

Telomeres and Telomerase: The Means to the End

Elizabeth H. Blackburn
Nobel Lecture 2009
Karolinska Institutet

Maize breeding

Ancient....



Téosite



Maïs primitif



Maïs actuel

Mayan corn god

.... modern

- cytogenetics
- chromosome discoveries



Barbara McClintock

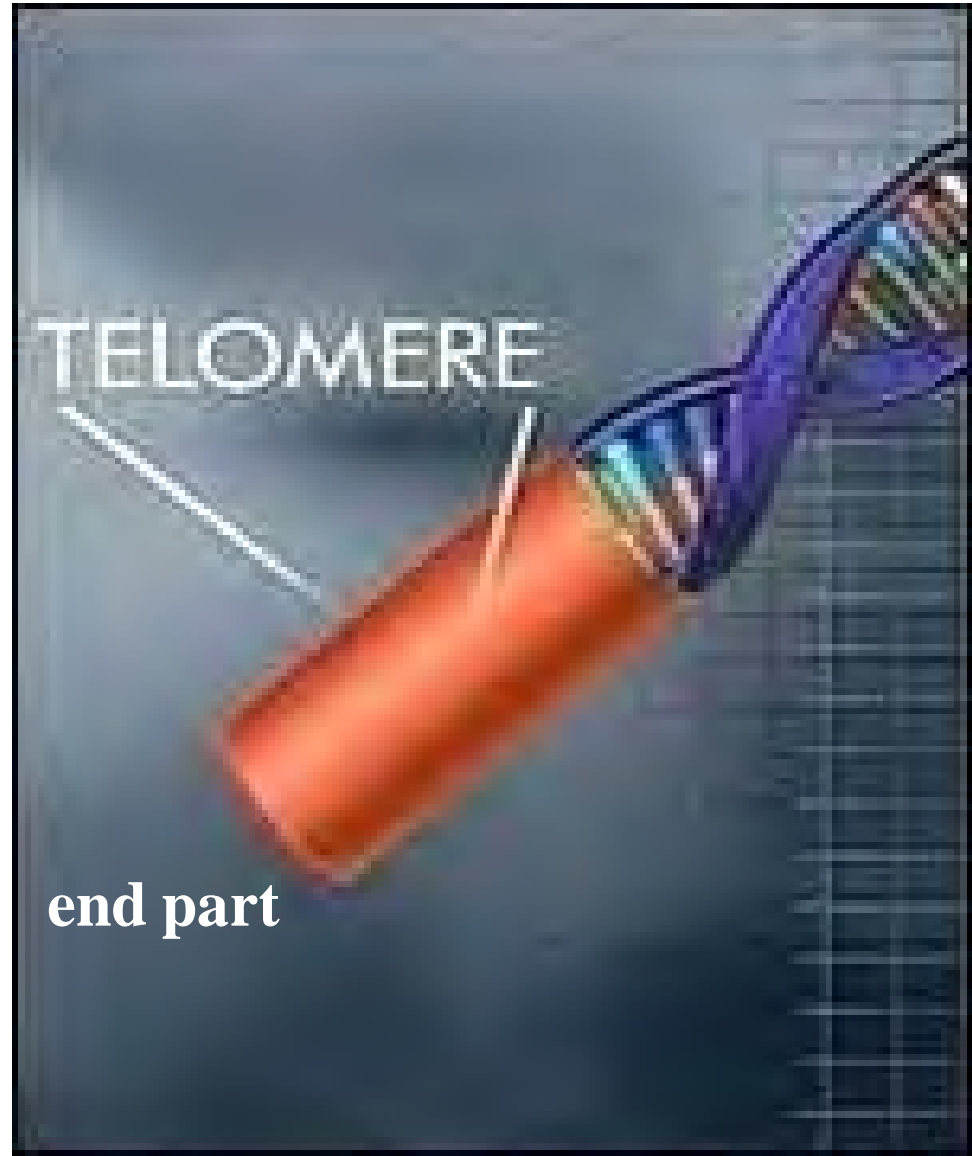


Cold Spring Harbor, 1947

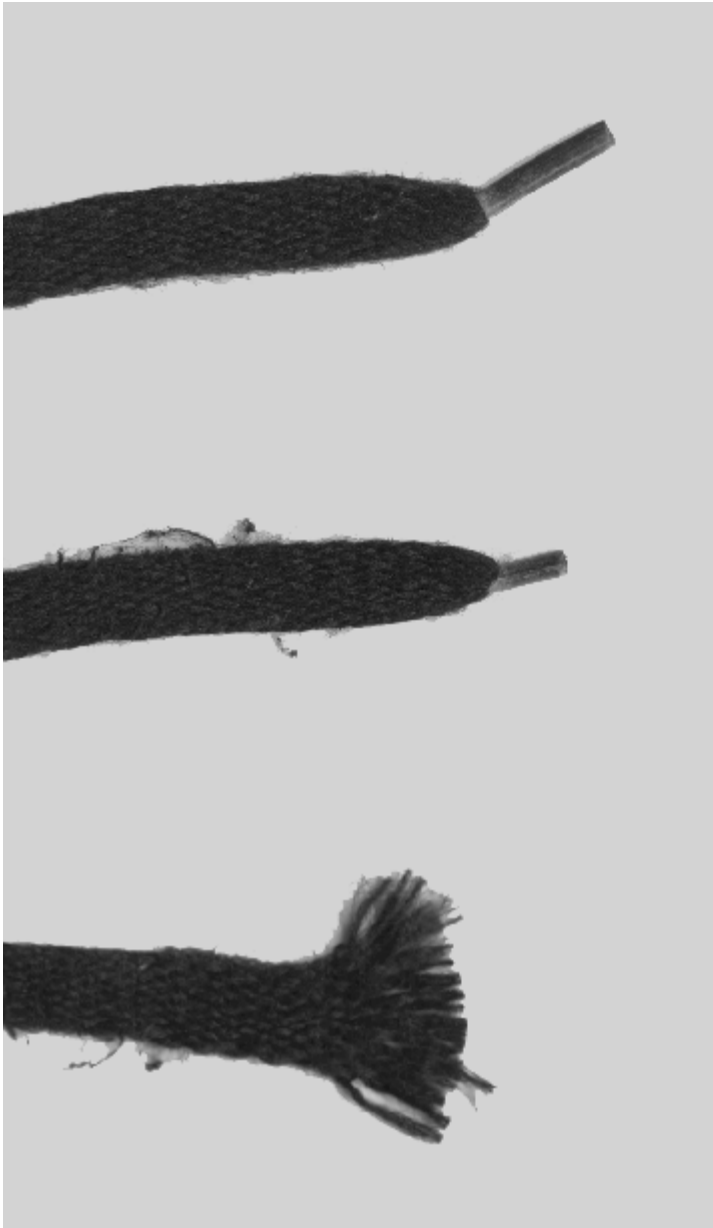
Telo-mere

(tel'uh mer or te lō mēr)

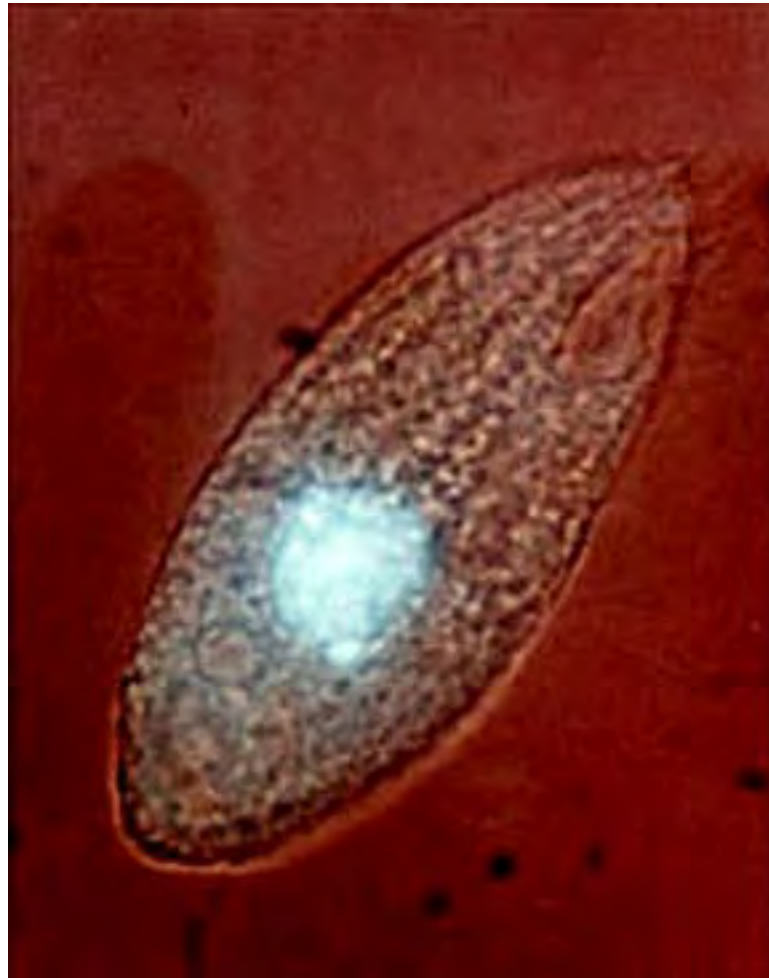
- named by Hermann Muller



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.



Common Shoelace, 10x.



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



Depurination of gel bands.

R1	1		
	2	C ₄ (A)	30 x 10 ⁶
	3	C ₄ (A)	15 x 10 ⁶

H in II (3 sup) C₄(A) 10 x 10⁶ Hae III 1 C₄(A) 2.5 x 10⁶
 (3 sub) C₄(A) 10 x 10⁶ 3 C₄(A) 1.4 x 10⁶

Note: M_w's of each pair of bands shows main labelled band is ± the size of unlabeled, C₄(A) containing band

This is evidence that each nick fragment is at end of molecule. I dimer-sized C₄(A)-containing fragments arise from either nicked / of rDNA or dimers

0.21 ml

M D ATP

ml

led 1hr 15°

20 µl EDTA 0.1M

20 µg/ml RNA

ed 1x

~10-15% incorporation

then fractions

over end/15 ml

stream, v 1/2 total

took up in 50 µl water + 5 µl

very good recovery

Note: unanalyzed 50 µl H₂O had only 0.7M (K₂PO₄) x 10⁶ x 1/10 = 14 µl 0.1M

extra bands seen may be EOR1* activity

Gel 20

Remaining 3 incorporated ³²P + SD₂ carrier RNA in 100 µl water. 4³ from EDTA ppt.

	Control	R1	R1*	H in I	Hae III	φD H in II
✓ DNA	2	5	5	5	5	17
10x EOR1 buffer	2	2	-	-	-	-
10x H in buffer	-	-	-	2	2	2
6.7M MgCl ₂	-	-	1.333M	-	-	-
β me	-	-	1 → 6mM	-	-	-
6.6mM Tris ACID pH 7.2	-	-	2 → 6.6mM	-	-	-
✓ H ₂ O	16	12.5	11	12	12	-
Eng	-	0.5	0.5	1	1	1

Incubate 1hr 37°

plus 4°

Then 1hr 37°

Add 1 µl 10% SDS

2 µl 0.2M EDTA

2 µl Bromophenol blue in sucrose to each.

1.4% agarose gel.

Bands eluted & dephosphorylated in situ.

ID: XAT + read

Beginning to piece together the first telomeric DNA sequence...

$T(A) \leq \frac{1}{4}$
 Pucccca 1
 AA or GA > 1
 PTTG 1
 PTTG 1
 GG or AG 3

CCCC AACCC CATA
 GGGG TTGGG GAT

PTTG
 AAC

GGGG TGGG TGGG T
 + GGGG TGGG TGGG T

CCCC AACCC C C C C A
 CCCC AACCC C C C C A

Quantitation
 incorrect for
 this sequence if
 both strands equal
 made.

7. Calculating no of ³²PAMP's incorporated per molecule of DNA → about
 20-30 ^{es} molecules per molecule of DNA (MW 13.6×10^6). No evidence that
 the DNA is saturated or 100% substituted at this pt.

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

3. With long incubations, get high rel am of C₄ at room temp + pane.
 C₄ also at 0° in high salt.

4. V. Short incubations - low C₄ rel to other simple depts.
 C₄ rel am^t increases = time (20'-90' at 4°)

5. C₄ found almost exclusively in small el frag 1.5×10^6 MW
 " Ban 2.2×10^6
 one hag frag 1.4×10^6
 in a heterogeneous hag frag $\approx 5 \times 10^5$ X Better
 ≈ 750 bpts. $\approx 4 \times 10^5$

6. If random incorporation, in high am^t of pane, find ~1% of A^t incorporated
 is in the sequence Puccca. This → ~650 bases (for 10-base repeat). or MW

7. Calculating no of ³²PAMP's incorporated per molecule of DNA → about
 20-30 ^{es} molecules per molecule of DNA (MW 13.6×10^6). No evidence that
 the DNA is saturated or 100% substituted at this pt.

Tetrahymena thermophila

- Contains abundant very short linear chromosomes

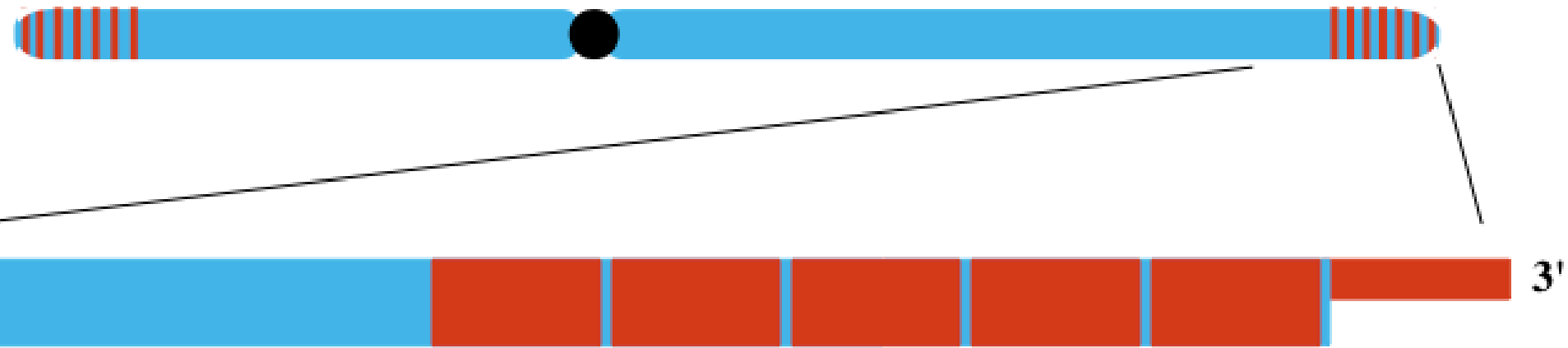
- They end in TTGGGG repeats.

Blackburn and Gall, 1978



Telomeric DNA contains simple tandem repeats

Chromosome with centromere



Tetrahymena

TTGGGG

Blackburn and Gall, 1978

S. cerevisiae

(TG)₁₋₆TG₂₋₃

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 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 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' GGGGGTTTGGGGTTTGGGGTTT 3' OH

Tetrahymena cell extract
↓
Mg⁺⁺
dGTP + TTP

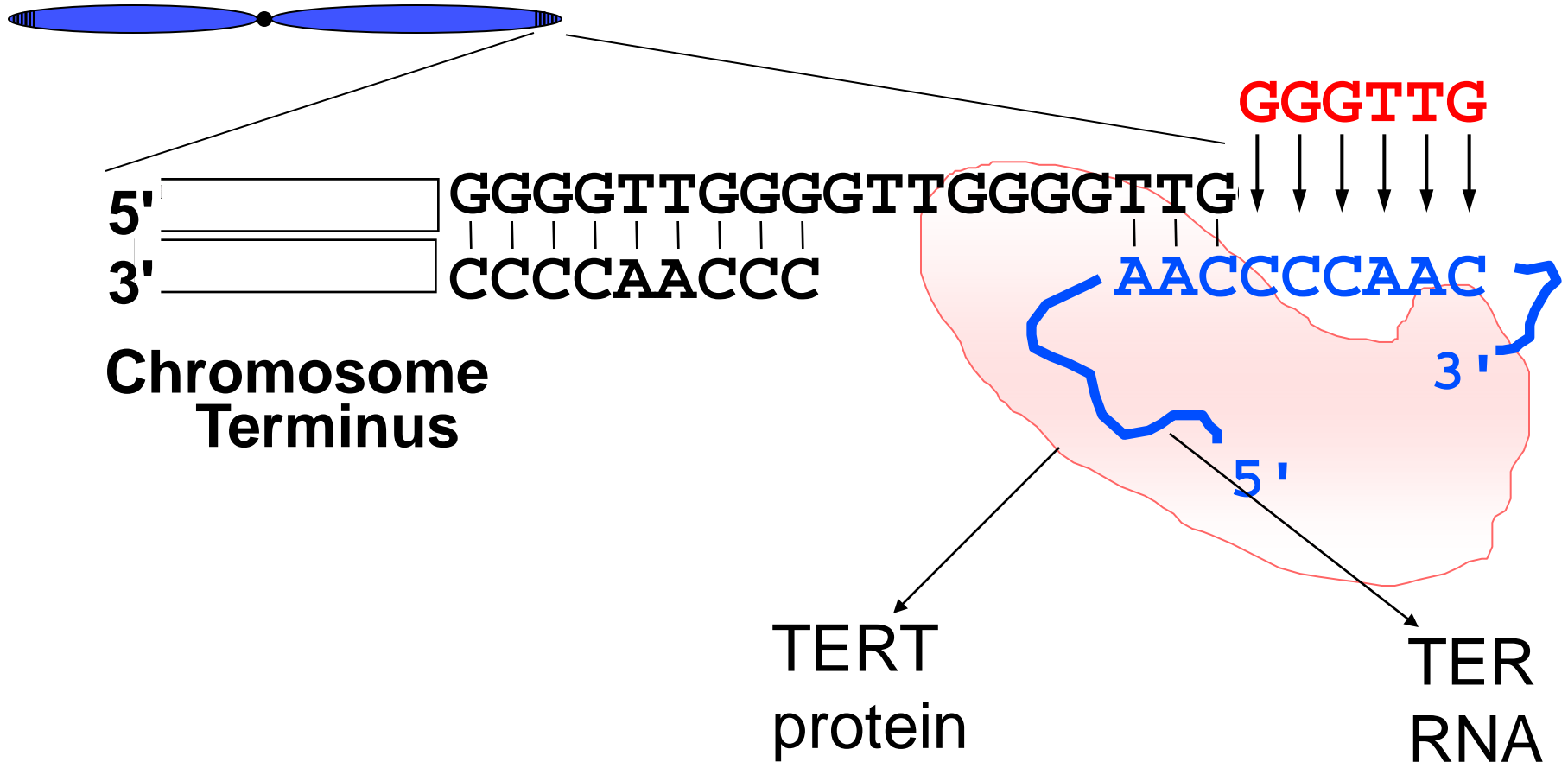
GGGGTTTGGGGTTTGGGGTTTGGGGTTTGG

Greider and Blackburn, 1985



The solution to telomere attrition

Telomerase:

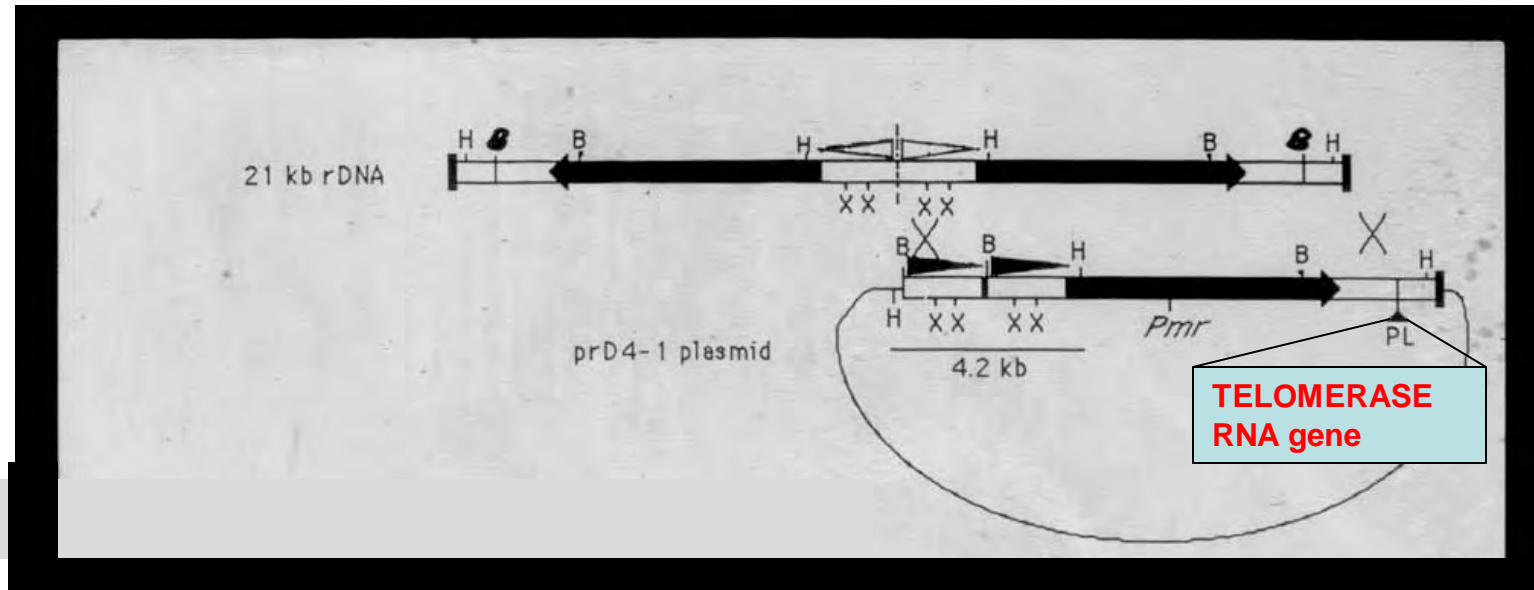


Blackburn lab members and friends at UC Berkeley 1986



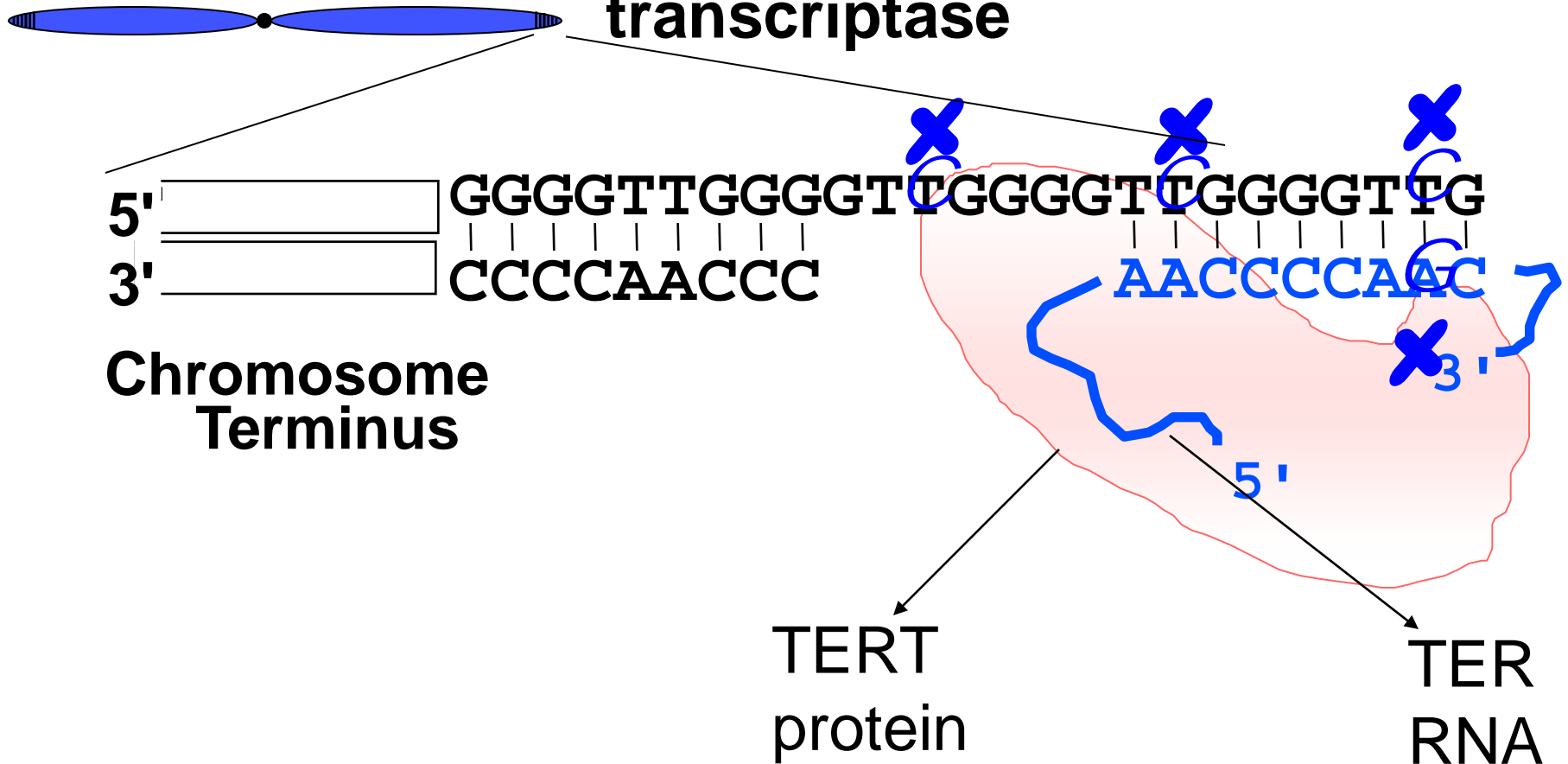
Ed Orias

A vector based on Tetrahymena rDNA replication properties



The solution to telomere attrition

Telomerase: a telomere-synthesizing reverse transcriptase



How do
Tetrahymena
cells
respond when telomerase is
nonfunctional?

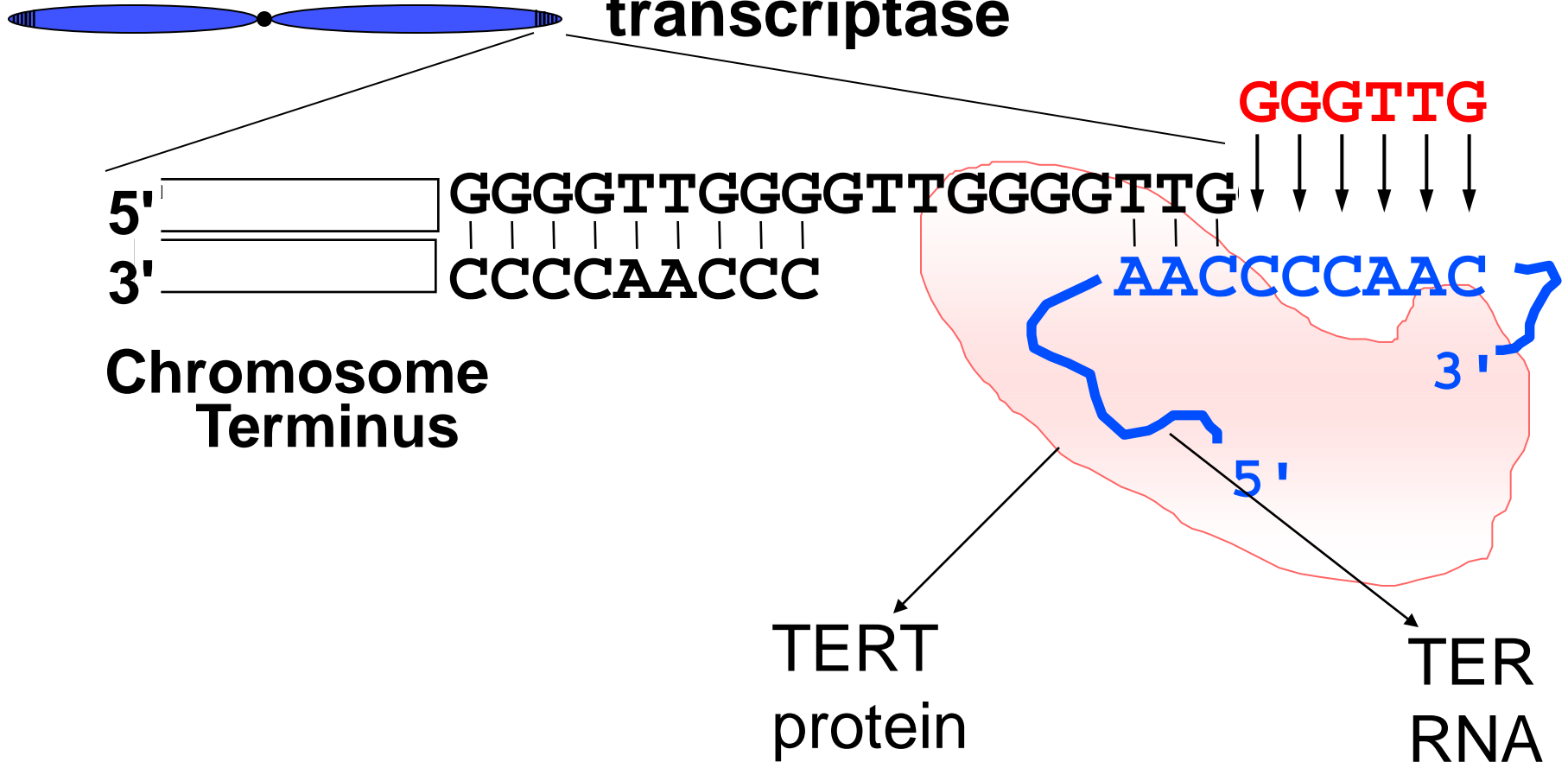
Guo-Liang Yu

John Bradley

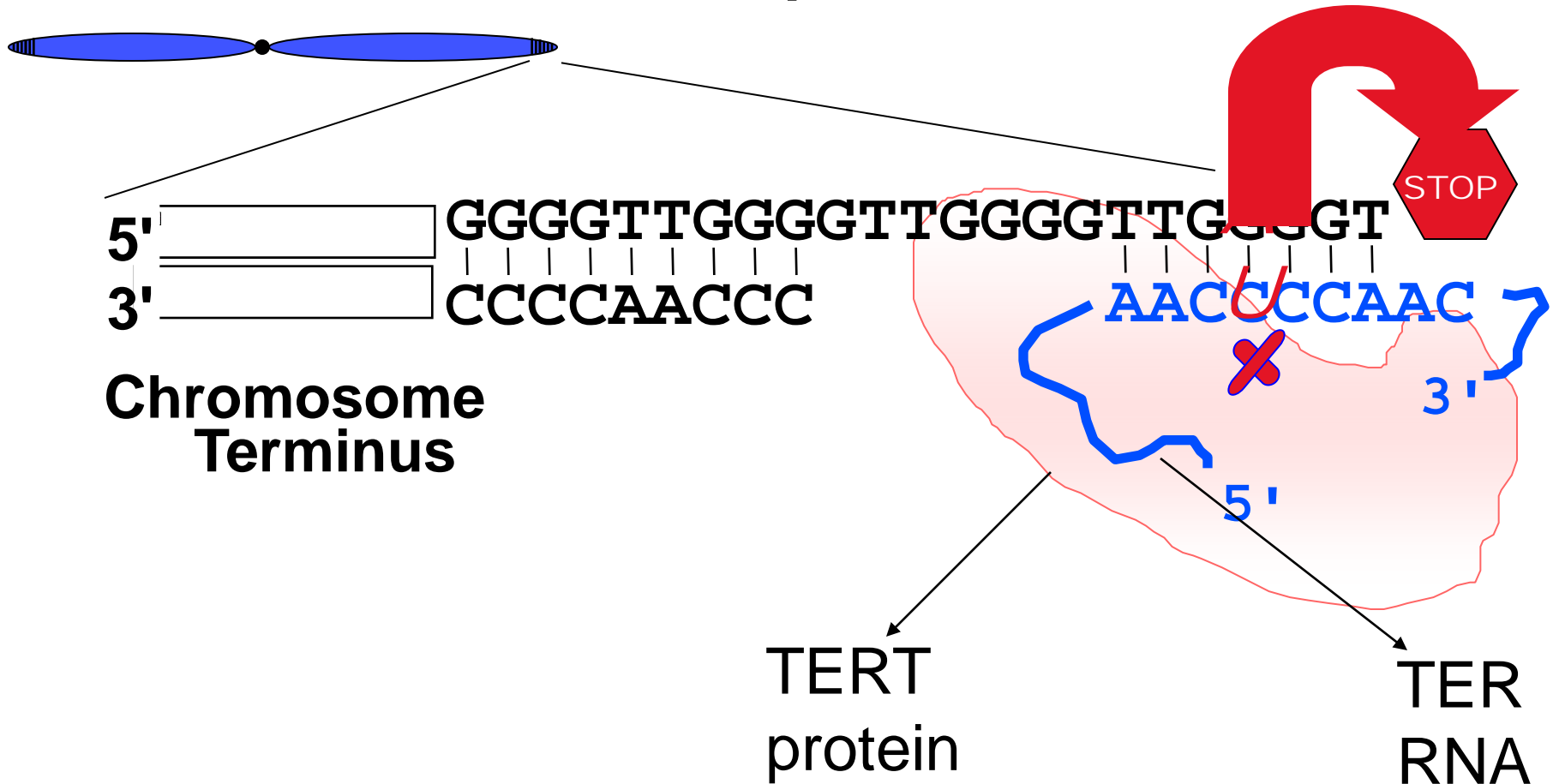
Laura Attardi

The solution to telomere attrition

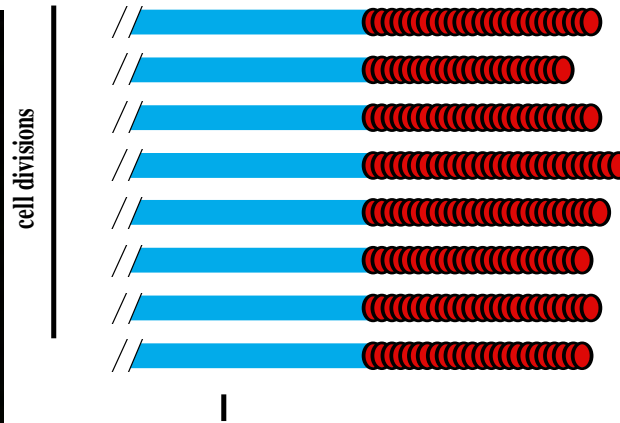
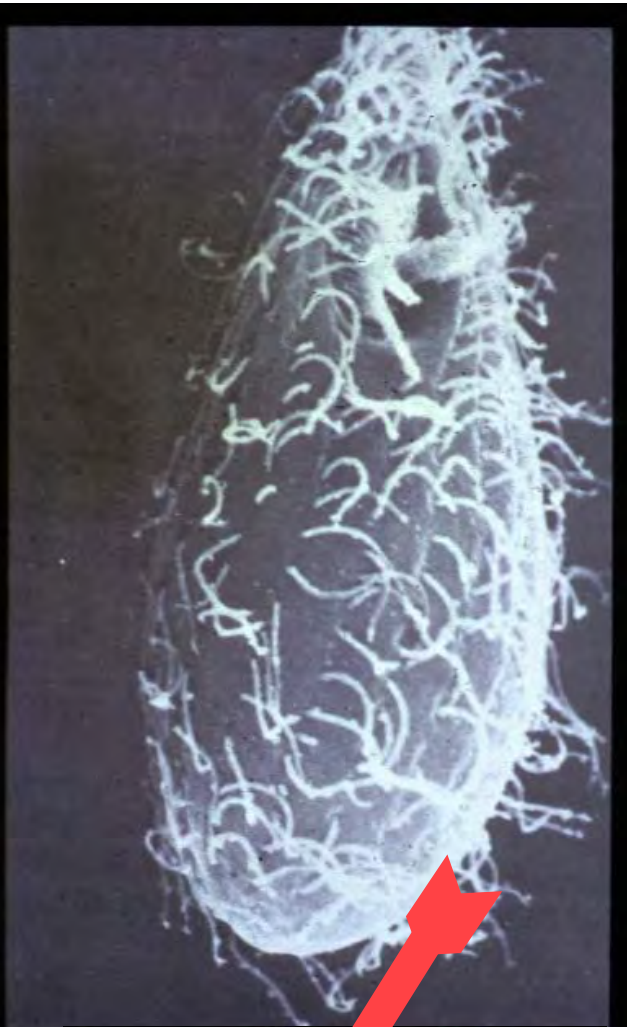
Telomerase: a telomere-synthesizing reverse transcriptase



A telomerase RNA mutant unable to copy the template



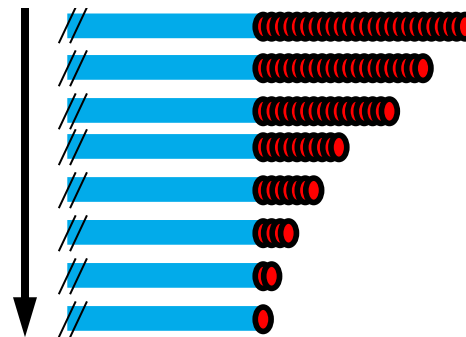
Tetrahymena thermophila



Telomeres
replenished by
telomerase

Cells are immortal

Plenty of telomerase



Genetically
kill telomerase ✘

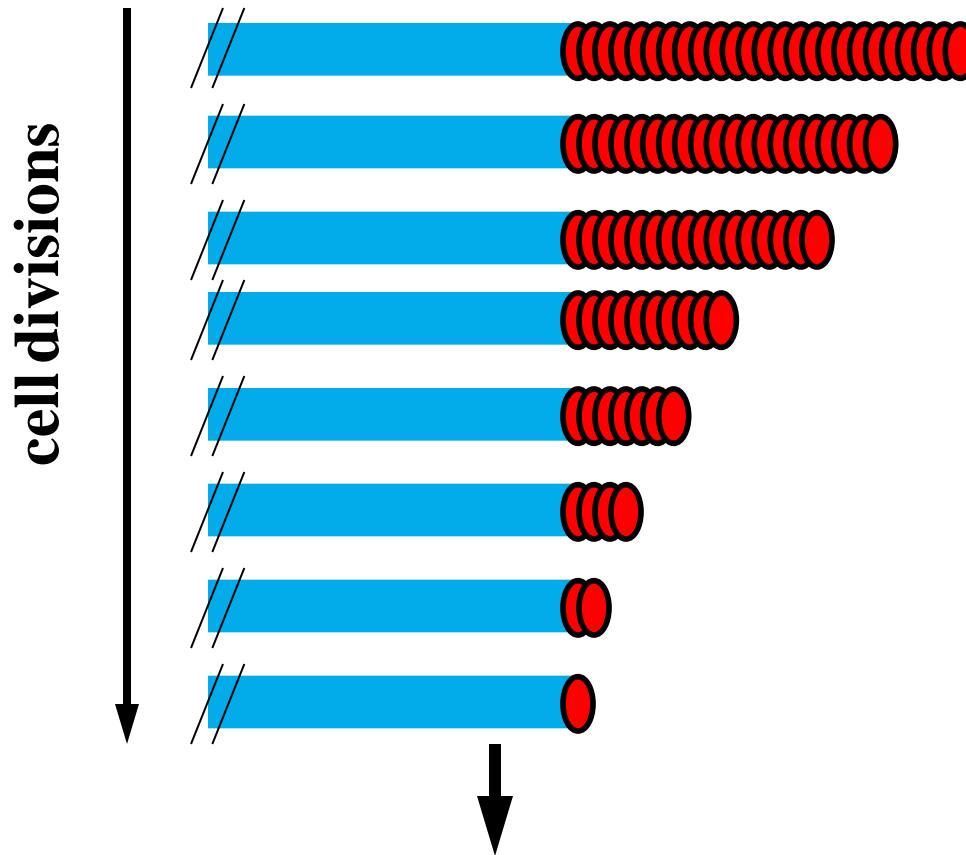
Telomeres progressively
shorten

Tetrahymena ceased divisions

They become "mortal"

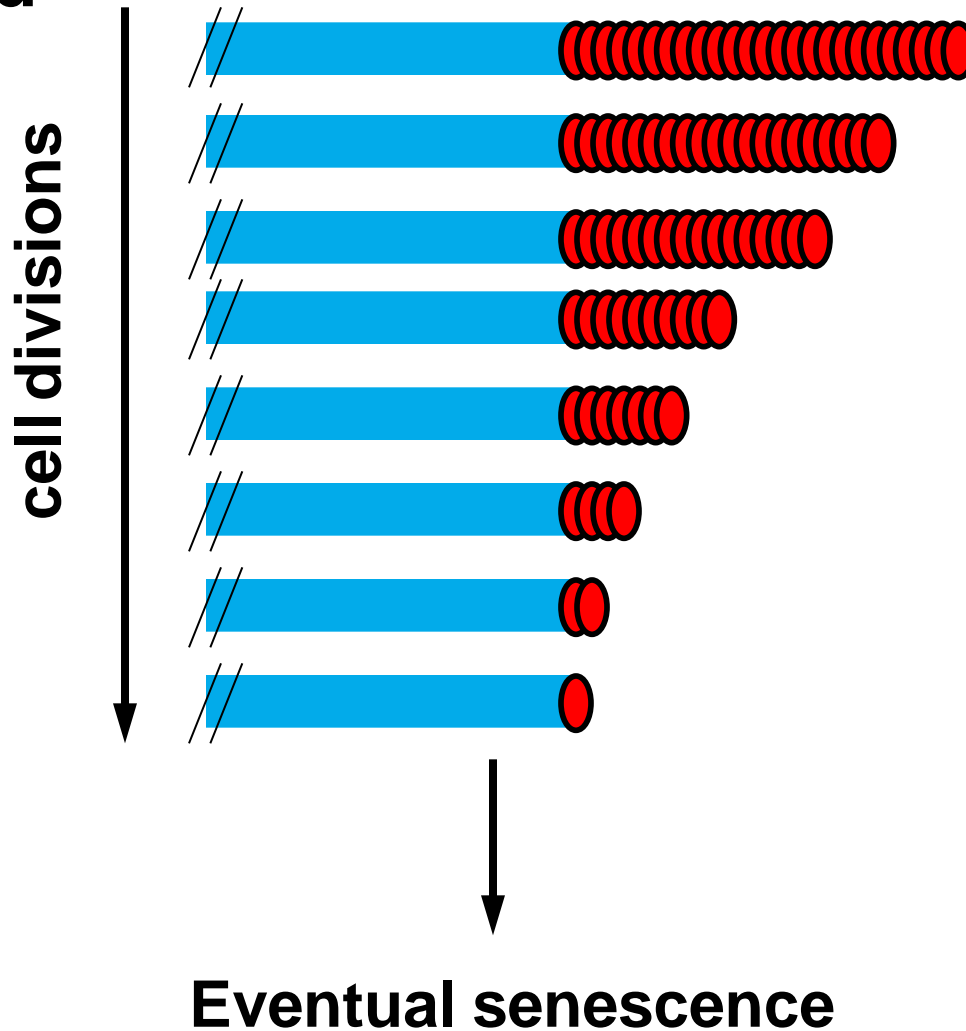
Yu et al, Nature 1990

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

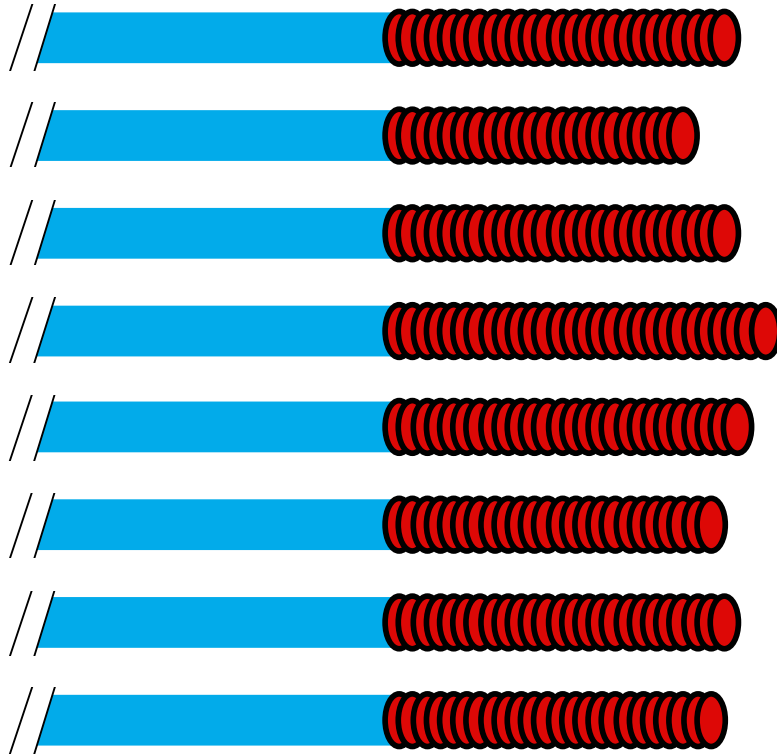


After a delay, senescence

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



cell divisions



|

No Senescence

Telomeres
replenished by
telomerase

*Tetrahymena
thermophila*

Immortal



Inactivate
telomerase



“Mortal”

How did
Tetrahymena
cells

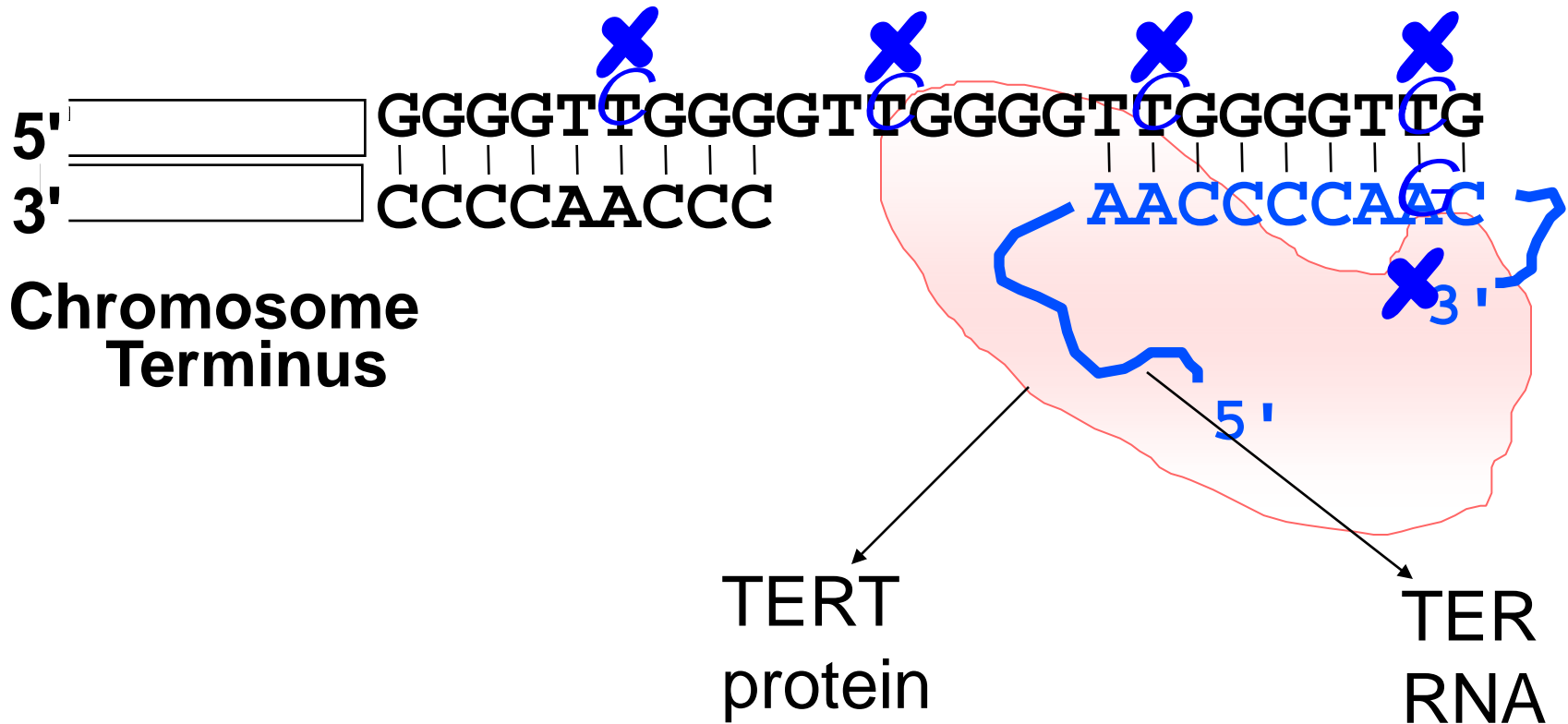
respond when telomerase is
forced to make the wrong DNA
sequence?

Guo-Liang Yu

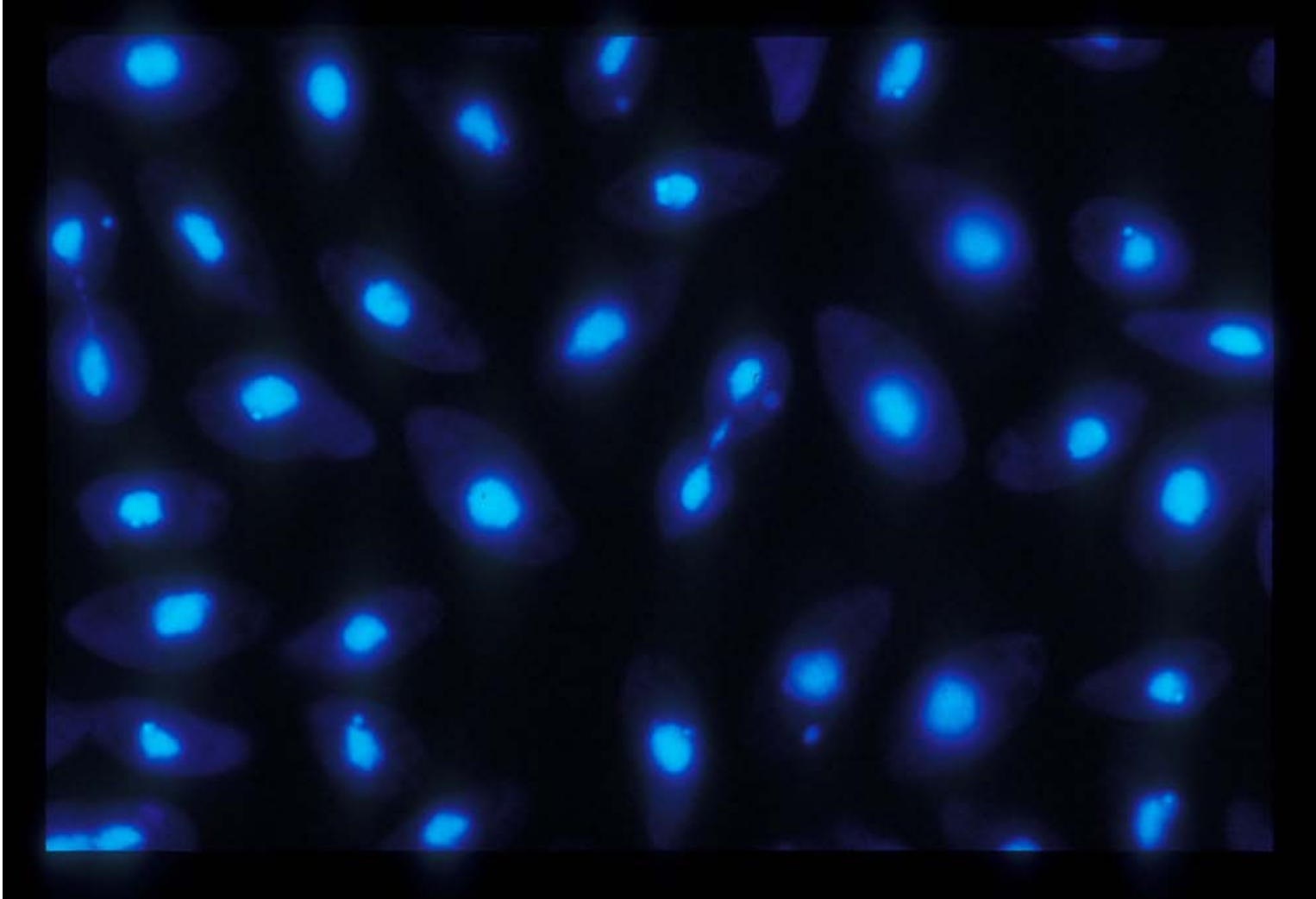
John Bradley

Laura Attardi

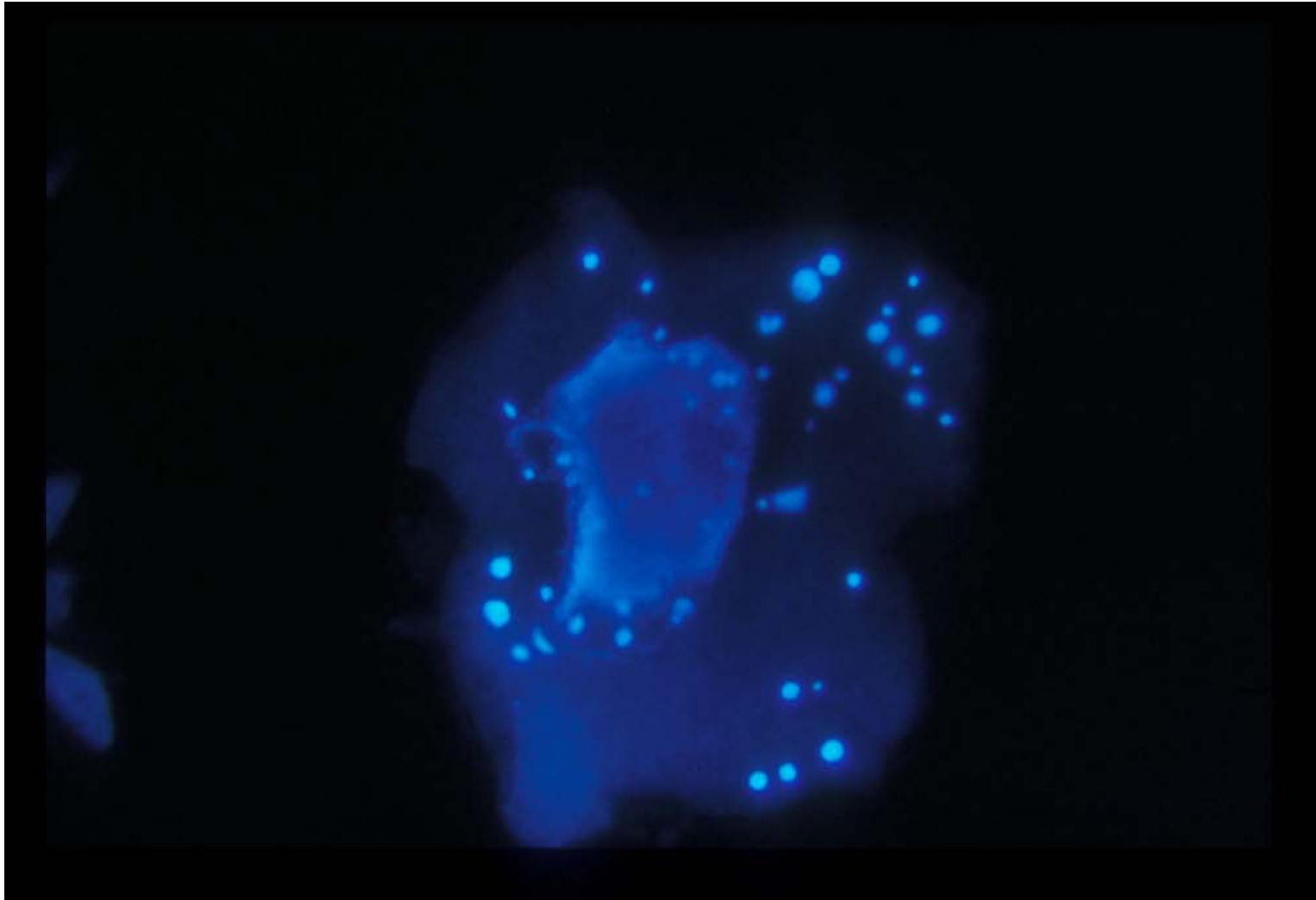
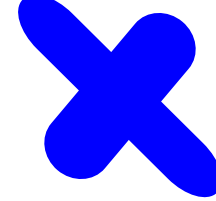
Telomerase: a telomere-synthesizing reverse transcriptase: the sequence matters



***Tetrahymena thermophila* WILD TYPE**

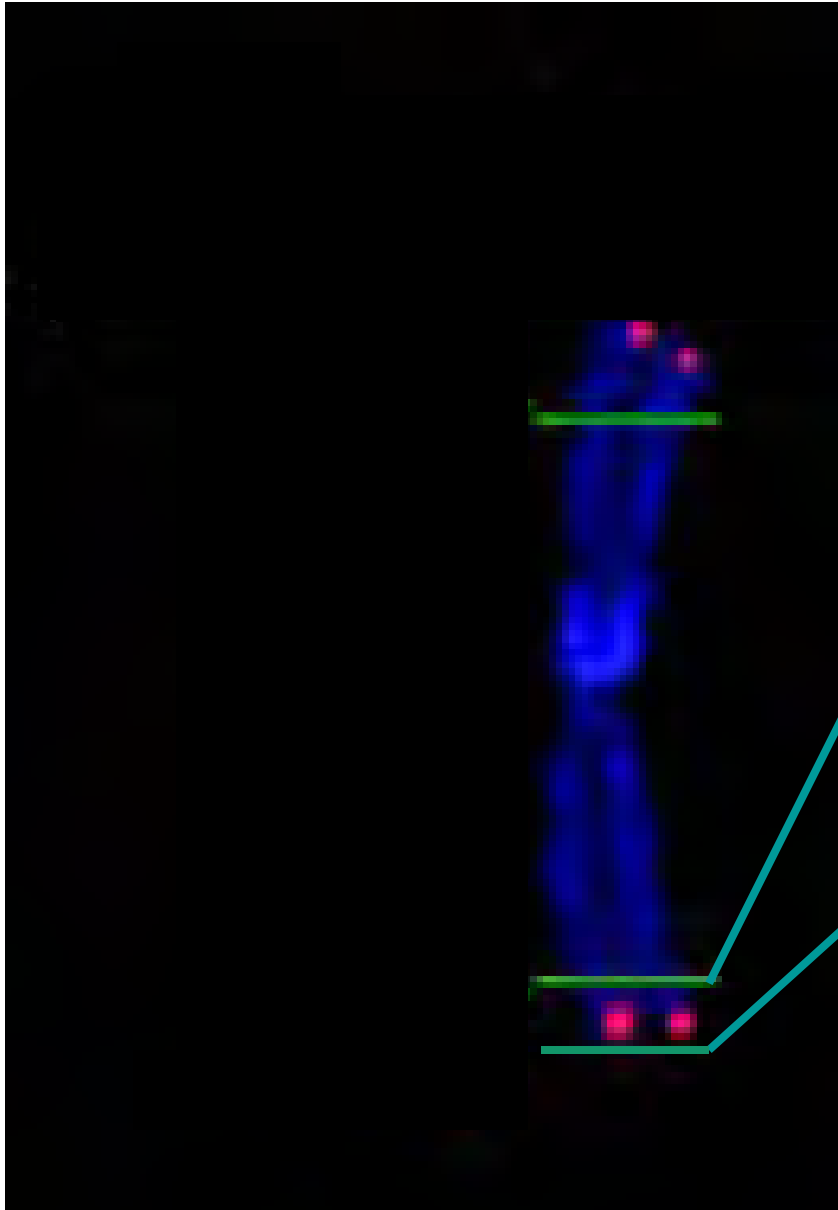


Tetrahymena thermophila
mutant-sequence telomeres



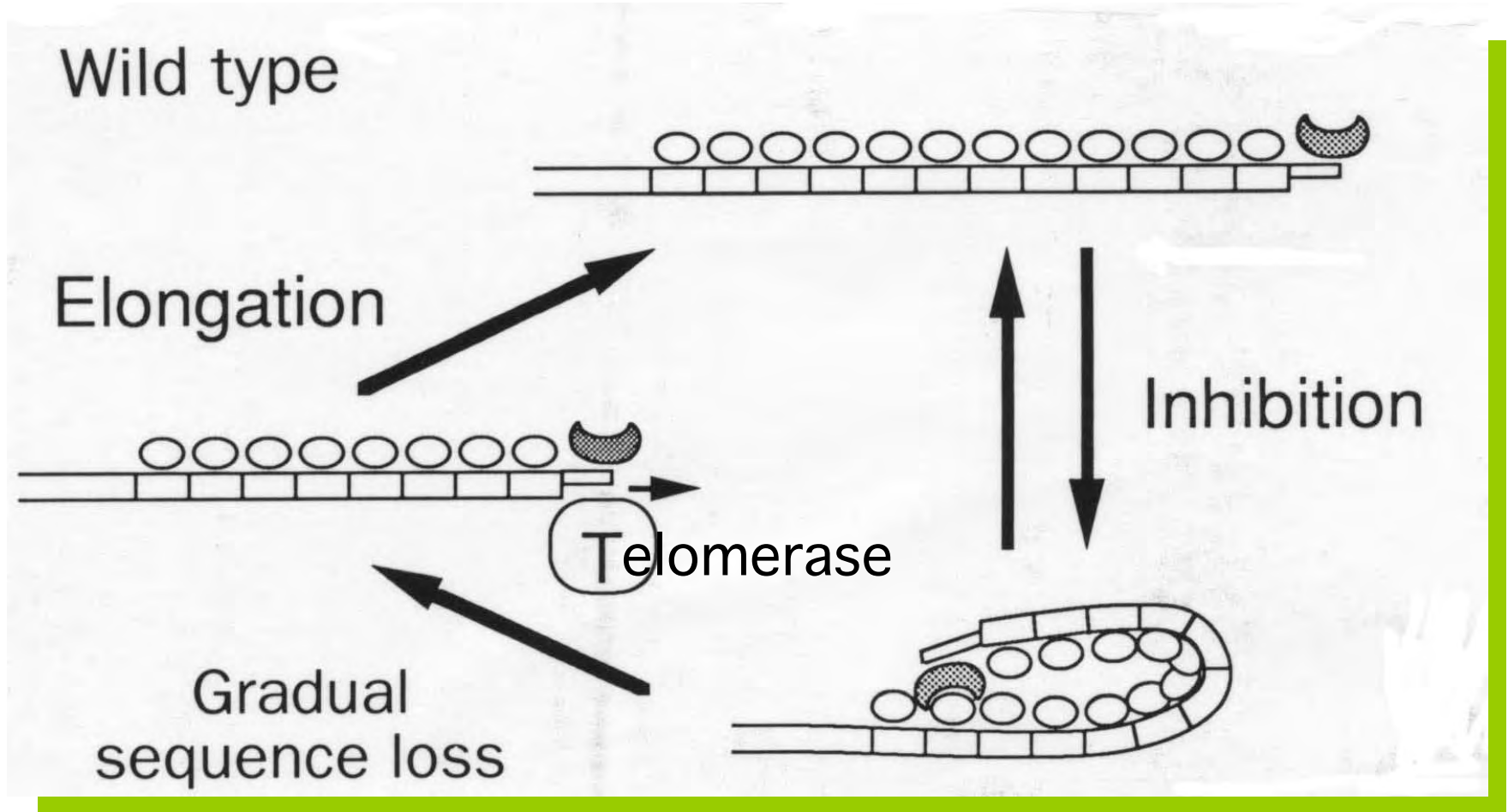
Cells rapidly lost viability!

Telomeres cap ends of chromosomes





Telomere dynamics: a homeostatic system



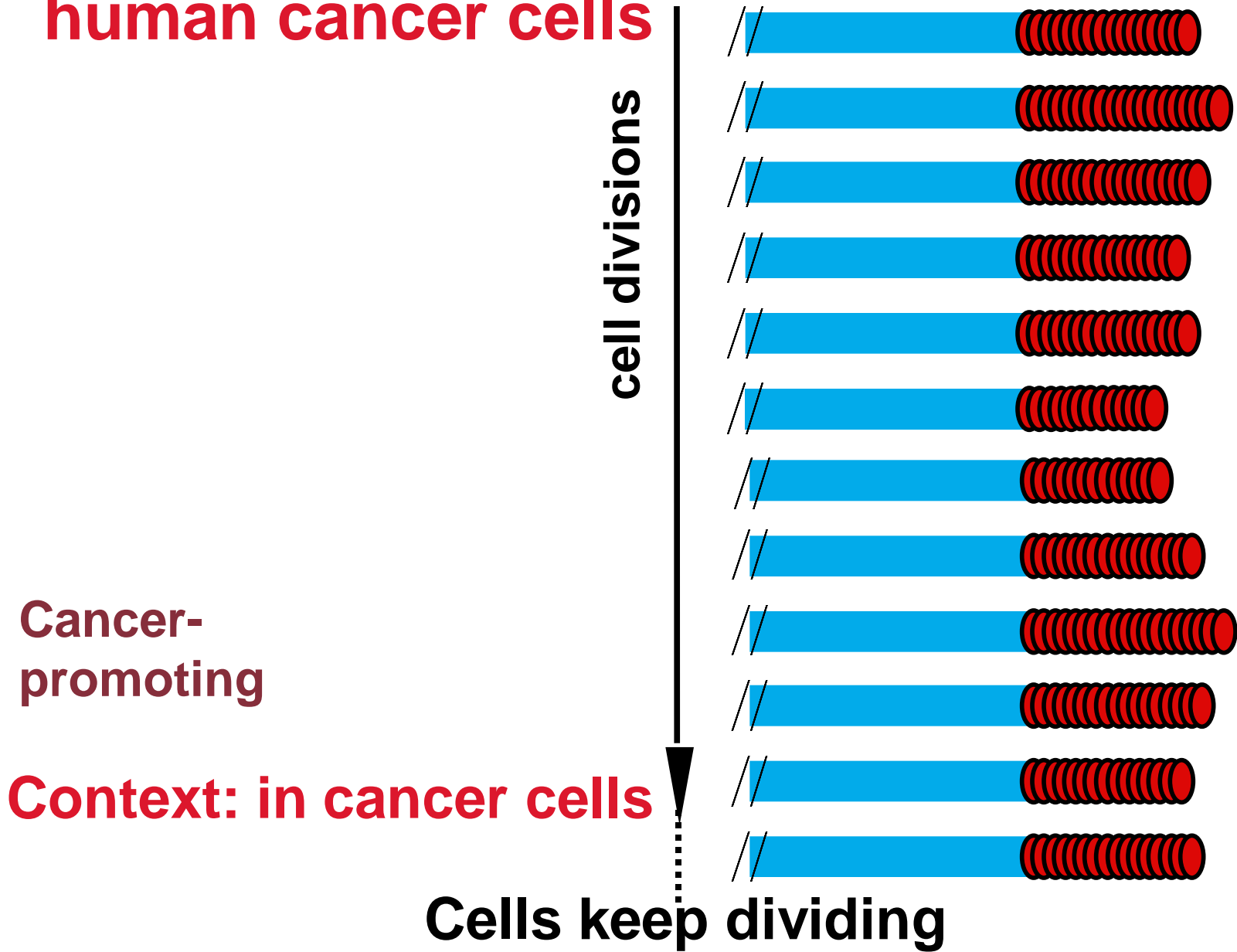
McEachern and Blackburn. Nature, 1995.

In humans?

In **cancer cells**

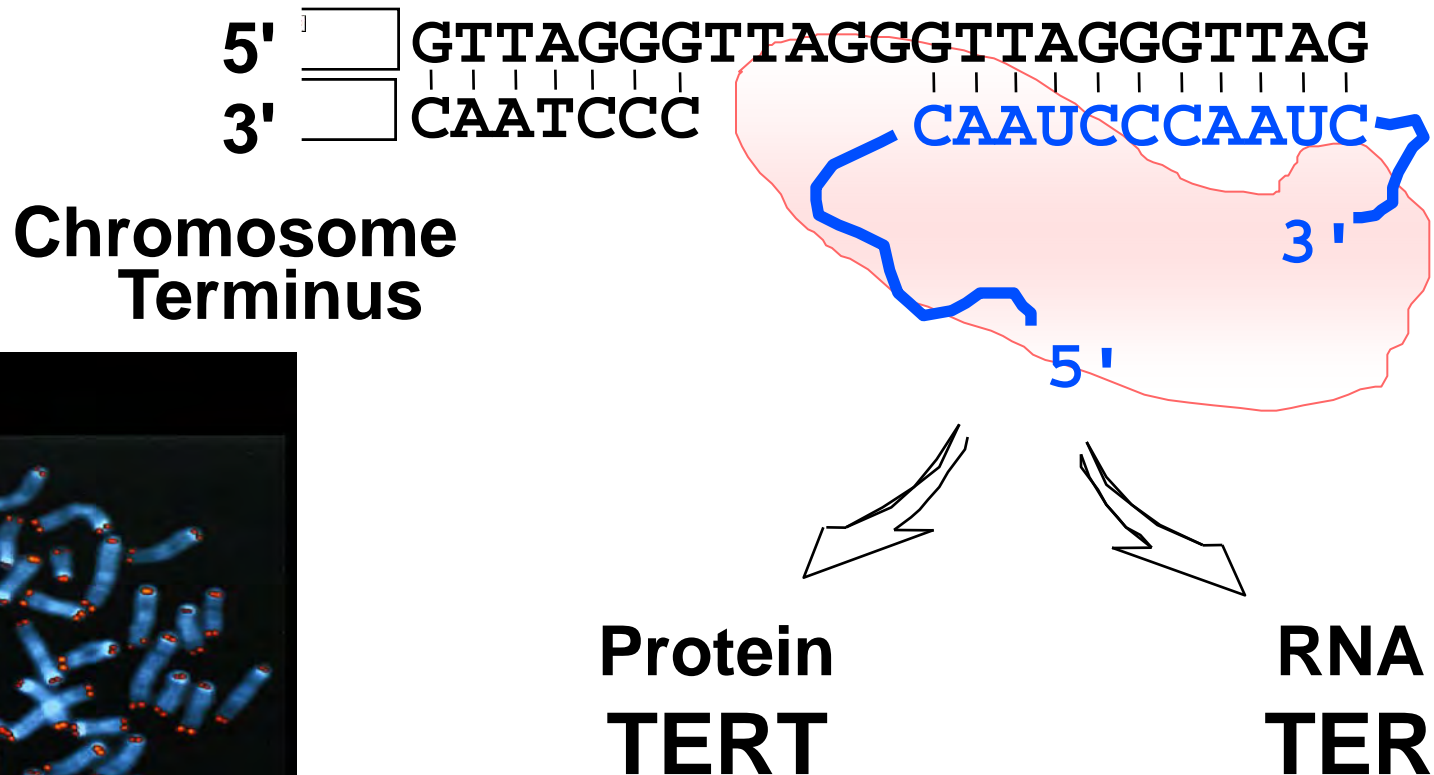
HIGH telomerase characterizes malignant human cancer cells

HIGH telomerase characterizes malignant human cancer cells

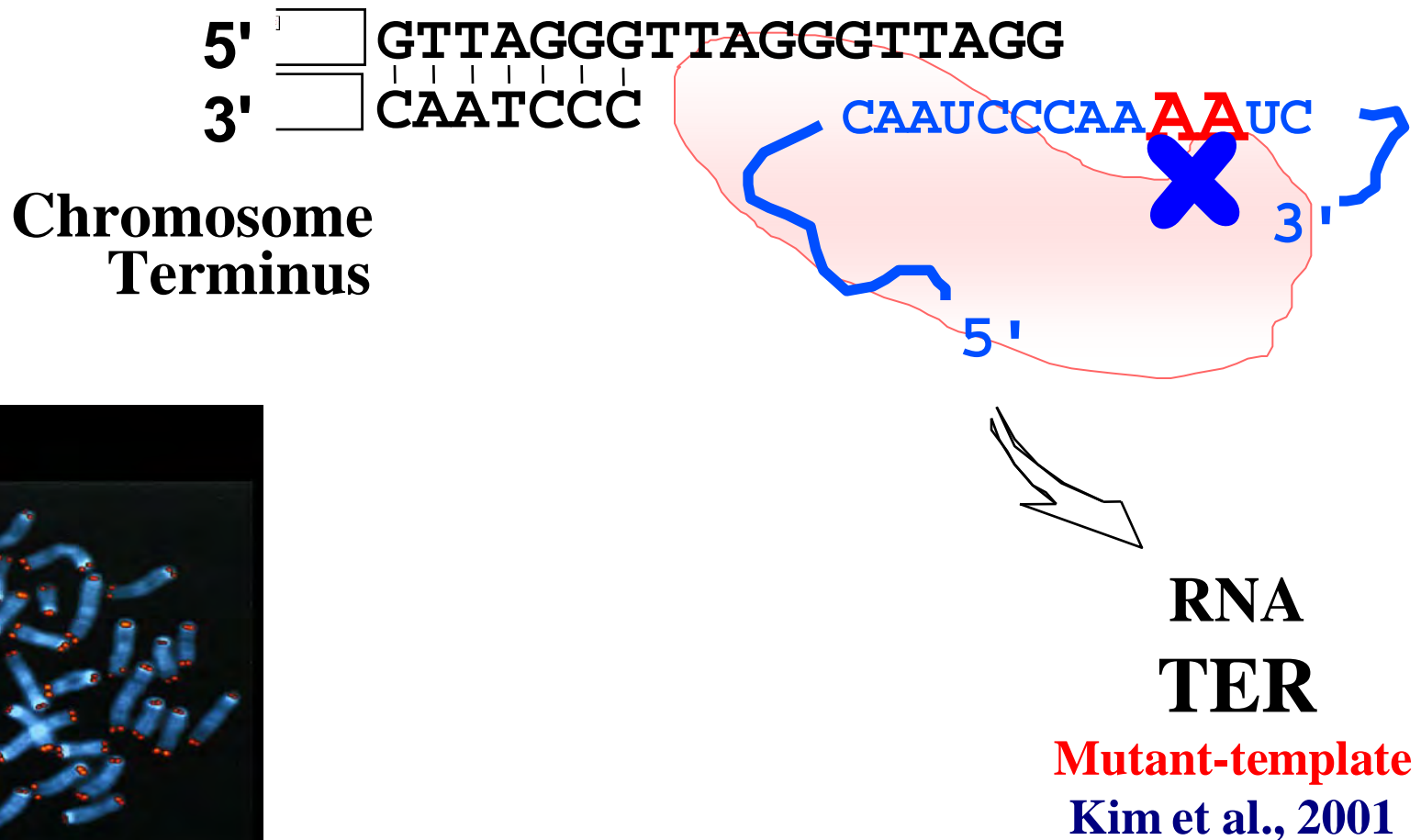


Exploiting the high telomerase of cancer cells to make toxic telomeric DNA

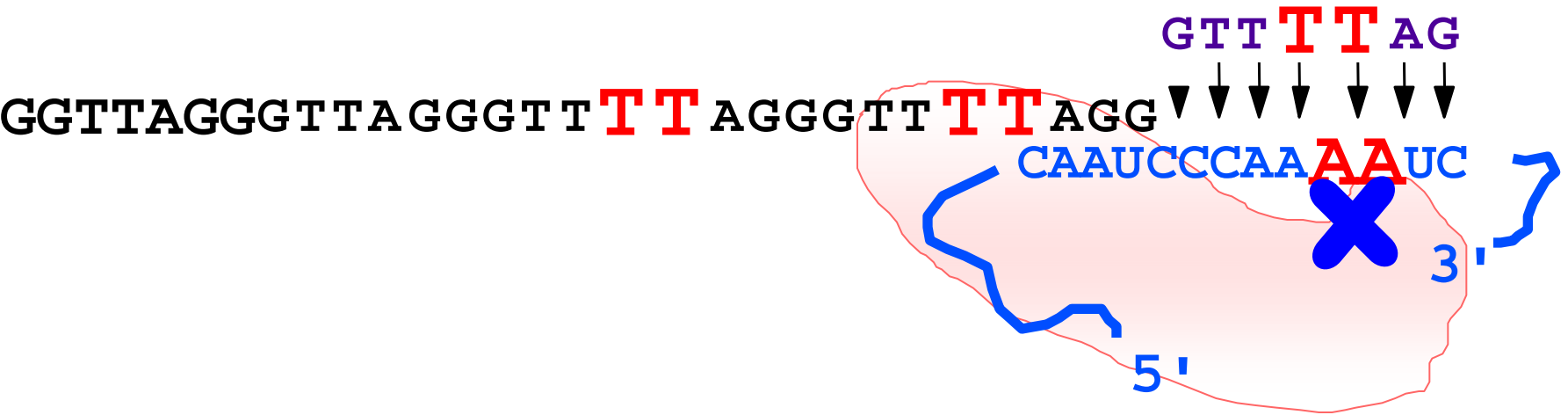
Human Telomerase



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



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

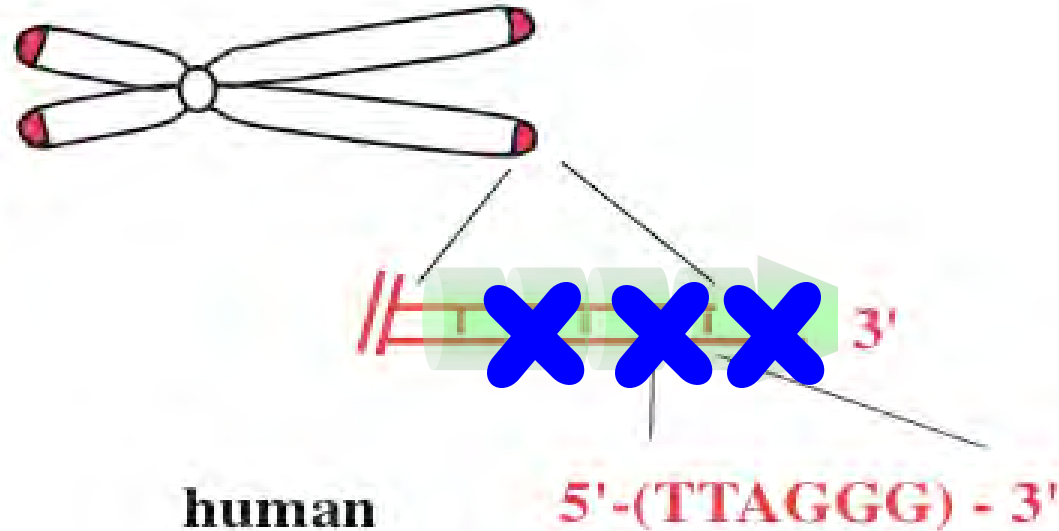


RNA
TER

Mutant-template
Kim et al., 2001



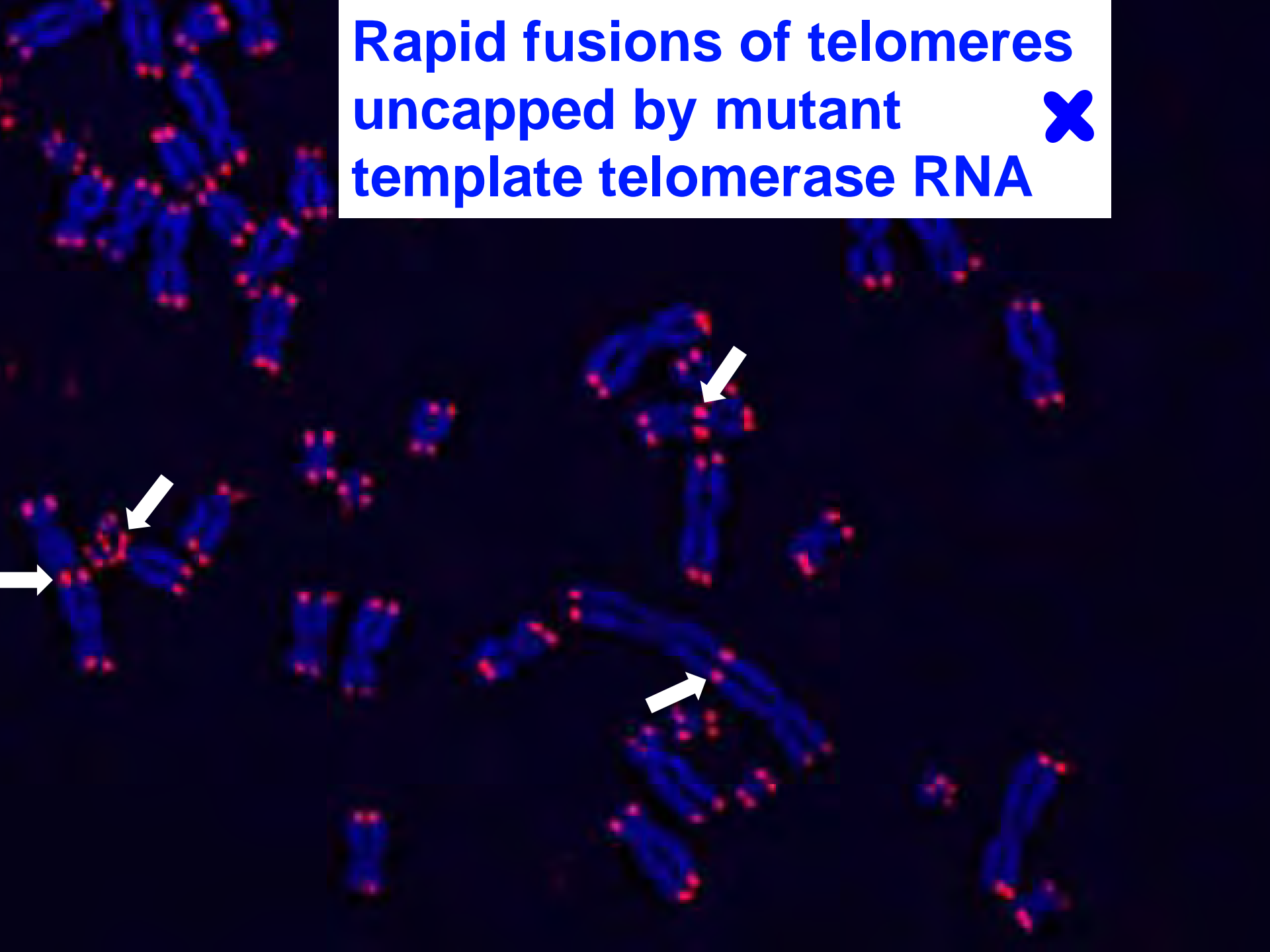
Exploiting the high telomerase of cancer cells to make toxic telomeric DNA



**Telomeric
Complex**

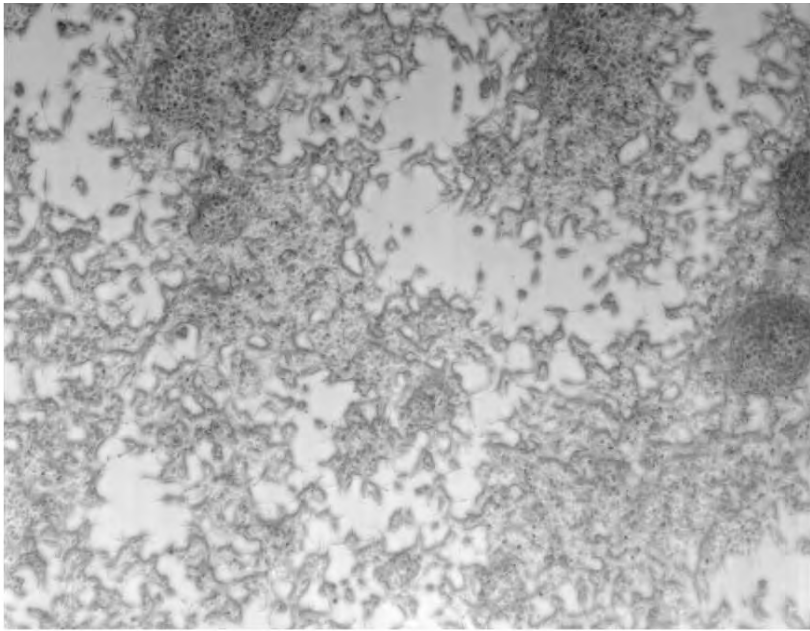
- Telomeric DNA
- Telomeric **sequence-specific** binding proteins

**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)

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*

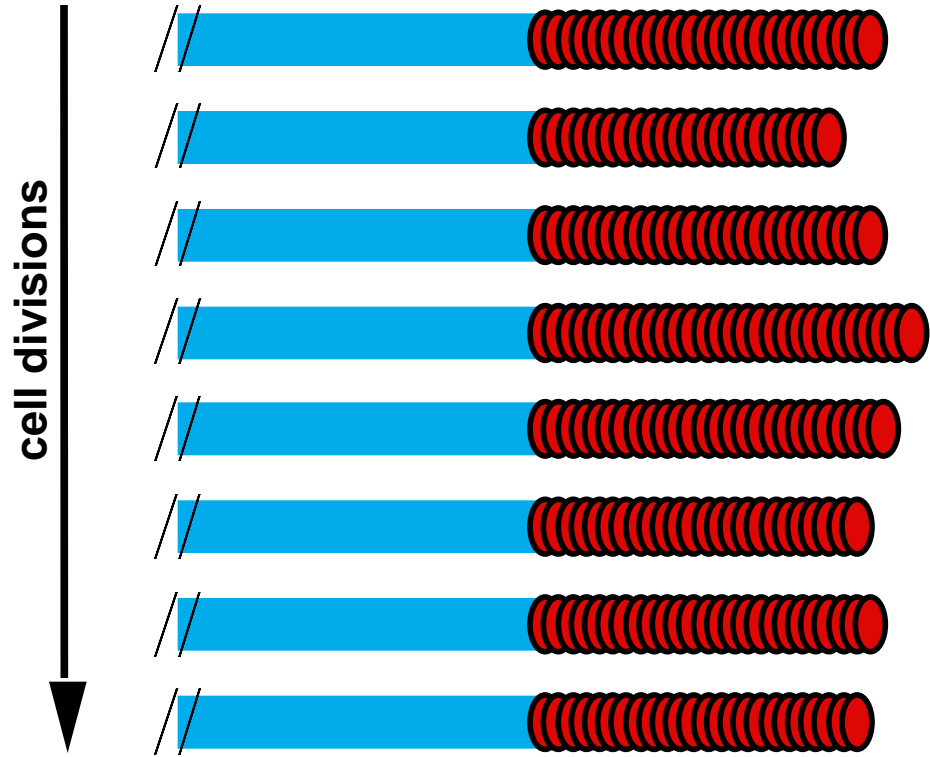
Immortal



Inactivate
telomerase



“Mortal”



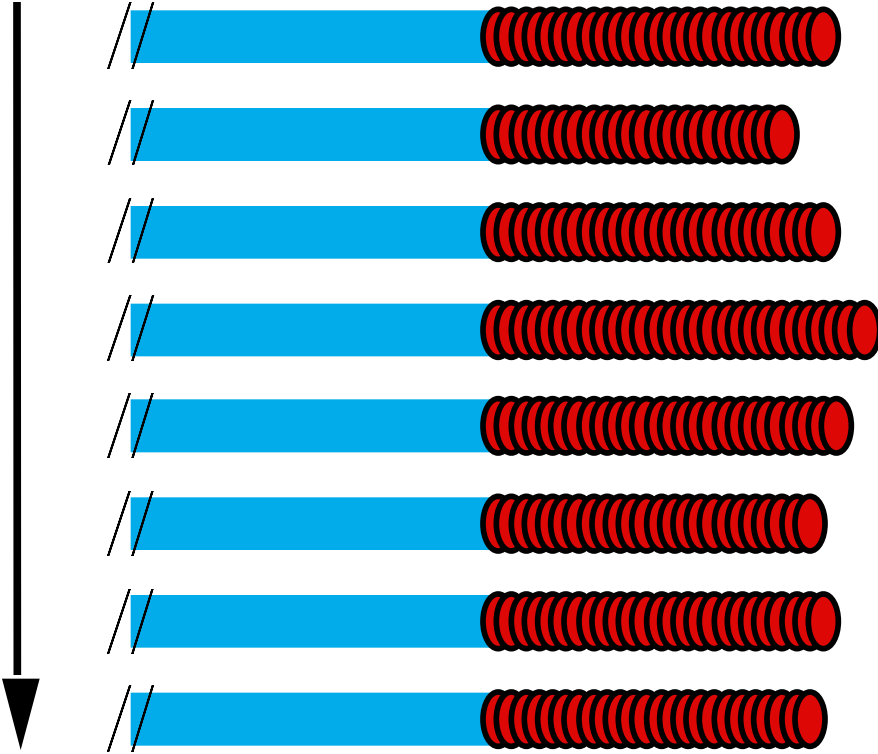
Telomeres
replenished by
telomerase

In humans

↓
Cells keep
dividing

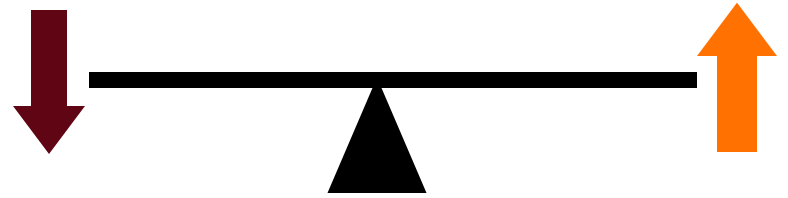
- Active: stem cells, germ cells
- Detectable: many normal adult cell types (quantifiable activity)
- Highly active: ~90% of human tumors

cell divisions



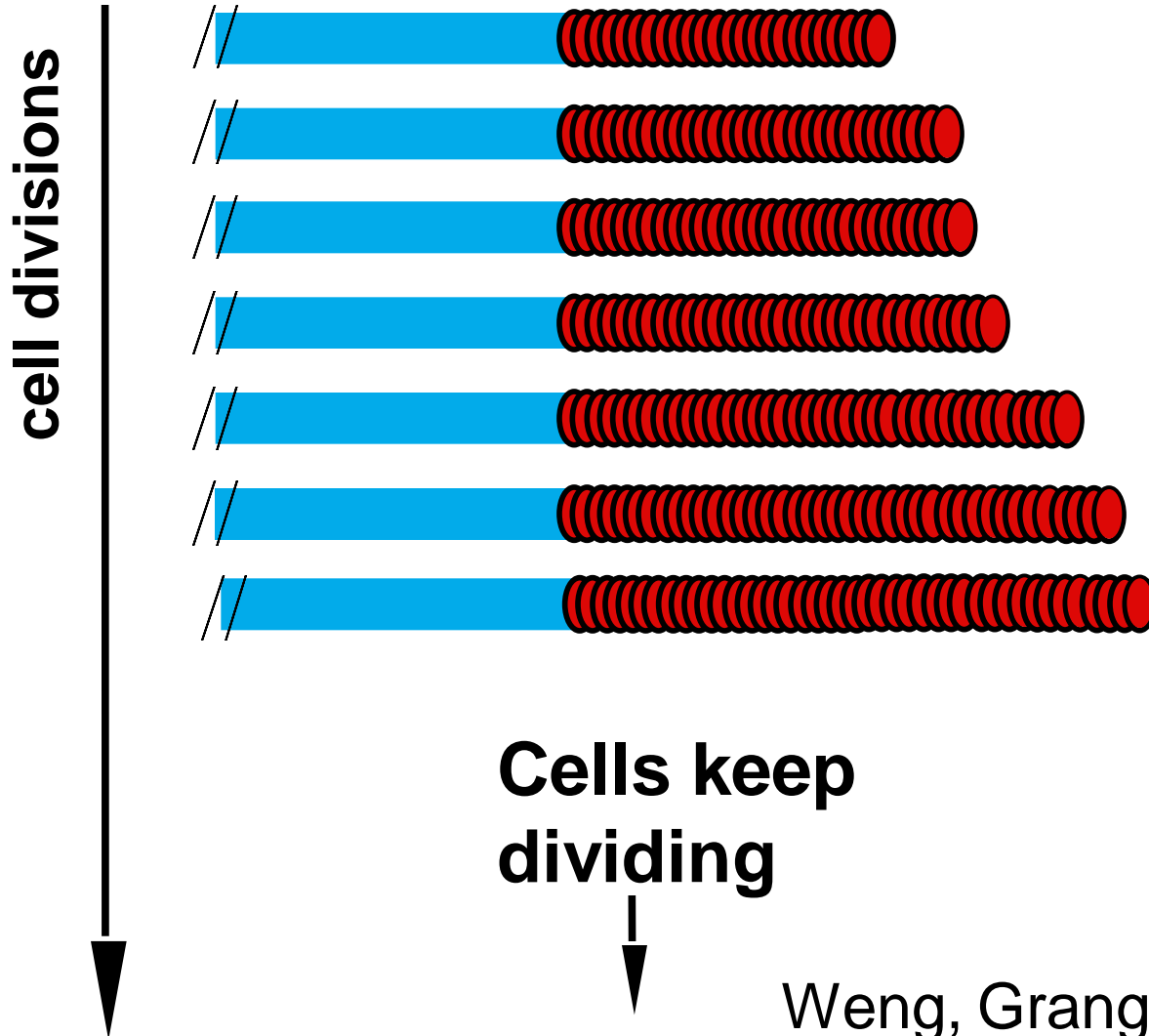
Cells keep dividing

Plenty of telomerase:



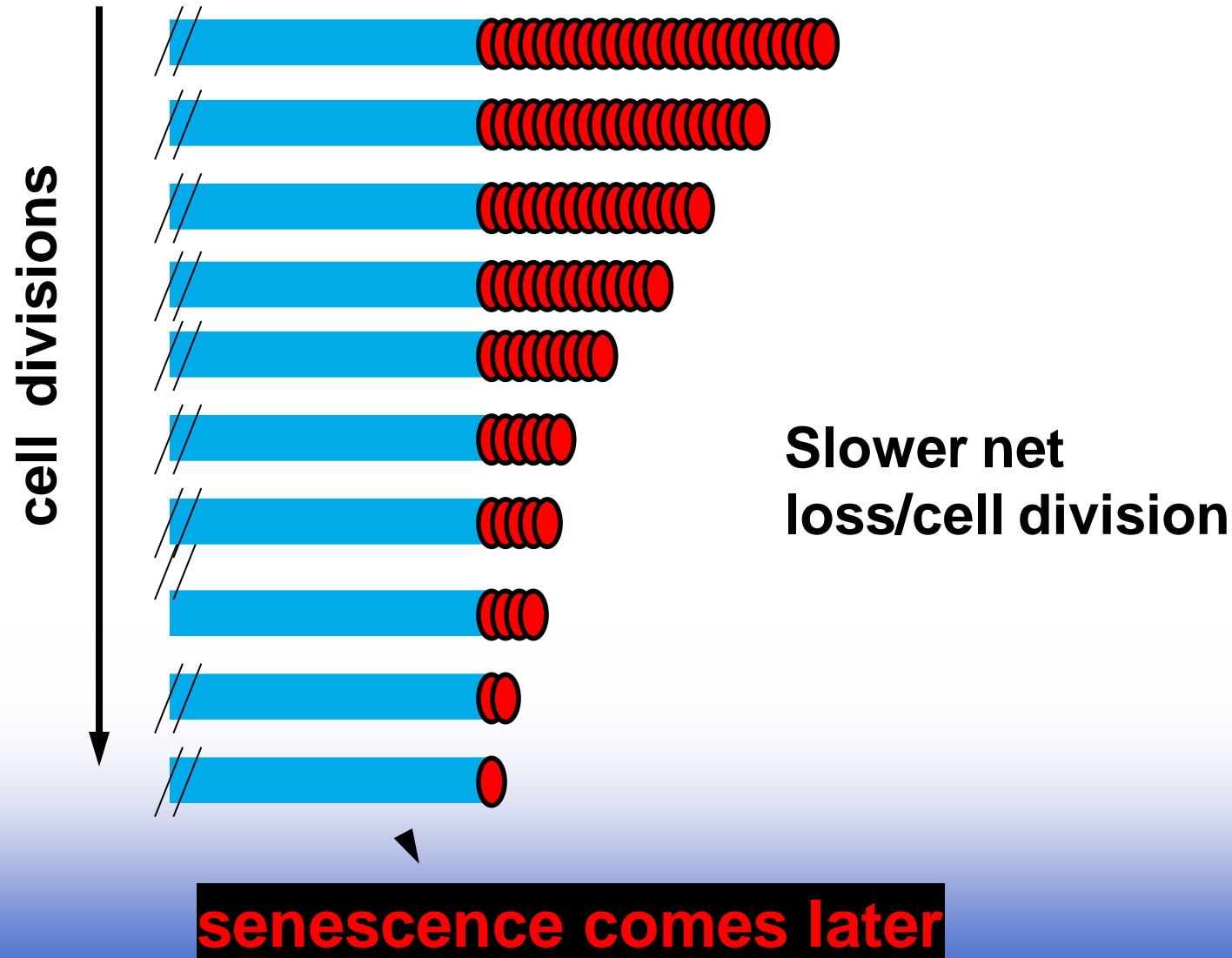
Addition and shortening stay balanced

Upregulated telomerase in humans: telomeres grow in vivo

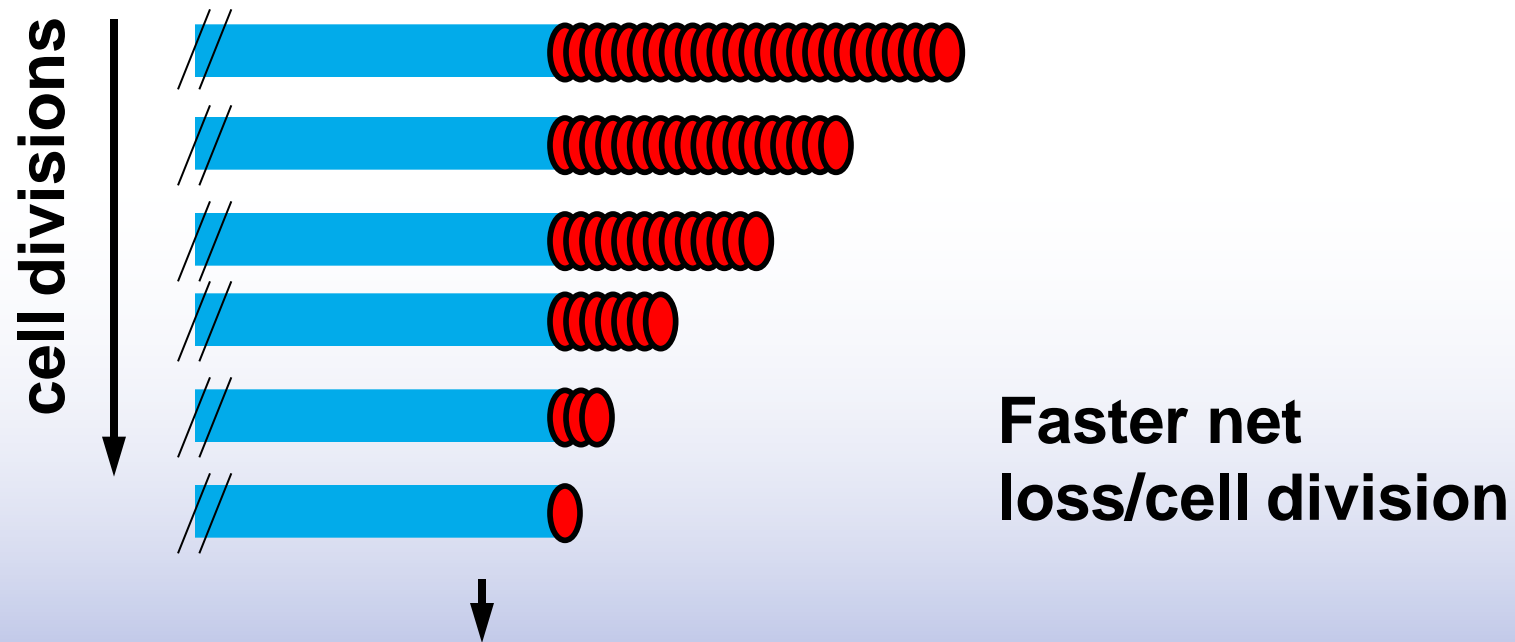


Weng, Granger and Hodes, 1997

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



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

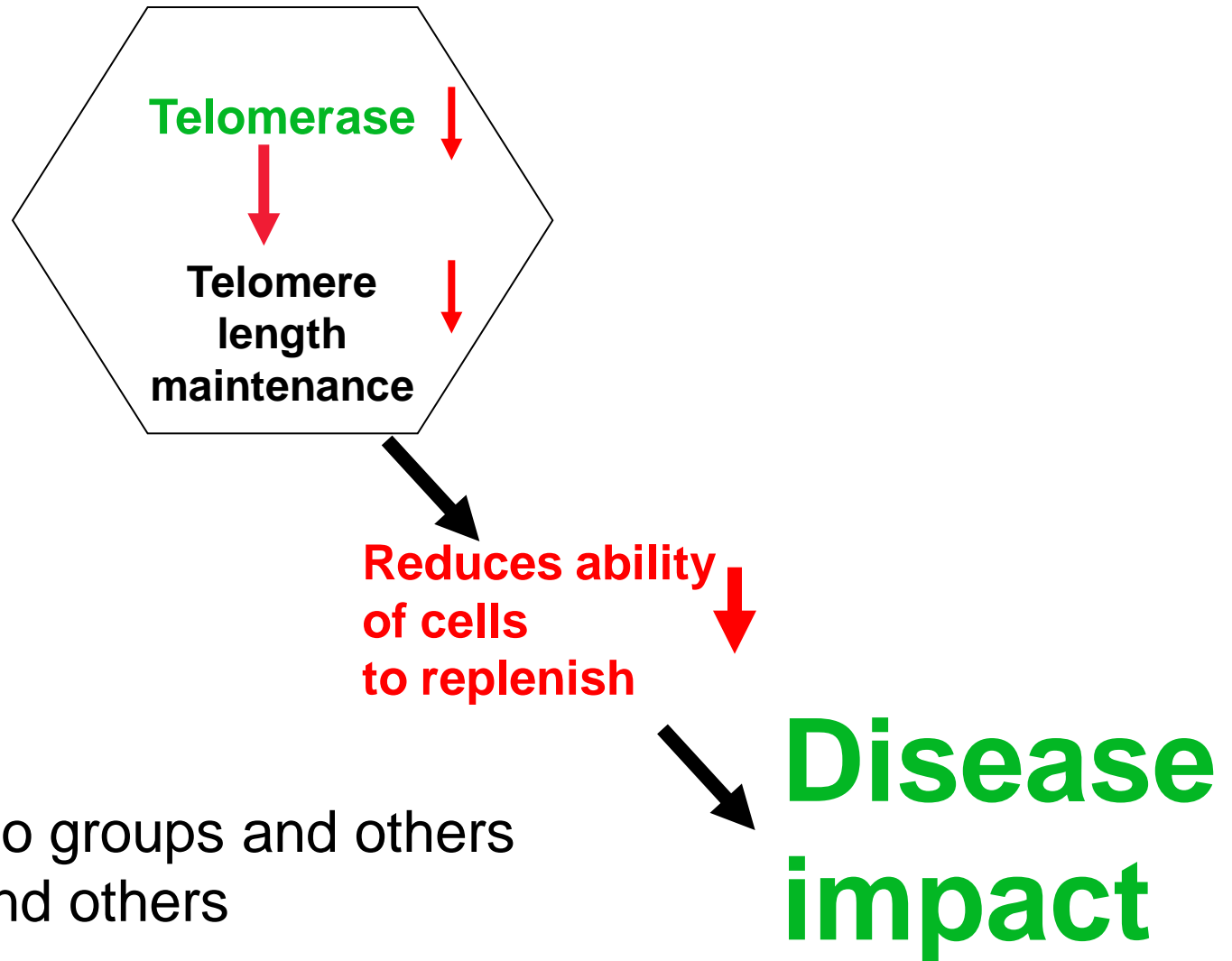


Senescence sooner

- genetic
- environment/life factors

Telomerase -

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

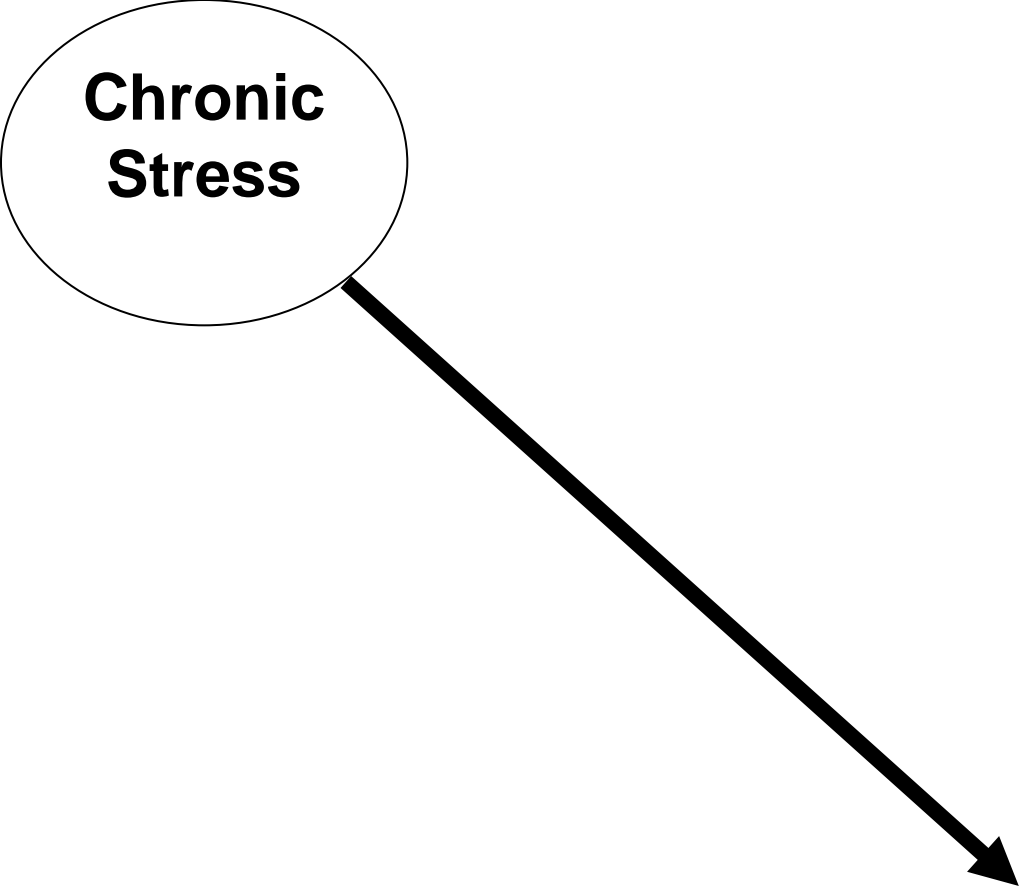


*Greider, DePinho groups and others

** Dokal group and others

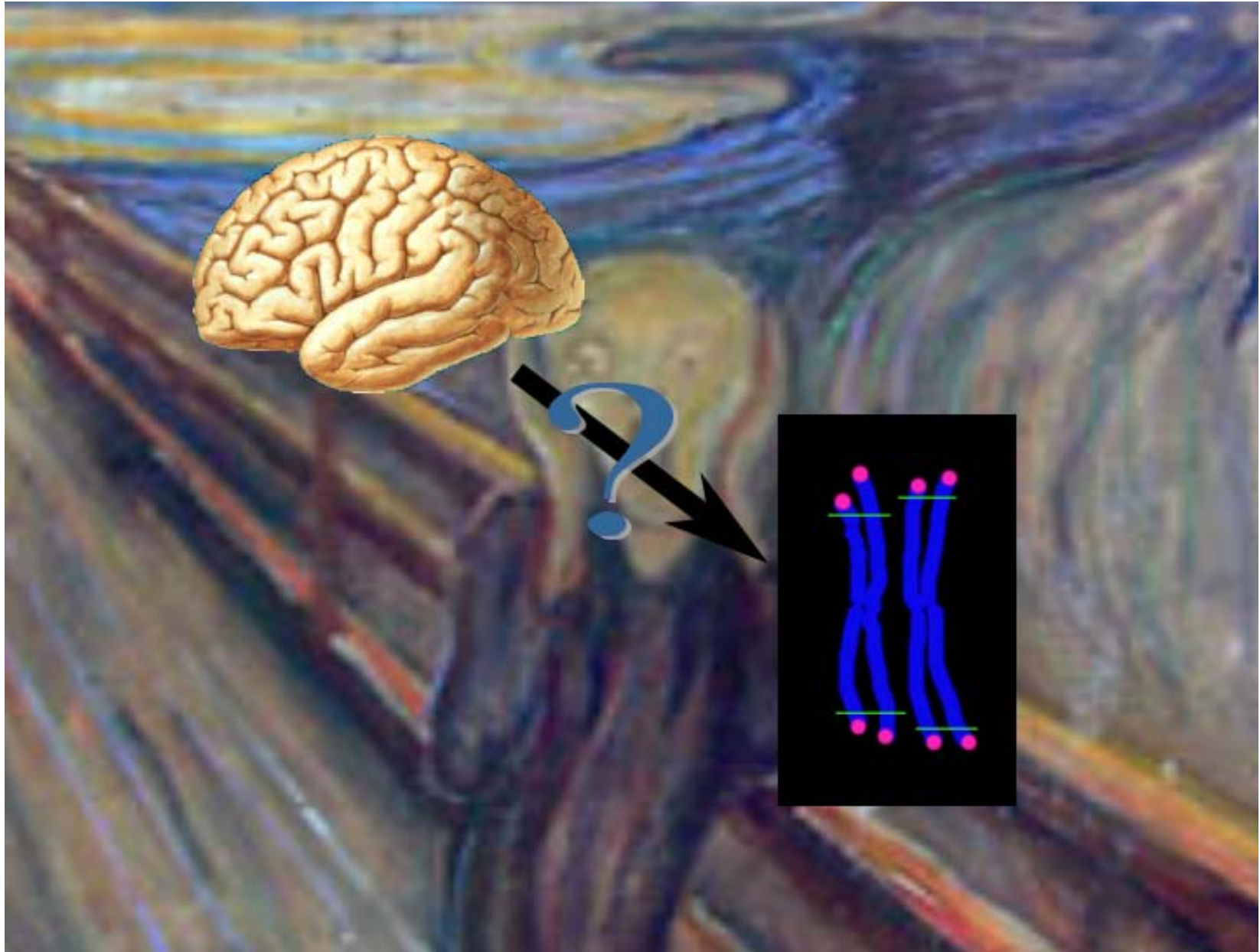
**Chronic psychological stress -
a known non-genetic determinant of human disease risk**

**Chronic
Stress**



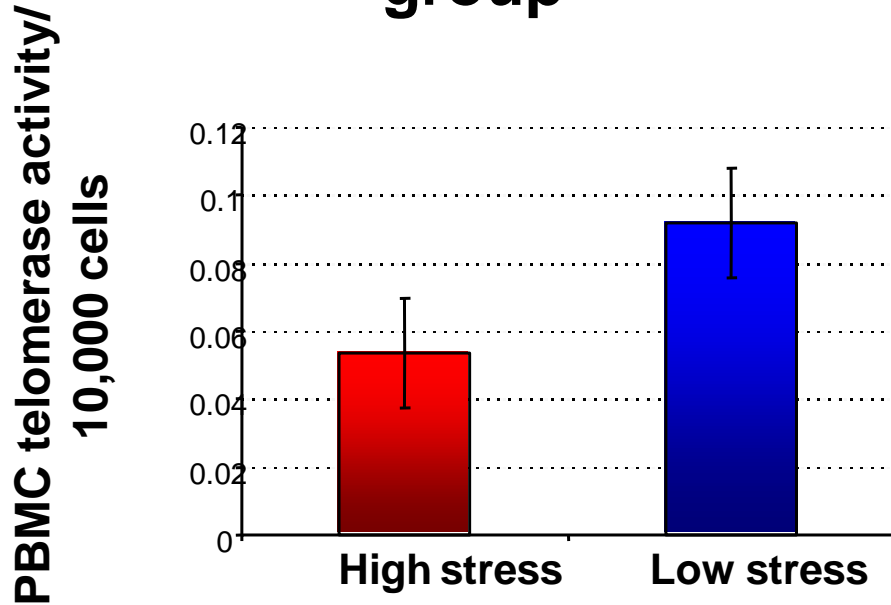
**Disease
impact**

Chronic psychological stress and telomeres



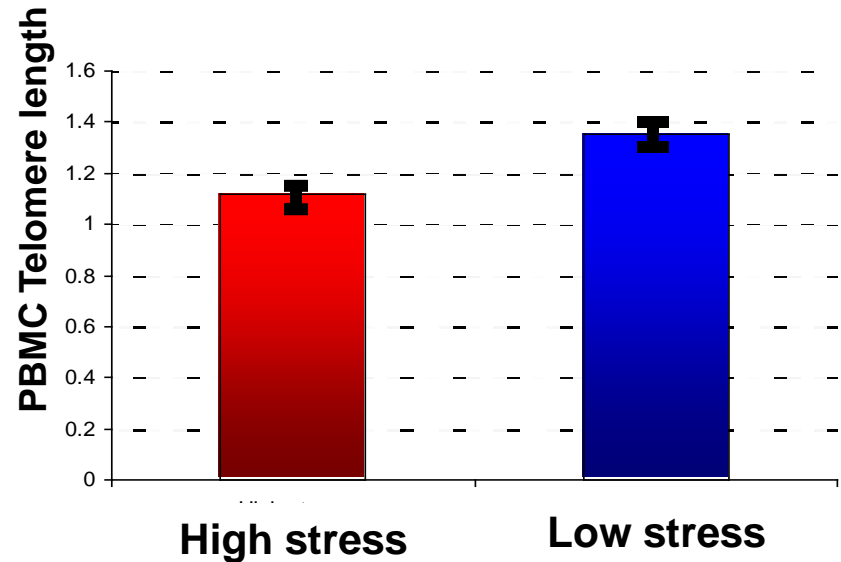
Caregiver mothers and chronic stress

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”)

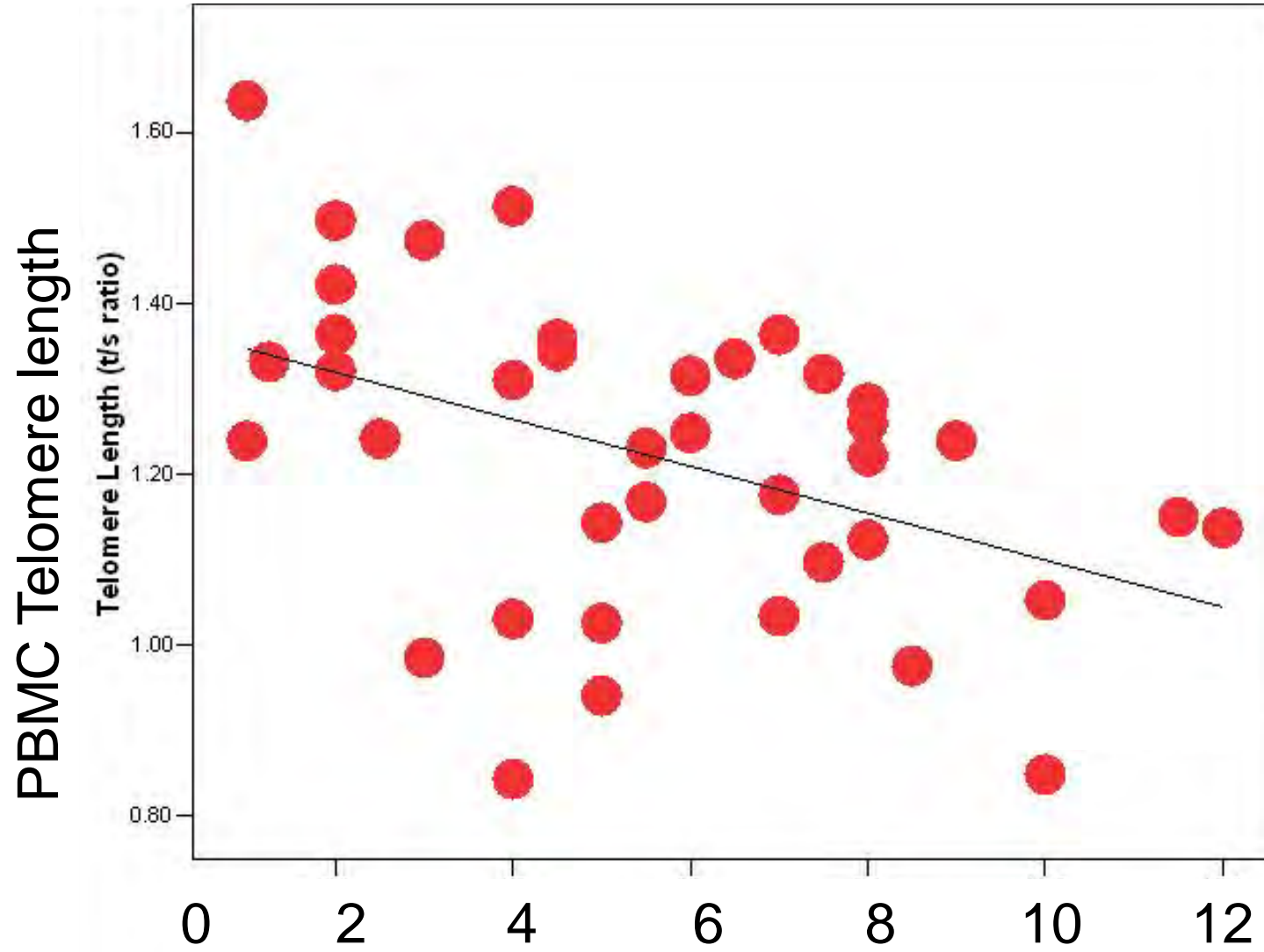


controlling for age and
body mass index:
 $F(3,27) = 12.8, p < .001$

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

Study 1: Caregiver mothers and chronic stress

Stressor Duration and Telomere length

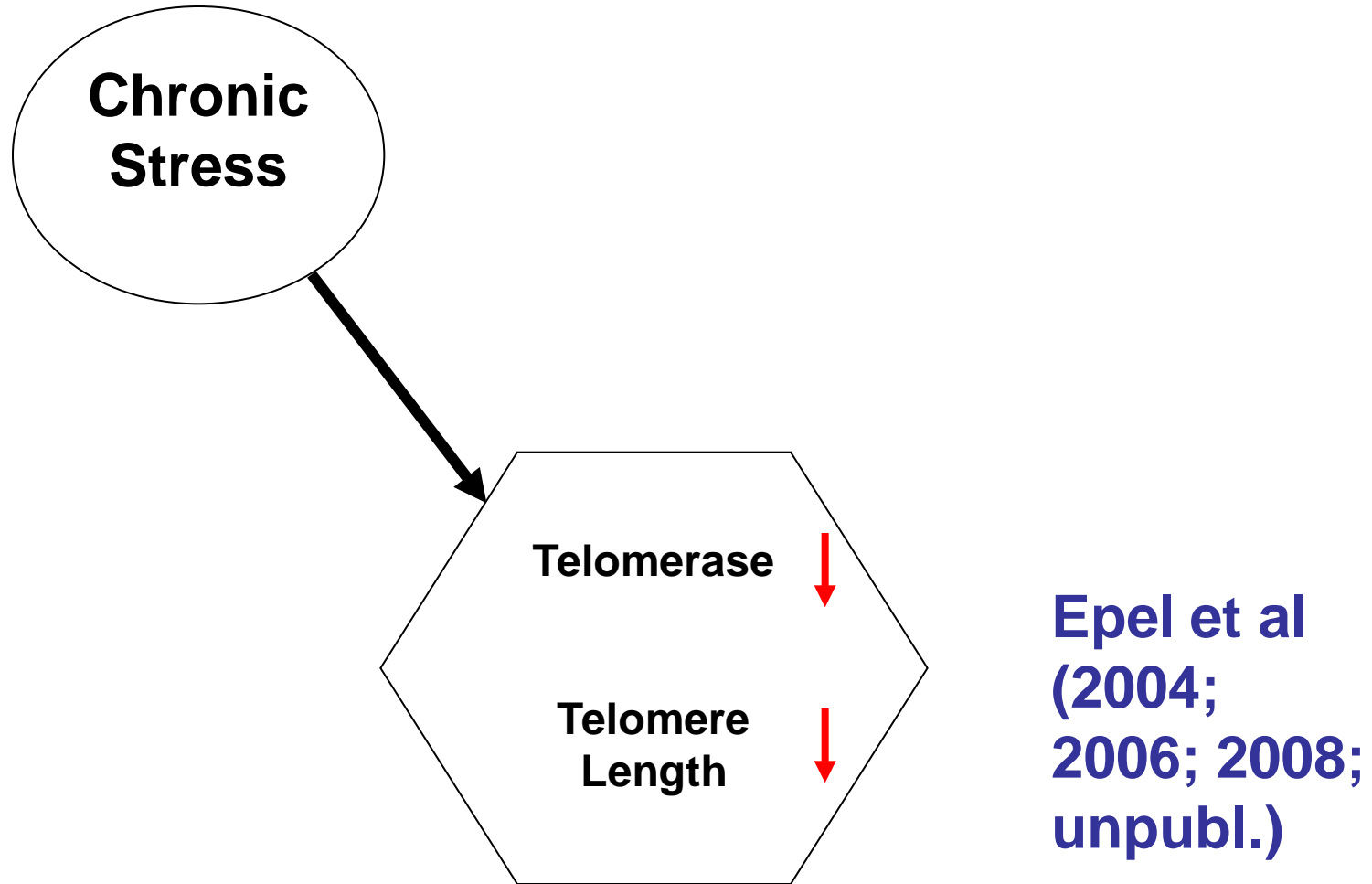


Study 1: Caregiver mothers and chronic stress

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

Chronic stress - reduces PBMC telomere length maintenance



**We and others have replicated these findings
in independent studies**

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

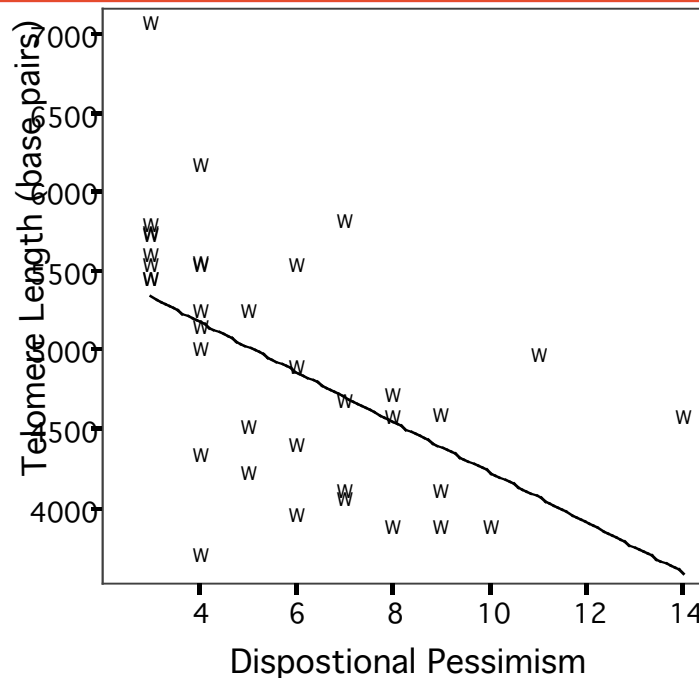


Figure 1. Association between dispositional pessimism and telomere length in post-menopausal women ($r = -.55$, $p = .001$; $n = 36$). This association appears independent of age, dispositional optimism, perceived stress, neuroticism, and health behaviors.

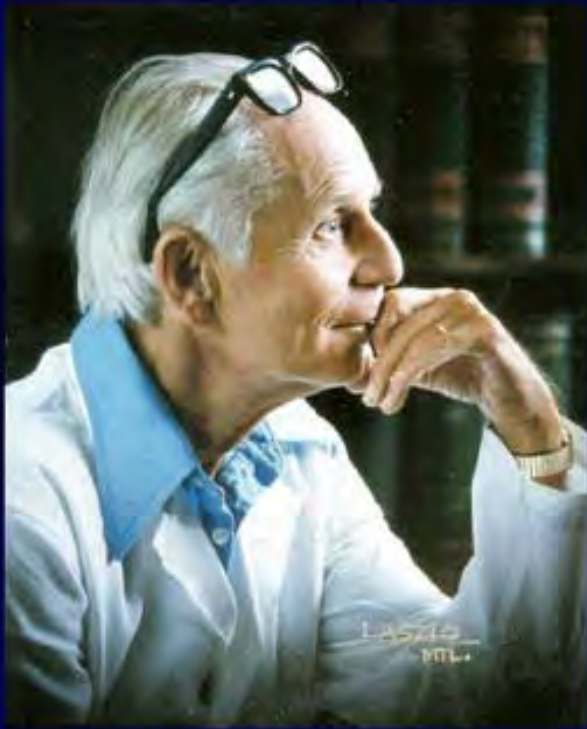
The relationship still remained excluding the one participant in the upper left quadrant, who was not a statistical outlier but who exhibited the longest mean TL ($r = -.53$, $p = .001$).

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..

HANS SELYE, MD, PhD



Picture taken by Laszlo, 1974

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

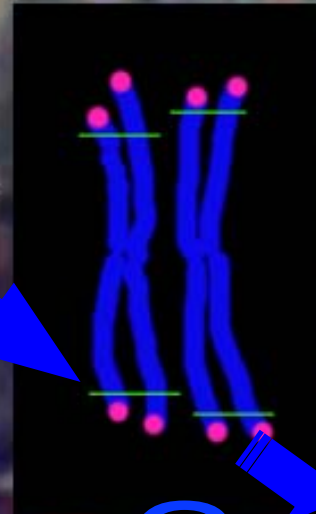
-Hans Selye

Chronic life stress wears down telomeres

Signal input



Signal
Integration and
processing



Disease
impact



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)

Brouillette, S. et al. (2003)

Benetos, A. et al. (2004)

Kurz, D. J. et al. (2006)

Starr et al (2007)

Brouillette 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

Gardner, J. P. et al. (2005)

- obesity and insulin resistance

DYNAMICS OF CHANGE?

- LONGITUDINAL STUDIES**

The Dogma was.....

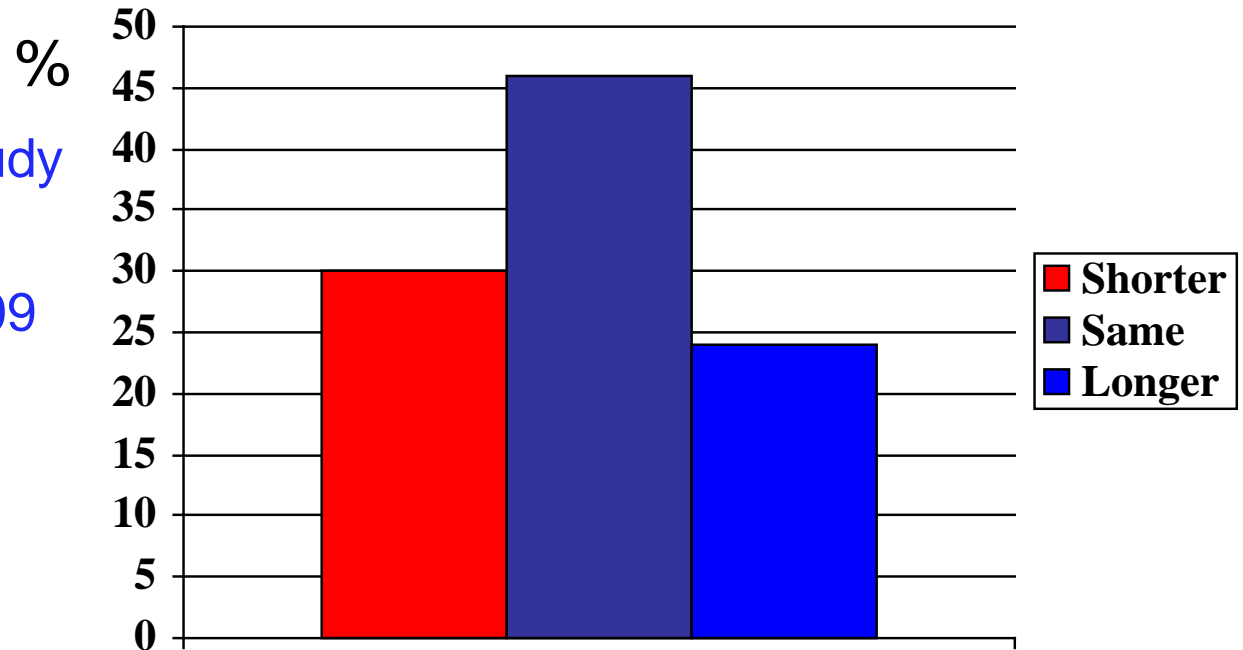
- Telomeres shorten over time, unidirectionally
 - Based entirely on cross sectional studies

The new findings

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

MacArthur Aging Study

Epel et al. Aging 2009



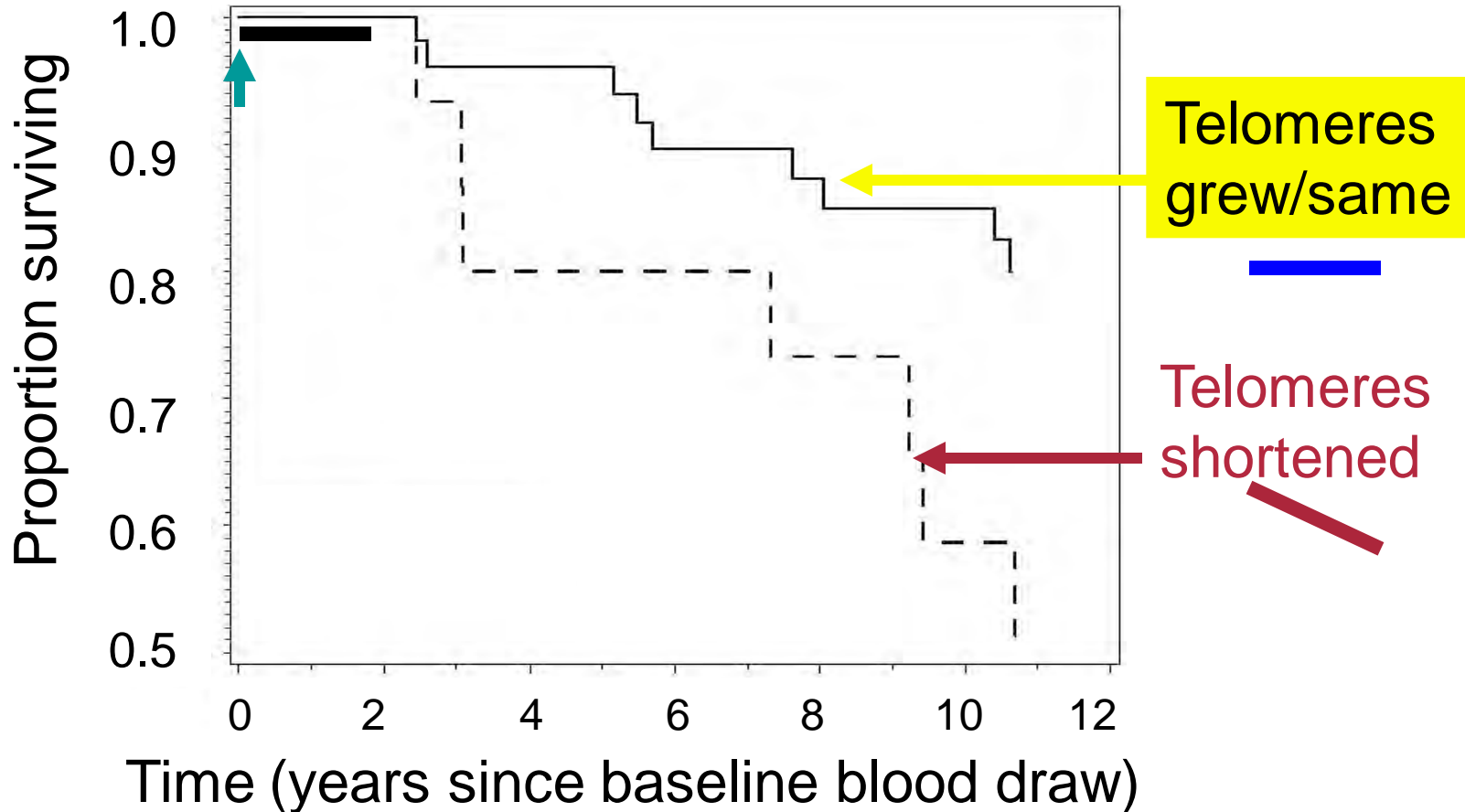
Also:

Nordfjäll K et al. PLoS Genet. 2009

Farzaneh-Far R, et al. PLOS One, in press.

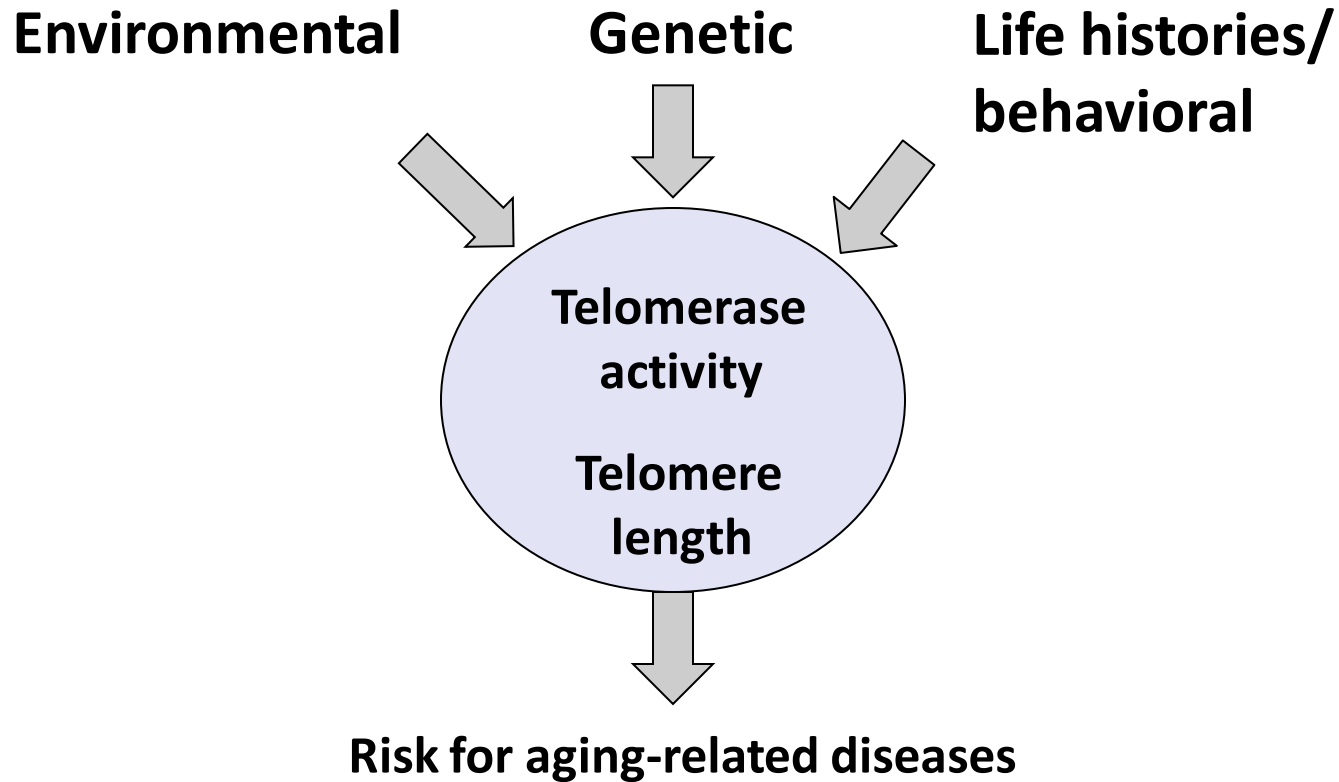
This opens the door for identifying malleable determinants of rate of change!

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).

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



Cancer
Mental disorder
Cardiovascular disease
Poor immune function

The journey...

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

to.....

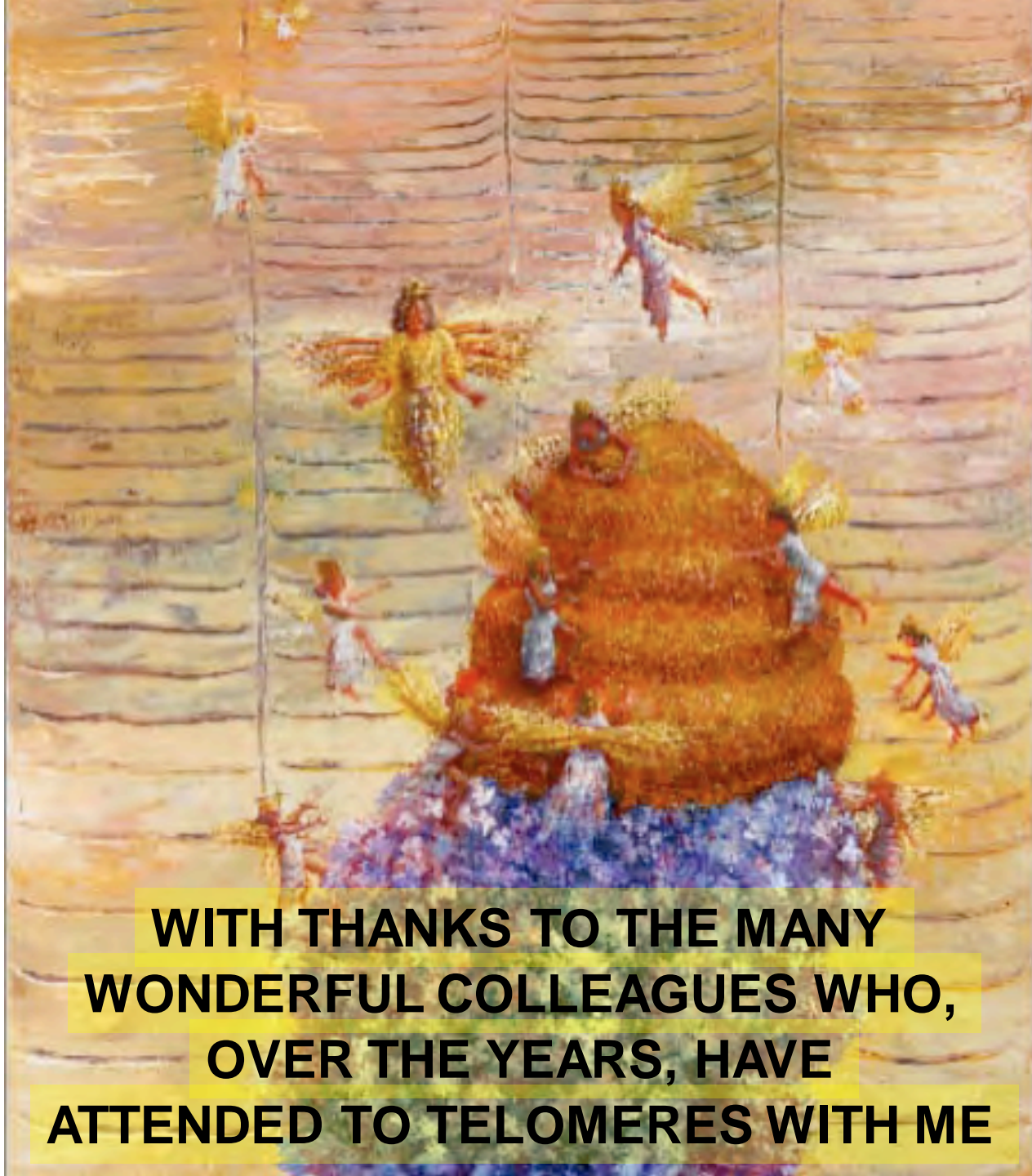
human life stories and their effects on telomere maintenance....

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*

