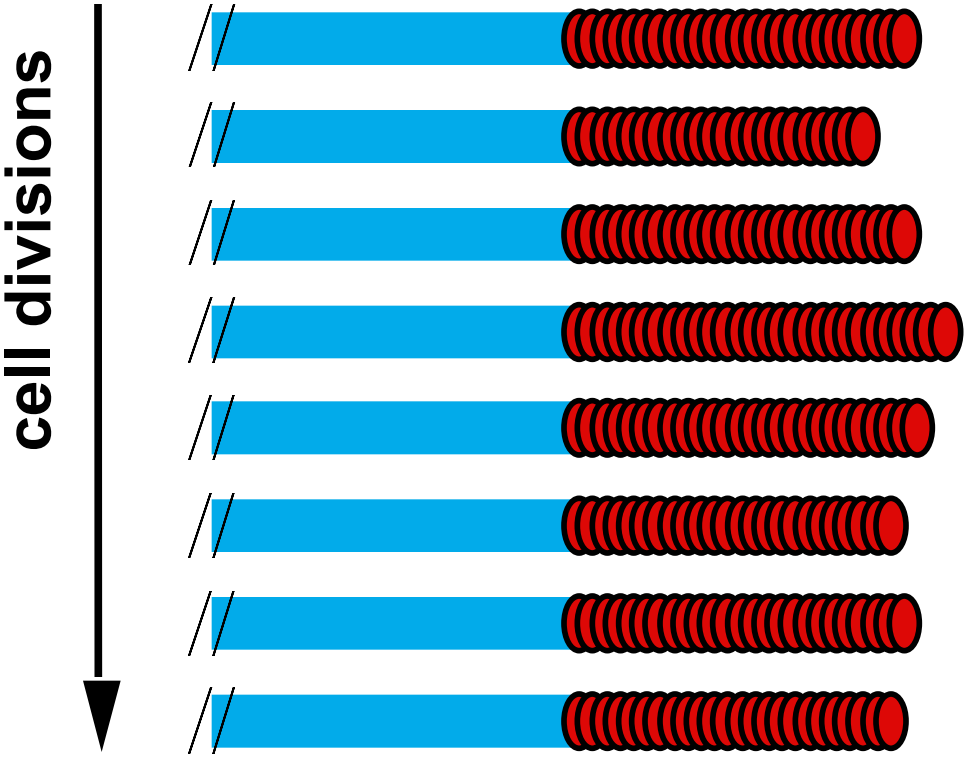
Yu et al, Nature 1990



Telomeres replenished by telomerase

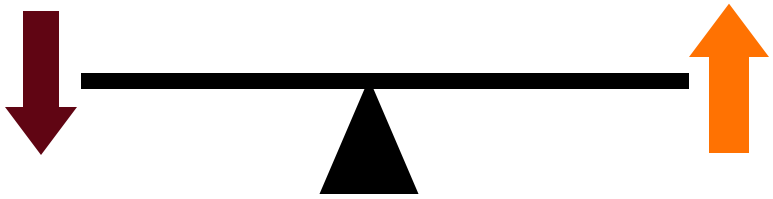
In humans

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



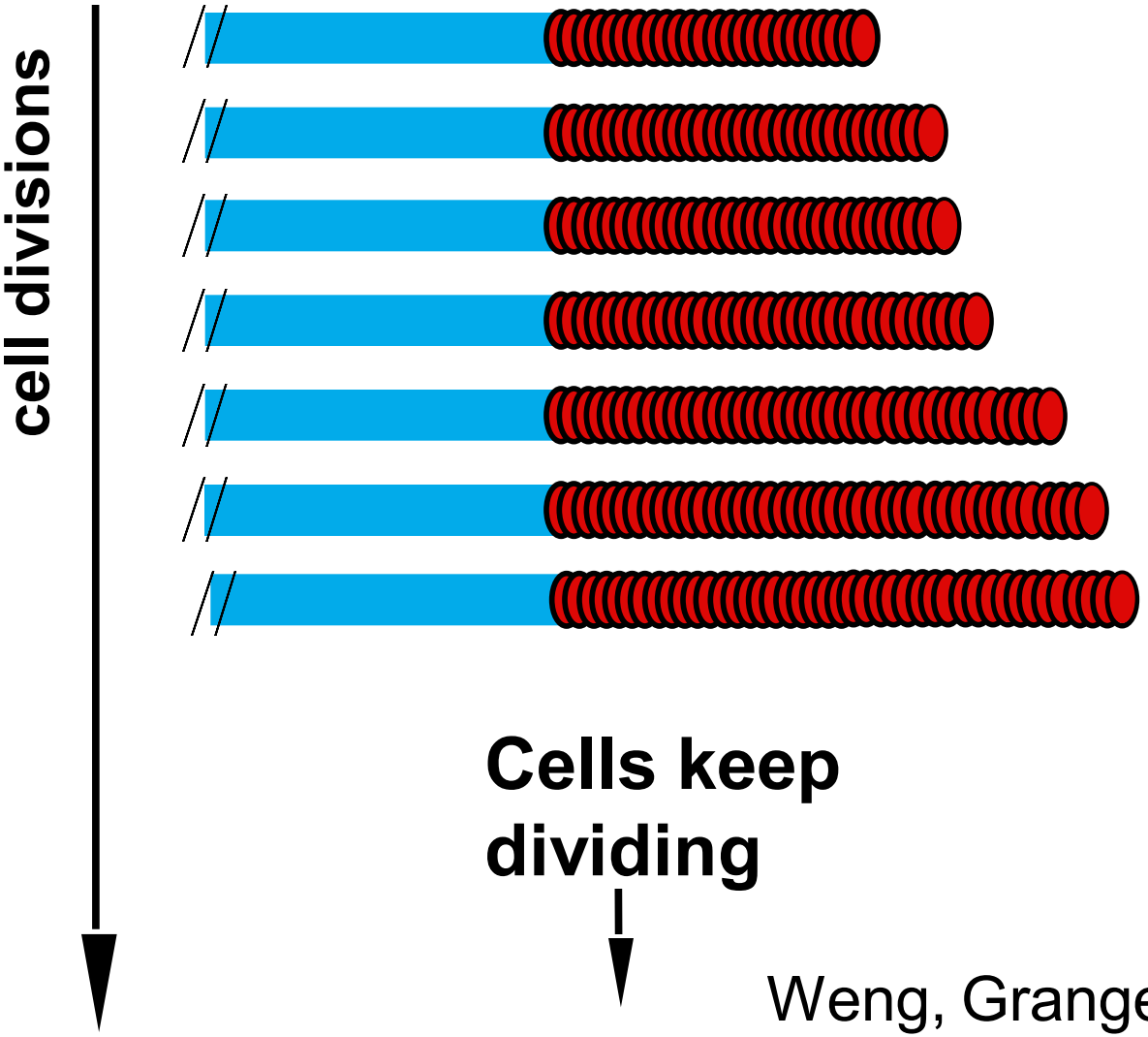
Cells keep dividing

Plenty of telomerase:



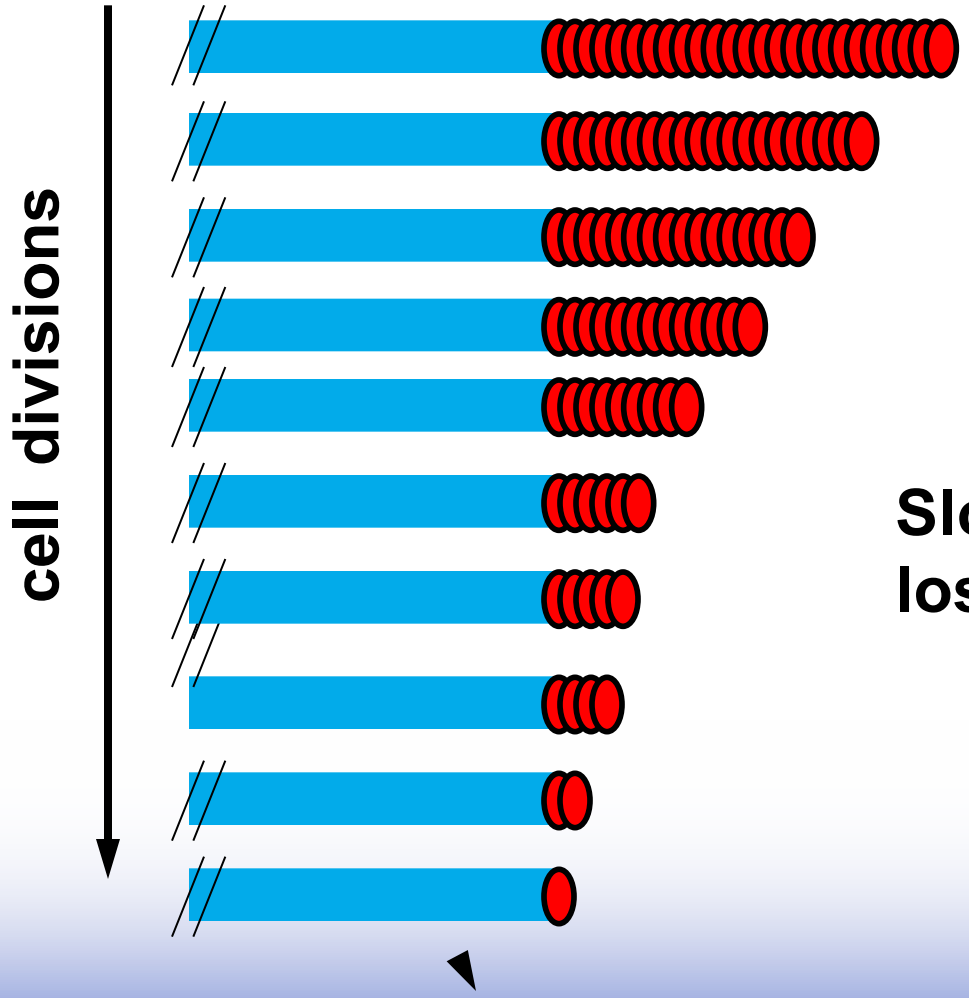
Addition and shortening stay balanced

Upregulated telomerase in humans: telomeres grow in vivo



Weng, Granger and Hodes, 1997

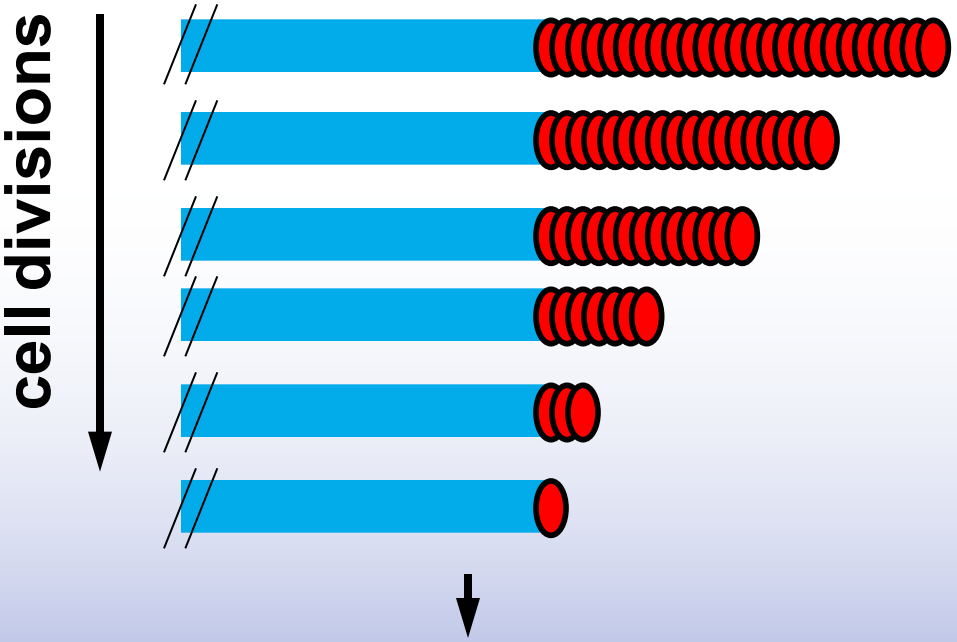
Predicted, if some telomerase:
Slow loss of DNA from chromosome ends



Slower net
loss/cell division

senescence comes later

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



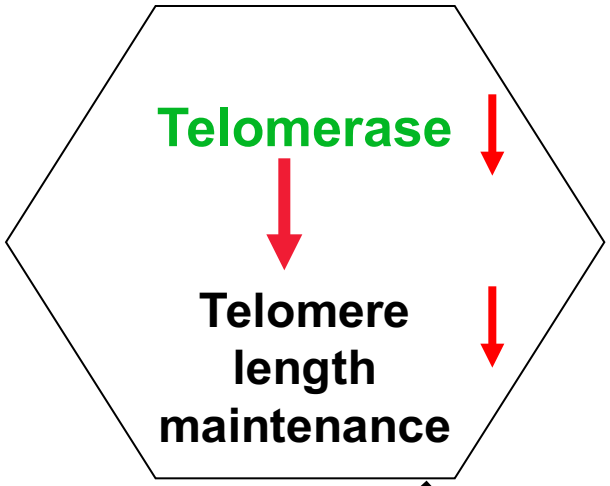
Faster net
loss/cell division

Senescence sooner

- genetic
- environment/life factors

Telomerase -

known genetic defects in telomerase genes cause disease risk in mice* and humans**



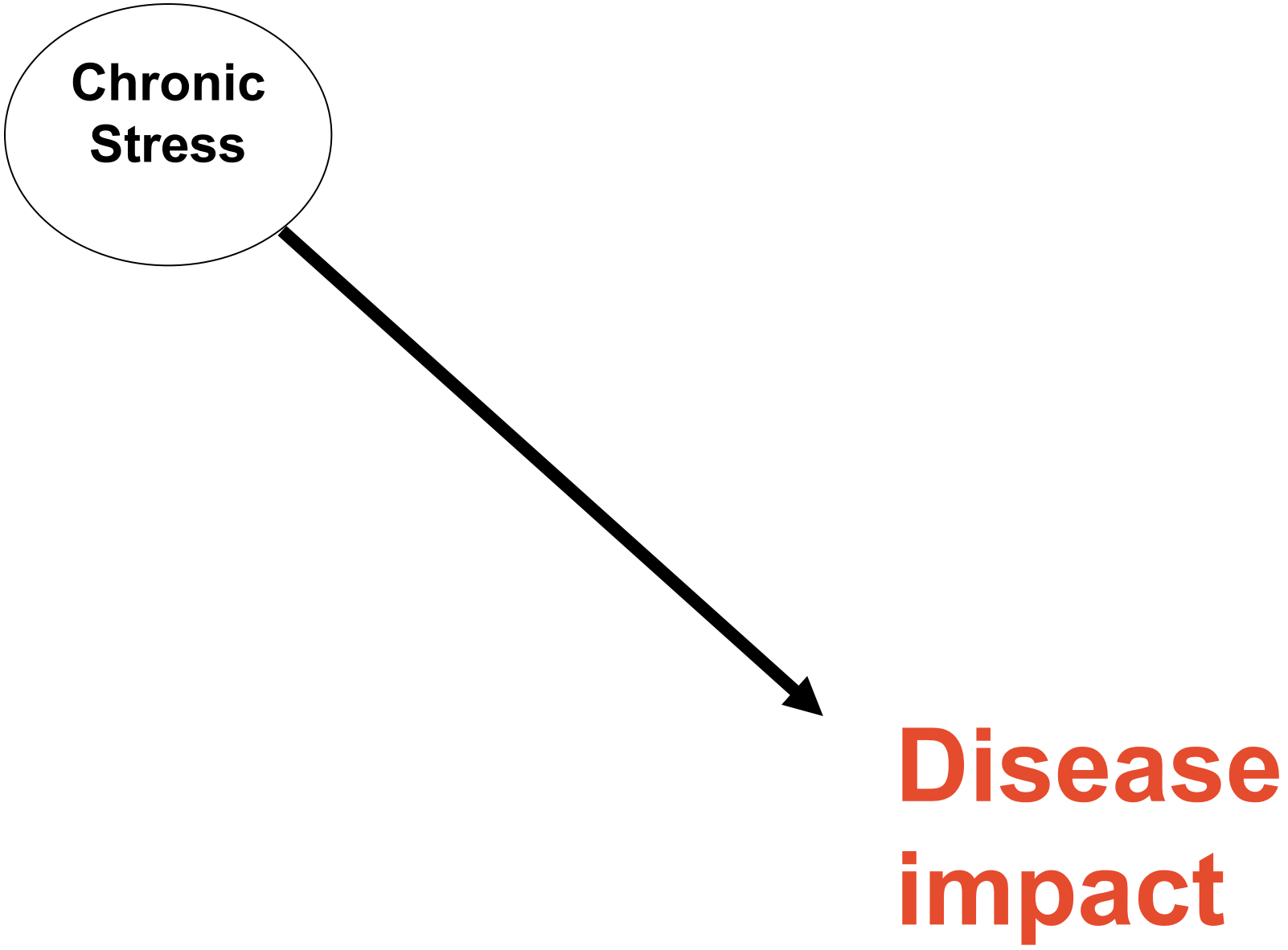
Reduces ability of cells to replenish

Disease impact

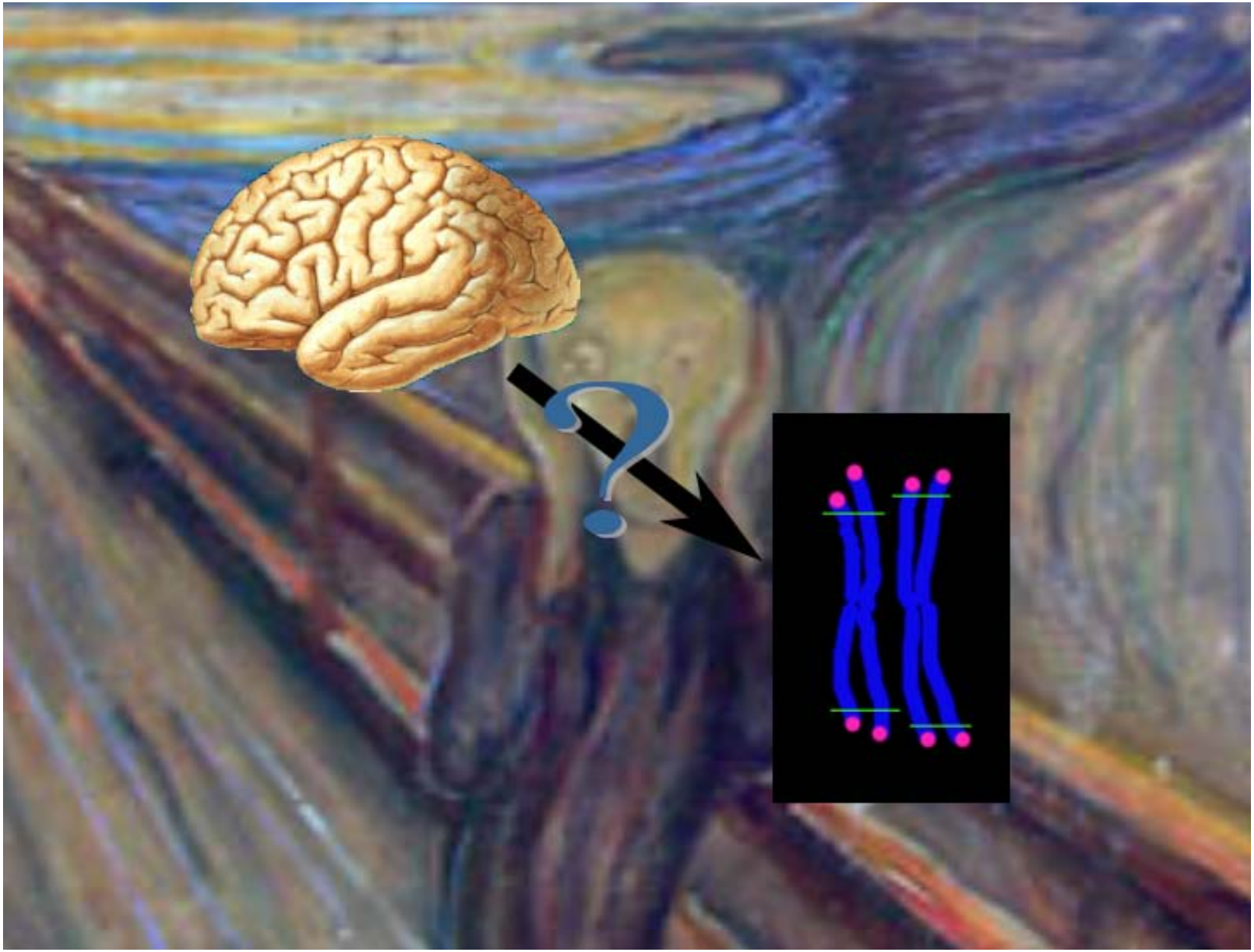
*Greider, DePinho groups and others

** Dokal group and others

Chronic psychological stress -
a known non-genetic determinant of human disease risk

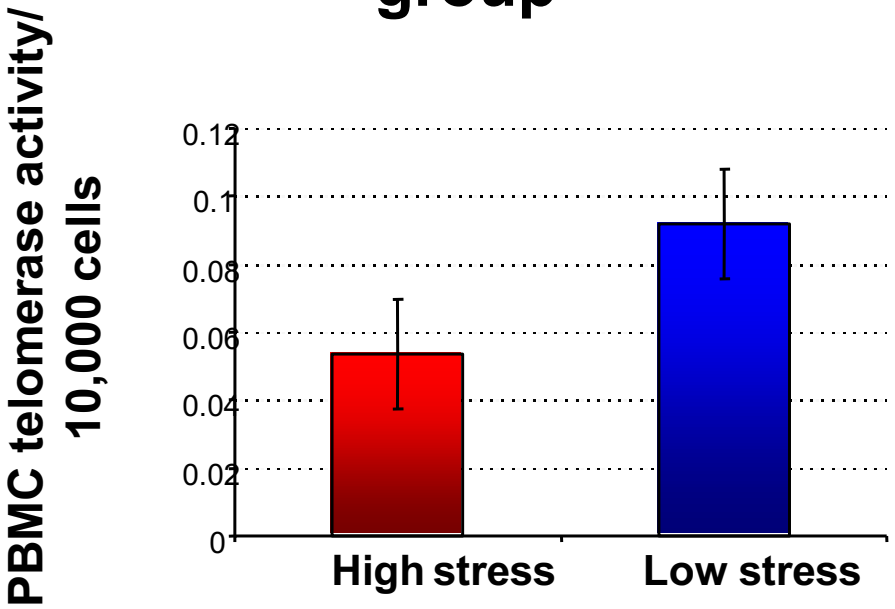


Chronic psychological stress and telomeres



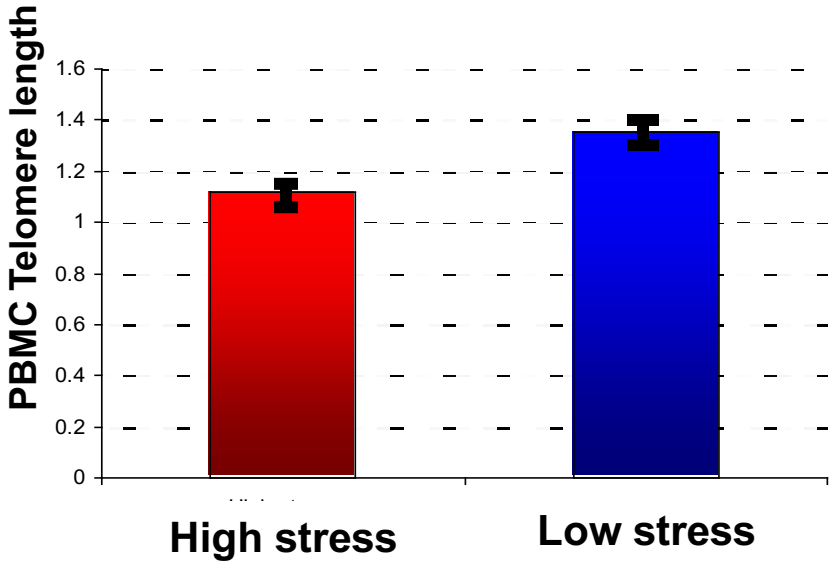
Caregiver mothers and chronic stress

Telomerase activity was ~ 50% lower in high stress group



M = .053, SE = .016 M = .092, SE = .016
p < .045

Telomeres were shorter in high stress group (equiv. 9 - 17 yrs of extra "aging")



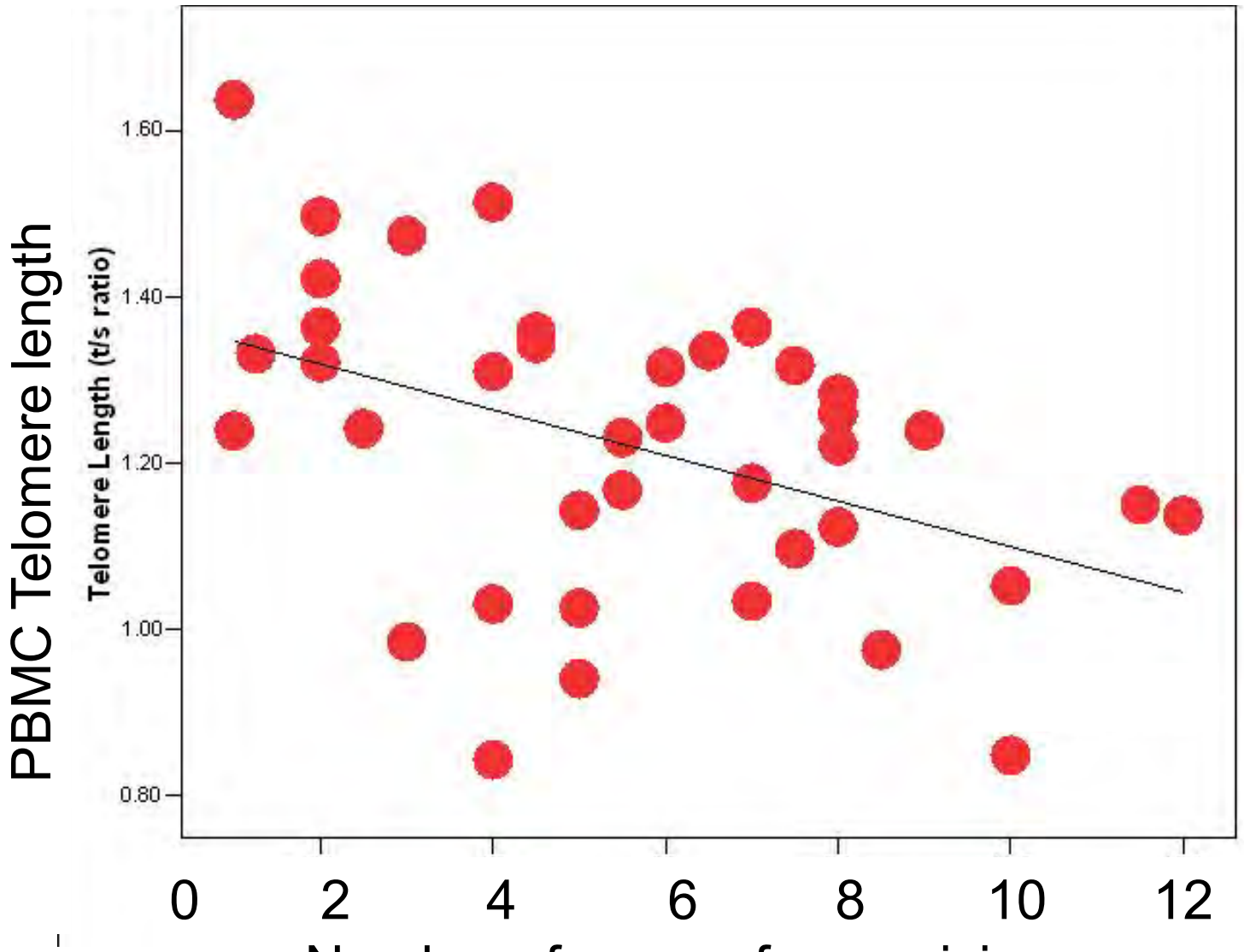
controlling for age and body mass index:
F(3,27) = 12.8, p < .001

The lowest and highest stress quartiles of the whole sample are compared.

Epel et al, 2004, PNAS

Study 1: Caregiver mothers and chronic stress

Stressor Duration and Telomere length



Epel et al, 2004, PNAS

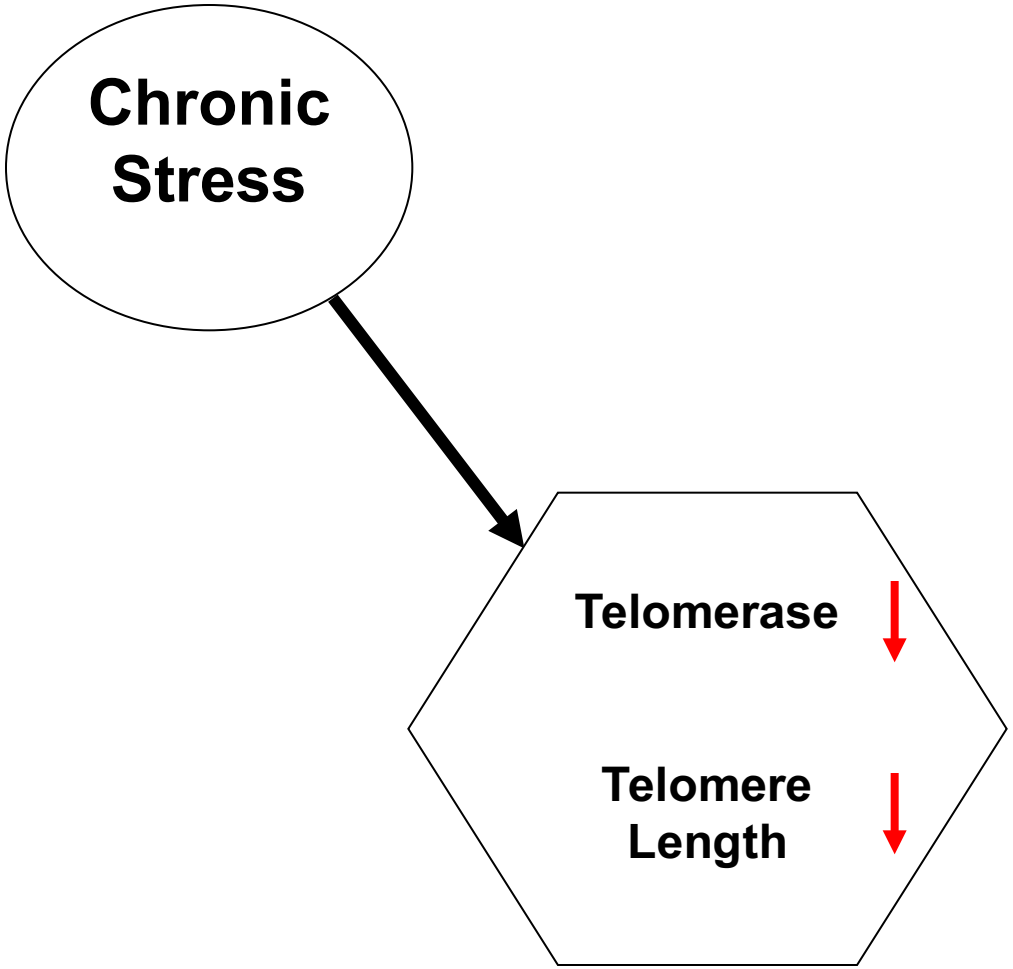
Study 1: Caregiver mothers and chronic stress

The more years the mothers had been in this objective stressor situation, the lower* were their PBMC telomere length and telomerase

*after correcting for all other available factors

Epel et al, 2004, PNAS

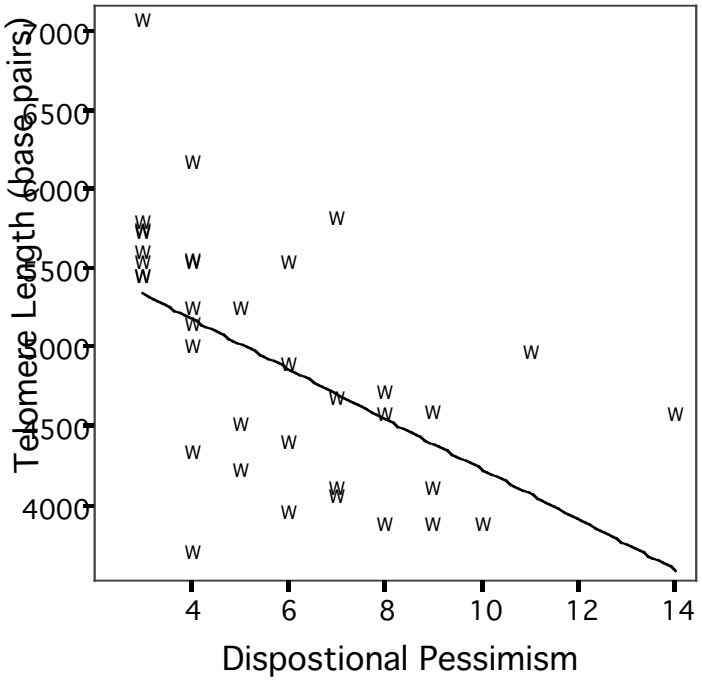
Chronic stress - reduces PBMC telomere length maintenance



Epel et al (2004; 2006; 2008; unpubl.)

We and others have replicated these findings in independent studies

Study 2: Post-menopausal women: Pessimism and shorter PBMC telomeres



P < .001

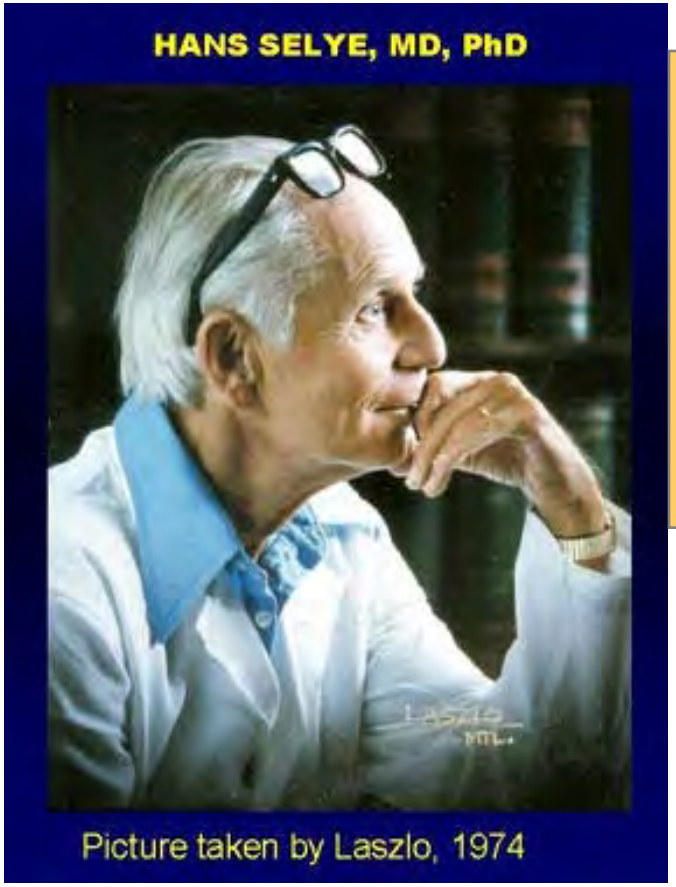
Figure 1. Association between dispositional pessimism and telomere length in post-menopausal women (r = -.55, p = .001; n = 36). This association appears independent of age, dispositional optimism, perceived stress, neuroticism, and health behaviors.

The relationship still remained excluding the one participant in the upper left quadrant, who was not a statistical outlier but who exhibited the longest mean TL (r = -.53, p = .001).

O'Donovan, A., Lin, J., Wolkowitz, O., Dhabhar, F.S., Tillie, J.M., Blackburn, E., and Epel, E. Brain, Behavior and Immunity, 2008

Chronic stress - reduces PBMC telomere length maintenance

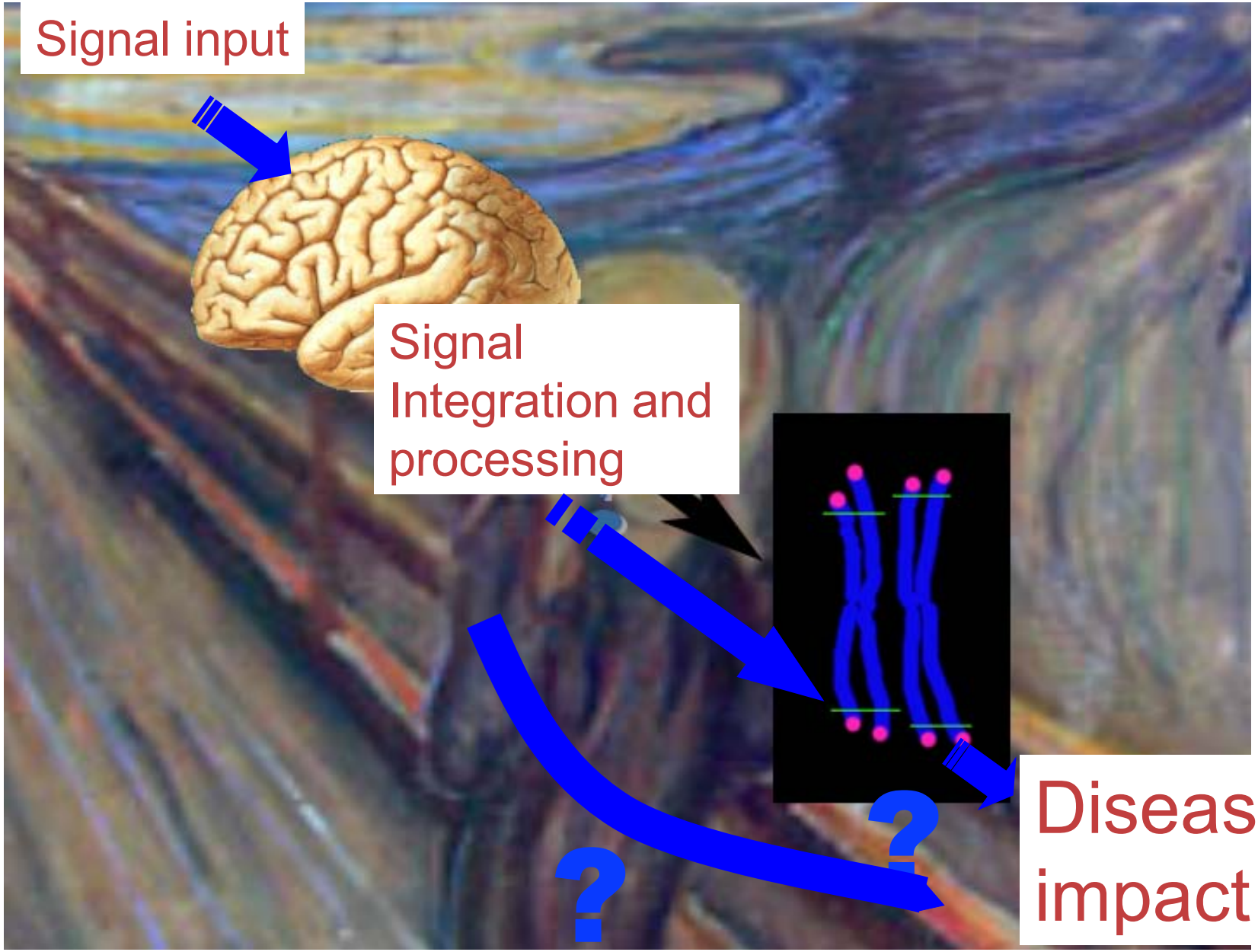
A SOBERING THOUGHT..



"Every stress leaves an indelible scar, and the organism pays for its survival after a stressful situation by becoming a little older."

-Hans Selye

Chronic life stress wears down telomeres



Major common diseases of aging have been linked to shorter telomeres

(many independent studies)

Telomerase mutations and diseases

Cancer risk

Vulliamy, T. et al. (2001)

Pulmonary fibrosis

Armanios, M. et al. (2007)

Telomere shortness links to common disease states

Cancer

Vulliamy, T. et al. (2001)
Risques et al; Joshua et al., Shen et al (2007)

Pulmonary fibrosis

Armanios, M. 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)

DYNAMICS OF CHANGE?

- LONGITUDINAL STUDIES

The Dogma was…..

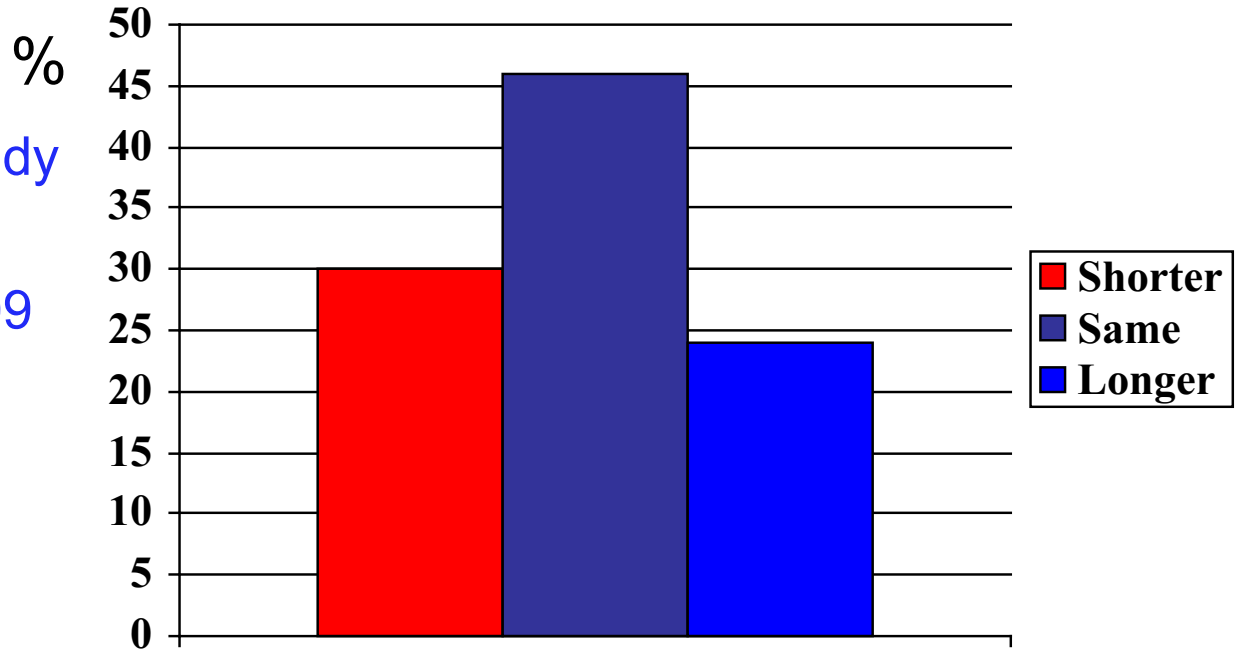
- Telomeres shorten over time, unidirectionally
  - Based entirely on cross sectional studies

The new findings

- Telomeres **lengthened** in ~1/4th of adults during 2.5 years

MacArthur Aging Study

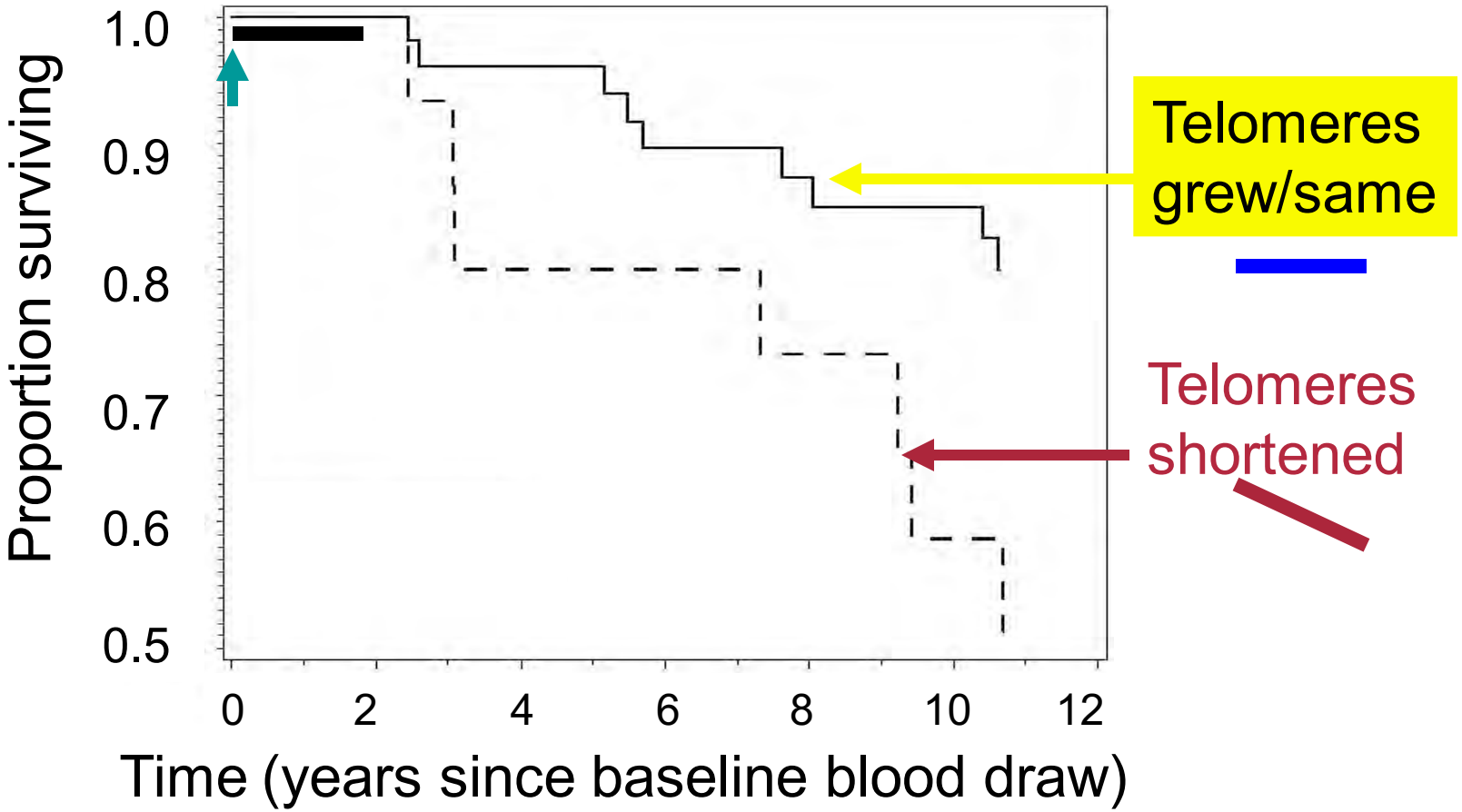
Epel et al. Aging 2009



Also:
Nordfjäll K et al. PLoS Genet. 2009
Farzaneh-Far R, et al. PLOS One, in press.

This opens the door for identifying malleable determinants of rate of change!

RATE of loss of telomere length predicts cardiovascular disease death in elderly men

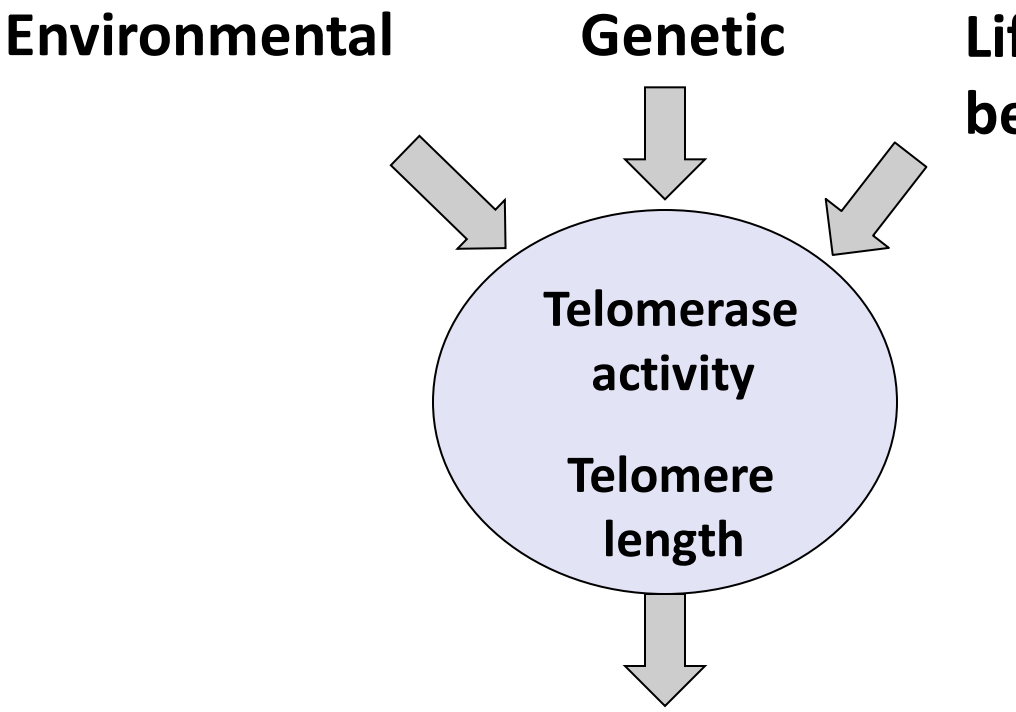


Those with telomere shortening (dashed line) over the initial 2.5 year period had 3.0 times greater likelihood of death over the 12 years since the baseline blood draw, compared to those without telomere shortening (solid line).

MacArthur Aging Study

Epel et al, 2009

Telomere length and telomerase: Biomarkers or causal mechanisms for aging-related disease



The journey…

From basic biological research on the molecular nature of telomeres and their maintenance mechanisms….

to…..

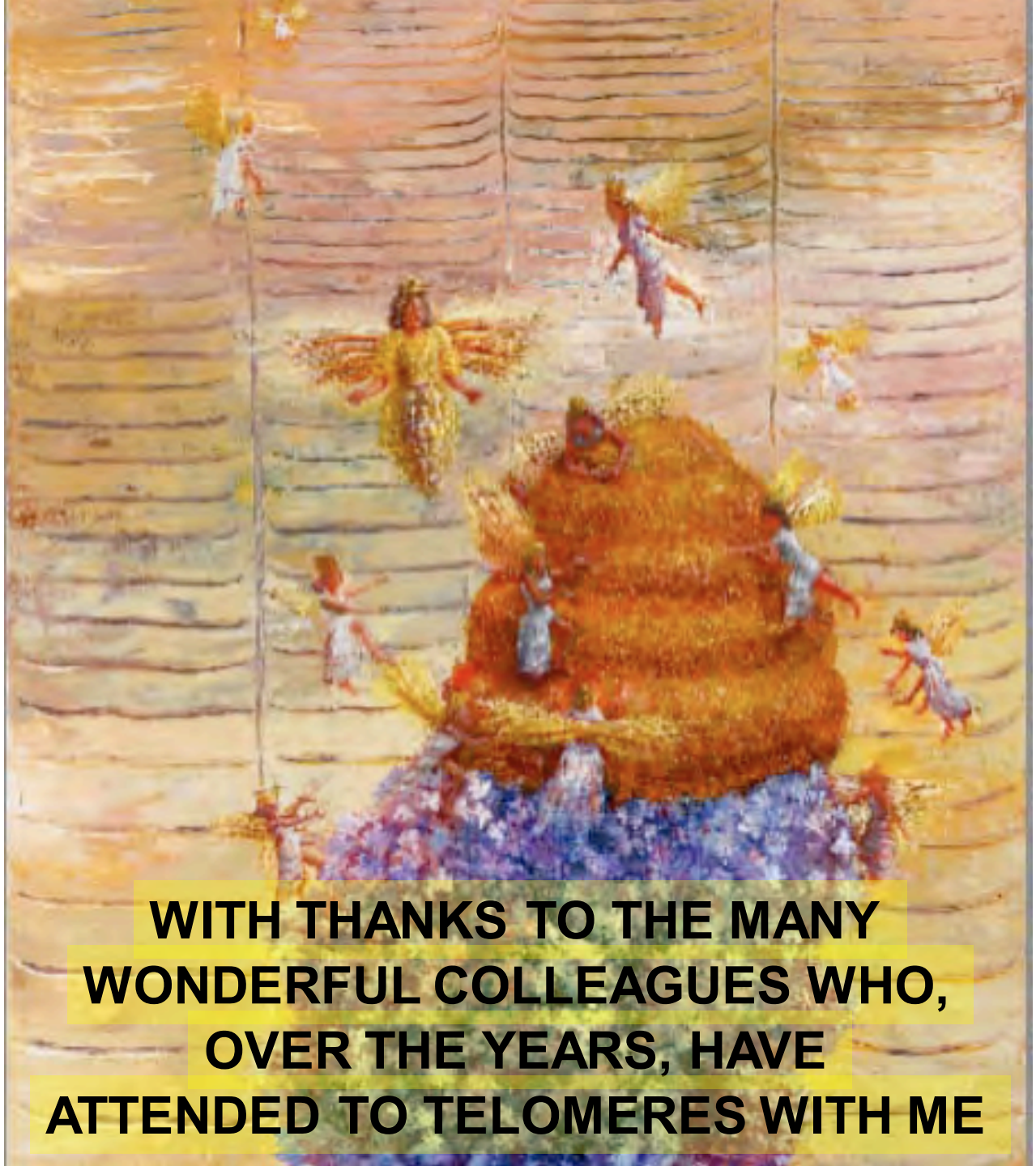
human life stories and their effects on telomere maintenance….

to….

in turn, the impact of telomeres on health and disease

SUMMARY

Correct telomere maintenance is crucial



WITH THANKS TO THE MANY WONDERFUL COLLEAGUES WHO, OVER THE YEARS, HAVE ATTENDED TO TELOMERES WITH ME

"Sumarian Bee Goddesses tending a telomere"

Julie Newdoll, 2008